



# IONIC BASIS OF GABA<sub>A</sub> RECEPTOR CHANNEL FUNCTION IN THE NERVOUS SYSTEM

KAI KAILA

*Department of Zoology, Division of Physiology, University of Helsinki, P.O. Box 17, SF-00014  
Helsinki, Finland*

## CONTENTS

|  |     |
|--|-----|
| Abbreviations  | 490 |
| 1. Introduction and background   | 490 |
| 1.1. GABA: an inhibitory transmitter substance   | 490 |
| 1.2. Targets of GABA   | 490 |
| 1.2.1. Central neurons   | 491 |
| 1.2.2. Glial cells   | 491 |
| 1.2.3. Peripheral actions of GABA in vertebrates   | 491 |
| 1.2.4. GABA as a link between neuronal and hormonal control systems  | 491 |
| 1.3. Receptors for GABA  | 491 |
| 1.4. Experimental approaches in the study of GABA action   | 492 |
| 1.5. Aims of the present review  | 493 |
| 2. Structural and pharmacological properties of GABA <sub>A</sub> receptors  | 494 |
| 2.1. Structure of the GABA <sub>A</sub> receptor   | 494 |
| 2.2. Heterogeneity of GABA <sub>A</sub> receptors  | 495 |
| 2.3. Agonists, antagonists and modulators  | 495 |
| 2.4. Criteria for the identification of responses mediated by GABA <sub>A</sub> receptors  | 496 |
| 3. Ionic selectivity, conductance and gating of GABA <sub>A</sub> receptor channels  | 497 |
| 3.1. Methods   | 497 |
| 3.2. Problems related to anion-substitution experiments  | 498 |
| 3.3. Permeability of GABA <sub>A</sub> receptor channels   | 499 |
| 3.3.1. Qualitative measurements of ion permeability: injection experiments   | 499 |
| 3.3.2. Relative anion permeabilities and the limiting diameter of GABA <sub>A</sub> receptor channels                            | 500 |
| 3.3.3. Factors affecting anion permeation  | 500 |
| 3.3.3.1. Halide permeability sequences   | 501 |
| 3.3.3.2. Conductance sequences, saturation and anion interaction   | 501 |
| 3.4. Single-channel conductances   | 502 |
| 3.5. Gating of GABA <sub>A</sub> receptor channels   | 503 |
| 3.5.1. Kinetics of single GABA <sub>A</sub> receptor channels  | 503 |
| 3.5.2. Channel lifetime as a determinant of IPSC duration  | 505 |
| 3.5.3. Desensitization and rundown of GABA <sub>A</sub> receptors  | 505 |
| 3.6. Ionic modulation of GABA <sub>A</sub> receptors   | 506 |
| 3.6.1. Ca <sup>2+</sup>  | 507 |
| 3.6.2. H <sup>+</sup>  | 507 |
| 3.6.3. Zn <sup>2+</sup>  | 508 |
| 4. Membrane potential changes mediated by GABA <sub>A</sub> receptors in intact cells  | 509 |
| 4.1. Methods in the study of cellular ion regulation   | 509 |
| 4.1.1. Ion-selective microelectrodes   | 510 |
| 4.1.2. Fluorescent indicators  | 511 |
| 4.2. Generation and maintenance of the driving force of currents mediated by GABA <sub>A</sub> receptor channels                 | 511 |
| 4.2.1. Properties of ion-transport mechanisms  | 512 |
| 4.2.2. Influence of Cl <sup>-</sup> and pH regulation on E <sub>GABA<sub>A</sub></sub>   | 512 |
| 4.3. Hyperpolarizing and depolarizing responses evoked by pharmacological and synaptic activation of GABA <sub>A</sub> receptors | 515 |
| 4.3.1. Crayfish preparations   | 515 |
| 4.3.1.1. Opener muscle fibre   | 515 |
| 4.3.1.2. Crayfish stretch-receptor neurons   | 517 |
| 4.3.2. Mammalian central neurons   | 517 |
| 4.3.2.1. Exogenous GABA  | 518 |
| 4.3.2.2. Synaptically-evoked responses   | 519 |
| 4.3.3. Neonatal neurons  | 522 |
| 4.3.4. Sympathetic and sensory neurons   | 522 |
| 4.3.5. Glial cells   | 523 |

|  |     |
|--|-----|
| 5. Ionic shifts mediated by GABA <sub>A</sub> receptors                          | 523 |
| 5.1. Net movements of Cl <sup>-</sup>  | 524 |
| 5.1.1. Changes in intracellular [Cl <sup>-</sup> ]                               | 524 |
| 5.1.2. Changes in extracellular [Cl <sup>-</sup> ]                               | 524 |
| 5.2. pH shifts   | 525 |
| 5.2.1. Changes in intracellular HCO <sub>3</sub> <sup>-</sup> and H <sup>+</sup> | 525 |
| 5.2.2. Changes in extracellular HCO <sub>3</sub> <sup>-</sup> and H <sup>+</sup> | 526 |
| Acknowledgements   | 527 |
| References   | 527 |

## **ABBREVIATIONS**

|   |  |       |  |
|---|--|-------|--|
| IPSP  | inhibitory postsynaptic potential  | BZD   | benzodiazepine   |
| IPSC  | inhibitory postsynaptic current  | DMCM  | methyl-4-ethyl-6,7-dimethoxy- $\beta$ -carboline-carboxylate         |
| IPSP <sub>A</sub> and IPSP <sub>B</sub>               | IPSP mediated by GABA <sub>A</sub> and GABA <sub>B</sub> receptors   | GABA  | $\gamma$ -aminobutyric acid  |
| $E_{IPSP}$ , $E_{IPSP_A}$ and $E_{IPSP_B}$            | reversal potentials of IPSP, IPSP <sub>A</sub> and IPSP <sub>B</sub>   | HEPES | <i>N</i> -2-hydroxyethylpiperazine- <i>N'</i> -2-ethanesulfonic acid |
| $E_{Cl^-}$ , $E_{HCO_3^-}$ , $E_H$ and $E_{Na^+}$     | equilibrium potentials of Cl <sup>-</sup> , HCO <sub>3</sub> <sup>-</sup> , H <sup>+</sup> and Na <sup>+</sup> | MQAE  | <i>N</i> -(6-methoxyquinolyl) aceto-ethyl ester                      |
| $E_m$   | membrane potential   | NMDA  | <i>N</i> -methyl-D-aspartate   |
| $E_r$   | reversal potential   | PiTX  | picrotoxin   |
| pH <sub>i</sub> , pH <sub>o</sub> and pH <sub>s</sub> | intracellular, extracellular and surface pH  | SNARF | seminalphthorhodafluor   |
| BCECF   | 2',7'( <i>bis</i> -carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester                                    | SPQ   | 6-methoxy- <i>N</i> -(3-sulfonatopropyl) quinolinium                 |
|   |  | THIP  | 4,5,6,7-tetrahydroisoxazolo [5,4- <i>c</i> ]pyridin-3-ol             |

## 1. INTRODUCTION

The concept of nervous inhibition as a physiological phenomenon in its own right—not merely as a lack of excitation—was central to a number of early theories on the integrative function of the nervous system (e.g. Sechenov, 1863; Foster, 1880; Sherrington, 1906), a long time before there was any knowledge about the physiological mechanisms underlying neuronal interactions (Sherrington, 1932; Brazier, 1959; Lloyd, 1961; Wiersma, 1961). Indeed, it was not until the early 1950s that the controversy about the electrical vs. chemical mechanism of synaptic transmission ended in favour of the latter (Eccles, 1964b). In view of the lack of information on the chemical nature of nervous transmission, it is, perhaps, not surprising that the first reports showing that  $\gamma$ -aminobutyric acid (GABA) is present at high concentrations in extracts of the mammalian brain (Roberts and Frankel, 1950; Awapara *et al.*, 1950) were of little interest to neurophysiologists. GABA was primarily viewed as a possible intermediary product of glutamate metabolism (see Obata, 1977; Roberts, 1986).

### 1.1. GABA: AN INHIBITORY TRANSMITTER SUBSTANCE

Tentative results suggesting an inhibitory role for GABA in the mammalian CNS came from experiments which showed that topically applied GABA had an inhibitory effect on the electrical activity of the brain (Hayashi and Nagai, 1956). Much more direct evidence was subsequently provided by Baze-  
more *et al.* (1957) who found that GABA was the main constituent of a brain extract (Factor I) which had previously been shown to exert an inhibitory influence on the firing of the crayfish stretch-receptor neuron (Florey, 1954). The status of GABA as an inhibitory transmitter was consolidated by further

work on crustacean preparations which showed that exogenous GABA closely mimicked the postsynaptic effects evoked by selective stimulation of inhibitory axons (Boistel and Fatt, 1958; Kuffler and Edwards, 1958; Takeuchi and Takeuchi, 1965); that GABA was present in large quantities in inhibitory but not in excitatory axons (Kravitz, 1963); and that GABA was, indeed, released in a calcium-dependent manner during inhibitory activity (Otsuka *et al.*, 1966).

Early experiments on mammalian spinal neurons revealed a powerful inhibitory effect of iontophoretically applied GABA, but this was considered a "non-specific depressant action", which was unrelated to the mechanisms of nervous inhibition (Curtis *et al.*, 1959). The first observations to suggest that GABA is an important inhibitory transmitter in the mammalian CNS were made by Krnjević and collaborators (Krnjević and Schwartz, 1967; Dreifuss *et al.*, 1969) who showed that the voltage responses evoked in cat cortical neurons by iontophoretically applied GABA and by inhibitory synaptic activity had a similar reversal potential under various experimental conditions.

## 1.2. TARGETS OF GABA

During the last few decades a vast amount of experimental work has shown that GABA is a transmitter substance with a mainly inhibitory action in the nervous systems of practically all multicellular animals (for reviews, see Gerschenfeld, 1973; Krnjević, 1974; Takeuchi, 1977; Nistri and Constanti, 1979; Walker and Holden-Dye, 1989; Dingledine *et al.*, 1988; Sivilotti and Nistri, 1991; Nicoll *et al.*, 1990). Virtually all neurons within the vertebrate CNS are sensitive to the application of GABA, although it should be noted here that exogenous GABA will activate both synaptic and extrasynaptic receptors.

### 1.2.1. Central neurons

Within the mammalian CNS, GABA is the major inhibitory transmitter at supraspinal inhibitory pathways (Krnjević, 1974; Fagg and Foster, 1983; Sivilotti and Nistri, 1991). Its importance is reflected by the fact that about 20–30% of synapses employ GABA as their transmitter (Bloom and Iversen, 1971; Somogyi, 1990). The output of the cerebellar cortex is purely GABAergic (Ito and Yoshida, 1966; Ito, 1984). A straightforward demonstration of the importance of GABA-mediated inhibition in the normal function of the CNS is that a decrease in the efficacy of central GABA-mediated inhibition (regardless of whether caused by kindling, by application of GABA antagonists or by selective destruction of GABAergic neurons) leads to neuronal hyperexcitability and seizures (Schwartzkroin, 1983; Krnjević, 1983; Alger, 1984). In line with this, disturbances in GABAergic transmission are implicated in a number of pathophysiological phenomena, including epilepsy. Various experimental conditions involving a long-term reduction of GABAergic inhibition have been used to provide model systems for the study of the causes of epilepsy and of the mechanisms of action of drugs used in its treatment (Dichter, 1989; Macdonald, 1989; Kapur and Lothman, 1989).

### 1.2.2. Glial cells

A recent finding is that glial cells (astrocytes and oligodendrocytes) are equipped with transmitter-sensitive ion channels, including those gated by GABA (Kettenmann *et al.*, 1987; Barres *et al.*, 1990; Barres, 1991; Berger *et al.*, 1992). The functional significance of glial transmitter receptors is presently unknown but it has been suggested that they might play a role in the regulation of local ionic activities within synaptic clefts.

### 1.2.3. Peripheral actions of GABA in vertebrates

In contrast to the situation regarding invertebrate species where much of the pioneering work on GABA-mediated inhibition was conducted on peripheral synapses, it was a common belief until fairly recently that in mammalian and other vertebrate species, GABA acts as a transmitter substance within the CNS only. In fact, the first observation of a peripheral action of GABA in a mammal was made by De Groat (1970) working on feline autonomic ganglia. By now, the idea of an exclusively central role of GABA has been superseded by a multitude of findings which show that enzymes for the synthesis and uptake of GABA, as well as GABA receptors (both GABA<sub>A</sub> and GABA<sub>B</sub>), are widely spread in the autonomic nervous system and in a number of neuroendocrine, endocrine and exocrine cells (for review, see Erdö and Wolff, 1990). Although a detailed description of peripheral GABAergic mechanisms is not within the scope of the present article, it may be of interest to note that GABA works as an inhibitory transmitter in certain non-neuronal cells *within* the endocrine system (Rorsman *et al.*, 1989; Sorenson *et al.*, 1991).

### 1.2.4. GABA as a link between neuronal and hormonal control systems

A wealth of evidence indicates that GABA acts as an important bilateral link between the nervous system and the endocrine system. One aspect of this interaction is that various neuroendocrine and endocrine cells receive a GABAergic innervation and show responses mediated by GABA<sub>A</sub> and GABA<sub>B</sub> receptors (e.g. Nicoll and Barker, 1971; Demeneix *et al.*, 1986; Williams *et al.*, 1989; Randle and Renaud, 1987; Peters *et al.*, 1989). On the other hand, certain steroid hormones and steroid hormone metabolites are capable of potentiating the inhibitory actions of GABA through a modulatory effect on the GABA<sub>A</sub> receptor (Majewska *et al.*, 1986; Morrow *et al.*, 1990; Twyman and Macdonald, 1992).

## 1.3. RECEPTORS FOR GABA

The molecule of GABA is conformationally very flexible and its ability to adopt different shapes is essential for its ability to bind to sterically distinct GABA recognition sites. By systematic variations of structural properties, a large number of conformationally restricted GABA analogs have been developed which act as selective agonists/substrates or antagonists/inhibitors at GABA<sub>A</sub> and GABA<sub>B</sub> receptors and at uptake systems in neuronal and glial cells (Krogsgaard-Larsen, 1980, 1988). Muscimol and baclofen are widely used selective agonists of GABA<sub>A</sub> and GABA<sub>B</sub> receptors (Fig. 1).

The early work on GABA suggested that its synaptic actions are mediated by a single class of receptors, which upon activation open up channels mainly permeable to chloride. This effect underlies the phenomenon now known as "conventional" or "classical" GABAergic inhibition, first described at synapses in the peripheral nervous system of crustaceans (Boistel and Fatt, 1958; Kuffler and Edwards, 1958; Takeuchi and Takeuchi, 1965, 1966b) and later found to operate at countless inhibitory synapses within the vertebrate brain. A similar ionic mode of action is also characteristic of strychnine-sensitive glycinergic inhibition in vertebrate neurons.

An important pharmacological criterion in the identification of responses mediated by the "classical" GABA-gated receptor-channel was its sensitivity to the blocking action of picrotoxin and, especially in vertebrate species, to bicuculline (Nistri and Constanti, 1979). Later, receptors which mediated effects of GABA that were insensitive to bicuculline and mimicked by baclofen (but not by muscimol) were detected in vertebrates, first in peripheral tissue preparations but subsequently also in the CNS (Hill and Bowery, 1981; Bowery *et al.*, 1980; Bowery, 1989). This led to the present nomenclature, where the classical GABA receptor and its integral anion channel is termed the GABA<sub>A</sub> receptor (or GABA<sub>A</sub> receptor channel), while the one activated by baclofen is the GABA<sub>B</sub> receptor. GABA receptors with functions similar to those of vertebrate GABA<sub>B</sub> receptors are present also in invertebrate nervous systems (Miwa *et al.*, 1990).

In evolutionary terms, GABA<sub>A</sub> and GABA<sub>B</sub> receptors have little in common. The GABA<sub>A</sub> receptor is

a member of the ligand-gated ion channel family of receptors, typified by the nicotinic acetylcholine receptor, while  $\text{GABA}_\text{B}$  receptors are functionally coupled to certain  $\text{K}^+$  and  $\text{Ca}^{2+}$  channels via GTP-binding proteins (G proteins) and/or other messengers. The electrophysiological responses mediated by  $\text{GABA}_\text{B}$  receptors are brought about by an increase in the  $\text{K}^+$  permeability or by a decrease in  $\text{Ca}^{2+}$  currents in the target cell (Dolphin, 1990; Nicoll *et al.*, 1990; Gage, 1992).

#### 1.4. EXPERIMENTAL APPROACHES IN THE STUDY OF GABA ACTION

A large number of techniques have contributed to our current understanding of the role of various ions in the physiological and biophysical actions of GABA. These include conventional voltage- and current-clamp techniques, fluctuation (noise) analysis, patch-clamping of whole cells and membrane fragments, ion-selective microelectrodes, tracer techniques and

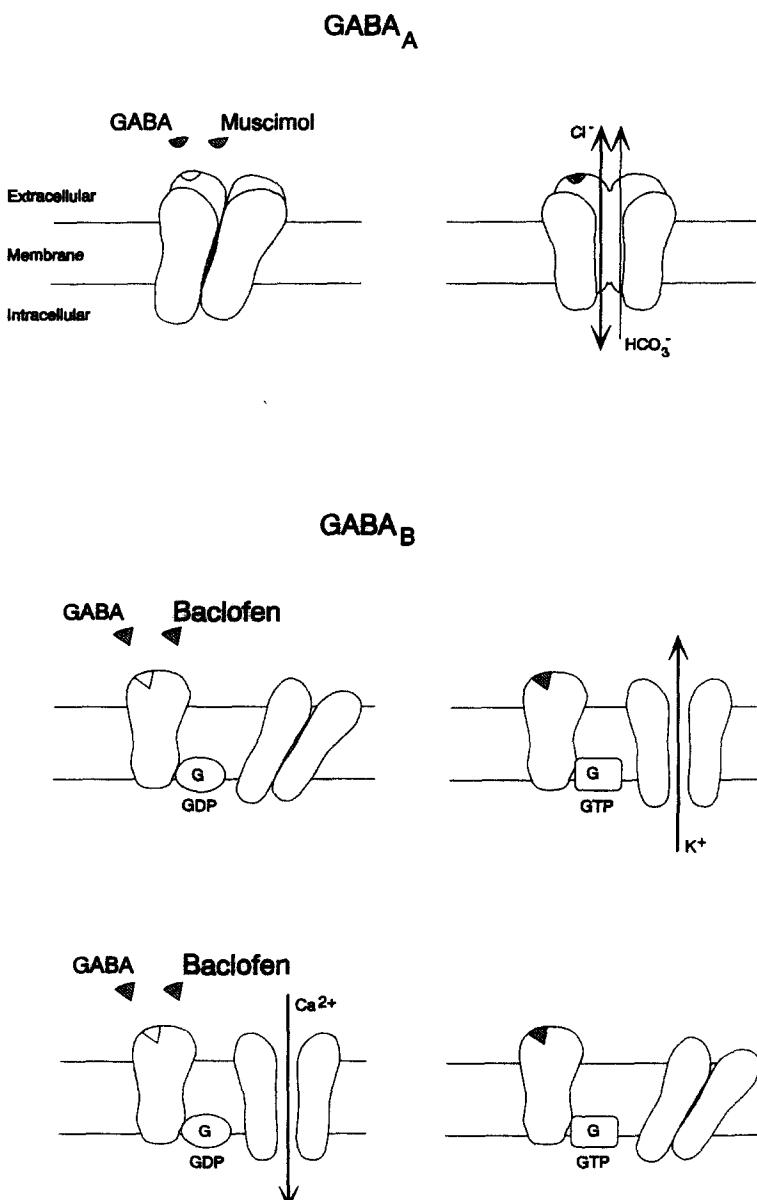


FIG. 1. A simplified scheme of the consequences of the activation of  $\text{GABA}_\text{A}$  and  $\text{GABA}_\text{B}$  receptors. The former leads to opening of an anion channel and the latter to opening of a  $\text{K}^+$  channel or to inhibition of a  $\text{Ca}^{2+}$  channel. The ion channel associated to the  $\text{GABA}_\text{A}$  receptor is an integral part of the receptor-channel complex, while the signal of the  $\text{GABA}_\text{B}$  receptor is initially transduced by a G protein. The arrows indicate movements of the permeant ions. At the level of the resting membrane potential,  $\text{Cl}^-$  can (depending on mechanisms regulating intracellular chloride) move either in or out across the  $\text{GABA}_\text{A}$  receptor channel, but the electrochemical gradient for  $\text{HCO}_3^-$  is always outwardly directed.

fluorescence (micro)spectrometry with ion indicators. Some of these techniques will be briefly described below in the relevant sections. An even larger diversity exists in the choice of research objects and it would not be meaningful (or even possible) to try and list them systematically, but a few examples might be mentioned to give an idea about the variety of preparations which have been of particular importance.

The central role played by crustacean preparations in the work on the ionic mechanisms involved in the actions of GABA (e.g. Boistel and Fatt, 1958; Takeuchi and Takeuchi, 1967) has been mainly due to the numerous technical advantages offered by them: the good viability of the preparations *in vitro*; the easy accessibility and clear-cut microanatomy of the inhibitory axons and the target (nerve or muscle) cells; the large size of the GABA-sensitive cells, which allow prolonged and stable recordings with intracellular microelectrodes (conventional as well as ion-selective); and the lack of significant barriers for drug delivery.

The early work carried out on crustacean preparations was paralleled by studies on GABAergic mechanisms in mammalian neurons *in vivo* (e.g. Kandel *et al.*, 1961; Krnjević and Schwartz, 1967). However, detailed investigations of synaptic function necessitate prolonged stable recordings, which are difficult to obtain *in vivo* where cardio-respiratory functions are bound to cause movements of brain tissue. In more recent times, the development of brain-slice techniques (Kerkut and Wheal, 1989; Dingledine, 1984a) has permitted detailed electrophysiological analysis *in vitro* of fully differentiated brain tissue of mammals and other vertebrates. Due to its laminar structure and well-defined neuronal circuitry, the transverse hippocampal brain slice has been a particularly useful preparation (Skrede and Westgaard, 1971; Johnston and Brown, 1984; Nicoll, 1988).

The choice of preparation largely dictates the means for experimental variation of the extracellular ionic environment and of drug application, i.e. whether done using iontophoresis, pneumatic (puff or microdrop) methods, bath application or with fast-perfusion ("concentration clamp") techniques. A drawback of brain slices is that, due to the relatively low volume fraction (about 20%) and tortuosity of the interstitial space, the equilibration of ions and drugs upon a change in their concentration in the perfusion medium is rather slow (Nicholson and Phillips, 1981; Nicholson and Rice, 1986). When working with transmitter compounds, this problem is accentuated by the presence of powerful uptake mechanisms (Nicholson and Rice, 1991).

Problems related to diffusion barriers are largely avoided in work on acutely isolated or cultured neurons and glial cells. Primary cell cultures obtained from dissociated neurons or glia (or both) maintained in culture establish synaptic connections which are readily accessible (e.g. Segal, 1983). Organotypic cultures (Gähwiler, 1981), where explants or slices of nervous tissue are cultivated for several weeks, are primary cultures which can, perhaps, be viewed as intermediates between brain slices and dispersed-cell cultures and their neuronal connection

patterns resemble those seen *in situ*. Due to the thinness of organotypic cultures, diffusional barriers are virtually absent and individual cells are easily visualized.

A serious problem with primary neuronal cultures is their heterogeneity (especially the variation seen between cells cultured in different laboratories) and limited life span. These difficulties do not arise in work on continuous tumour cell lines. However, the possibility of abnormal or uncontrolled variation in the patterns of gene expression in cells of both primary and continuous cultures makes them, in several respects, inferior to acutely isolated native cells.

### 1.5. AIMS OF THE PRESENT REVIEW

As stated by Dingledine (1984b) in a review 10 years ago, the ionic mechanism of the action of GABA in the mammalian hippocampus is uncertain. Although this statement mainly referred to the dendritic responses mediated by GABA<sub>A</sub> receptors in hippocampal pyramidal neurons, it is not far fetched to claim that it holds to practically all (both synaptic and pharmacologic) GABA<sub>A</sub> responses in vertebrate central neurons under physiological conditions.

A number of indirect approaches (usually measurements of reversal potentials) have been used in attempts to analyze the ionic basis of GABA<sub>A</sub> receptor-mediated responses. However, taken together, work of this type reveals a genuine egg-and-hen problem: in the study of neuronal Cl<sup>-</sup> regulation, the reversal potential of GABA<sub>A</sub> receptor-mediated currents and voltage responses ( $E_{GABA,A}$ ) has been frequently used as an estimate of  $E_{Cl^-}$  (the chloride equilibrium potential). Information of this kind has, thereafter, been used by other authors in attempts to interpret GABA-evoked responses in terms of ionic mechanisms. This paradoxical situation is a simple consequence of the scarcity of direct information on ion concentrations within native mammalian central neurons. It is also apparent here that the classical work by Eccles and coworkers (Coombs *et al.*, 1955; Eccles, 1964b) on spinal (glycinergic) inhibitory mechanisms has had an enormous impact on the theoretical concepts employed in studies of GABAergic inhibition in the brain. Hence, it is perhaps not surprising that the expression *hyperpolarizing inhibition* is frequently used in papers where the actual measurements show that most (if not all) fast GABAergic inhibitory postsynaptic potentials (IPSPs) are depolarizing!

An examination of the relevant literature reveals that the polarity and amplitude of voltage transients evoked by GABA<sub>A</sub> receptors show an enormous diversity. A marked use-dependency of GABAergic effects has also frequently been reported and it has recently become clear that use-dependent changes in the efficacy of GABAergic inhibition play a central role in various plasticity mechanisms (Davies *et al.*, 1991; Alger, 1991; see also Wigström and Gustafsson, 1983) as well as in pathological manifestations within the nervous system. Although presynaptic GABA<sub>B</sub> autoreceptors seem often to be largely responsible for use-dependent changes in the efficacy of inhibition (e.g. Deisz and Prince, 1989; Davies *et al.*, 1990), it is likely that an activity-induced dissipation of ionic gradients can contribute to the loss of inhibitory

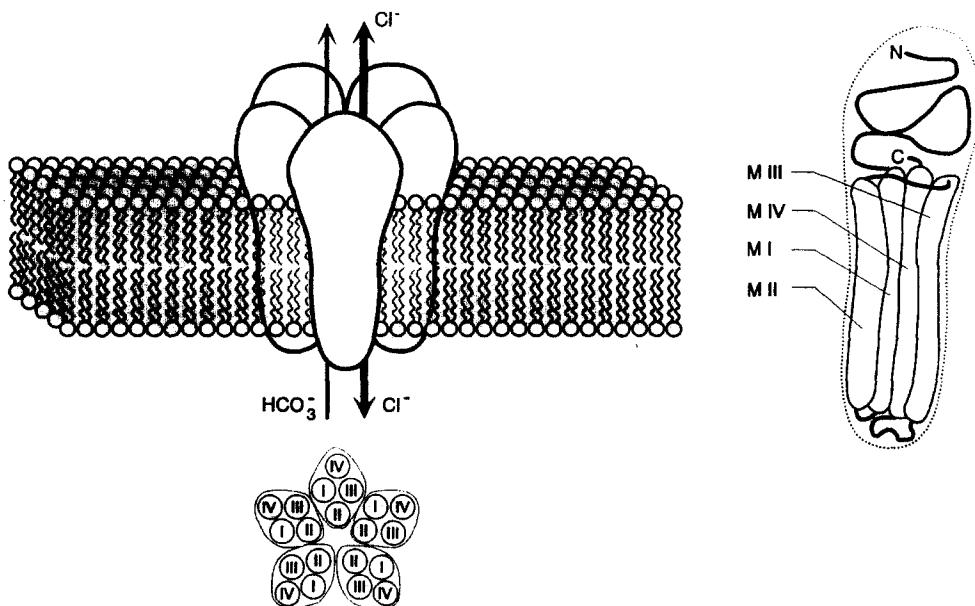


FIG. 2. Diagram of the structure of the GABA<sub>A</sub> receptor channel. The left part of the figure shows a complete pentameric receptor-channel complex partly embedded in the lipid matrix of the cell membrane. A single subunit with its four membrane-spanning domains (M I–M IV) is shown on the right. Note critical positioning of M II in relation to the transmembrane channel depicted in the cross-sectional scheme on the bottom (for references, see text).

drive. On a much wider temporal scale, i.e. during prenatal and early postnatal development, GABA<sub>A</sub> receptor-mediated synaptic transmission undergoes dramatic changes (Swann *et al.*, 1989; Ben-Ari *et al.*, 1990; Luhmann and Prince, 1991). For instance, in the neonatal rat hippocampus, most of the excitatory transmission is mediated by GABA<sub>A</sub> receptors and the available evidence suggests that GABA exerts a trophic action through membrane depolarization which promotes an increase in intracellular Ca<sup>2+</sup> (see Cherubini *et al.*, 1991).

The main theme of the present article is to examine the role of the ionic selectivity of GABA<sub>A</sub> receptor channels and of the properties of the cellular ion regulatory mechanisms, in the shaping of the diverse patterns of GABA<sub>A</sub> receptor-mediated responses. It is worth noting here also that ions do not always act merely as carriers of charge across ionic channels. There are numerous cases where the functional properties of membrane channels are modulated by physiological changes in local ion concentrations. As will be discussed below, such effects are likely to be important in the modulation of GABA<sub>A</sub> receptors.

## 2. STRUCTURAL AND PHARMACOLOGICAL PROPERTIES OF GABA<sub>A</sub> RECEPTORS

Mammalian central neurons express a larger fraction of the genome than any other cell type within the organism (Milner and Sutcliffe, 1983) and recent times have witnessed an incredible rate of expansion of molecular biological techniques and knowledge within the field of neurobiology. These techniques have been successfully combined with electrophysio-

logical methods which has resulted in an incarnation of purely hypothetical models of ion-channel function into the chemistry of structure and allosteric regulation of protein molecules. *Xenopus* oocytes (e.g. Schofield *et al.*, 1987) and transformed human embryonic kidney cells (e.g. Pritchett *et al.*, 1988) have been extensively used for transient expression of mRNAs and cDNAs coding for GABA<sub>A</sub> receptor subunits. Inducible, stable expression has been obtained in cultured hamster ovary cells (Moss *et al.*, 1990).

### 2.1. STRUCTURE OF THE GABA<sub>A</sub> RECEPTOR

The GABA<sub>A</sub> receptor is a hetero-oligomeric protein molecule composed of several distinct polypeptide types,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\rho$  (for review, see Stephenson, 1988; Olsen and Tobin, 1990; Wisden and Seuberg, 1992). Cloning of the GABA<sub>A</sub> receptor channel showed that it belongs to the gene superfamily of ligand-gated ion channels (Schofield *et al.*, 1987; Barnard *et al.*, 1987). The members of this superfamily (which include nicotinic acetylcholine, GABA<sub>A</sub>, glycine and 5HT<sub>3</sub> receptors) are thought to be built in the manner depicted in Fig. 2. Both structural and functional investigations suggest that the different properties of various ligand-gated receptor channels are based on variation in a small number of key amino acid side chains on a common polypeptide backbone. A single GABA<sub>A</sub> receptor consists presumably of five subunits, each contributing to the walls of the transmembrane ion channel. The subunits each have four putative membrane-spanning hydrophobic sequences (M I–M IV).

There is much evidence, including mutagenesis

experiments on nicotinic ACh receptors, that MII provides a key contribution to the walls of the transmembrane ion channel. Indeed, the introduction by site-directed mutagenesis of three amino acids from the MII segment of glycine or GABA<sub>A</sub> receptors into the MII segment of  $\alpha_7$  nicotinic receptor converted its ionic selectivity from cationic to anionic (Galzi *et al.*, 1992).

It has been suggested that channel opening is the result of concerted tilting of the subunits, that is, rotation about an axis parallel to the membrane plane (Unwin, 1989). The selection among monovalent anions is predominantly due to the action of the ionic filter located in the most constricted part of the channel (Bormann *et al.*, 1987; see also Unwin, 1989; Sakmann, 1992).

## 2.2. HETEROGENEITY OF GABA<sub>A</sub> RECEPTORS

cDNA cloning has revealed the existence of a number of distinct subunit isoforms ( $\alpha_1, \alpha_2, \alpha_3 \dots \beta_1, \beta_2 \dots$  etc.). Based on the number of subunits and their isoforms, there is an immense number of theoretically possible ways to build distinct GABA<sub>A</sub> receptors. Although it is probable that only a fraction of this potential variability manifests itself in native receptors (Wisden and Seuberg, 1992), this structural heterogeneity has turned out to be of much importance since GABA<sub>A</sub> receptors with distinct subunit combinations have been found to exhibit considerable functional variation. In addition to variations in their pharmacology, differences in the intrinsic electrophysiological properties (gating, conductance) have also been reported (e.g. Verdoorn *et al.*, 1990; Sigel *et al.*, 1990).

There is much evidence indicating considerable regional differences in the expression of GABA<sub>A</sub> receptor subtypes in the mammalian brain and the possibility exists that heterogeneous populations of GABA<sub>A</sub> receptors may be expressed in individual neurons or glial cells (Wisden *et al.*, 1988; Olsen and Tobin, 1990). This situation is made even more complex by alterations in subunit expression during development (Killisch *et al.*, 1991; Meinecke and Rakic, 1990; Poulter *et al.*, 1992).

Until recently, it was not known to what extent the "conventional" invertebrate and vertebrate GABA-gated anion channels are homologous, although the astonishing similarity in the electrophysiological and biophysical properties of at least certain well-studied crustacean receptor channels and their mammalian counterparts suggests a high degree of structural homology (see Section 3.3). A striking similarity between vertebrate and (at least some) invertebrate subunits is evident from coexpression experiments which have shown that molluscan  $\beta$  subunits are capable of replacing mammalian  $\beta$  subunits thus forming chimeric receptors with mammalian  $\alpha$  subunits (Harvey *et al.*, 1991; Darlison, 1992).

## 2.3. AGONISTS, ANTAGONISTS AND MODULATORS

With regard to allosteric modulation of its activity, the versatility of vertebrate GABA<sub>A</sub> receptors seems to surpass all other ligand-gated channels except, perhaps, the N-methyl-D-aspartate (NMDA) sensi-

tive subtype of glutamate receptors (see Nicoll *et al.*, 1990; Gasic and Hollmann, 1992). A large number of allosteric modulatory sites that bind both endogenous and exogenous ligands (hormones, drugs) have been structurally and functionally characterized. In addition, the GABA<sub>A</sub> receptor is modulated by phosphorylation factors and by certain cations, notably H<sup>+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup> (Section 3.5.3). Combined biochemical, electrophysiological and pharmacological studies on brain tissue and on expression systems using cDNA encoding various GABA<sub>A</sub> receptor subunits have demonstrated a striking variability in the localization of various physiologically and pharmacologically important binding sites in different subunits and subunit combinations.

Work on homomeric recombinant receptors has shown that there is no particular subunit which is obligatory for channel gating by GABA. However, marked differences in the affinity and cooperativity of GABA binding among receptors composed from various types (and stoichiometries) of subunits have been observed (Levitin *et al.*, 1988; Sigel *et al.*, 1990; Verdoorn *et al.*, 1990). Native GABA<sub>A</sub> receptors are thought to be activated by the cooperative action of two molecules of GABA (Takeuchi and Takeuchi, 1967; Dudel, 1977; Bormann and Clapham, 1985; Akaike *et al.*, 1986) which has not been evident in some of the recombinant receptors (e.g. Levitan *et al.*, 1988). The presence of a  $\gamma_3$  subunit in place of  $\gamma_2$  in  $\alpha_1\beta_2\gamma_3$  leads to a marked decrease in the affinity of agonists (Herb *et al.*, 1992).

Bicuculline (Curtis *et al.*, 1971) is a competitive GABA<sub>A</sub> antagonist acting at the recognition site (particularly in vertebrates) while picrotoxin (PiTX) is a mainly noncompetitive antagonist that acts at another binding site on the receptor complex (Takeuchi and Takeuchi, 1969; Barker *et al.*, 1983; Newland and Cull-Candy, 1992).

GABA<sub>A</sub> receptors are subject to modulation by a number of allosteric ligands, including steroids (Majewska *et al.*, 1986), barbiturates (Study and Barker, 1981) and benzodiazepines (Haefely, 1984). All these compounds act at distinct recognition sites on the receptor (Puia *et al.*, 1990; Twyman and Macdonald, 1992) and there is evidence that, *in situ*, the steroid and benzodiazepine sites can bind both exogenous and endogenous ligands (e.g. Majewska *et al.*, 1986; Alho *et al.*, 1985; Unseld *et al.*, 1990).

The benzodiazepine (BZD) site is peculiar in that it allows both an enhancement and a reduction of GABA<sub>A</sub> receptor function (Haefely, 1984; Vicini *et al.*, 1988). The BZD group of allosteric modulators comprises three functionally different types of compounds. *BZD agonists* (or positive modulators; e.g. diazepam) enhance the conductance evoked by activation of GABA<sub>A</sub> receptors; *inverse agonists* (negative modulators, e.g.  $\beta$ -carbolines such as DMCM (methyl-4-ethyl-6,7-dimethoxy- $\beta$ -carboline-carboxylate)) have the opposite effect; and competitive *BZD antagonists* lack an effect of either kind but they block the effects caused by agonists and inverse agonists. The agonist-antagonist terminology (cf. Richards *et al.*, 1986) has been subject to criticism (Costa *et al.*, 1988), mainly because the allosteric modulators have no effect on the GABA<sub>A</sub> receptor in the absence of GABA. With regard to the actions of benzo-

diazepines on recombinant  $\text{GABA}_A$  receptors, a  $\gamma$  subunit variant ( $\gamma_2$ ) has to be coexpressed with certain  $\alpha$  subunits in order to reproduce the pharmacological profiles of native receptors (Pritchett *et al.*, 1988; von Blankenstein *et al.*, 1990). Recent work suggests that a single histidine residue is essential for BZD agonist binding (Wieland *et al.*, 1992).

Large variations from conventional BZD pharmacology (which is mainly applicable to vertebrate neurons) is evident among different animal species and cell types, as well as during development. For instance, in frog sensory neurons, flumazenil (a widely-used BZD antagonist), slightly enhances GABA responses (Yakushiji *et al.*, 1989a); the inverse BZD agonist, DBCM, produces an enhancement of effects mediated by native  $\text{GABA}_A$  receptors in cultured rat astrocytes (Backus *et al.*, 1988); and some mammalian recombinant receptors (of the type  $\alpha_1\beta_1$ ) have been shown to respond to BZDs, but in this case the action of all three kinds of BZDs has been of the agonist type (Malherbe *et al.*, 1990). In contrast to observations on adult rats, BZD agonists do not potentiate responses to exogenous GABA in neonatal hippocampal neurons (Rovira and Ben-Ari, 1991).

An important consequence of observations of functional and pharmacological differences among cloned receptors with defined subunit compositions is that they are likely to provide markers for electrophysiological investigations of the subunit composition of native  $\text{GABA}_A$  receptors (cf. Draguhn *et al.*, 1990; Smart *et al.*, 1991).

#### 2.4. CRITERIA FOR THE IDENTIFICATION OF RESPONSES MEDIATED BY $\text{GABA}_A$ RECEPTORS

Classification of transmitter receptors and receptor subtypes must ultimately rely on their molecular biological properties. However, in electrophysiological experiments, pharmacological interventions will remain important in the identification of physiological responses mediated by GABA receptors and their subtypes. An examination of the criteria used to identify GABA receptors is of particular importance in the present review which attempts to assimilate results from studies on both vertebrates and invertebrates.

Invertebrate  $\text{GABA}_A$  receptors are generally rather insensitive to the competitive GABA antagonist, bicuculline (Takeuchi and Onodera, 1972) and most of those examined are not affected by benzodiazepines (Simmonds, 1983; Walker and Holden-Dye, 1989; but see Sattelle *et al.*, 1988; Zaman *et al.*, 1992). In the light of the available information, a set of diagnostic criteria for the functional identification of electrophysiological responses mediated by  $\text{GABA}_A$  receptor channels include the following:

(i) GABA and muscimol act as agonists to induce a conductance.

(ii) The GABA/muscimol-induced conductance is blocked by picrotoxin.

(iii) Under physiological conditions, the GABA/muscimol-induced conductance is mainly due to  $\text{Cl}^-$ .

As a sterically rigid  $\text{GABA}_A$  agonist, muscimol is a poor substrate for GABA uptake (Krosgaard-Larsen, 1988) and, in comparison to exogenous GABA, it is therefore expected to induce voltage and

current responses more specifically related to  $\text{GABA}_A$  receptor activation (e.g. Kaila *et al.*, 1992b). The advantage of using the non-competitive antagonist PiTX as a tool for identification of  $\text{GABA}_A$  receptor-mediated responses is underscored by the fact that, in terms of subunit isoform requirements, the PiTX binding site is relatively nonspecific (Sigel *et al.*, 1990).

While it is a well known fact that most vertebrate  $\text{GABA}_A$  receptors share a number of additional properties including benzodiazepine modulation, inhibition by bicuculline and potentiation by pentobarbital, there are frequent deviations from this pattern.

With regard to the diagnostic criteria (i–iii) above, it is likely that any receptor satisfying all of them is a  $\text{GABA}_A$  receptor. However, the converse is not necessarily true (note that necessary criteria do not define a logical identity—one would need to know both the necessary *and* sufficient criteria to establish a true identity). In other words, it is fully possible that receptors do exist, which on molecular biological grounds belong to the  $\text{GABA}_A$  class, but which do not satisfy the above set of functional criteria.

In this context, it is interesting to note that certain GABA receptors in vertebrate visual pathways exhibit unique pharmacological properties. An aberrant GABA receptor was found by Sivilotti and Nistri (1989) in the frog optic tectum. Although the GABA-evoked response mediated by this receptor was dependent on  $\text{Cl}^-$ , mimicked by muscimol and blocked by PiTX, it was not affected by bicuculline and BZDs (and not by baclofen, either). Likewise, a  $\text{GABA}_A$  receptor from bovine retina, expressed in *Xenopus* oocytes, was insensitive to bicuculline (and to the  $\text{GABA}_B$  agonist, baclofen) and showed no modulation by barbiturates or benzodiazepines (Polenzani *et al.*, 1991; Woodward *et al.*, 1992). The response mediated by this receptor was a  $\text{Cl}^-$  current that was blocked by picrotoxin and showed little desensitization. GABA responses that are insensitive to both bicuculline and baclofen have been termed  $\text{GABA}_C$  by Johnston (e.g. Drew *et al.*, 1984). Recent studies employing *Xenopus* oocytes (Shimada *et al.*, 1992) indicate that the pharmacologic profile of  $\text{GABA}_C$  receptors is attributable to expression of the  $\rho_1$  GABA receptor subunit (Cutting *et al.*, 1992). A comparison of the structure of the  $\rho_1$  cDNA has revealed that, among known ligand-gated channels, its closest homology is with other vertebrate  $\text{GABA}_A$  receptor subunits, although it is more divergent from this group than any other member (Cutting *et al.*, 1992). On purely structural grounds it would therefore be appropriate to use a parsimonious classification where the  $\text{GABA}_C$  receptor is referred to as  $\text{GABA}_{A,\rho_1}$  (Shimada *et al.*, 1992).

From an evolutionary point of view, it may be of interest that several of the pharmacological properties as well as the absence of prominent desensitization that are typical for  $\text{GABA}_C$  receptors are also characteristic of certain well studied crayfish GABA receptors (Takeuchi and Takeuchi, 1967, 1969; Takeuchi and Onodera, 1972). As to the agonist selectivity of crustacean  $\text{GABA}_A$  receptor channels, experiments on excised patches of crayfish muscle fibers suggested rather surprisingly that glutamate is a potent GABA agonist and that glutamate and

GABA show competition in the activation of the receptor (Franke *et al.*, 1986). These findings are in sharp contradiction to previous results (e.g. Takeuchi and Takeuchi, 1965) which had not revealed any effect of glutamate on GABA-evoked currents. Due to the central role played by crayfish preparations in work on the mechanisms of GABA-mediated inhibition, a re-examination of the action of glutamate in intact cells was found worth conducting (Pasternack *et al.*, 1992b). The results showed that even under conditions known to block glutamate uptake, the dependence on concentration of the GABA-induced Cl<sup>-</sup> conductance was not detectably affected by the continuous presence of a high concentration (up to 1 mM) of glutamate. The cause of the aberrant results obtained in the patch-clamp experiments (see also Zufall *et al.*, 1988) are not clear, but they may have resulted from the enzymatic procedures that were required to obtain patches suitable for gigaseal formation from crayfish muscle fibers.

### 3. IONIC SELECTIVITY, CONDUCTANCE AND GATING OF GABA<sub>A</sub> RECEPTOR CHANNELS

#### 3.1. METHODS

The biophysical aspects of GABA<sub>A</sub> receptor function have been examined using a number of different approaches, including microelectrode voltage-clamp and voltage-jump measurements as well as fluctuation analysis and patch clamping. Since the two latter techniques have permitted extensive investigations of receptor channel function at its microscopic level, they will be briefly described here.

*Fluctuation analysis* (or noise analysis) has its origin in the observation made by Katz and Miledi (1970) that the depolarization induced by acetylcholine at the frog neuromuscular junction was linked to an increase in the base line voltage noise. This observation suggested that the number of open channels fluctuates in a random manner as the transmitter molecules collide with their receptors. The above technique allowed estimation of the amplitude of the elementary voltage contributions provided by a single open channel. A major advance in the technique of fluctuation analysis was made by Anderson and Stevens (1973) who used voltage clamping to measure current fluctuations instead of voltage fluctuations. This increases the recording bandwidth since the low-pass filtering effect due to membrane capacitance is eliminated. At a low agonist concentration, the *single-channel current* (*i*) is obtained from the ratio (variance of the agonist-induced current)/(mean agonist-induced current) and the *mean single-channel conductance* (*g*) is then given by  $g = i/(\text{driving force})$ , where the driving force ( $= E_m - E_r$ ) is the difference between the potential ( $E_m$ ) at which the membrane is clamped and the reversal potential ( $E_r$ ) of the transmitter-activated current.

Information about the time course of channel behaviour can be obtained by examining the spectral composition of the current noise seen at a low agonist concentration. To this end, the power density of the

agonist-induced current noise (expressed as  $A^2/\text{Hz}$ ) is plotted against frequency (usually on double-logarithmic coordinates). Assuming a single open state and that the transition from the open to the closed state is a first-order process, then the mean open times are exponentially distributed and the mean channel open time is identical to the channel closing time constant (Mathers and Barker, 1982). The *mean channel open time* ( $\tau$ ) is now obtained from the half-power frequency ( $f_c$ ):  $\tau = 1/(2\pi f_c)$ .

While fluctuation analysis provides an indirect way to examine the behaviour of single transmitter-gated channels, *patch-clamping* (Hamill *et al.*, 1981) is a means for real-time monitoring of elementary currents mediated by individual channels. In this technique, the patch electrode (a blunt micropipette with a smooth tip) is brought into contact with the membrane surface and, often following the application of slight suction, an electrically tight contact ("gigaohm seal") is formed between the pipette and the membrane. This permits direct low-noise recording of the currents mediated by individual channels located in the membrane patch within the opening of the tip of the patch electrode. The frequency resolution of patch clamp recordings is better than that of fluctuation measurements and patch clamping is also better suited for resolving channel conductance substates.

Several recording configurations have been used. In the *cell-attached* (or *on-cell*) configuration, the patch of membrane delineated by the gigaseal remains intact and continuous with the rest of the plasma membrane. In two other configurations, the patch under study is detached from the rest of the plasma membrane. *Inside-out* patches have their cytoplasmic surface and *outside-out* patches their extracellular surface, exposed to the bathing solution. The advantages of using isolated patches include an accurate control of membrane voltage, as well as the possibility of controlling the ionic environment on either side of the membrane and of examining the effects of various intracellularly and extracellularly acting substances.

In its *whole-cell voltage clamp* mode, the patch-clamp technique is widely used to examine macroscopic currents. This configuration is achieved by rupturing the membrane patch at the suction pipette tip while leaving the pipette in contact with the cell. The low-resistance access to the intracellular compartment thus formed provides an excellent route for single-electrode voltage- and current-clamping. The whole-cell configuration is often applicable to very small cells which do not tolerate conventional transmural microelectrodes. The fact that intracellular ions and other diffusible particles will eventually equilibrate with the pipette filling solution can be viewed as an advantage if control of the composition of the intracellular milieu is desired. However, the washout of intracellular second messengers and related compounds into the pipette filling solution often leads to deleterious effects, including channel rundown, if the missing factors cannot be experimentally compensated for. The problem of inadvertent intracellular dialysis has been largely circumvented by the development of the perforated patch technique (Horn and Marty, 1988; Korn *et al.*, 1991), which allows

recordings to be made from metabolically intact cells. This technique takes advantage of pore-forming antibiotics (such as nystatin and amphotericin) which are incorporated into the electrode filling solution. These exogenous channels yield upon gigaseal formation a good access resistance across the otherwise intact cell membrane. A point to note here is, however, that due to the permeability properties of nystatin and amphotericin channels, this technique does not preserve the original transmembrane  $\text{Cl}^-$  and  $\text{H}^+$  (and hence,  $\text{HCO}_3^-$ ) electrochemical gradients.

In single-channel experiments, a clean and clearly visible cell membrane is advantageous for gigaseal formation and therefore much of this work has been performed on enzymatically isolated or cultured cells. Recently, techniques have been developed that allow patch-clamping of neurons in brain slices. When making gigaseal recordings, a jet of physiological solution can be applied from a pipette onto the cell under study for mechanical cleaning of part of its surface and suction is used to remove the resulting debris (Edwards *et al.*, 1989; Sakmann *et al.*, 1989). However, somewhat surprisingly, stable whole-cell recordings can be routinely obtained by inserting an electrode blindly into the tissue (Blanton *et al.*, 1989; Coleman and Miller, 1989).

### 3.2. PROBLEMS RELATED TO ANION-SUBSTITUTION EXPERIMENTS

Ideally, the ions chosen as tools to probe the biophysical properties of membrane channels should have no effects other than those acting on the selectivity and transport mechanisms of the channel under study. This is, however, a requirement which is not often likely to be fully satisfied, especially if a large series of ions are to be tested. The difficulties related to the use of various anions as  $\text{Cl}^-$  substituents can arise from multiple causes. A trivial problem frequently encountered is the capability of some anions (e.g. gluconate) to chelate  $\text{Ca}^{2+}$  (Edwards, 1982).

A problem (or a set of interrelated problems) which has often been ignored arises when anions of weak acids, such as formate, acetate and propionate, are employed as  $\text{Cl}^-$  substituents. It is generally known that exposure of cells to a solution containing anions ( $\text{A}^-$ ) of a weak acid leads to a prompt fall in intracellular pH ( $\text{pH}_i$ ) (Roos and Boron, 1981). This is due to the high membrane permeability of the neutral (protonated) species (HA), which leads to an influx of HA until its intracellular concentration equals the extracellular one. Following equilibration of the neutral form, the intracellular anion concentration is determined by the transmembrane pH gradient so that the equilibrium potential of the weak-acid anion equals that of  $\text{H}^+$  and the latter is much more positive than the resting membrane potential (Kaila and Voipio, 1990). This means that a powerful intracellular accumulation of  $\text{A}^-$  takes place even if the plasma membrane provides no conductance pathway or carrier for the anion. Hence, it is highly unlikely that, in whole-cell recordings done under bi-anionic conditions with extracellular weak-acid anions, the intracellular ionic milieu is fully controlled by the filling solution of the recording pipette. An intracellular accumulation of the weak-

acid anions will take place until an unidentified steady-state is reached. Conversely, it may be hard to establish a situation where the internal concentration of a weak-acid anion is strictly controlled by intracellular perfusion, since under such conditions a continuous efflux of HA across the cell membrane tends to produce a fall in the intracellular  $\text{A}^-$  concentration. Problems analogous to those above are bound to arise in experiments on isolated membrane patches bathed in asymmetric solutions.

It may be worthwhile adding that the above considerations apply also to bi-ionic experimental conditions where the permeability of  $\text{HCO}_3^-$  is being investigated. The mobile neutral species in this case is  $\text{CO}_2$ , which has an extremely high permeability in cell membranes and therefore readily promotes a dissipation of the  $\text{HCO}_3^-$  gradient. However, if  $\text{CO}_2$  is at equilibrium across the cell membrane and the transmembrane pH gradient is known, measurements of bicarbonate permeability can be made with no source of error related to an ill-defined  $\text{HCO}_3^-$  distribution (Kaila and Voipio, 1987; Kaila *et al.*, 1989).

Another problem related to experiments with weak-acid anions under non-equilibrium conditions for HA is that the net flux of HA across the plasma membrane gives rise to changes in solution pH close to the membrane surface. For instance in crayfish muscle fibres, exposure to 30 mM acetate leads to an alkalosis at the external surface of the plasma membrane of about one pH unit (Mason *et al.*, 1990). In a study on bullfrog sensory neurons, Akaike *et al.* (1989a) examined the effects of a series of anions on GABA-activated currents. An intriguing finding was that substitution of extracellular  $\text{Cl}^-$  by an equivalent amount (120 mM) of acetate suppressed the maximum inward current carried by  $\text{Cl}^-$  without any effect on the affinity of agonist binding. In addition, formate, acetate and butyrate dramatically increased the rate of desensitization. The authors explain these effects on the basis of a direct action of the organic anions on the  $\text{GABA}_A$  receptor. However, an additional possibility is that the extracellular application of a high concentration of weak-acid anions produced an alkaline shift in the unstirred water layer on the external membrane surface which, in view of the pH-sensitivity of  $\text{GABA}_A$  receptors (Section 3.6.2), might well give rise to the observed effects. A corollary of the above considerations is that weak-acid anions should never be used as "inert" anions in experiments where they are simply needed in order to replace part or all of the external or internal  $\text{Cl}^-$ . In some preparations at least, methanesulfonate seems to be a suitable  $\text{Cl}^-$  substitute as it has no direct effect on pH, and it does not, either, bind calcium (cf. Kaila *et al.*, 1989).

An additional problem in ion-substitution experiments is the possible toxicity of a given anion. For instance, fluoride produces an irreversible deterioration of GABA-evoked currents in frog sensory neurons (Akaike *et al.*, 1989a). Foreign anions (including  $\text{I}^-$ ,  $\text{Br}^-$  and  $\text{SCN}^-$ ) may also alter the kinetics of GABA-gated currents (Onodera and Takeuchi, 1979; Robertson, 1989; Akaike *et al.*, 1989a).

Quite apart from specific problems related to anion

substitution experiments, a situation which may be commonplace but is particularly worrying arises in experiments where a patch pipette with a CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>-free filling solution is used to record from a neuron that is bathed in a conventional physiological solution equilibrated with CO<sub>2</sub>. Despite the high permeability of CO<sub>2</sub>, one cannot assume that the internal HCO<sub>3</sub><sup>-</sup> level reaches a value set by the non-CO<sub>2</sub> buffer (e.g. HEPES) of the artificial intracellular solution. This is because for each HCO<sub>3</sub><sup>-</sup> ion entering in the form of CO<sub>2</sub>, one proton is released within the cell, which means that the pH of the intracellular perfusion solution will reach an undefined steady-state level more acid than the initial one. This kind of a problem is accentuated if the cell under study is equipped with an intracellular carbonic anhydrase, which seems to be the case with hippocampal pyramidal neurons (Pasternack *et al.*, 1993), dorsal root ganglion cells (Droz and Kazimierczak, 1987; Kazimierczak *et al.*, 1986) and some other neurons (Voipio *et al.*, 1991; Neubauer, 1991).

### 3.3. PERMEABILITY OF GABA<sub>A</sub> RECEPTOR CHANNELS

Ion channels are never perfectly selective so as to allow the passage of one species of ions only. A large quantitative variation exists among the capabilities of different channels to select for certain ions and to discriminate against others. Most Cl<sup>-</sup> channels in excitable cells do not show a high degree of selectivity when examined in solutions containing various small monovalent anions, such as other halides, or NO<sub>3</sub><sup>-</sup> and SCN<sup>-</sup>. In fact, some neuronal voltage-dependent Cl<sup>-</sup> channels are considerably permeable even to monovalent cations (Franciolini and Nonner, 1987). The ion permeation mechanism in these channels is peculiar in that it involves an activated complex of a fixed anionic site within the channel, an extrinsic mobile cation attracted by the fixed negative charge and a permeant extrinsic anion.

In early work on the ion selectivity of postsynaptic channels gated by glycine and GABA, their hyperpolarizing action was taken as evidence for a significant K<sup>+</sup> permeability, although the evidence to support this view was far from conclusive (Coombs *et al.*, 1955; Eccles *et al.*, 1964a,b; Eccles, 1964b). This incorrect conclusion was largely due to ignoring the possibility of a nonequilibrium distribution of Cl<sup>-</sup> across neuronal membranes. The early work on crustacean preparations did not provide any support for a K<sup>+</sup> permeability of GABA<sub>A</sub> receptor channels (Boistel and Fatt, 1958; Takeuchi and Takeuchi, 1967) and this is also the case with their mammalian counterparts (e.g. Bormann *et al.*, 1987).

As will be described below, GABA<sub>A</sub> channels are permeable to a large number of small monovalent anions. It has been suggested that channels which are composed of four subunits show the highest ion selectivity, while those with six subunits (e.g. the nicotinic acetylcholine receptor channel) are poorly selective (Unwin, 1989). The relatively low anion selectivity of the GABA<sub>A</sub> receptor channel is in agreement with its postulated five-subunit structure. However, at the moment, there is no information on the possible influence of subunit composition on the anion selectivity of GABA<sub>A</sub> receptors.

The selectivity of GABA<sub>A</sub> receptor channels has been examined in a variety of preparations, including muscle fibers and neurons of crustaceans (Boistel and Fatt, 1958; Takeuchi and Takeuchi, 1966b, 1967, 1971; Motokizawa *et al.*, 1969; Ozawa and Tsuda, 1973; Kaila and Voipio, 1987; Kaila *et al.*, 1989; Mason *et al.*, 1990) and insects (Scott and Duce, 1986; Pinnock *et al.*, 1988); dorsal root ganglion neurons of frogs (Inomata *et al.*, 1986; Akaike *et al.*, 1989a), rats and cats (Gallagher *et al.*, 1978; Robertson, 1989); cultured mouse spinal neurons (Bormann *et al.*, 1987); as well as mammalian hippocampal (Eccles *et al.*, 1977; Biscoe and Duchen, 1985) and neocortical (Kelly *et al.*, 1969; Kaila *et al.*, 1993) neurons. All these studies are consistent with the view that, under physiological conditions, the conductance mediated by GABA<sub>A</sub> receptors is largely due to Cl<sup>-</sup> ions. However, recent studies of the permeability properties of GABA<sub>A</sub> receptors have shown that a significant, inwardly-directed component of the GABA-gated anion current is carried by HCO<sub>3</sub><sup>-</sup> ions (Kaila and Voipio, 1987; Kaila *et al.*, 1989, 1993; Voipio *et al.*, 1991). An important consequence of the HCO<sub>3</sub><sup>-</sup> permeability is that it can lead to a depolarizing total current mediated by GABA<sub>A</sub> receptors even in the presence of an outwardly directed (hyperpolarizing) current component carried by Cl<sup>-</sup>.

#### 3.3.1. Qualitative measurements of ion permeability: injection experiments

The first systematic investigations of the ionic selectivity of postsynaptic inhibitory anion channels were conducted on cat motoneurons *in situ*, which were loaded with various probe anions present in the microelectrode filling solution (Coombs *et al.*, 1955; Araki *et al.*, 1961; Ito *et al.*, 1962). In these experiments it was found that not only injection of Cl<sup>-</sup> but also of bromide, nitrate and thiocyanate changed the hyperpolarizing IPSP into a depolarizing response. In contrast to this, injection of anions such as phosphate, acetate and glutamate had no effect on the IPSP. The inhibitory channels studied in the above papers are gated by glycine, but since intracellular injection of chloride is still being used as a diagnostic test for Cl<sup>-</sup>-mediated responses, it may be worthwhile discussing this approach in some detail.

An unavoidable leak of the electrode filling solution takes place in intracellular experiments with conventional microelectrodes (Coombs *et al.*, 1955; Blatt and Slayman, 1983) and this can lead to a significant increase in the intracellular concentration of Cl<sup>-</sup>. The rate of Cl<sup>-</sup> loading can, of course, be either increased or decreased by means of an iontophoretic current of suitable polarity and strength. In the experiments by Eccles and coworkers, the permeability of a given anion that was included in the electrode filling solution was judged by its ability to produce a positive shift in the reversal potential of the inhibitory postsynaptic potential ( $E_{IPSP}$ ). A subsequent examination of the effects of 33 species of anions (Araki *et al.*, 1961; Ito *et al.*, 1962) suggested that there was a clear dichotomy between two classes of anions: the permeant ones which mimicked the action of Cl<sup>-</sup> and the impermeant ones which had

little effect on  $E_{IPSP}$ . The permeant anions (such as  $\text{Br}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{SCN}^-$ ) had a "hydrated size" of 1.2–1.3 times that of  $\text{Cl}^-$  while the impermeant ones (e.g.  $\text{I}^-$ , methylsulphate, citrate) were larger (for a criticism of the concept of hydrated ionic size, see Hille, 1992). This implied that the selectivity filter of glycine-gated  $\text{Cl}^-$  channels in mammalian neurons has a diameter of about 0.29–0.33 nm. Similar results were obtained in experiments on anion channels gated by GABA in hippocampal CA1 pyramidal cells (Eccles *et al.*, 1977).

A theoretical concept which dominated the design and interpretation of the above experiments was that, when open, the inhibitory channels are simple aqueous pores and that their anion selectivity is strictly determined by their limiting (or critical) pore diameter (Eccles, 1964b). This view, known as the molecular sieve hypothesis, has been modified in the light of observations which have shown that while an absolute cut-off effect takes place at a fixed maximum ionic size, channel geometry is not sufficient to account for the observed quantitative differences in the permeabilities among those anions that are capable of traversing the channel (Takeuchi and Takeuchi, 1967; Bormann *et al.*, 1987).

Another assumption inherent in the injection experiments described above was, of course, that a significant intracellular accumulation of the test anions takes place. This assumption may not be valid in the case of the frequently used weak-acid anions, since they can exit the cell in an electrically neutral manner (see Section 3.2). Indeed, as shown by Thomas (1976), if  $\text{HCO}_3^-$  is injected in the nominal absence of  $\text{CO}_2$ , there is no intracellular accumulation of bicarbonate and the main end result is an increase in  $\text{pH}_i$ . An analogous situation will be faced in experiments with other weak-acid anions. In fact, the "anomalously" high permeability of formate in inhibitory postsynaptic channels (Eccles, 1964b) repeatedly observed in injection measurements (Araki *et al.*, 1961; Kerkut and Thomas, 1964) may be at least partly due to the low membrane permeability of formic acid, which favours the intracellular accumulation of its conjugate anion. Indeed, in experiments where the transmembrane distribution of nondissociated formic acid has been at equilibrium,  $\text{GABA}_A$  receptor channels do not show an unpredictably high permeability to formate (Mason *et al.*, 1990).

### 3.3.2. Relative anion permeabilities and the limiting diameter of $\text{GABA}_A$ receptor channels

A quantitative examination of the anion permeability of  $\text{GABA}_A$  receptor channels is motivated by the fact that, under physiological conditions, the voltage responses, currents and conductances mediated by  $\text{GABA}_A$  receptors are due to channel-mediated movements of both  $\text{Cl}^-$  and  $\text{HCO}_3^-$  ions (Kaila and Voipio, 1987; Kaila *et al.*, 1989, 1993). In addition, measurements of the relative ion permeability of ion channels, including  $\text{GABA}_A$  receptors, have provided important information on their structural and biophysical properties (e.g. Bormann *et al.*, 1987).

The relative permeability of a given anion ( $\text{A}^-$ ) vs.  $\text{Cl}^-$  can be calculated on the basis of the Goldman–Hodgkin–Katz voltage equation:

$$E_r = -\frac{RT}{F} \ln \frac{[\text{Cl}^-]_o + P_A/P_{\text{Cl}}[\text{A}^-]_o}{[\text{Cl}^-]_i + P_A/P_{\text{Cl}}[\text{A}^-]_i} \quad (1)$$

where  $E_r$  is the reversal potential of the channel-mediated current;  $P_A/P_{\text{Cl}}$  is the relative permeability of  $\text{A}^-$  vs.  $\text{Cl}^-$ ;  $R$ ,  $T$  and  $F$  have their usual meanings; and the subscripts o and i indicate the ionic concentrations at the external and internal sides of the membrane. A standard approach is to measure the reversal potential of the channel-mediated current under bi-ionic conditions, where one only of the two ionic species is present on each side of the membrane.

When considering geometrical factors relevant for selective permeability, a simplistic model of a transmembrane channel is a pore which permits passage of ions that are smaller than its minimum diameter. Since large polyatomic anions are poorly hydrated, their effective size can be estimated from space-filling models (such as the Pauling–Corey–Kaltung model) or from their Stokes diameter, which is determined by their limiting conductivity in water. Working on acetylcholine receptor channels of frog muscle, Hille and associates (Dwyer *et al.*, 1980) measured the relative permeability (vs.  $\text{Na}^+$ ) of a large series of monovalent cations including large organic molecules. Molecular models of the permeant ions indicated that the smallest cross-section of the open pore is at least as large as a square of  $0.65 \text{ nm} \times 0.65 \text{ nm}$ . In an analogous manner, Bormann *et al.* (1987) examined the limiting diameter of the  $\text{GABA}_A$  receptor channel in experiments on cultured mouse spinal neurons on the basis of reversal-potential measurements in whole-cell recordings under bi-ionic conditions. The apparent size of a given probe anion was calculated using the Einstein–Stokes equation (Robinson and Stokes, 1959):

$$(2) d = 18.36/\lambda_o$$

where  $d$  is the Stokes diameter (in nm) of the anion and  $\lambda_o$  its limiting conductivity (S/cm) in water. The relative permeabilities (vs.  $\text{Cl}^-$ ) of a series of anions indicated that  $\text{GABA}_A$  channels of cultured mouse spinal neurons have a limiting diameter of 0.56 nm, which was slightly larger than the estimate for the strychnine-sensitive glycine receptor (0.52 nm) obtained in the same study. Permeability measurements carried out on frog sensory neurons (Inomata *et al.*, 1986; Akaike *et al.*, 1989a) and crayfish muscle fibers (Mason *et al.*, 1990) have yielded similar estimates (around 0.5 nm) for the limiting diameter of  $\text{GABA}$ -gated anion channels. Figure 3 shows the dependence on Stokes diameter of the relative permeabilities of polyatomic (weak-acid) anions in mouse (Bormann *et al.*, 1987) and frog (Akaike *et al.*, 1989a) neurons and in crayfish muscle fibres (Kaila and Voipio, 1987; Kaila *et al.*, 1989; Mason *et al.*, 1990). Since bicarbonate is the only physiologically relevant anion besides  $\text{Cl}^-$  that is permeable across  $\text{GABA}$ -gated anion channels, it is worth noting that the relative permeability of  $\text{HCO}_3^-$  vs.  $\text{Cl}^-$  is rather high, amounting to 0.2–0.3 in the preparations examined in the above papers.

### 3.3.3. Factors affecting anion permeation

Work on ion channels has shown that the simple pore concept does not account for their selectivity and transport properties. One of the earliest observations in this direction was made by Takeuchi and Takeuchi (1967, 1971). Having first demonstrated that the GABA-gated channels of crayfish opener muscle fibres show a graded permeability to various small anions, the Takeuchis made the following important observations and conclusions:

(i) For a series of anions, neither the permeability sequence nor the conductance sequence was identical to the sequence of the free mobilities of the anions in solution, which indicated that the permeating anions do not experience a simple water-filled pore with a restricted size as predicted by the original molecular-sieve hypothesis. These observations implied that the permeant ions interact with anion-binding sites within the channel while passing through.

(ii) A further observation which was not quantitatively compatible with the molecular-sieve hypothesis was that the GABA-activated conductance decreased when 25 or 50% of Cl<sup>-</sup> was substituted by certain foreign anions (such as SCN<sup>-</sup> and I<sup>-</sup>), but it increased again if a higher proportion of Cl<sup>-</sup> was replaced. Such an "anomalous mole fraction dependence" could be explained on the basis of an interaction among permeating anions within the channel and it implied that the channel contains more than one binding site. These conclusions have been fully verified and extended in more recent work, as will be described below.

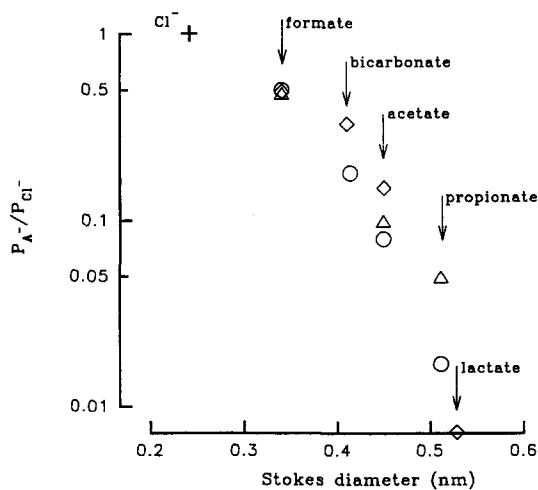


FIG. 3. Dependence on Stokes diameter of the permeability of polyatomic weak-acid anions in GABA<sub>A</sub> receptor channels. Data from cultured mouse spinal neurons (circles; Bormann *et al.*, 1987), frog sensory neurons (triangles; Akaike *et al.*, 1989a) and crayfish muscle fibers (diamonds; Kaila *et al.*, 1989; Mason *et al.*, 1990). The permeability of lactate was below detection limit (Mason *et al.*, 1990). The data suggest a rather similar limiting diameter for the GABA<sub>A</sub> receptor channels in all three preparations, estimated as 0.56 nm in mouse neurons (Bormann *et al.*, 1987).

#### 3.3.3.1. Halide permeability sequences

A well-known theory related to ion selectivity was developed by Eisenman (1962) on the basis of his work on the equilibrium selectivity properties of ion-selective glasses (see Diamond and Wright, 1969). In Eisenman's theory, equilibrium selectivity is explained on the basis of two factors: the hydration energy of a given ion; and the electrostatic (coulombic) energy of attraction between the ion and fixed charges (of opposite polarity) at the selectivity site. If the site has a very high electric field strength, the ion-site interaction will be governed by the electrostatic energy and the affinity of such a site will increase with a decrease in ionic radius. If the site has a very low field strength, then the ion-site attractions are much weaker than the hydration energies, and, consequently, the site will favour ions with a large radius. The success of Eisenman's theory was evident from its capability to correctly predict that from 120 distinct selectivity sequences (permutations) which could be written for the 5 alkali metal cations (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup> and Cs<sup>+</sup>), only 11 exist in nature (for review, see Diamond and Wright, 1969; Eisenman and Horn, 1983).

An approach of the kind described above can be extended to the four halide anions F<sup>-</sup>, Br<sup>-</sup>, Cl<sup>-</sup> and I<sup>-</sup> but, of course, one must now consider interactions between mobile anions and fixed cationic sites. A low field strength of a positively charged binding site leads to the halide selectivity sequence I<sup>-</sup> > Br<sup>-</sup> > Cl<sup>-</sup> > F<sup>-</sup>, while a high field strength gives an exactly opposite sequence (Wright and Diamond, 1977). The anion permeability sequence SCN<sup>-</sup> > I<sup>-</sup> > Br<sup>-</sup> > Cl<sup>-</sup> > F<sup>-</sup> observed in GABA<sub>A</sub> receptor channels in various cells (Takeuchi and Takeuchi, 1971; Bormann *et al.*, 1987; Akaike *et al.*, 1989a; Robertson, 1989) is indicative of a weak electrostatic interaction between permeant anions and fixed cationic binding sites within the channel. In other words, the permeability of a sufficiently small anion is mainly determined by its hydration energy. However, the permeability sequences of the small anions do not strictly reflect their free-solution mobilities (Br<sup>-</sup> > I<sup>-</sup> > Cl<sup>-</sup> > SCN<sup>-</sup> > F<sup>-</sup>; see Robinson and Stokes, 1959), which is in agreement with the idea that the open GABA<sub>A</sub> channel is not a simple water-filled pore.

#### 3.3.3.2. Conductance sequences, saturation and anion interaction

When examining various small monovalent anions, Bormann *et al.* (1987) found that their single-channel conductance was nearly in the reverse order (Cl<sup>-</sup> > Br<sup>-</sup> > I<sup>-</sup> > SCN<sup>-</sup> > F<sup>-</sup>) to the permeability sequence (see above) which indicated that the rate of ion transport across the channel was limited by the binding of anions inside the channel. An interaction between permeant ions and fixed charges within the channel was also evident from the saturation of single-channel conductance at a high Cl<sup>-</sup> concentration, with half-saturation taking place at 155 mM (Bormann *et al.*, 1987). Furthermore, in symmetrical mixtures of SCN<sup>-</sup> and Cl<sup>-</sup>, keeping the total anion concentration at 145 mM, the single-channel conduc-

TABLE I. SINGLE-CHANNEL CONDUCTANCES\* OF GABA<sub>A</sub> RECEPTOR CHANNELS

| Cell type  | Single channel conductance§ (pS) |         |       |    |    | Reference                          |
|--|----------------------------------|---------|-------|----|----|------------------------------------|
| Cultured mouse spinal neuron                               | 30*                              | 12      | 19    | 44 |    | Bormann <i>et al.</i> , 1987       |
| Cultured rat spinal neuron                                 | 20*                              | 29*     | 13    | 39 | 71 | Smith <i>et al.</i> , 1989         |
| Cultured chick cerebral neurons                            | 26*                              | 13      | 18    |    |    | Mistry and Hablitz, 1990           |
| Cultured rat cerebellar Purkinje cell                      | 28–30*                           | 19–20   |       |    |    | Llano <i>et al.</i> , 1988         |
| Cultured rat astrocyte                                     | 29*                              | 3       | 4     | 12 | 21 | Bormann and Kettenmann, 1988       |
| Rat superior cervical ganglion neuron                      | 30*                              | 7–36†   |       |    |    | Newland <i>et al.</i> , 1991       |
| Rat hippocampal granule cell                               | 14*                              | and 23* |       |    |    | Edwards <i>et al.</i> , 1990       |
| Goldfish retinal ganglion cell                             | 16*                              | 7–9     | 11–13 |    |    | Cohen <i>et al.</i> , 1989         |
| Rat melanotroph  | 26*                              | 14      |       |    |    | Schneggenburger and Konnerth, 1992 |
| Bovine adrenal chromaffin cell                             | 45*                              | 11      | 18    | 31 |    | Bormann and Clapham, 1985          |
| Cloned GABA <sub>A</sub> receptors in kidney cell culture: |                                  |         |       |    |    | Verdoorn <i>et al.</i> , 1990      |
| $\alpha_1^*\beta_2^*$                                      |                                  | 11*     |       |    |    |                                    |
| $\alpha_1^*\gamma_2^*$                                     |                                  | 31*     | 17    |    |    |                                    |

§Measured with an equal Cl<sup>-</sup> concentration (about 150 mM) on both membrane faces.

\*Main conductance state indicated by asterisk.

†Many substates within this conductance range.

tance showed an anomalous mole-fraction dependence, which indicates that GABA<sub>A</sub> receptor channels contain at least two positively charged anion binding sites. The model put forward by the above authors to account for the transport properties of the GABA<sub>A</sub> channel incorporates two energy wells (i.e. binding sites) for penetrating anions. A parallel investigation of strychnine-sensitive glycine receptors channels revealed a high degree of similarity between these two types of ligand-gated channels (Bormann *et al.*, 1987). In terms of transport characteristics, the most obvious difference between them is the larger main state conductance of the glycine receptor (see also Mathers and Barker, 1982).

Finally, it may be appropriate to note that virtually all presently available information on the permeability and transport characteristics of GABA<sub>A</sub> receptor channels deals with their main conductance state(s).

#### 3.4. SINGLE-CHANNEL CONDUCTANCES

The first measurements of currents gated by single mammalian GABA<sub>A</sub> receptors were made using fluctuation analysis in two-microelectrode voltage-clamp experiments on cultured mouse neurons (Barker and Mathers, 1981; Study and Barker, 1981; Barker *et al.*, 1982). The mean single channel conductance was about 15–20 pS (see also Mathers and Barker, 1982; Segal and Barker, 1984a,b). Measurements of current noise in intact crayfish muscle fibres yielded a mean single channel conductance of about 9 pS (Dudel *et al.*, 1980). A comparison of several GABA analogs showed that the elementary conductance of the GABA-gated anion channel does not depend on agonist structure, but channel gating does (Barker and Mathers, 1981; see below).

Single-channel conductances measured from intact cells can be useful in several ways. For instance, they have been used to estimate the number of inhibitory channels involved in the generation of unitary and miniature IPSPs (Dudel *et al.*, 1977; Segal and Barker, 1984b; Collingridge *et al.*, 1984; Edwards *et al.*, 1990). In addition, the question whether physiological and pharmacological modulating fac-

tors act on channel conductance or on gating, or both, can be examined using intact cells. However, on the basis of such experiments, it is difficult if not impossible to make meaningful comparisons of the biophysical characteristics of individual channels from different preparations.

Experiments on excised patches or intracellularly perfused cells permit an accurate control of membrane voltage and of the distribution of Cl<sup>-</sup>. It has become customary to examine the single-channel conductance of GABA<sub>A</sub> receptors with an equal concentration of Cl<sup>-</sup> (140–150 mM) on both sides of the cell membrane, which yields data that can be compared among channels from various sources. An additional advantage gained in using a symmetrical solution is that it provides a simple means for finding out whether rectification (a regular characteristic of currents mediated by GABA<sub>A</sub> receptor channels under physiological conditions; see below) is due to a voltage-sensitivity of channel gating or conductance, or to the asymmetrical Cl<sup>-</sup> distribution across the intact cell membrane (Goldman or constant-field rectification).

Patch-clamp studies with symmetrical Cl<sup>-</sup> solutions have directly demonstrated that (perhaps with a few exceptions), the conductance of a single GABA<sub>A</sub> receptor channel in its open state does not rectify (e.g. Bormann *et al.*, 1987; Bormann and Kettenmann, 1988; Macdonald *et al.*, 1989a; but see Weiss, 1988). However, the channels can exhibit several conductance states (see Fig. 5). A rigorous quantification of the relative frequency of occurrence of the different conductance substates can be hampered by a low frequency of some states as well as by the fact that both agonist type and concentration may have an effect on their distribution (Bormann *et al.*, 1987; Smith *et al.*, 1989; Mistry and Hablitz, 1990; Yasui *et al.*, 1985; Newland *et al.*, 1991). However, a main state at around 30 pS is frequently observed in different types of cells, at least in cultured ones (e.g. Bormann *et al.*, 1987). Table 1 provides a collection of conductance levels observed in various kinds of cells.

In measurements on outside-out patches taken from the soma membrane of granule cells in rat

hippocampal slices, Edwards *et al.* (1990) noticed that the proportion of single-channel currents with two amplitude levels varied between different patches from almost pure populations of either subtype to various combinations of both subtypes. This variation suggested that the two single-channel conductances (23 and 14 pS) reflected the expression of two receptor subtypes, rather than the activation of two substates of one receptor. This scarcity or virtual absence of substates may point to an important difference between experiments done on cultured and native neurons (Edwards *et al.*, 1990; see also Yasui *et al.*, 1985). However, exposure of outside-out patches from isolated sympathetic neurons to a relatively high GABA concentration (50  $\mu$ M) induced channel openings to many—often ill-defined—conductance states ranging from 7 to 36 pS with a main state at 30 pS (Newland *et al.*, 1991).

Interestingly, studies on cloned GABA<sub>A</sub> receptors have shown that their conductance depends on sub-unit composition. Recombinant rat receptors of the type  $\alpha_1\beta_2$  had a conductance main state of 11 pS while the main state of the  $\alpha_1\gamma_2$  receptors was 31 pS with a subconductance state of 17 pS (Verdoorn *et al.*, 1990).

### 3.5. GATING OF GABA<sub>A</sub> RECEPTOR CHANNELS

Much of the conceptual framework in investigations of the kinetic properties of receptor channels gated by amino acid transmitters, including GABA, has been derived from work carried out on the nicotinic acetylcholine receptor (see Katz and Thesleff, 1957; Katz and Miledi, 1970, 1973; Magleby and Stevens, 1972; Anderson and Stevens, 1973; Colquhoun and Sakmann, 1981; Sakmann, 1992). The kinetic properties of ligand-gated channels have been examined using perturbation techniques as well as measurements of current fluctuations or single-channel currents under equilibrium conditions. With regard to the latter methods, it is important to note that kinetic parameters can be measured under stationary conditions (Stevens, 1973; Magleby and Stevens, 1972; Mathers and Barker, 1982). Perturbation studies of channel kinetics involve experimental manipulations that produce an instantaneous change (relative to the time course of the gating processes) in the average number of open channels. Release of endogenous transmitter either spontaneously or following stimulation produces an abrupt, short-lived increase in the transmitter concentration within the synaptic cleft (e.g. Dudel, 1977; Segal and Barker, 1984b; Faber and Korn, 1980; Edwards *et al.*, 1990). An exogenous agonist can be applied and removed with a time constant of a few milliseconds using fast perfusion techniques (Akaike *et al.*, 1986; Franke *et al.*, 1987). In addition, due to the voltage-sensitivity of the agonist–receptor interaction (Magleby and Stevens, 1972), a perturbation can be produced by a step change in membrane potential during maintained agonist application (Dudel, 1977; Mathers and Barker, 1982).

All of these techniques have inherent limitations. For instance, receptor desensitization (Katz and Thesleff, 1957) which can take place under steady-state conditions may have an influence on the kinetic

parameters measured under stationary conditions (cf. Akaike *et al.*, 1986; Mathers, 1991; Frosch *et al.*, 1992). On the other hand, perturbation studies always involve an analysis of the ensemble response of a large number of channels.

#### 3.5.1. Kinetics of single GABA<sub>A</sub> receptor channels

Fluctuation analysis applied to cultured neurons showed that the power spectra of GABA-induced current fluctuations could be fitted by a single Lorentzian curve with a time constant (which is an estimate of mean channel lifetime) varying between 20 and 30 msec (Study and Barker, 1981; Barker *et al.*, 1982; Segal and Barker, 1984a). In preparations as varied as hippocampal neurons and crayfish muscle fibers, mean channel lifetime has been shown to increase with depolarization (Dudel *et al.*, 1980; Segal and Barker, 1984a). Evidently, the gating of GABA<sub>A</sub> receptor channels is influenced by membrane voltage. An important finding, also paralleling those previously made in work on the nicotinic ACh receptor, was that different GABA<sub>A</sub> receptor agonists activate different channel lifetimes (Barker and Mathers, 1981; see also Mistry and Hablitz, 1990). In mouse spinal neurons, the mean lifetime of GABA-induced events was about 30 msec while those induced by muscimol had a duration about twice as long (Barker and Mathers, 1981). This result has important implications in the study of channel kinetics, as it indicates that the unbinding of the agonist coincides with the end of channel lifetime.

Work done using noise analysis has shown that more than one Lorentzian components are often needed to fit the spectra of agonist-induced current noise. Two time constants were needed to account for the kinetics of GABA-gated channels in crayfish muscle (Dudel *et al.*, 1980) and similar findings have been made in mammalian preparations. For instance, in cultured rat cerebellar neurons, two voltage-dependent time constants (about 2 and 25 msec at  $-110$  mV) are required for a good fit (Cull-Candy and Usowicz, 1989). Evidently then, the GABA<sub>A</sub> receptor channel can pass current in several states.

The meaning of the term *channel lifetime* as originally used in noise analysis was not, as such, applicable to patch-clamp recordings of GABA<sub>A</sub> receptor-mediated single-channel currents which showed that the elementary currents have a burst-like appearance, i.e. they are interrupted by short closing gaps (Sakmann *et al.*, 1983; Bormann and Clapham, 1985; Bormann and Kettenmann, 1988; Macdonald *et al.*, 1989a; Twyman *et al.*, 1990; Mistry and Hablitz, 1990). In view of the cooperativity of the binding of two GABA molecules seen in virtually all native GABA<sub>A</sub> receptors (e.g. Takeuchi and Takeuchi, 1967; Dudel, 1977; Akaike *et al.*, 1986; Bormann and Clapham, 1985), a reaction scheme of the kind depicted in Fig. 4 can be used to describe the time course of channel gating (Sakmann *et al.*, 1983; Bormann and Clapham, 1985; cf. del Castillo and Katz, 1957). Here, two GABA molecules (A) bind to the receptor (R) in a stepwise manner to form the complex A<sub>2</sub>R which then changes its conformation to A<sub>2</sub>R\*, the open channel (see also Bormann, 1988a,b). Recordings from adrenal chromaffin cells (Bormann

and Clapham, 1985) and cultured astrocytes (Bormann and Kettenmann, 1988) gave rather similar values for the kinetic parameters in the two cell types, with  $\alpha$  (the rate constant for channel closing) equal to 167 and 140 sec<sup>-1</sup>,  $\beta$  (for channel opening) 467 and 833 sec<sup>-1</sup> and  $k_{-1}$  123 and 208 sec<sup>-1</sup>, respectively.

It should be noted that the scheme in Fig. 4 is highly simplified in that it does not take into account the possibility of more than one open state. Indeed, it is a frequent observation that a single exponential does not produce an adequate fit for the distribution of open state durations, which means that the GABA<sub>A</sub> receptor channel has two or more open states with different off-rate constants (see Sakmann *et al.*, 1983; Weiss, 1988; Macdonald *et al.*, 1989a). Agonist concentration can also have an influence on mean open time by affecting the likelihood of different open states (Macdonald *et al.*, 1989a). It is possible that the short-lived openings frequently detected in patch clamp recordings arise from the monoliganded state of the receptor (Sakmann *et al.*, 1983; Bormann and Clapham, 1985; Twyman *et al.*, 1990). Various schemes for GABA<sub>A</sub> receptor gating have been published (e.g. Weiss *et al.*, 1988; Weiss and Magleby, 1989; Macdonald *et al.*, 1989a; Twyman *et al.*, 1990).

Figure 5 shows specimen recordings of GABA<sub>A</sub> receptor-mediated single channel currents evoked in an outside-out patch of a chick cerebellar neuron by exposure to GABA and the GABA<sub>A</sub> agonists mus-

cimol and isoguvacine (Mistry and Hablitz, 1990). In addition to a main-state conductance of about 28 pS activated by all agonists (which included also THIP, 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol), subconductance states of about 13–15 pS and 18–19 pS were seen with application of each agonist, especially of muscimol and isoguvacine.

With regard to the voltage sensitivity of gating, work on chick neurons has suggested that channel open probability, but not duration, increases upon depolarization (Weiss, 1988). A higher open probability at depolarized potentials has also been observed in cultured mouse neurons (Bormann *et al.*, 1987).

It is of interest to note that allosteric modulators of GABA<sub>A</sub> receptors act on channel kinetics with no effect on elementary conductances (however, effects on gating may produce changes in the distribution of various conductance states). For instance, BZD agonists (positive modulators) augment the current gated by GABA<sub>A</sub> receptors mainly by increasing the frequency of channel activation (Study and Barker, 1981). Barbiturates such as pentobarbital also enhance GABA<sub>A</sub> responses (Ransom and Barker, 1975) but in this case the modulatory mechanism is to prolong burst duration, which was initially seen as an increase in mean channel lifetime in noise measurements (Study and Barker, 1981). A detailed patch-clamp analysis on cultured mouse spinal neurons showed that the effect of phenobarbital and pentobarbital was to increase the channel open time related

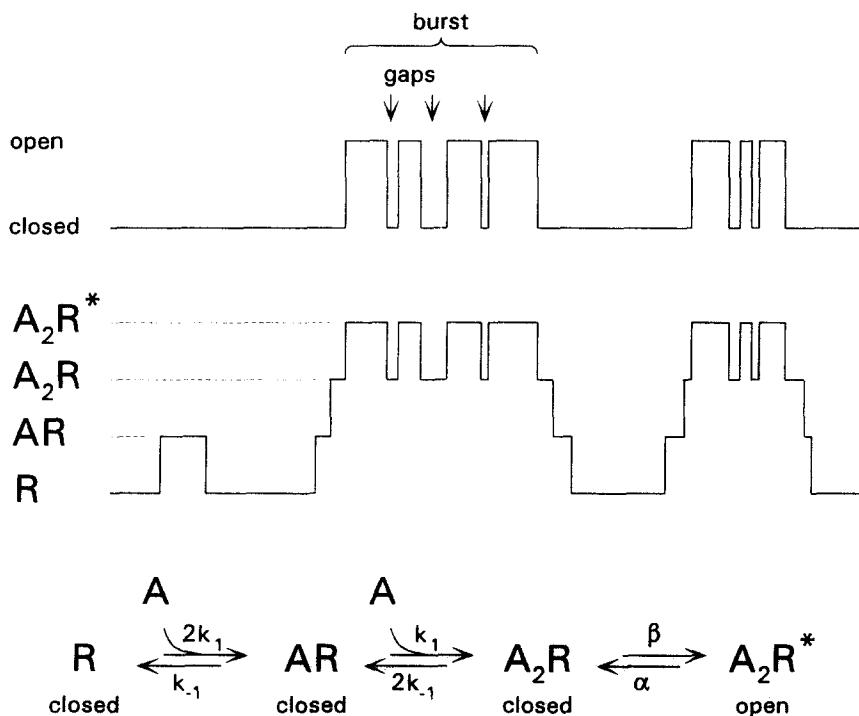


FIG. 4. Gating of the GABA<sub>A</sub> receptor channel. Two agonist molecules (A) bind to the receptor (R) in a stepwise manner to form the complex A<sub>2</sub>R which then repeatedly adopts conformation A<sub>2</sub>R\*, the open channel. This is seen as bursts (fast openings interrupted by gaps) which often last for tens of milliseconds. The rate constants  $k_1$  and  $k_{-1}$  are usually assumed to be identical in both binding steps.  $\alpha$  and  $\beta$  are the rate constants for channel closing and opening, respectively. The above scheme does not take into account the possibility of more than one open state. (Modified after Bormann, 1988a; Hille, 1992.)

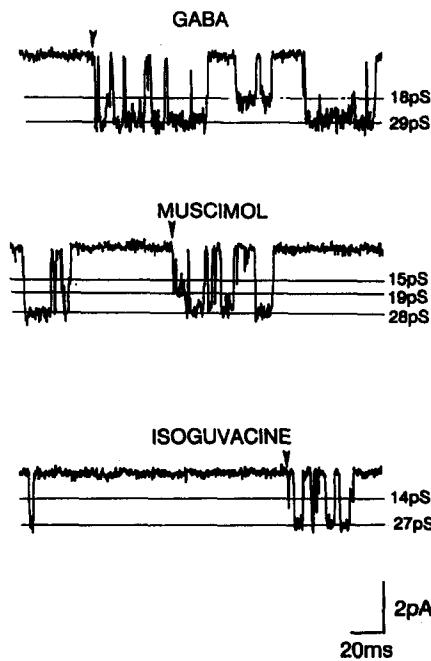


FIG. 5. GABA<sub>A</sub> receptor mediated single-channel currents recorded from an outside-out membrane patch from a cultured chick cerebral neuron in a symmetrical (about 150 mM) Cl<sup>-</sup> solution (holding potential -80 mV). GABA (500 nM), muscimol (250 nM) and isoguvacine (500 nM) activate openings to a main conductance state of 27–29 pS and to a subconductance state of 18–19 pS with occasional direct transitions between the two open states. A third (14 pS) conductance state is seen during the application of muscimol. (Reproduced by permission from Mistry and Hablitz, 1990.)

to the main conductance state (27 pS) and to shift the frequency histograms of channel open times to longer durations. This increased the relative frequency of long bursts (which contained multiple long openings) and decreased the relative frequency of shorter bursts (which contained shorter openings) (Macdonald *et al.*, 1989b). In addition to the above actions, at high concentrations barbiturates can act as GABA<sub>A</sub> agonists (Mathers and Barker, 1982).

Structurally different steroids have been shown to potentiate or antagonize GABA<sub>A</sub> receptor-mediated responses (for references, see Twyman and Macdonald, 1992). For instance, in cultured rat hippocampal and spinal neurons the effects of certain steroids (including metabolites of progesterone and deoxycorticosterone) resemble those of barbiturates (Majewska *et al.*, 1986). Patch-clamp experiments on mouse spinal neurons (Twyman and Macdonald, 1992) showed that the mechanism whereby neurosteroids (androsterone and pregnanolone) induce a prolongation of average open and burst durations was similar to that of barbiturates examined in the same neurons (Macdonald *et al.*, 1989b) but, in addition, an increase in channel opening frequency was evident.

Experiments on dissociated sympathetic neurons of the rat indicated that the blocking action of picrotoxin on GABA<sub>A</sub> receptor-mediated currents was due

to a decrease in the frequency of channel openings (Newland and Cull-Candy, 1992). PiTX had no effect on the main-state conductance (see also Barker *et al.*, 1983). The observations were consistent with a mechanism whereby picrotoxin binds preferentially to an agonist-bound form of the receptor and stabilizes an agonist-bound closed state (Newland and Cull-Candy, 1992).

### 3.5.2. Channel lifetime as a determinant of IPSC duration

In a number of preparations measurements of the decay time constant of the inhibitory postsynaptic current (IPSC) and of the mean lifetime of postsynaptic GABA<sub>A</sub> receptor channels have indicated that they are equal in magnitude and show a similar dependence on membrane voltage, such that a hyperpolarization induces an increase in the rate of IPSC decay and a decrease in mean channel lifetime (Dudel *et al.*, 1977; Onodera and Takeuchi, 1979; Dudel *et al.*, 1980). Observations of this kind are consistent with a "single shot" mechanism (Faber and Korn, 1980), where the relaxation kinetics of a population of inhibitory channels that is activated only once accounts for the duration of the IPSC. This appears to be a general feature of GABA<sub>A</sub>-mediated inhibition in various kinds of preparations, at least under conditions where the uptake mechanisms assisting in the clearance of transmitter within the synaptic cleft are functional (e.g. Segal and Barker, 1984b; Collingridge *et al.*, 1984; Barker and Harrison, 1988). However, pharmacological inhibition of GABA uptake can lead to a dramatic increase in IPSC duration (Thompson and Gähwiler, 1992; Roepstorff and Lambert, 1992).

In agreement with their actions observed at the single channel level described above, barbiturates induce a prolongation of the decay phase of GABA<sub>A</sub>-mediated IPSCs (e.g. Segal and Barker, 1984b; Collingridge *et al.*, 1984) while BZD agonists increase the IPSC amplitude (Segal and Barker, 1984b).

### 3.5.3. Desensitization and rundown of GABA<sub>A</sub> receptors

In recordings of macroscopic currents mediated by ligand-gated channels, receptor desensitization is seen as a time-dependent decrease in conductance which takes place in the continuous presence of an agonist, particularly at high concentrations (Katz and Thesleff, 1957). Functionally, desensitization of ligand-gated channels is equivalent to the inactivation of voltage-sensitive channels.

With some exceptions (e.g. Takeuchi and Takeuchi, 1967; Cherubini *et al.*, 1990; Polenzani *et al.*, 1991), prominent desensitization of GABA<sub>A</sub> receptors induced by application of an exogenous agonist has been observed in most cell types examined, including not only neurons (e.g. Dreifuss *et al.*, 1969; Numann and Wong, 1984; Thompson and Gähwiler, 1989c; Frosch *et al.*, 1992; Oh and Dichter, 1992) but also glial (Bormann and Kettenmann, 1988; Weiss and Magleby, 1989) and chromaffin cells (Bormann and Clapham, 1985) as well as various

types of crustacean muscle fibers (e.g. Dudel and Hatt, 1976; Atwood, 1976).

Even in a single cell, desensitization can involve temporally distinct processes. However, desensitization of GABA<sub>A</sub> receptors is always much slower than their activation. To take an example, in experiments on frog sensory neurons conducted by means of a fast perfusion technique, activation of GABA<sub>A</sub> receptor-mediated current followed a single exponential with a time constant of 5 msec at low concentrations ( $10^{-6}$  M) while at a concentration one order of magnitude higher, the response consisted of a double exponential which included a slower, concentration-dependent time constant (about 50–200 msec) (Akaike *et al.*, 1986). The time constant of desensitization of the fast kinetic component was about 3 sec and that of the slower one was more than 10 sec.

The rate of recovery from the desensitized state is slow and highly variable among various cell types. In the above experiments on frog neurons, the recovery rate of the fast component was lower (time constant about 90 sec) than that of the slow one, which recovered with a time constant of about 20 sec (Akaike *et al.*, 1986). Sometimes recovery is incomplete, which indicates that receptor rundown (discussed below) has occurred during the experiment.

A temporal heterogeneity of desensitization of macroscopic currents in single neurons may suggest the presence of heterogeneous populations of GABA<sub>A</sub> receptors (Yasui *et al.*, 1985; Akaike *et al.*, 1986). Although functional heterogeneity of gating behaviour does not necessarily imply structural heterogeneity of the channel population (Newland *et al.*, 1991), it is interesting to note here that recombinant GABA<sub>A</sub> receptors with variable subunit compositions also fall into different groups with regard to the time course and extent of desensitization. The  $\beta_2$  subunit seems to be largely responsible for desensitization and rundown characteristics (Levitin *et al.*, 1988; Verdoorn *et al.*, 1990; Mathers, 1991; Moss *et al.*, 1992). It may be relevant to note here also that GABA<sub>A</sub> currents in neonatal rat hippocampal neurons show little desensitization (Cherubini *et al.*, 1990).

Desensitization of GABA<sub>A</sub> receptors in cultured rat cortical and hippocampal neurons has been shown to be sensitive to membrane potential, becoming much slower as the membrane is depolarized (Frosch *et al.*, 1992; Oh and Dichter, 1992). This contrasts with observations on rat retinal ganglion cells (Tauck *et al.*, 1988) and frog sensory neurons (Akaike *et al.*, 1986) where membrane voltage does not have a significant influence on the rate of desensitization. In acutely isolated rat hippocampal neurons, desensitization was found to be enhanced when the intracellular Cl<sup>-</sup> concentration was elevated (Huguenard and Alger, 1986). If this turns out to be a general property of GABA<sub>A</sub> receptor desensitization, it will obviously complicate the interpretation of the actions of membrane potential on desensitization in intact cells.

The view that receptor desensitization is directly linked to the binding of GABA to its receptor (and not, for instance, to charge transfer across the channel) is supported by a number of observations, including those showing that a submaximal concentration of the competitive GABA<sub>A</sub> antagonist, bicu-

culline, slows desensitization in a manner consistent with a decrease in receptor occupancy, while a low concentration of the non-competitive antagonist, picrotoxinin, has no effect on the time course of the GABA-induced current response (Frosch *et al.*, 1992). Benzodiazepines have been shown to accelerate desensitization of GABA<sub>A</sub> receptors, but it is not clear to what extent this effect is attributable to an increase in receptor occupancy or to an allosteric modulatory influence on desensitization *per se* (Bormann and Clapham, 1985; Mierlak and Farb, 1988; Yakushiji *et al.*, 1989b; Frosch *et al.*, 1992).

In contrast to observations on certain glutamatergic synapses (Dudel *et al.*, 1988; Trussell and Fischbach, 1989), GABA<sub>A</sub> receptor desensitization is far too slow to play a role in fast synaptic transmission in any preparation studied so far. It may, however, be of functional significance during tonic activation of GABA<sub>A</sub> receptors which may take place under physiological (cf. Frosch *et al.*, 1992) and especially, under pathophysiological conditions (cf. Lutz, 1992), as well as upon application of GABA uptake blockers. However, at the moment there is little evidence to suggest a role for desensitization in frequency-dependent depression of GABA<sub>A</sub> mediated IPSPs (IPSP<sub>AS</sub>) in central neurons (Wong and Watkins, 1982; Thompson and Gähwiler, 1989c).

Finally, it should be emphasized here that long-term application of GABA and repetitive synaptic activity often lead to gradual changes in GABA<sub>A</sub> receptor-mediated responses which may or may not involve receptor desensitization. A reversible attenuation of voltage responses can result from a passive redistribution of ions which leads to a decrease in the driving force of currents gated by GABA<sub>A</sub> receptor channels (e.g. Dudel, 1977, 1978; Wong and Watkins, 1982; Numann and Wong, 1984; Segal and Barker, 1984a; Huguenard and Alger, 1986; Ashwood *et al.*, 1987; Akaike *et al.*, 1987a; Thompson and Gähwiler, 1989c). A decrease (often irreversible) in GABA<sub>A</sub> receptor-mediated currents can also be secondary to rundown of receptors. This has been frequently observed in experiments involving intracellular perfusion, but can be offset by a low (buffered) Ca<sup>2+</sup> concentration and by adding Mg<sup>2+</sup> and ATP in the perfusion solution (Inoue *et al.*, 1986; Stelzer *et al.*, 1988; Gyenes *et al.*, 1988; Chen *et al.*, 1990; Llano *et al.*, 1991; Mouginot *et al.*, 1991; Marchenko, 1991; see also Harrison and Lambert, 1989; Browning *et al.*, 1990). The Ca<sup>2+</sup>-dependent receptor rundown is speeded up by intracellular phosphatase perfusion (Stelzer *et al.*, 1988; Chen *et al.*, 1990). A considerable variability is evident in the effects of various phosphorylation factors (cf. Ticku and Mehta, 1990; Moss *et al.*, 1992) and, in some cases, they may affect GABA<sub>A</sub> receptor rundown or genuine desensitization, or both (Tehrani *et al.*, 1989; Moss *et al.*, 1992; Leidenheimer *et al.*, 1992).

### 3.6. IONIC MODULATION OF GABA<sub>A</sub> RECEPTORS

Nervous activity can lead to substantial changes in the concentrations of various ions within neurons as well as in the interstitial space, which constitutes about one fifth of the total volume of the parenchyma

in vertebrate CNS tissue (Nicholson, 1980). Most of the activity-induced ionic transients in both the intra- and extracellular compartments are highly localized (see Section 5) and, with regard to GABA<sub>A</sub>-mediated inhibition, ionic shifts can have two kinds of physiological consequences. First, they may produce changes in the IPSP driving force thereby leading to changes in the efficacy of inhibition. In addition to this, certain cations—including H<sup>+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup>—are known to exert a direct modulatory action on GABA<sub>A</sub> receptors. There is also evidence that GABA<sub>A</sub> receptors are directly involved in the hyperexcitability which is associated with hypomagnesemia (El-Beheiry and Puil, 1990).

### 3.6.1. Ca<sup>2+</sup>

While variations in extracellular Ca<sup>2+</sup> have not been found to have a marked influence on GABA<sub>A</sub> receptors (Takeuchi and Takeuchi, 1971; Akaike *et al.*, 1989b), intracellular Ca<sup>2+</sup> exerts significant modulatory actions. In frog sensory neurons, GABA<sub>A</sub> receptor-mediated responses are suppressed if application of GABA is preceded by a single Ca<sup>2+</sup> spike (Inoue *et al.*, 1986). Voltage-clamp experiments showed that this effect involved a transient decrease in GABA<sub>A</sub> receptor affinity with a magnitude related to the integral of the Ca<sup>2+</sup> current. Single-channel recordings revealed a decrease in the duration of the open time of the GABA-gated channel with no change in conductance (Behrends *et al.*, 1988).

Intracellular perfusion of hippocampal neurons has also revealed a clear dependence of GABA<sub>A</sub> receptor function on Ca<sup>2+</sup> (Chen *et al.*, 1990). In these cells, the Ca<sup>2+</sup>-dependent down regulation of the GABA-gated conductance was apparently mediated by phosphorylation factors.

Ca<sup>2+</sup> influx through voltage-dependent Ca<sup>2+</sup> channels appears not to affect the amplitude of GABA<sub>A</sub> receptor-mediated currents in hippocampal CA3 pyramidal neurons in organotypic culture (Thompson and Gähwiler, 1989c) or in cultured porcine melanotrophs (Mouginot *et al.*, 1991). However, in the latter preparation, a rise in free intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) triggered by release from intracellular stores can—depending on the base-line level of [Ca<sup>2+</sup>]<sub>i</sub>—either enhance or inhibit the activity of GABA<sub>A</sub> receptors. This finding could be accounted for on the basis of the bell-shaped dependence of GABA<sub>A</sub> receptor activity on [Ca<sup>2+</sup>]<sub>i</sub>, with a maximum at around 10<sup>-7</sup> M (Taleb *et al.*, 1987).

From a functional point of view, the sensitivity to [Ca<sup>2+</sup>]<sub>i</sub> of GABA<sub>A</sub>-mediated inhibition is interesting, since it provides an intracellular link between excitatory and inhibitory synaptic inputs (Stelzer *et al.*, 1987; Marchenko, 1991). The pronounced cellular compartmentalization of [Ca<sup>2+</sup>]<sub>i</sub> (e.g. Connor *et al.*, 1988; Regehr *et al.*, 1989; Müller and Connor, 1991; Guthrie *et al.*, 1991; Llinas *et al.*, 1992; see also Miller, 1987) adds to the versatility of a calcium-based modulatory action. A Ca<sup>2+</sup>-dependent suppression of GABA<sub>A</sub> responses might well be of importance in pathophysiological phenomena such as epileptogenesis (Inoue *et al.*, 1986; Stelzer *et al.*, 1987) and during anoxic/ischaemic conditions (Duchen,

1989; Siesjö and Bengtsson, 1989; Duchen *et al.*, 1990).

Work by Llano *et al.* (1991) on rat Purkinje neurons in cerebellar slices has revealed a complex modulatory influence of postsynaptic intracellular Ca<sup>2+</sup> on GABAergic inhibition. Currents due to exogenous GABA were enhanced in a Ca<sup>2+</sup>-sensitive manner following a train of postsynaptic depolarizing pulses, but, in sharp contrast to this, spontaneous inhibitory currents were reduced. The potentiation of the effect of exogenous GABA had a half recovery time (20 sec) which was much faster than that related to the inhibition of spontaneous currents (about 4 min). The authors explain the potentiation on the basis of an action of [Ca<sup>2+</sup>]<sub>i</sub> on GABA<sub>A</sub> receptors within the postsynaptic neuron and evidence is provided for the view that the inhibition of the synaptically-evoked responses is due to a presynaptic action of a retrograde messenger produced in the postsynaptic cell in response to a rise in [Ca<sup>2+</sup>]<sub>i</sub> (Llano *et al.*, 1991). A similar mechanism seems to operate in the rat hippocampus (Pitler and Alger, 1992).

In a study on rat cerebellar slices, Kano *et al.* (1992) identified a novel form of neural plasticity where activation of an excitatory synaptic input induces a potentiation of GABAergic inputs to the same cell. An increase in postsynaptic [Ca<sup>2+</sup>]<sub>i</sub> in Purkinje cells brought about by stimulation of climbing fibres produced, after a delay of several minutes, a long-lasting potentiation of responses mediated by both synaptic and pharmacological activation of GABA<sub>A</sub> receptors.

### 3.6.2. H<sup>+</sup>

While intracellular but not extracellular Ca<sup>2+</sup> seems to affect GABA<sub>A</sub> receptor function, the opposite is true for H<sup>+</sup>. Early work on crayfish muscle fibers showed that a decrease in extracellular pH (pH<sub>o</sub>) produced an increase in the GABA-gated Cl<sup>-</sup> conductance while an alkaline pH<sub>o</sub> had the opposite effect (Takeuchi and Takeuchi, 1967). The effect of pH<sub>o</sub> is not linked to a shift in the concentration-conductance curve which indicates that H<sup>+</sup> does not influence the affinity of the GABA<sub>A</sub> receptor channel (Takeuchi and Takeuchi, 1967; Smart and Constanti, 1982; Pasternack *et al.*, 1992a). A decrease in pH<sub>o</sub> was found to slow down the IPSC decay rate implying that at least part of the effect of H<sup>+</sup> on the action of bath-applied GABA was attributable to an increase in mean channel lifetime (Onodera and Takeuchi, 1979). A rise in pH<sub>o</sub> also leads to more pronounced desensitization (Pasternack *et al.*, 1992a).

In a manner qualitatively similar to that seen in crustacean preparations, extracellular H<sup>+</sup> ions have been shown to enhance GABA<sub>A</sub>-mediated responses in cat dorsal root ganglia (Gallagher *et al.*, 1983) and in cultured hippocampal neurons (Tang *et al.*, 1990). In contrast to this, iontophoretic application of protons on cultured mouse spinal neurons (Gruol *et al.*, 1980), as well as lowering the external pH from 7.4 to 6.2 in experiments on rat sympathetic neurons (Smart, 1992), decreased GABA-evoked currents. In contrast to the influence of pH<sub>o</sub>, intracellular H<sup>+</sup> ions have little effect on the function of GABA<sub>A</sub> receptors

in the preparations examined so far (Tang *et al.*, 1990; Pasternack *et al.*, 1992a). Evidently then, protons modulate GABA<sub>A</sub> receptors through an action at a site (or sites) in the extracellular domain of the GABA<sub>A</sub> receptor, but the qualitative influence of H<sup>+</sup> is variable among different types of neurons. More information on this topic is required to establish whether the type of H<sup>+</sup> sensitivity might be used for electrophysiological fingerprinting of structural features of GABA<sub>A</sub> receptors.

In functional terms, the facilitatory action of external H<sup>+</sup> ions on GABA<sub>A</sub> receptor-mediated responses is intriguing, since it is qualitatively opposite to their blocking effect on glutamate-gated channels, especially on the NMDA receptor (Tang *et al.*, 1990; Traynelis and Cull-Candy, 1991). A functional synergism resulting from the opposite modulatory actions of H<sup>+</sup> on inhibitory and excitatory ligand-gated channels may explain the frequent observations that alkaline and acid shifts in pH<sub>o</sub> lead to a respective increase and a decrease in neuronal excitability (e.g. Aram and Lodge, 1987; Jarolimek *et al.*, 1989).

In crayfish muscle fibres, application of GABA has been shown to evoke a fall in intracellular pH that is coupled to an alkaline shift on the extracellular surface of the plasma membrane (Kaila and Voipio, 1987; Kaila *et al.*, 1990). These pH shifts are caused by a net movement of HCO<sub>3</sub><sup>-</sup> across GABA<sub>A</sub> receptor channels (see also Kaila *et al.*, 1989; Voipio *et al.*, 1991). There is strong evidence that (in terms of the underlying mechanism) identical pH shifts are mediated by GABA<sub>A</sub> receptors in the rat hippocampus (Kaila *et al.*, 1992a). In view of the H<sup>+</sup> sensitivity of GABA<sub>A</sub> receptors, the possibility emerges that the channel-mediated movements of acid-base equivalents exert a modulatory action on the underlying inhibitory conductance mechanism (Kaila *et al.*, 1990). While this hypothesis has not yet been subject to experimental testing, there is evidence that an analogous kind of fast proton modulation plays a role in excitatory transmission in the hippocampus (Taira *et al.*, 1993).

The pH<sub>o</sub> sensitivity of GABA<sub>A</sub> receptors might also play a (protective) role under pathophysiological conditions such as anoxia and ischaemia (cf. Lutz, 1992) which are known to be associated with large acid shifts within the interstitial space (Hansen, 1985; Siesjö, 1988). In this context, it is of interest to note that there is evidence that anoxic block of GABAergic inhibition in the mammalian brain (Fujiwara *et al.*, 1987) is due to a presynaptic effect (Krnjević *et al.*, 1991). In keeping with a possible enhancement of GABA<sub>A</sub>-receptor mediated responses by H<sup>+</sup>, the effect of exogenous GABA was enhanced during anoxia in hippocampal slices (Krnjević *et al.*, 1991). It is, of course, also possible that both the pre- and postsynaptic actions of anoxia were mediated by changes in intracellular Ca<sup>2+</sup> (see above).

### 3.6.3. Zn<sup>2+</sup>

Certain neurons in the CNS—particularly within limbic and neocortical regions—sequester large amounts of Zn<sup>2+</sup> in their presynaptic boutons, which results in Zn<sup>2+</sup> being released during synaptic activity (Assaf and Chung, 1984; Frederickson and Danscher,

1990). Zinc has a number of effects on neuronal function but, with regard to synaptic transmission, experiments on hippocampal neurons have indicated that zinc may block both excitatory (especially NMDA receptor-mediated) and inhibitory responses (Westbrook and Mayer, 1987; see also Sahara *et al.*, 1991). It has been suggested that spontaneous GABA-mediated giant depolarizing potentials (GDPs; see Section 4.3.3) that occur in the neonate rat hippocampus are induced by endogenously released Zn<sup>2+</sup> ions as a result of their blocking action on both pre- and postsynaptic GABA<sub>B</sub> receptors (Xie and Smart, 1991; but see Cherubini *et al.*, 1991; Lambert *et al.*, 1992).

Work on lobster muscle fibres showed that lowering external pH antagonized the blocking action of zinc on the GABA-gated Cl<sup>-</sup> conductance, which suggested that H<sup>+</sup> and Zn<sup>2+</sup> bind to the same site, possibly to an imidazole group as indicated by pH titration of the GABA-evoked conductance (Smart and Constanti, 1982). However, this type of competition between the two cations has not been observed in mammalian neurons (Smart, 1992).

The GABA<sub>A</sub> receptor blocking action of Zn<sup>2+</sup> shows large variability among vertebrate neurons. No blocking effect was found in experiments on pyramidal neurons in the pyriform cortex (Smart and Constanti, 1983) and in rat cortical neurons (Wright, 1984), while a clear antagonism of GABA responses by Zn<sup>2+</sup> is seen in frog sensory neurons (Akaike *et al.*, 1987b), cat primary afferents (Curtis and Gynther, 1987) and in cultured mouse hippocampal neurons (Westbrook and Mayer, 1987; Mayer and Vyklicky, 1989). Rather than reflecting experimental factors, this variability is likely to result from genuine differences among different cell types (Smart and Constanti, 1990; Smart, 1990; Legendre and Westbrook, 1991). However, a striking dependence of the action of Zn<sup>2+</sup> on developmental stage within one cell type has been demonstrated by Smart (1992) who found that cultured embryonic sympathetic neurons of the rat were more sensitive than cultured adult neurons to the GABA<sub>A</sub> blocking action of zinc. A similar dependence of Zn<sup>2+</sup> on developmental stage was seen in experiments on embryonic neurons maintained in culture for 40–50 days.

Work on recombinant GABA<sub>A</sub> receptors has shown that the presence of a  $\gamma$  unit leads to an insensitivity to the action of Zn<sup>2+</sup> (Draguhn *et al.*, 1990; Smart *et al.*, 1991). However, the observations that GABA<sub>A</sub> receptor-mediated responses of neurons in neonatal rat hippocampal slices are insensitive to both benzodiazepines and zinc (Rovira and Ben-Ari, 1991; Smart *et al.*, 1991) suggest that there are GABA<sub>A</sub> receptor subtypes devoid of a  $\gamma$  subunit which, nevertheless, are insensitive to Zn<sup>2+</sup> (Smart, 1992).

The GABA<sub>A</sub> antagonistic action of zinc is non-competitive (Smart and Constanti, 1982, 1990; Legendre and Westbrook, 1991). No change in single-channel conductance has been detected and the main effect seems to be a decrease in channel opening frequency (Legendre and Westbrook, 1991; Smart, 1992). Experiments with various drugs support the conclusion that Zn<sup>2+</sup> acts on a binding site that is independent of those that mediate

the actions of picrotoxin, benzodiazepines, steroids and barbiturates (Smart and Constanti, 1982; Legende and Westbrook, 1991; Smart, 1992).

#### 4. MEMBRANE POTENTIAL CHANGES MEDIATED BY GABA<sub>A</sub> RECEPTORS IN INTACT CELLS

With regard to the concept of the ionic basis of GABA<sub>A</sub> receptor-mediated inhibition, present neurophysiological orthodoxy is largely based on a mesh of circular arguments: GABA-induced changes in membrane potential are often interpreted using estimates of the Cl<sup>-</sup> equilibrium potential which, in turn, have been deduced in previous work on the basis of the reversal potential of GABA-mediated voltage and current responses! No doubt, under a variety of experimental conditions the reversal potentials of responses evoked by pharmacological and synaptic activation of GABA<sub>A</sub> receptors ( $E_{GABA\text{-}A}$  and  $E_{IPSP\text{-}A}$ ) behave qualitatively in a manner predicted by the identity  $E_{GABA\text{-}A} = E_{IPSP\text{-}A} = E_{Cl^-}$ . Indeed, a vast number of papers report a positive shift in  $E_{GABA\text{-}A}$  following intracellular injection of Cl<sup>-</sup> and this can still be regarded as a useful criterion for the identification of a GABA<sub>A</sub> receptor-mediated current. An additional standard test has been to look for a positive shift in  $E_{GABA\text{-}A}$  in response to a partial replacement of the extracellular Cl<sup>-</sup> by an impermeant anion. However, as will be shown below, neither type of experiment is valid in examining whether a GABA<sub>A</sub> response is solely mediated by Cl<sup>-</sup>.

A further corollary of the postulated identity  $E_{GABA\text{-}A} = E_{IPSP\text{-}A} = E_{Cl^-}$  is that a GABA<sub>A</sub> receptor-mediated hyperpolarization to a level more negative than the resting membrane potential indicates the presence of an active Cl<sup>-</sup> extrusion mechanism in the cell under study, while a depolarization can be taken as evidence for a Cl<sup>-</sup> uptake system in the target cell. As will be evident from what follows, the former line of thought cannot lead to a qualitative error: in a resting cell under physiological conditions, it is thermodynamically impossible to obtain a GABA<sub>A</sub>-mediated hyperpolarization in the absence of Cl<sup>-</sup> extrusion. However, the converse is not true—net extrusion of Cl<sup>-</sup> will not necessarily give rise to a hyperpolarizing GABA<sub>A</sub> response (Kaila *et al.*, 1989, 1993; Kaila and Voipio, 1990).

The fact that predictions based on an identity between  $E_{GABA\text{-}A}$ ,  $E_{IPSP\text{-}A}$  and  $E_{Cl^-}$  are prone not only to quantitative but also to qualitative errors is a consequence of the rather high relative permeability of HCO<sub>3</sub><sup>-</sup> vs Cl<sup>-</sup> which amounts to about 0.2–0.3 in both invertebrate and vertebrate GABA<sub>A</sub> receptor channels (Section 3.3.2). It is important to note here also that due to active regulation of intracellular pH, a steep outwardly directed plasmalemmal HCO<sub>3</sub><sup>-</sup> electrochemical gradient exists in cells at a physiological CO<sub>2</sub> partial pressure and the intracellular concentration of HCO<sub>3</sub><sup>-</sup> is often higher than that of Cl<sup>-</sup> (cf. Kaila *et al.*, 1989, 1993; Voipio *et al.*, 1991). Hence, as will be shown below, in cells with a low [Cl<sup>-</sup>]<sub>i</sub>,  $E_{Cl^-}$  is substantially more negative than  $E_{GABA\text{-}A}$ .

Of course, experiments deliberately carried out in the absence of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> *in vitro* may allow an

examination of GABA<sub>A</sub> responses that are mediated by Cl<sup>-</sup> ions only, but it should be emphasized that omitting bicarbonate from the physiological solution does not only abolish the HCO<sub>3</sub><sup>-</sup>-mediated current component—it is also likely to have an influence on various mechanisms involved in cellular ionic homeostasis, including those responsible for the regulation of intracellular Cl<sup>-</sup> (Hoffmann and Simonsen, 1989; Russell and Boron, 1990; Kimelberg, 1990). A further point that deserves attention is that getting rid of bicarbonate is not always feasible even when working with nominally CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>-free solutions. For instance, in brain slices kept in an interface chamber where CO<sub>2</sub> equilibration should be fast, the CO<sub>2</sub> concentration in the centre of the slice can be much higher than the ambient one, even in the absence of stimulation (Voipio and Kaila, 1993).

Most of the uncertainty regarding the ionic basis of GABA<sub>A</sub> receptor-mediated voltage responses in neurons under physiological conditions is due to the scarcity of direct information on the concentrations of intracellular Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> (cf. Kaila and Voipio, 1990). In what follows, the mechanisms that generate and maintain the driving forces of the two physiologically relevant anions that are permeant in GABA<sub>A</sub> receptor channels are briefly discussed. Thereafter, the membrane potential changes evoked upon pharmacological or synaptic activation of GABA<sub>A</sub> receptors in intact cells are examined. No attempt is made here to provide a systematic treatment of all types of cells that have been shown to generate GABA<sub>A</sub> receptor-mediated voltage responses. The aim is—by focussing on a few representative examples—to provide an idea about what is known, which GABA-mediated actions can be explained in the light of our present knowledge and what needs to be (re)examined in future work.

#### 4.1. METHODS IN THE STUDY OF CELLULAR ION REGULATION

With regard to the mechanisms underlying GABA<sub>A</sub> receptor-mediated actions, measurements of intracellular (and extracellular) ionic concentrations can provide information related to topics including:

- (i) the identity of ions which act as charge carriers under physiological conditions;
- (ii) the ion-regulatory mechanisms responsible for the generation and maintenance of the driving forces of the permeant ions;
- (iii) the effect of GABAergic transmission and exogenous GABA on intracellular and extracellular ion concentrations;
- (iv) the influence of activity-induced changes in interstitial and intracellular ion levels on GABAergic mechanisms.

The main focus in this section will be on item (ii), that is, on the transport phenomena that shape the voltage responses mediated by GABA<sub>A</sub> receptors in intact cells.

The first direct demonstrations of a nonpassive Cl<sup>-</sup> distribution across neuronal membranes came from work on squid giant axons, which take up Cl<sup>-</sup> (Keynes, 1963) and from molluscan neurons that

extrude  $\text{Cl}^-$  (Russel and Brown, 1972). At present, most of the solid data on neuronal  $\text{Cl}^-$  regulation is still based on work on the squid axon (Russell, 1983; Russell and Boron, 1990) and, with regard to the vertebrate CNS,  $\text{Cl}^-$  regulation has been studied more extensively in glial cells (Kimmelberg, 1990; Kettenmann, 1990) than in neurons.

Unlike the situation with  $\text{Cl}^-$ , at the moment there is no method that permits reliable direct measurements of the intracellular  $\text{HCO}_3^-$  concentration (cf. Wietasch and Kraig, 1991; Voipio and Kaila, 1992) and the latter has to be derived from measurements of  $\text{pH}_i$ . Under a constant partial pressure of  $\text{CO}_2$ , the equilibrium concentration can be obtained from

(3)  $[\text{HCO}_3^-]_i = 10^{\text{pH}_i - \text{pH}_o} \times [\text{HCO}_3^-]_o$ , where the subscripts  $i$  and  $o$  refer to intracellular and extracellular. As shown in Fig. 6,  $[\text{HCO}_3^-]_i$  reaches rather high levels at  $\text{CO}_2$  and  $\text{pH}_i$  levels that are physiologically relevant.

Present knowledge on cellular ionic regulation is based on experiments with intracellular ion-selective microelectrodes, radiotracer techniques and optical indicators. Each of these techniques has its advantages and drawbacks, but it is quite obvious that the ion-electrode and fluorescence techniques hold most promise in work aimed at elucidating the ionic mechanisms underlying neuronal and glial responses to transmitters.

#### 4.1.1. Ion-selective microelectrodes

Two kinds of ion-selective microelectrodes (solid-state and liquid-membrane electrodes) have been used to monitor intracellular ionic activities. In solid-state ion-selective microelectrodes (Thomas, 1978) the ion-sensing tip is usually constructed from ion-selective glass and this type of electrode was employed in much of the classical work on cellular pH regulation (e.g. Thomas, 1976, 1984). A solid-state  $\text{Cl}^-$ -

selective microelectrode based on  $\text{Ag}/\text{AgCl}$  has been used for measurements of intraneuronal  $\text{Cl}^-$  (Neild and Thomas, 1974).

Although the solid-state glass membrane pH electrodes often show an extremely high ionic selectivity and little sensitivity to drugs that are used as tools in the study of transport phenomena, they are hard to construct and it is virtually impossible to miniaturize them sufficiently to obtain electrodes compatible with work on single vertebrate neurons and glial cells. Due to the clearcut methods that have been developed for the construction of liquid-membrane electrodes (Ammann, 1986), most of the ion-selective microelectrodes that are currently used are of this type. In these electrodes, the ion-sensing element within the electrode tip is a liquid membrane column which contains a charged ion exchanger (e.g. for  $\text{Cl}^-$ ) or a neutral ion carrier (e.g. for  $\text{H}^+$ ) which renders the membrane a Nernstian type of electrical behaviour when exposed to environments with variable concentrations (or, more accurately, thermodynamic activities) of the relevant ion.

Potentiometric monitoring of ionic activities in biological preparations requires that the changes in the local electrical potential (measured by means of a reference electrode) are subtracted from the signal of ion-selective microelectrode, which yields a differential signal that can be calibrated in terms of ion activity. The reference electrode is either a separate conventional microelectrode, or its equivalent incorporated as one channel in a double-barrelled (or multibarrelled) micropipette.

Electrode measurements of  $[\text{Cl}^-]_i$  have been carried out on neurons (e.g. Ascher *et al.*, 1976; Deisz and Lux, 1982; Bührle and Sonnhof, 1985; Ballanyi and Grafe, 1985; Alvarez-Leefmans *et al.*, 1988; Kaila *et al.*, 1992b) and on glial cells (Ballanyi *et al.*, 1987; Hoppe and Kettenmann, 1989a,b). With regard to  $\text{Cl}^-$ , the available liquid-membrane solutions are not quite as satisfactory as those selective for certain cations (e.g.  $\text{H}^+$ ), but a careful analysis of the effects of interfering anions enables quantitatively meaningful measurements of  $[\text{Cl}^-]_i$ . In this context, it might be noted that measurements of  $E_{\text{Cl}}$  that are completely unaffected by nonidealities in  $\text{Cl}^-$ -electrode performance have been done by measuring the reversal potential of instantaneous net fluxes of  $\text{Cl}^-$  evoked by pulses of GABA applied under voltage-clamp conditions (Kaila *et al.*, 1989, 1992b). To obtain the reversal potential of the channel-mediated net  $\text{Cl}^-$  flux (which must equal  $E_{\text{Cl}}$ ), it is sufficient to find out the reversal potential of the differential voltage signals of the  $\text{Cl}^-$ -selective microelectrode evoked by brief exposures of the preparation to GABA. This approach will, in fact, give an accurate estimate of  $E_{\text{Cl}}$  without any calibration of the  $\text{Cl}^-$ -selective electrode.

Membrane solutions that yield an excellent selectivity to  $\text{H}^+$ -sensitive microelectrodes are readily available. The work on neurons and glial cells done with  $\text{H}^+$ -selective liquid-membrane microelectrodes includes experiments on invertebrates (e.g. Deitmer and Schlueter, 1987; Voipio *et al.*, 1991; Schwiening and Thomas, 1992), on lampreys (Chesler, 1986) and on vertebrates (e.g. Endres *et al.*, 1986; Kettenmann and Schlueter, 1988; Chesler and Kraig, 1989; Wuttke and

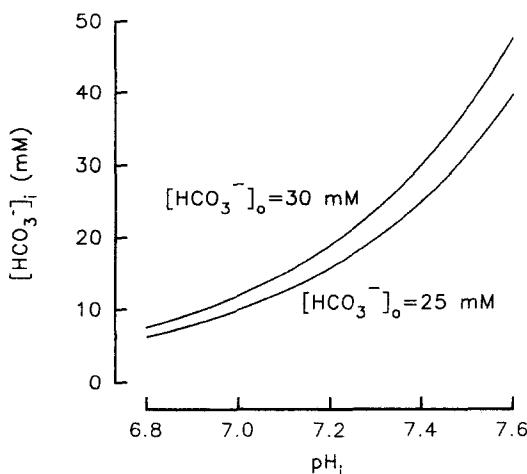


FIG. 6. Dependence of the intracellular bicarbonate concentration ( $[\text{HCO}_3^-]_i$ ) on  $\text{pH}_i$  in the presence of 25 and 30 mM extracellular bicarbonate ( $[\text{HCO}_3^-]_o$ ), calculated on the basis of Eq. 3. The two extracellular concentrations are close to those prevailing in solutions equilibrated with 5%  $\text{CO}_2 + 95\% \text{ O}_2$  (or 95% air) at a temperature of 35°C and 20°C, respectively (solution pH,  $\text{pH}_o = 7.4$ ).

Walz, 1990). An assumption (whether explicit or implicit) usually done when estimating bicarbonate concentrations from pH and  $P_{CO_2}$  under conditions *in vitro* is that the CO<sub>2</sub> partial pressure within the preparation under study is identical to the ambient one (usually equivalent to that of a physiological solution equilibrated with 5% CO<sub>2</sub> + 95% O<sub>2</sub> gas mixture). However, as already mentioned above, the P<sub>CO<sub>2</sub></sub> level within brain slices *in vitro* is not constant (Voipio and Kaila, 1992). This implies that while a fixed P<sub>CO<sub>2</sub></sub> is probably a justified assumption in work on isolated cells, one has to recognize the possibility of significant P<sub>CO<sub>2</sub></sub> gradients and transients in experiments on isolated nervous tissue.

Due to their relatively large tip diameter, ion-selective microelectrodes are not ideally suited for intracellular work on small cells such as mammalian neurons. However, once successfully carried out, electrode measurements of intracellular ion activities are easy to quantify.

At first glance, making extracellular measurements of interstitial ion activities in central nervous tissue may seem much easier than work on intracellular ions, but the interpretation of the results is more complicated. The tip diameter of the double-barrel microelectrodes that are used to record ion activities within the interstitial compartment is typically a few micrometres, i.e. about two orders of magnitude larger than the width of the extracellular clefts. Hence, the region around the microelectrode tip is bound to smooth out much of the spatial as well as temporal variation in the interstitial ion concentrations. However, if this characteristic of the electrode technique is kept in mind, it is evident that meaningful measurements of the composition of the interstitial fluid under various conditions can be (and certainly have been!) carried out.

#### 4.1.2. Fluorescent indicators

In comparison with the situation regarding cations such as H<sup>+</sup> and Ca<sup>2+</sup>, data on intracellular Cl<sup>-</sup> concentrations obtained using fluorescent indicators are still rather scanty. This is mainly due to the lack, until recently, of indicators with suitable biological and physicochemical properties. SPQ (methoxy-sulfonatopropyl quinolinium) and some closely related compounds (such as MQAE, methoxyquinolyl acetoethyl ester) are fluorescent dyes that have been used for measurements of [Cl<sup>-</sup>]<sub>i</sub> (Verkman *et al.*, 1989; Chao *et al.*, 1990; Inoue *et al.*, 1991). While these indicators have several attractive properties, their main weaknesses include the rather fast rate of leakage from cells and the fact that they are not suitable for ratiometric measurements (see below).

Measurement of pH<sub>i</sub> by means of intracellular fluorescence indicators has become a routine technique following the introduction of membrane permeable (acetoxymethyl ester) derivatives of ion indicators, which are trapped within cells following their conversion into the much less permeable (free acid) form by intracellular esterases (Tsien, 1989). At present, BCECF [2',7'-(bis-carboxyethyl)-5,6-carboxyfluorescein] and carboxy-SNARF (carboxy-semaphorhodafiuor) are mainly used for fluorescence measurements of pH<sub>i</sub> (e.g. Jean *et al.*, 1986; Gaillard

and Dupont, 1990; Kopito *et al.*, 1989; Tolkovsky and Richards, 1987; Buckler and Vaughan-Jones, 1990; Raley-Susman *et al.*, 1991; Pocock and Richards, 1992; Pasternack *et al.*, 1993). In ratiometric measurements with indicators such as BCECF which has a pH sensitive excitation spectrum, excitation is done alternating two wavelengths and the emission is measured at a single wavelength. In the case of SNARF, the emission spectrum is sensitive to pH and hence, a single wavelength is used for excitation and emission is measured at two wavelengths. With both types of indicators, the two emission signals are used to obtain a pH sensitive ratio which has the advantage of being unaffected by spatial differences and temporal changes in total fluorescence, which can result from various factors, including a nonuniform thickness of the cell examined as well as dye bleaching or leakage out of the cell.

A drawback of the fluorescent indicators which are loaded in their ester form is that they may accumulate within cells into compartments with ionic concentrations that differ from those prevailing in the cytosol. While this kind of bias does not necessarily invalidate all the information obtained, it has the unfortunate consequence that the apparent intracellular ionic concentration measured cannot be used to estimate the transmembrane gradient of the ion under study. Problems related to ionic compartmentalization are particularly acute in measurements of intracellular Ca<sup>2+</sup> (cf. Müller and Connor, 1991; Regehr and Tank, 1992; Llinás *et al.*, 1992), but it is likely that this is a serious problem with all dyes that are loaded in their neutral liposoluble ester form, including the H<sup>+</sup> indicators BCECF and SNARF. In fact, a new generation of dextrane-bound fluorescence indicators that are impermeant have recently become commercially available. In a way then, a circle has closed: initially, the pursuit for indicators that are readily accumulated within cells was motivated by the difficulties encountered when loading cells with various poorly permeant ion indicators.

#### 4.2. GENERATION AND MAINTENANCE OF THE DRIVING FORCE OF CURRENTS MEDIATED BY GABA<sub>A</sub> RECEPTOR CHANNELS

Since GABA<sub>A</sub> receptor-mediated currents are carried by Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> ions, this section will focus on the cellular mechanisms involved in the regulation of these two anions. Due to the tight coupling between HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup> which is mediated by both physicochemical and physiological mechanisms, the general features of intracellular pH regulation will be briefly described below.

In general terms, the electrochemical gradient of a given ionic species is set by the activity of plasmalemmal transport mechanisms and by conductive pathways (i.e. channels) which tend to dissipate the gradient. Under steady-state conditions, the total net flux of a given ion on active transport mechanisms is—by definition—equal in magnitude and opposite to the net flux mediated by leak pathways. (This statement must, however, be slightly modified in the case of H<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> equivalents which can be produced *de novo*, and sometimes consumed, within cells.)

In vertebrate central neurons at rest, the rate of conductive leakage of  $\text{Cl}^-$  appears to be very low (Thompson *et al.*, 1988a; Thompson and Gähwiler, 1989b). In fact, the neuronal "background" chloride channels (cf. Francolini and Petris, 1990) which are often seen after a delay in excised patches probably play no functional role in intact cells and their activity may be due to the washing-off of a regulatory component that keeps the channel in its closed state under conditions *in situ* (Blatz, 1991). However, activation of transmitter-gated channels may give rise to substantial  $\text{Cl}^-$  shifts (Section 5.1). In addition, considerable net fluxes of  $\text{Cl}^-$  are likely to take place across  $\text{Ca}^{2+}$ -dependent and voltage-dependent chloride channels during neuronal activity (Mayer *et al.*, 1990; Scott *et al.*, 1988; Madison *et al.*, 1986; Chesnoy-Marchais, 1990). Although "resting" glial cells have traditionally been considered almost exclusively permeable to  $\text{K}^+$ , there is evidence for the presence of channels permeable to other ions as well, including  $\text{Cl}^-$  (see Kettenmann, 1990; Ransom and Sontheimer, 1992).

There is not much data on the resting  $\text{HCO}_3^-$  (and  $\text{H}^+$ ) permeability of vertebrate neurons, but an extrapolation from invertebrate studies (Thomas, 1984; Kaila *et al.*, 1990) would suggest that conductive acid-base leakage is small at rest. However, activation of transmitter-gated channels appears to lead to large transmembrane movements of  $\text{HCO}_3^-$  and  $\text{H}^+$  (Chesler and Kaila, 1992; Section 5.2). In fact, it has been postulated that one of the primary tasks of acid-extrusion mechanisms in excitable cells is to deal with channel-mediated intracellular acid loads (Kaila *et al.*, 1990).

#### 4.2.1. Properties of ion-transport mechanisms

With regard to their energy input, ion transport mechanisms can be classified into (i) *primary active transport systems* (transport ATPases) which are directly fuelled by the hydrolysis of ATP and (ii) *secondary active transport systems* which energize a vectorial movement of the *driven* ion by a parallel movement (cotransport) or an opposite movement (countertransport or exchange) of a *driving* ion (see Stein, 1986). For instance, the energy required for secondary active extrusion or uptake of  $\text{Cl}^-$  is taken from the gradients of  $\text{K}^+$  or  $\text{Na}^+$  ( $\text{K}^+-\text{Cl}^-$  cotransport and  $\text{Na}^+-\text{K}^+-\text{Cl}^-$  cotransport, respectively). The ultimate source of energy used by these secondary transport mechanisms can be traced back to the activity of the  $\text{Na}^+-\text{K}^+$  ATPase which maintains the plasmalemmal  $\text{Na}^+$  and  $\text{K}^+$  gradients.

On a purely thermodynamic basis it is possible to define the *equilibrium conditions* for a given transport system, which then also allows a prediction of the *direction* of net transport as well as the conditions for its *reversal*. For instance, under physiological conditions the activity of a 1:1  $\text{K}^+-\text{Cl}^-$  cotransport system leads to net extrusion of  $\text{Cl}^-$  and, at equilibrium,  $E_{\text{Cl}}$  would equal  $E_K$  (the  $\text{K}^+$  equilibrium potential). However, it is worth emphasizing that thermodynamic considerations do not permit conclusions on the turnover rate of any transport mechanism. Indeed, a common misconception is that the amount of ions transported on a co- or countertrans-

porter is monotonically related to the deviation of the ionic gradients from the equilibrium of the system in such a way that a large disequilibrium automatically leads to a high turnover rate of transport. However, it has been repeatedly demonstrated in various systems that the rate depends on factors which control the *degree of activation* of the transporter (Aronson, 1985; Ganz *et al.*, 1989; Jean *et al.*, 1985; Gaillard and Dupont, 1990; Russell and Boron, 1990). Such mechanisms often involve an allosteric modulation of the transporter.

In its inactive state the turnover rate of a transport system is (by definition) zero despite a possible large thermodynamic disequilibrium of the transporter. For instance, the  $\text{Na}^+-\text{H}^+$  exchanger, which has a major influence on  $\text{pH}_i$  in various cells, is activated by intracellular  $\text{H}^+$  ions (Aronson, 1985). The exchanger is usually switched off at  $\text{pH}_i$  values much more acid than those predicted for an equilibrium state of the system, which would request that the equilibrium potential of protons ( $E_H$ ) equals that of  $\text{Na}^+$  ( $E_{\text{Na}}$ ) and, hence,  $\text{pH}_i$  would attain a level of about 8 or higher. Another kinetic aspect which is particularly important in ion-depletion experiments is that, due to kinetic constraints such as a limited affinity to one or more of the transported ions, an activated transport system may not be able to reach its thermodynamic equilibrium even under conditions where there are no other (active or passive) movements of the substrate ions.

Some ion transport mechanisms (such as the  $\text{K}^+-\text{Cl}^-$  and  $\text{Na}^+-\text{K}^+-\text{Cl}^-$  cotransporters) are electroneutral, that is, their activity does not directly generate any membrane current. Others are electrogenic, for instance, the  $\text{Na}^+-\text{HCO}_3^-$  cotransporter which has a  $\text{Na}^+:\text{HCO}_3^-$  stoichiometry of 1:2 or 1:3 (Deitmer and Schlue, 1989; Newman and Astion, 1991). A frequent confusion in the use of thermodynamic and kinetic considerations is encountered in statements where a voltage-dependence of the turnover rate of a transport mechanism is taken as a diagnostic criterion for its electrogenic nature. However, the electrogenic nature of a given transport mechanism implies only that it *produces* a net current. It is important to note here that a voltage dependence of a net transport rate can result from an influence on the kinetics of the underlying mechanism. Hence, the turnover rate of an electroneutral transport process may well show a sensitivity to voltage; and an electrogenic mechanism may have a voltage sensitivity opposite to what one might predict on the basis of its net charge-transfer characteristics.

#### 4.2.2. Influence of $\text{Cl}^-$ and pH regulation on $E_{\text{GABA}_A}$

A summary of the transport mechanisms involved in the membrane traffic of  $\text{Cl}^-$  and  $\text{H}^+/\text{HCO}_3^-$  in the nervous system is shown in Fig. 7 and some aspects of ion transport in neurons and glial cells will be briefly reviewed below. However, a summary of the *immediate* influence of various ion transport mechanisms on  $\text{GABA}_A$  receptor-mediated current can be incorporated into three statements:

- (i) net extrusion of  $\text{Cl}^-$  generates a source of outward (hyperpolarizing) current;

|                      | a.<br>Cl <sup>-</sup> ATPase | b.<br>K <sup>+</sup> -Cl <sup>-</sup><br>cotransport | c.<br>Na <sup>+</sup> -K <sup>+</sup> -Cl <sup>-</sup><br>cotransport | d.<br>Cl <sup>-</sup> -HCO <sub>3</sub> <sup>-</sup><br>exchange | e.<br>Na <sup>+</sup> -dependent<br>Cl <sup>-</sup> -HCO <sub>3</sub> <sup>-</sup><br>exchange | f.<br>Na <sup>+</sup> -H <sup>+</sup><br>exchange | g.<br>Na <sup>+</sup> -HCO <sub>3</sub> <sup>-</sup><br>cotransport |
|----------------------|------------------------------|--|---|--|--|---|---|
| Influence on         |                              |  |   |  |  |   |   |
| $E_{\text{Cl}^-}$    | neg                          | neg  | pos   | pos  | neg  | -   | -   |
| $E_{\text{HCO}_3^-}$ | -                            | -  | -   | neg  | pos  | pos   | pos   |
| $E_{\text{GABA}_A}$  | neg                          | neg  | pos   | pos  | neg  | pos   | pos   |

FIG. 7. Membrane ion-transport mechanisms involved in the regulation of intracellular Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup>. (a-g) depict the stoichiometries and directions of various types of primary (a) and secondary active (b-g) transporters (for further details, see text). The immediate influence of an increase in the activity of a given transport system on  $E_{\text{Cl}^-}$ ,  $E_{\text{HCO}_3^-}$ , and  $E_{\text{GABA}_A}$  is indicated in the bottom part of the figure, where pos indicates a positive and neg a negative voltage shift.

(ii) net uptake of Cl<sup>-</sup> generates a source of inward (depolarizing) current;

(iii) in the presence of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>, net extrusion of acid equivalents (extrusion of H<sup>+</sup> or uptake of HCO<sub>3</sub><sup>-</sup>) generates a source of inward current.

While statements (i) and (ii) can be regarded as self-evident, the role of pH (i.e. H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup>) regulation (statement (iii)) is worth examining in more detail. As discussed earlier on, GABA<sub>A</sub> receptor channels in both vertebrates and invertebrates show a considerable permeability to HCO<sub>3</sub><sup>-</sup>. When considering intact cells, it is important to note that, under a constant CO<sub>2</sub> partial pressure, the equilibrium potentials of HCO<sub>3</sub><sup>-</sup> ( $E_{\text{HCO}_3^-}$ ) and H<sup>+</sup> ( $E_{\text{H}^+}$ ) are identical (Kaila and Voipio, 1990). Most neurons and glial cells maintain a steady-state pH<sub>i</sub> level (usually about 7.1–7.2) that is about one unit more alkaline than what would be expected on the basis of a passive distribution of H<sup>+</sup> (or HCO<sub>3</sub><sup>-</sup>) ions (Thomas, 1984; Chesler, 1990; Schlue *et al.*, 1991). Hence,  $E_{\text{HCO}_3^-}$  is about 60 mV more positive than the resting membrane potential, which means that a net efflux of bicarbonate across GABA<sub>A</sub> receptor channels is driven by a steep electrochemical gradient (Kaila and Voipio, 1987, 1990). This leads to the generation of an inward current (and it also has a direct influence on intracellular and extracellular pH; Section 5.2). As will be emphasized below, the electrical consequences of channel-mediated movements of HCO<sub>3</sub><sup>-</sup> are of particular importance in cells that maintain a relatively low intracellular Cl<sup>-</sup> concentration.

A number of the ion transport mechanisms depicted in Fig. 7 are thought to coexist in various combinations in different types of neurons and glial cells and it is obvious that their joint action determines the Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> electrochemical gradients. However, it may be instructive to consider the qualitative influence on  $E_{\text{GABA}_A}$  of the individual mechanisms separately, as has been done in Fig. 7. The

following account deals mainly with those aspects of the transport mechanisms that may contribute to an understanding of their role in the generation of GABA<sub>A</sub> receptor-mediated voltage responses and net ionic shifts. The letters below refer to those in Fig. 7.

(a) Primary active transport of Cl<sup>-</sup> takes place in a number of cell types outside the nervous system and evidence obtained using the fluorescent indicators MQAE and SPQ, and ethacrynic acid (a compound reported to inhibit Cl<sup>-</sup> ATPase activity), suggests that it is involved in extrusion of Cl<sup>-</sup> in cultured hippocampal neurons (Inoue *et al.*, 1991; see also Inagaki *et al.*, 1987; Shiroya *et al.*, 1989). As is evident from Fig. 7, when working against a finite leak, an increase in the turnover rate of this mechanism will lead to a negative shift in  $E_{\text{Cl}^-}$  (with no effect on  $E_{\text{HCO}_3^-}$ ) and, hence, to a negative shift in  $E_{\text{GABA}_A}$ . There is, as yet, not much information on the presence and functional significance of primary active transport of chloride in nervous tissue.

(b) K<sup>+</sup>-Cl<sup>-</sup> cotransport is thought to be responsible for the generation of an inwardly directed U<sup>-</sup> electrochemical gradient in the crayfish stretch-receptor neuron (Deisz and Lux, 1982; Aickin *et al.*, 1982) as well as for Cl<sup>-</sup> extrusion in mammalian neocortical and hippocampal neurons (Thompson *et al.*, 1988a,b; Thompson and Gähwiler, 1989b; see also Alvarez-Leefmans, 1990). Due to the 1:1 stoichiometry of this transport mechanism and to the outwardly directed K<sup>+</sup> gradient mediated by the activity of the Na<sup>+</sup>-K<sup>+</sup> ATPase, the activity of a K<sup>+</sup>-Cl<sup>-</sup> cotransport mechanism alone in resting tissue will (in a manner qualitatively similar to primary active Cl<sup>-</sup> transport) produce an  $E_{\text{Cl}^-}$  more negative than the resting potential. However, it is worth recalling that large increases in the extracellular K<sup>+</sup> concentration are observed following neuronal activity both *in vivo* and *in vitro*. Maximum

changes from a base-line  $K^+$  concentration (usually about 3–4 mM) within the interstitial space peak at about 10 mM (Somjen, 1979; Sykova, 1983). Since  $K^+$  acts as the driving ion in  $K^+-Cl^-$  cotransport, such increases in the extracellular  $K^+$  concentration can lead to a large increase in the intracellular  $[Cl^-]$  in both neuronal and glial cells and hence, to a positive shift in  $E_{GABA_A}$  (Thompson *et al.*, 1988a,b; Thompson and Gähwiler, 1989b; Kettenmann, 1990; see also Barker and Ransom, 1978; Wong and Watkins, 1982; Michelson and Wong, 1991). Due to the similarity of equilibrium thermodynamics related to  $K^+-Cl^-$  cotransport and to a Donnan distribution of the two ions involved (cf. Alvarez-Leefmans, 1990), experiments comparing the effects of depolarizing current and a high external  $K^+$  can be used to decide whether an effect on  $E_{Cl^-}$  seen upon a change in extracellular  $K^+$  is due to the cotransport system or to  $Cl^-$  channels, or both.

In various cells, the  $K^+-Cl^-$  cotransporter is sensitive to the blocking action of sulfamoylbenzoic acid derivatives such as furosemide and bumetanide as well as to stilbenes such as SITS and DIDS (Alvarez-Leefmans, 1990). Furosemide is perhaps the drug most frequently used to inhibit  $K^+-Cl^-$  cotransport in neurons (Nicoll, 1978; Scappaticci *et al.*, 1981; Misgeld *et al.*, 1986; Thompson *et al.*, 1988a; Thompson and Gähwiler, 1989b). A difficulty often encountered in the use of the above compounds is that they interfere with other anion transport mechanisms (including those that transport  $HCO_3^-$ ) and they can also block anion-selective channels, including those gated by GABA (Wojtowicz and Nicoll, 1982; Gallagher *et al.*, 1983; Inoue, 1985; Inomata *et al.*, 1988). In fact, the latter effect has sometimes been the only one observed (e.g. Nicoll, 1978; Gallagher *et al.*, 1983), which gives weight to the argument that pharmacological interventions are often far from sufficient in the identification of ionic transport mechanisms. However, furosemide seems not to block  $GABA_A$  receptor-mediated IPSPs ( $IPSP_A$ s) in neocortical and hippocampal slices (Thompson *et al.*, 1988a; Thompson and Gähwiler, 1989b), but it should be noted that a partial block of postsynaptic  $GABA_A$  receptor channels might well be masked by a furosemide-induced increase in overall excitability which will lead to the recruitment of additional inhibitory interneurons (Thompson and Gähwiler, 1989b; but see also Lerma and Martín del Río, 1992).

A different approach to inhibit  $Cl^-$  extrusion on  $K^+-Cl^-$  cotransport involves application of cations that compete with  $K^+$ . In the crayfish stretch-receptor neuron, a series of monovalent cations were found to decrease the  $IPSP_A$  driving force in the order  $Rb^+ > NH_4^+ > K^+ > Cs^+$  (Aickin *et al.*, 1982). Since these experiments were done in the absence of  $HCO_3^-$ , it may be safe to conclude that this shift was mainly due to a similar shift in  $E_{Cl^-}$ . In particular, the capability of rather low concentrations of  $NH_4^+$  to inhibit neuronal  $Cl^-$  extrusion (Ascher *et al.*, 1976) which appears to result in a block of hyperpolarizing IPSPs (Lux, 1971; Llinás *et al.*, 1974; Iles and Jack, 1980; Aickin *et al.*, 1982; Mazda *et al.*, 1990) may explain the convulsant action of ammonia in the mammalian CNS (Raabe and Gummit, 1975; Raabe,

1990; but see Nicoll, 1978; Alger and Nicoll, 1983; Fan *et al.*, 1990).

(c)  $Na^+-K^+-Cl^-$  cotransport has been identified in certain neurons and glial cells (Ballanyi and Grafe, 1985; Alvarez-Leefmans *et al.*, 1988; Alvarez-Leefmans, 1990; Kimelberg, 1990; Kettenmann, 1990) and it catalyzes an electroneutral uptake of  $Cl^-$ . It is highly likely that the strongly depolarizing responses mediated by  $GABA_A$  receptor channels in vertebrate sensory neurons result from the operation of the  $Na^+-K^+-Cl^-$  cotransporter (Section 4.3.4). In most cells where it is present it appears to have a stoichiometry of  $1Na^+:1K^+:2Cl^-$ , with the notable (and well-studied) exception of the squid giant axon, where the stoichiometry is 2:1:3 (Russell, 1983; Russell and Boron, 1990). The relatively specific blocking effect of bumetanide makes it currently the drug of choice in pharmacological manipulations of this transport system (e.g. Altamirano and Russell, 1987; Alvarez-Leefmans *et al.*, 1988).

(d)  $Cl^-$ - $HCO_3^-$  exchange mediates an electroneutral exchange of  $Cl^-$  and  $HCO_3^-$  across the cell membrane. Sodium-independent  $Cl^-$ - $HCO_3^-$  exchange systems have been identified in both neurons (Kopito *et al.*, 1989; Gaillard and Dupont, 1990; Pocock and Richards, 1992; see also Kay *et al.*, 1991) and glial cells (Kimelberg, 1990; Kettenmann, 1990). Unlike the  $Cl^-$  transport mechanisms described so far (a–c above), the activity of  $Cl^-$ - $HCO_3^-$  exchange exerts a direct influence on both of the two anions that are physiologically permeable in  $GABA_A$  receptor channels. In the transport mode depicted in Fig. 7, the direct effect on  $E_{GABA_A}$  of activation of  $Cl^-$ - $HCO_3^-$  exchange is a positive shift, since a 1:1 exchange of intracellular  $HCO_3^-$  for  $Cl^-$  leads to an increase in the “effective total concentration” (given by the denominator in Eqn. 1) of the intracellular permeant anions. A further factor that has to be taken into account when considering the impact of exchange ratios involving  $HCO_3^-$  is that, unlike  $Cl^-$ , changes in intracellular bicarbonate are attenuated by the intracellular non- $CO_2$  pH buffering power.

(e)  $Na^+$ -dependent  $Cl^-$ - $HCO_3^-$  exchange is an electroneutral transport system that was first identified in squid giant axons and molluscan neurons (Russell and Boron, 1976; Thomas, 1977) and subsequently found to be present in other invertebrate neurons (Moody, 1981; Moser, 1985; Schwiening and Thomas, 1992). It resembles the  $Cl^-$ - $HCO_3^-$  exchanger described above in that it translocates both  $Cl^-$  and  $HCO_3^-$ . However, due to the steepness of the  $Na^+$  gradient, this mechanism mediates a net extrusion of  $Cl^-$  linked to a simultaneous uptake of  $HCO_3^-$  and extrusion of  $H^+$  under physiological conditions. Despite the  $Cl^-:(H^+$  equivalent) stoichiometry of 1:2, the immediate consequence of an increase in the activity of this mechanism is a negative shift in  $E_{GABA_A}$  (cf. Eqn. 1; and note that all available estimates of the  $HCO_3^-:Cl^-$  permeability ratio are < 0.5 and that intracellular buffering acts on  $HCO_3^-$ ).

The present context offers a good opportunity to comment on the fact that, while it is possible to deduce the “immediate” action of a given transport mechanism *per se* on  $E_{GABA_A}$  on the basis of its stoichiometry, an analogous approach cannot be

always used when examining a cell under steady-state conditions. To give an example, the only mechanism that regulates pH<sub>i</sub> (and hence, [HCO<sub>3</sub><sup>-</sup>]) in crayfish opener muscle fibres is the Na<sup>+</sup>-dependent Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanger. However, due to the high background Cl<sup>-</sup> conductance of the muscle membrane, E<sub>Cl</sub> is effectively clamped at the level of the resting membrane potential (E<sub>Cl</sub> = E<sub>m</sub>). Consequently, the extrusion of Cl<sup>-</sup> on the exchanger is of no consequence in setting the driving force of the GABA<sub>A</sub> receptor-mediated current. However, in a bicarbonate-containing solution the uptake of HCO<sub>3</sub><sup>-</sup> on the exchanger leads to an E<sub>HCO</sub>, which is about -10 mV, i.e. much more positive than the resting membrane potential. As a result, E<sub>GABA,A</sub> is more positive than resting E<sub>m</sub> (see Kaila *et al.*, 1989, 1990).

(f) Na<sup>+</sup>-H<sup>+</sup> exchange is a well characterized acid extrusion mechanism that is present in virtually all animal cells, including neurons and glia (for reviews, see Thomas, 1984; Frelin *et al.*, 1988; Hoffmann and Simonsen, 1989; Clark and Limbird, 1991; Schlue *et al.*, 1991). Unlike the transport systems described above, it has no direct influence on the transmembrane Cl<sup>-</sup> distribution. In several kinds of cells, it is the main mechanism assisting the recovery of pH<sub>i</sub> following an acid load. The operation of this exchanger is not dependent on the presence of bicarbonate, but in the presence of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> a net increase in pH<sub>i</sub> brought about by Na<sup>+</sup>-H<sup>+</sup> exchange will, of course, give rise to an increase in the intracellular HCO<sub>3</sub><sup>-</sup> concentration. As indicated in Fig. 7, activation of the exchanger will lead to a positive shift in E<sub>HCO</sub>, and, at a fixed Cl<sup>-</sup> distribution, in a positive shift in E<sub>GABA,A</sub>. In this context one should recall, however, that a fast coupling between the concentrations of H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> necessitates the presence of the enzyme carbonic anhydrase (Maren, 1984; Dodgson, 1991). At present there is evidence that intracellular carbonic anhydrase is present not only in certain glial cells (especially in oligodendrocytes) but also in nerve cells including sensory and hippocampal neurons (Droz and Kazimierczak, 1987; Voipio *et al.*, 1991; Neubauer, 1991; Cammer, 1991; Pasternack *et al.*, 1993).

(g) Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup> cotransport is an electrogenic mechanism that takes up HCO<sub>3</sub><sup>-</sup> with a Na<sup>+</sup>:HCO<sub>3</sub><sup>-</sup> stoichiometry of 1:2 (Deitmer and Schlue, 1989) or 1:3 (Newman and Astion, 1991). Originally detected in epithelial cells (Boron and Boulpaep, 1983), it has been recently found to play a major role in pH<sub>i</sub> regulation in glial cells and to render pH<sub>i</sub> sensitive to membrane potential (Astion and Orkand, 1988; Kettenmann and Schlue, 1988; Deitmer and Schlue, 1989; Deitmer, 1991). With the exception of a study on cultured cerebellar granule cells (Pocock and Richards, 1992), neuronal expression of this transporter has not been reported. The intracellular accumulation of HCO<sub>3</sub><sup>-</sup> mediated by this system is expected to lead (at a fixed Cl<sup>-</sup> distribution) to a depolarizing shift in E<sub>GABA,A</sub>. However, intracellular Cl<sup>-</sup> in glial cells is usually rather high which precludes a significant influence on membrane potential of a possible HCO<sub>3</sub><sup>-</sup> component of GABA<sub>A</sub>-mediated currents. Despite this, a channel-mediated net efflux of HCO<sub>3</sub><sup>-</sup> down its electrochem-

ical gradient may give rise to GABA-induced pH shifts (Kaila *et al.*, 1991).

#### 4.3. HYPERPOLARIZING AND DEPOLARIZING RESPONSES EVOKED BY PHARMACOLOGICAL AND SYNAPTIC ACTIVATION OF GABA<sub>A</sub> RECEPTORS

Activation of GABA<sub>A</sub> receptors evokes electrical responses in a vast number of cell types, including all vertebrate central neurons, glial cells and various kinds of nerve and muscle cells of invertebrates. The examination below of some of the relevant work begins from studies on the crayfish leg opener muscle fibre since this is so far the only intact cell where the ionic basis of the voltage response mediated by GABA<sub>A</sub> receptors in the presence of HCO<sub>3</sub><sup>-</sup> has been quantitatively determined (Kaila and Voipio, 1987; Kaila *et al.*, 1989). A review of these results is followed by a description of GABA-mediated effects in the crayfish stretch-receptor neuron, which was the first preparation where Cl<sup>-</sup>-selective microelectrodes were successfully used for a direct test of the hypothesis that hyperpolarizing IPSPs are due to a nonequilibrium distribution of Cl<sup>-</sup> (Deisz and Lux, 1982). Thereafter, much of the concepts and knowledge related to the basic mechanisms that have been worked out in these two crustacean preparations will be used to examine GABA<sub>A</sub> receptor-mediated responses in vertebrate neurons and glial cells.

##### 4.3.1. Crayfish preparations

###### 4.3.1.1. Opener muscle fibre

The first experiments designed to gain information on the possible physiological role of HCO<sub>3</sub><sup>-</sup> in the actions of GABA were conducted on crayfish muscle fibres (Kaila and Voipio, 1987; Kaila *et al.*, 1989, 1990). The opener muscle fibres of the crayfish leg were eminently suited for the examination of the ionic basis of GABA action. Due to their large size, they permitted stable impalements (often lasting 4–8 hr) with five intracellular microelectrodes: three conventional ones for voltage and current clamping plus two ion-selective microelectrodes, one selective for Cl<sup>-</sup> and one for H<sup>+</sup> (used to estimate HCO<sub>3</sub><sup>-</sup>). In addition to this, a blunt H<sup>+</sup>-selective microelectrode was placed on the surface of the muscle fibre in order to monitor the pH in the unstirred water layer surrounding the fibre (surface pH, pH<sub>s</sub>), which was found to be directly affected by GABA-induced net movements of HCO<sub>3</sub><sup>-</sup>. The crayfish preparation has also the advantage that the GABA<sub>A</sub> receptors show only a small amount of desensitization even when activated by near-saturating agonist concentrations (Takeuchi and Takeuchi, 1967) so that a given fraction of the available conductance can be tonically activated by bath-applied GABA. Unlike neurons, the muscle fibres are not surrounded by glial cells, which excludes the possibility of any glial contribution to the responses examined. In retrospect, an additional definitive advantage of the opener muscle fiber is that—unlike the stretch receptor neuron (Kaila *et al.*, 1992b)—it does not generate a dis-

cernible uptake current when exposed to GABA (see below).

The background  $\text{Cl}^-$  permeability of the opener muscle fibre is high, which leads to an  $E_{\text{Cl}}$  that is virtually identical to the resting membrane potential under steady-state conditions (Kaila *et al.*, 1989). Extrusion of acid from the opener muscle fibre is achieved by  $\text{Na}^+$ -dependent  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange (Galler and Moser, 1986; Kaila *et al.*, 1990). In the experiments where the actions of GABA were examined, the preparations were kept in a solution equilibrated with 5%  $\text{CO}_2$  + 95% air, pH 7.4. Under these conditions, control  $\text{pH}_i$  was about 7.25,  $E_{\text{HCO}_3}$  was  $-9 \text{ mV}$  and (with a resting membrane potential of  $-75 \text{ mV}$ ) the  $\text{HCO}_3^-$  outward driving force was  $66 \text{ mV}$  (Kaila *et al.*, 1990).

The main findings concerning the role of  $\text{HCO}_3^-$  in the effect of GABA on membrane potential are evident in the recording shown in Fig. 8. In the presence of  $\text{HCO}_3^-$ , the GABA-evoked conductance increase is linked to a large (up to 20 mV) depolarization, to an increase in  $[\text{Cl}^-]_i$  and to a fall in  $[\text{HCO}_3^-]_i$ . The fact that picrotoxin completely blocks all three effects of GABA clearly showed that they are due to channel activation and not to some other mechanism, e.g. GABA uptake. A key finding in experiments of the above kind was that the GABA-induced depolarization was linked to a channel-mediated influx of  $\text{Cl}^-$ , i.e. the depolarization was opposed by an outwardly directed current component

carried by  $\text{Cl}^-$ . The GABA-induced increase in  $[\text{Cl}^-]_i$  was blocked by clamping the membrane potential at its resting level (Fig. 8) which indicated that the channel-mediated net movement of  $\text{Cl}^-$  was a response to the depolarization driven by another ion.

The conclusion that the inward current leading to the GABA-induced depolarization was carried by  $\text{HCO}_3^-$  was supported by a number of observations (Kaila *et al.*, 1989). The depolarization showed a dependence on  $\text{HCO}_3^-$  and it was strongly enhanced in the complete absence of both intracellular and extracellular  $\text{Cl}^-$ . In addition to this, a picrotoxin-sensitive,  $\text{HCO}_3^-$ -dependent GABA-gated current with a reversal potential equal to  $E_{\text{HCO}_3}$  (which was measured independently using  $\text{H}^+$ -selective microelectrodes), was observed following depletion of extra- and intracellular  $\text{Cl}^-$ . Simultaneous direct measurements of  $E_{\text{Cl}}$  and  $E_{\text{GABA}}$  under voltage-clamp conditions showed that, in the presence of  $\text{HCO}_3^-$ ,  $E_{\text{Cl}}$  was about 15 mV more negative than  $E_{\text{GABA}}$  (Kaila *et al.*, 1989). These experiments yielded a relative permeability of  $\text{HCO}_3^-$  vs  $\text{Cl}^-$  of 0.33. As noted earlier on, so far the measurements of the  $\text{HCO}_3^-$  permeability carried out on the crayfish opener muscle are the only ones where  $\text{CO}_2$  has been at equilibrium across the cell membrane and hence, problems related to an accurate control of the  $\text{HCO}_3^-$  gradient (cf. Section 3.2) are effectively circumvented.

#### Measurements of the GABA-activated conductance

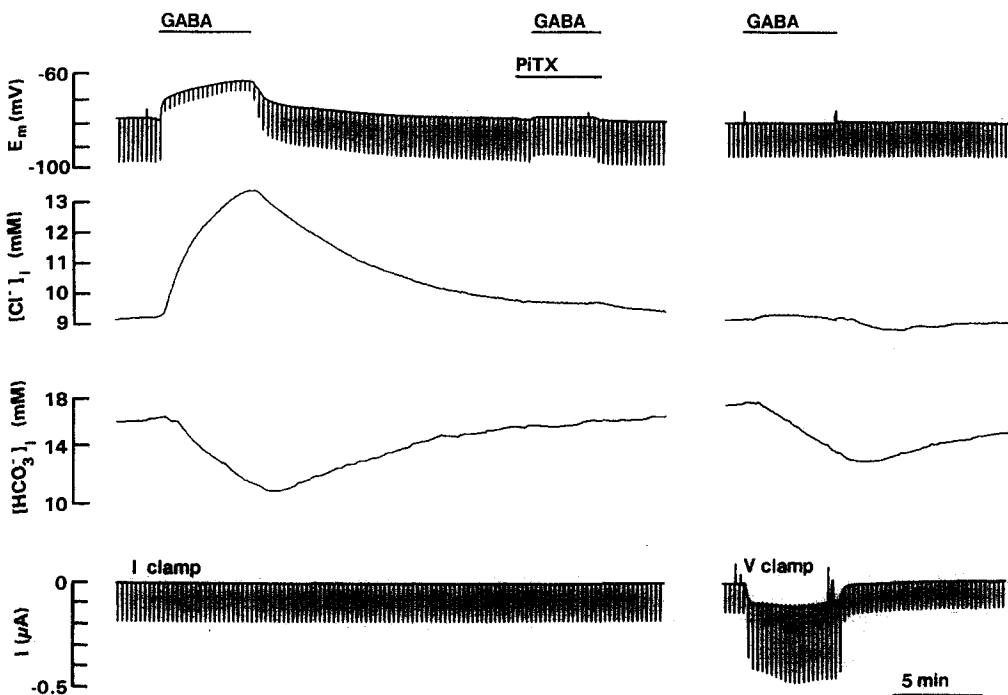


FIG. 8. Experiment on a crayfish muscle fibre illustrating the effects of GABA (0.2 mM) on membrane potential and current, as well as on  $[\text{Cl}^-]_i$  and  $[\text{HCO}_3^-]_i$  (estimated on the basis of  $\text{pH}_i$ ). Under current-clamp conditions, GABA produces a large depolarization which is coupled to an increase in intracellular  $\text{Cl}^-$  and to a decrease in  $[\text{HCO}_3^-]_i$ . All three effects of GABA are inhibited by picrotoxin (PITX, 0.1 mM). As shown in the final part of the recording, clamping the membrane potential at its resting level blocks the GABA-induced chloride shift, but not the fall in  $[\text{HCO}_3^-]_i$ . Now, the current carried by  $\text{HCO}_3^-$  is seen as an inward shift in holding current (modified from Kaila *et al.*, 1989).

under conditions where only one of the two permeant anions was present yielded (assuming constant-field conditions) an estimate of about 0.4 for the relative HCO<sub>3</sub><sup>-</sup> permeability. The fact that the reversal-potential measurements gave a slightly lower value can be explained by assuming that Cl<sup>-</sup> ions interact more strongly than HCO<sub>3</sub><sup>-</sup> ions with binding sites within the GABA<sub>A</sub> receptor channel (cf. Section 3.3.3).

As could be predicted on the basis of the above results, HCO<sub>3</sub><sup>-</sup> induces a large positive shift in the GABA<sub>A</sub> receptor-mediated IPSP evoked by stimulation of the inhibitory nerve in the opener muscle fibre (Golan *et al.*, 1991).

#### 4.3.1.2. Crayfish stretch-receptor neurons

Observations on the effects of NH<sub>4</sub><sup>+</sup> ions on IPSPs in mammalian neurons suggested that a channel-mediated influx of Cl<sup>-</sup> was responsible for the hyperpolarization that was often associated with postsynaptic inhibition (Lux, 1971; Llinás *et al.*, 1974). Direct evidence for a nonequilibrium distribution of Cl<sup>-</sup>, maintained by active chloride extrusion, was subsequently obtained in pioneering work on the crayfish stretch-receptor neuron (Deisz and Lux, 1982; Aickin *et al.*, 1982). Based on the effects of furosemide and extracellularly applied cations (including NH<sub>4</sub><sup>+</sup>), it was concluded that extrusion of Cl<sup>-</sup> in the stretch-receptor neuron was mediated by K<sup>+</sup>-Cl<sup>-</sup> cotransport.

The above experiments on the crayfish stretch-receptor neuron were carried out in the absence of HCO<sub>3</sub><sup>-</sup>. However, even under these conditions, E<sub>GABA</sub> does not equal E<sub>Cl</sub>, as has recently been demonstrated (Kaila *et al.*, 1992b). Experiments with a two-microelectrode current/voltage clamp and Cl<sup>-</sup>-selective microelectrodes showed that E<sub>Cl</sub> (estimated under voltage-clamp conditions by measuring the reversal potential of the instantaneous Cl<sup>-</sup> fluxes evoked by brief pulses of GABA) was more negative than the resting membrane potential. However, despite the inwardly directed Cl<sup>-</sup> electrochemical gradient, application of GABA produced a mono- or biphasic depolarization, often with a prominent initial depolarizing component followed by a transient shift to a more negative level. In some neurons, an additional depolarizing phase was seen upon washout of GABA. Receptor desensitization was negligible and played no role in the above effects of GABA. In contrast to the large Cl<sup>-</sup>-dependent conductance increase evoked by GABA, the depolarizing current component was not inhibited by PiTX, but it was blocked in the absence of Na<sup>+</sup> and following the application of nipecotic acid, an inhibitor of GABA uptake (Krogsgaard-Larsen, 1988). Unlike the PiTX-sensitive Cl<sup>-</sup> mediated outward current, the PiTX-resistant inward current was immediately activated by low concentrations of GABA and it had a reversal potential of +30 to +60 mV.

All the findings above, as well as the lack of a significant inward current following application of the specific GABA<sub>A</sub> agonist, muscimol, indicated that the PiTX-resistant current was caused by the operation of an electrogenic GABA uptake system in the stretch-receptor neuron (Kaila *et al.*, 1992b). Whether an electrogenic uptake mechanism directly

contributes to GABA-evoked voltage responses in other kinds of neurons which receive a GABAergic innervation is presently not known.

It may be advisable to restate that all of the work on the stretch-receptor neuron discussed above was carried out in nominally HCO<sub>3</sub><sup>-</sup>-free solutions. In agreement with the results obtained in the opener muscular fibres, a change from a HCO<sub>3</sub><sup>-</sup>-free solution to one equilibrated with 5% CO<sub>2</sub> in air induces a positive shift in E<sub>GABA</sub> in the stretch-receptor neuron (Voipio *et al.*, 1991). The conclusion that this shift was, indeed, attributable to a bicarbonate conductance gated by GABA was supported by the observations that, following complete depletion of both extra- and intracellular Cl<sup>-</sup>, GABA produced a large PiTX-sensitive HCO<sub>3</sub><sup>-</sup>-dependent depolarization which was sufficient to elicit firing of action potentials. In other words, with HCO<sub>3</sub><sup>-</sup> as the dominant permeable anion, GABA acted in the manner of an excitatory transmitter.

To summarize, in the crayfish stretch-receptor neuron, two GABA-activated current components exist which tend to make the reversal potential of GABA action more positive than E<sub>Cl</sub>: the HCO<sub>3</sub><sup>-</sup> current mediated by GABA<sub>A</sub> receptor channels and the GABA uptake current. This implies that, despite the absence of GABA receptor channels other than those of the A type, E<sub>IPSP-A</sub> and E<sub>GABA</sub> are not equal in the stretch-receptor neuron. In fact, this conclusion can be made on the basis of the finding that, due to the different affinities of the receptor channels and the uptake system (cf. Deisz and Dose, 1983), E<sub>GABA</sub> shows a clear concentration dependence in the stretch receptor neuron, such that at low concentrations E<sub>GABA</sub> approaches the extremely positive reversal potential of the uptake mechanism. However, there is reason to believe that a significant neuronal uptake current is seen only upon application of exogenous GABA (Kaila *et al.*, 1992b).

#### 4.3.2. Mammalian central neurons

In textbooks of neurophysiology, it is customary to regard a hyperpolarizing action as a characteristic feature of "conventional" (GABA<sub>A</sub> receptor-mediated) postsynaptic inhibition in the mammalian CNS. This, however, is a strong generalization that is mainly based on an extrapolation from the classical studies by Eccles and coworkers on the (glycinergic) mechanism of postsynaptic inhibition in mammalian motoneurons (Coombs *et al.*, 1955; Eccles, 1964b) and from the early work on GABA-mediated inhibition carried out on neocortical and hippocampal neurons *in vivo* (Kandel *et al.*, 1961; Krnjević and Schwartz, 1967; Eccles *et al.*, 1977). It is a well known fact that both synaptic and pharmacological activation of GABA<sub>A</sub> receptors can lead to complex effects on the membrane potential in various types of mammalian central neurons and, in addition to hyperpolarizations, depolarizing as well as bi- and multiphasic voltage responses mediated by GABA<sub>A</sub> receptors have frequently been encountered. The depolarizing responses mediated by GABA<sub>A</sub> receptors in adult central neurons are usually not large enough to exceed the neuronal firing threshold and hence, they are compatible with a genuine inhibitory

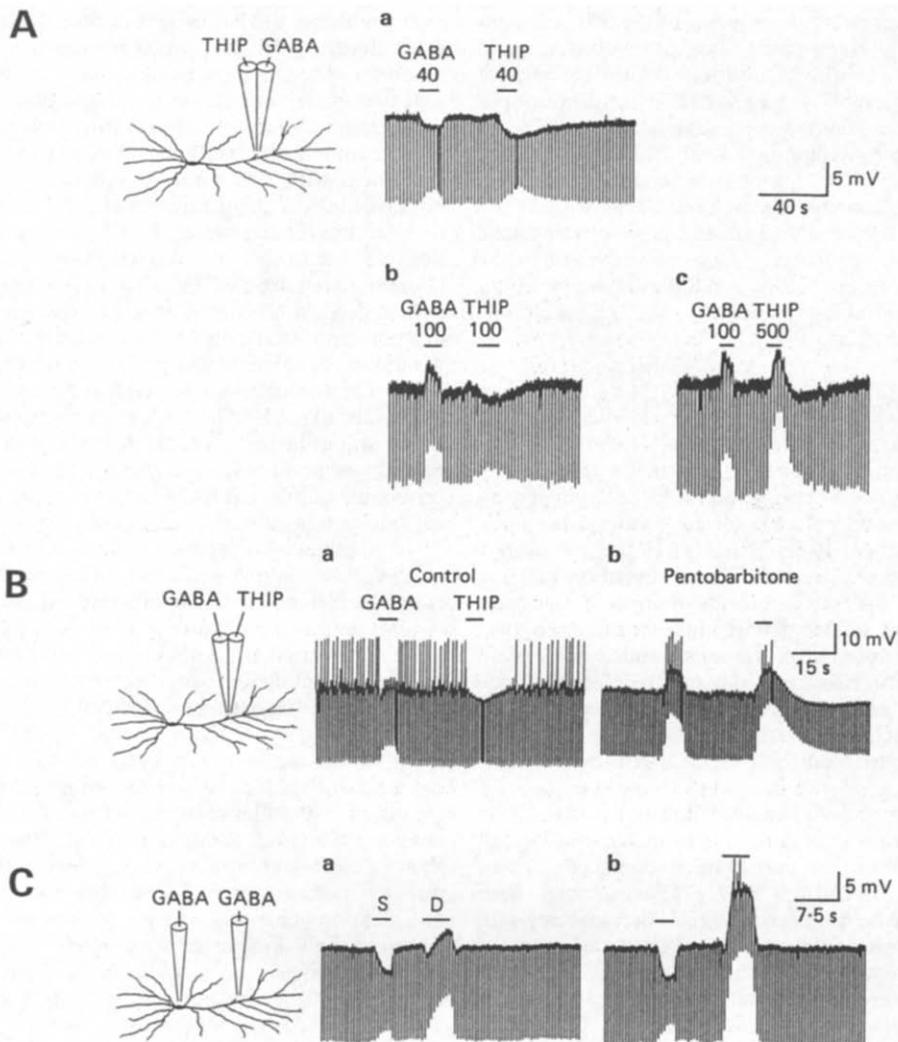


FIG. 9. Voltage and conductance changes evoked by iontophoretic application of GABA and THIP on the somatic and dendritic region of rat hippocampal pyramidal neurons (CA1 region). Schematic drawing of ionophoretic electrode placement shown on the left. (A)(a) A relatively weak ionophoretic current (indicated in nA) evokes hyperpolarizing dendritic responses to both GABA and THIP. (b) Recording from another neuron shows a depolarizing GABA response and a hyperpolarizing THIP response upon equal ionophoretic currents. (c) The current for the THIP response had to be increased five-fold to produce a depolarization equal to that evoked by GABA. (B) Hyperpolarizing dendritic GABA and THIP responses (a) are converted into depolarizations following application of 100  $\mu$ M pentobarbital (b). Ionophoretic current 200 nA throughout. (C) Hyperpolarizing somatic (S) response and depolarizing dendritic (D) response (a) are potentiated by 100  $\mu$ M pentobarbital. The somatic and dendritic responses were induced with ionophoretic currents of 2 and 25 nA, respectively. (Reproduced by permission from Alger and Nicoll, 1982b.)

action based on the associated conductance increase which can shunt the much more intense depolarizations elicited by excitatory input (e.g. Staley and Mody, 1992). However, a recent study shows that a strongly depolarizing (and hence excitatory) coupling mediated by  $GABA_A$  receptors synchronizes the firing of inhibitory interneurons in the adult hippocampus (Michelson and Wong, 1991).

#### 4.3.2.1. Exogenous GABA

Detailed work done under conditions *in vitro* has revealed both hyperpolarizing and depolarizing as well as biphasic and multiphasic responses mediated by  $GABA_A$  receptors in adult neurons in the hippocampus, neocortex, thalamus and lateral geniculate nucleus (Alger and Nicoll, 1979; Andersen *et al.*, 1980; Djørup *et al.*, 1981; Thalmann *et al.*, 1981;

Rovira *et al.*, 1984; Scharfman and Sarvey, 1985; Huguenard and Alger, 1986; Thomson, 1988; Crunelli *et al.*, 1988; Higashi *et al.*, 1991; Luhmann and Prince, 1991). Moderately depolarizing responses have been observed in the supraoptic nucleus (Ogata, 1987), in the olfactory cortex (Brown and Scholfield, 1979) and in striatal neurons (Mercuri *et al.*, 1991). An interesting aspect of this variability is that it is not entirely due to differences in cell type but large quantitative and qualitative differences have been observed in somatic vs. dendritic regions in neurons of a given kind (Fig. 9).

A frequently-cited work by Barker and Ransom (1978) on mouse spinal and cerebellar-brainstem neurons grown in tissue culture showed that the Cl<sup>-</sup>-dependent voltage responses that were evoked by iontophoretic application of GABA at the cell body were hyperpolarizing while those at peripheral processes were either hyperpolarizing, depolarizing or a combination of both. All of these responses were associated with a large increase in membrane conductance. A further observation of interest was that in about half of the cells tested, a prolonged or repeated application of GABA (or glycine) transformed the hyperpolarizing GABA responses into depolarizations with little effect on the associated conductance increase. It is obvious that while a simple dissipation of the Cl<sup>-</sup> electrochemical gradient in response to a maintained conductance might well explain an abolishment of the driving force underlying the GABA-evoked current (cf. Barker and Ransom, 1978), such an explanation cannot account for a reversal in the polarity of a current that is solely carried by Cl<sup>-</sup>. However, a reversal of current polarity of the above kind can be explained if one assumes that in the above experiments, part of the GABA-induced current was carried by HCO<sub>3</sub><sup>-</sup> (cf. Kaila *et al.*, 1989).

In a manner resembling that later seen in hippocampal neurons (e.g. Alger and Nicoll, 1982b), pentobarbital converted the pure hyperpolarizations evoked by GABA in mouse spinal neurons into biphasic responses (Barker and Ransom, 1978). This, in itself, may suggest the presence of two types of functionally different GABA<sub>A</sub> receptors (Alger and Nicoll, 1982b). Nevertheless, the situation is complicated by unpublished observations (reported on p. 350 in Barker and Ransom, 1978) showing that, in the presence of picrotoxin, the GABA-evoked multiphasic responses were converted into pure depolarizations. In the crayfish stretch-receptor neuron, a similar effect has been shown to be due to the operation of an inward current component mediated by Na<sup>+</sup>-dependent electrogenic uptake of GABA (Kaila *et al.*, 1992b; see also Malchow and Ripps, 1990). Taken together, the effects of GABA reported by Barker and Ransom (1978) may involve a GABA<sub>A</sub> receptor-mediated current carried by Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> as well as an inwardly-directed uptake current.

Hyperpolarizing, depolarizing and biphasic responses have been frequently observed upon pharmacological activation of GABA<sub>A</sub> receptors in hippocampal pyramidal neurons (Alger and Nicoll, 1979, 1982b; Andersen *et al.*, 1980; Djørup *et al.*, 1981; Thalmann *et al.*, 1981; Wong and Watkins, 1982; Huguenard and Alger, 1986). Again, the somatic response is a hyperpolarization while dendritic re-

sponses are more depolarizing (Fig. 9). Application of pentobarbital enhances the depolarizing response much more than the hyperpolarizing one, while diazepam does the opposite (Alger and Nicoll, 1982b). However, it is important to point out that hyperpolarizing responses can also be generated in the dendrites (that is, they are not exclusively limited to the cell body; Fig. 9) and, in fact, there seems to be a dependence on concentration such that high concentrations of GABA tend to produce more depolarizing (or less hyperpolarizing) responses (Andersen *et al.*, 1980; Alger and Nicoll, 1982b; Wong and Watkins, 1982; Newberry and Nicoll, 1985). As also shown in Fig. 9, the GABA<sub>A</sub> agonist, THIP, evokes both hyperpolarizing and depolarizing responses in hippocampal neurons, which suggests that the depolarizing action of GABA cannot be explained on the basis of an electrogenic uptake mechanism. In the light of the present knowledge, there seems not to be any simple explanation to account for all the above observations, but it is possible to postulate the involvement of two types of receptors with distinct pharmacological properties (Alger and Nicoll, 1982b) and ionic selectivities.

The idea (originally put forward but rejected by Andersen *et al.*, 1980) that qualitative differences of the above kind in the somatic and dendritic responses to GABA might arise from the presence of a Cl<sup>-</sup> uptake mechanism in the dendrites with Cl<sup>-</sup> extrusion taking place in the soma will be dealt with in detail below in connection with synaptically-evoked GABA<sub>A</sub> responses. However, the concentration dependence of the polarity of the GABA-mediated response and the effects of pentobarbital (Fig. 9), are not easily explained on the basis of a difference between somatic and dendritic Cl<sup>-</sup> driving forces.

#### 4.3.2.2. Synaptically-evoked responses

In a variety of standard experimental situations (particularly in experiments on brain slices) synchronous neuronal activity evoked by extracellular stimuli leads to a stereotyped pattern of postsynaptic responses that can be recorded intracellularly: a fast EPSP most likely mediated by glutamate, followed by a fast and a slow IPSP (e.g. Alger and Nicoll, 1982a; Connors *et al.*, 1982, 1988; Howe *et al.*, 1987; Crunelli *et al.*, 1988; Higashi *et al.*, 1991; Lacaille, 1991). There is much evidence favouring the conclusion that the fast IPSP (IPSP<sub>A</sub>, which usually peaks within tens of milliseconds) is mediated by anion conductance gated by GABA<sub>A</sub> receptors while the slower one (IPSP<sub>B</sub>, which peaks with a delay of 100–200 msec) is due to a K<sup>+</sup> conductance controlled by GABA<sub>B</sub> receptors via G proteins (reviewed by Nicoll *et al.*, 1990; Alger, 1991; Sivilotti and Nistri, 1991; Connors, 1992). Figure 10A illustrates such EPSP-IPSP sequences evoked under current-clamp conditions in a rat neocortical neuron.

The compound postsynaptic potentials in neurons in brain slices are usually produced by monosynaptic and multisynaptic pathways which include various types of inhibitory interneurons (Andersen, 1990; Somogyi, 1990; Connors, 1992). A convenient way to study monosynaptic IPSPs and IPSCs in isolation is to work on hippocampal slices exposed to glutamate

antagonists, with stimuli applied in the stratum radiatum (area CA1) close to an intracellular recording site in the cell body layer (Collingridge *et al.*, 1988; Davies *et al.*, 1990). In addition to studies of isolated IPSPs and IPSCs, this experimental design has allowed an analysis of extracellular field potentials (Lambert *et al.*, 1991) and ionic shifts (Kaila *et al.*, 1992a) directly related to GABAergic inhibition.

The fast GABAergic IPSP<sub>A</sub>s seen *in vivo* are hyperpolarizing in both the neocortex and hippocampus (Kandel *et al.*, 1961; Krnjević and Schwartz, 1967). However, strongly hyperpolarizing fast IPSP<sub>A</sub>s are rarely seen in neocortical neurons recorded in slices (Connors *et al.*, 1982; Howe *et al.*, 1987; Deisz and Prince, 1989; Kaila *et al.*, 1993). This discrepancy may be due to neuronal damage and a consequent reduction of the resting  $E_m$  following impalements under conditions *in vivo*. In hippocampal pyramidal neurons, synaptic activation of GABA<sub>A</sub> receptors evokes both hyperpolarizing (mainly somatic) and depolarizing (mainly dendritic) responses (Alger and Nicoll, 1982a; Perreault and Avoli, 1991) while purely depolarizing responses are observed in granule cells of the dentate gyrus (Misgeld *et al.*, 1986).

The complex pattern described above of voltage transients evoked by pharmacological activation of GABA<sub>A</sub> receptors in hippocampal pyramidal neurons is largely matched by inhibitory synaptic responses. Working on rat brain slices, Alger and Nicoll (1982b) found that the reversal potential of the IPSP mediated by recurrent inhibition (IPSP<sub>A</sub>, evoked by stimulation of pyramidal axons) was similar to that of the somatic (hyperpolarizing) GABA response. The orthodromically evoked IPSP (seen upon stimulation of the Schaffer collaterals) is a more complex response which is now known to contain an early (IPSP<sub>A</sub>) and a late (IPSP<sub>B</sub>) component. Following application of pentobarbital, the early IPSP consisted of an initial fast hyperpolarizing phase followed by a depolarizing component which was also mediated by GABA<sub>A</sub> receptors (Alger and Nicoll, 1982a). In view of the influence of pentobarbital on the actions of exogenous GABA (Alger and Nicoll, 1982b), this result can be explained by assuming that the GABA<sub>A</sub> receptors mediating the depolarizing response are normally activated by synaptic release of GABA on dendrites, but that the very powerful overlapping somatic hyperpolarizing IPSP<sub>A</sub> and the slow IPSP<sub>B</sub> mask this dendritic response (Nicoll *et al.*, 1990). Such an interpretation is consistent with the observations that presynaptic potentiation of transmission induced by 4-aminopyridine (Perreault and Avoli, 1991) leads to the generation of depolarizing IPSP<sub>A</sub>s (Avoli and Perreault, 1987; Avoli *et al.*, 1988). The above explanation (Nicoll *et al.*, 1990) has superseded the original assumption (Alger and Nicoll, 1982b) that the depolarizing response is mediated by dendritic extrasynaptic receptors.

The ionic basis of the IPSP<sub>A</sub>s was examined by Misgeld *et al.* (1986) in experiments on CA3 pyramidal neurons and dentate gyrus granule cells in guinea pig hippocampal slices. These two types of neurons were found to generate a hyperpolarizing and a depolarizing IPSP<sub>A</sub>, respectively. The key finding made by the above authors was that intracellular injection of Cl<sup>-</sup> shifted the fast IPSP in both cell types into a depolar-

izing direction and application of furosemide (0.5–2 mM) decreased the recovery from the injection. In addition to this, both GABA-induced hyperpolarizing responses (seen in pyramidal cell somata) and depolarizing responses (seen in pyramidal dendrites and granule cells) were reduced in the presence of furosemide, with no detectable change in the parallel increase in conductance. In order to explain these results, the authors proposed that the hippocampal neurons examined are equipped with both an inwardly directed and an outwardly directed Cl<sup>-</sup> transport mechanism. An additional assumption that had to be done is that the two types of Cl<sup>-</sup> transport mechanisms are homogeneously distributed in the granule cells while in the CA3 pyramidal neurons, the chloride uptake mechanism is mainly localized in the dendrites and the extrusion mechanism in the cell body (cf. Andersen *et al.*, 1980). Assuming that the resting membrane potential of the somatic region of pyramidal neurons is not higher than that prevailing in the dendrites, the above explanation implies the presence of an intracellular Cl<sup>-</sup> gradient, which leads to a constant Cl<sup>-</sup> flux from the dendrites to the soma.

If correct, the dual-pump hypothesis of Misgeld *et al.* (1986) would imply that a large fraction of CNS neurons continuously pump in chloride into their dendrites and extrude it from the soma. However, it is worth restating here that a difference in the polarity of the Cl<sup>-</sup> driving force in somatic and dendritic areas does not explain the fact that both hyperpolarizing and depolarizing responses can be evoked by dendritic application of GABA, nor does it account for the frequently observed concentration dependence of the polarity of the voltage responses (Barker and Ransom, 1978; Andersen *et al.*, 1980; Djørup *et al.*, 1981; Alger and Nicoll, 1982b; Wong and Watkins, 1982; Newberry and Nicoll, 1985). The view that GABA<sub>A</sub> receptor-mediated voltage responses in hippocampal neurons can be explained in terms of the Cl<sup>-</sup> driving force only is also at variance with results from work on rat hippocampal slices involving laminar analysis of extracellular field IPSPs associated with monosynaptic GABA-mediated transmission (Lambert *et al.*, 1991). One of the main findings in this study was that the current sinks associated with depolarizing IPSP<sub>A</sub>s evoked in the presence of 4-aminopyridine (cf. Avoli *et al.*, 1988; Perreault and Avoli, 1991) were localized in the same dendritic area as the current sources which were associated with hyperpolarizing IPSP<sub>A</sub>s seen under control conditions (Lambert *et al.*, 1991). The authors note that their observations are not consistent with the idea that depolarizing IPSP<sub>A</sub>s are caused by an inward current carried by Cl<sup>-</sup>. A possible explanation is that two types of GABA<sub>A</sub> receptor channels with distinct ionic permeabilities coexist in the dendrites (see also Nicoll *et al.*, 1990). In addition to its lower sensitivity to GABA and higher sensitivity to GABA<sub>A</sub> antagonists (Wong and Watkins, 1982; Alger and Nicoll, 1982b; Thomson, 1988), the GABA<sub>A</sub> receptor channel type responsible for the depolarizing action might have a higher permeability to HCO<sub>3</sub><sup>-</sup> than the hyperpolarizing one. A high HCO<sub>3</sub><sup>-</sup> permeability of the dendritic GABA<sub>A</sub> receptor channel would explain the depolarizing action of GABA and make the assumption of an intracellular Cl<sup>-</sup> gradient obsolete. Another possibility is that pH<sub>i</sub>

within dendrites is higher than in the soma, which would lead to a more positive  $E_{GABA_A}$  in the dendrites (cf. Figs 6 and 10D).

The idea that HCO<sub>3</sub><sup>-</sup> ions are involved in the generation of GABA<sub>A</sub> receptor-mediated responses in mammalian neurons under physiological conditions has not been addressed in experimental work until very recently in a study on rat neocortical slices (Kaila *et al.*, 1993). This work was motivated by the fact that there is much evidence—albeit indirect—that mammalian neocortical neurons are equipped with a powerful Cl<sup>-</sup> extrusion mechanism, most likely a K<sup>+</sup>-Cl<sup>-</sup> cotransporter (Thompson *et al.*, 1988a,b; Thompson and Gähwiler, 1989a,b). However, a number of studies on neocortical neurons have shown that  $E_{IPSP_A}$  is often more positive than the resting membrane potential (Connors *et al.*, 1982;

Deisz and Prince, 1989; Howe *et al.*, 1987). It is also of interest to note that spontaneous IPSP<sub>A</sub>s recorded with acetate-filled microelectrodes in neocortical neurons *in vitro* are depolarizing (Connors *et al.*, 1982). In view of the widely-accepted equality  $E_{IPSP_A} = E_{Cl^-}$ , it is rather surprising that the obvious paradox of depolarizing GABA<sub>A</sub> responses in neurons that actively extrude Cl<sup>-</sup> has attracted little attention.

The possible contribution of HCO<sub>3</sub><sup>-</sup> to IPSP<sub>A</sub>s was studied using acetate and methanesulfonate-filled intracellular microelectrodes (Kaila *et al.*, 1993). In most neurons, IPSP<sub>A</sub> was depolarizing with a mean reversal potential about 6 mV more positive than the resting membrane potential (see Fig. 10A,B). An examination of the temporal relationships between EPSPs and IPSP<sub>A</sub>s indicated that a contamination of

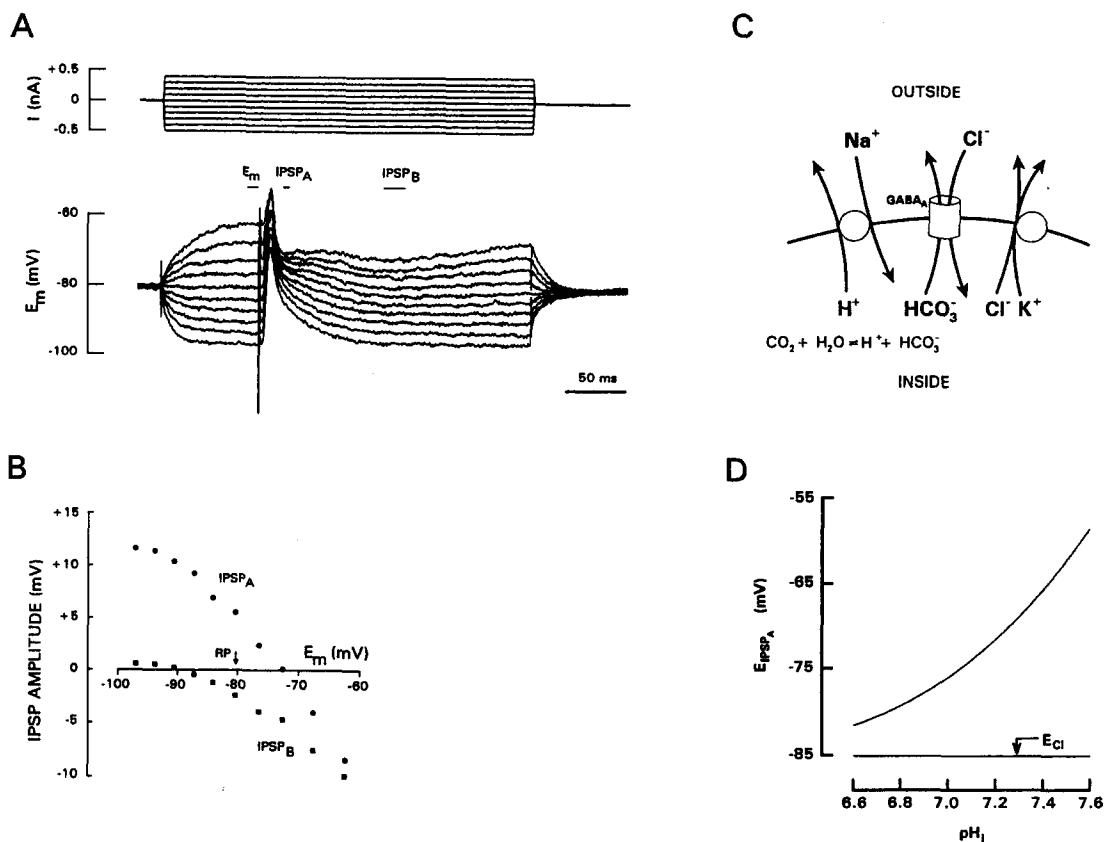


FIG. 10. Synaptic responses and the ionic mechanism of IPSP<sub>A</sub> generation in rat neocortical neurons. (A) Recording from a pyramidal neuron in a neocortical slice showing synaptic responses evoked at various levels of membrane potential (bottom traces) set by current injection (top traces). Time windows used for the measurements of IPSP<sub>A</sub>, IPSP<sub>B</sub> and membrane potential ( $E_m$ ) are indicated with horizontal bars. (B) The amplitudes of the inhibitory postsynaptic potentials shown in (A) have been plotted against membrane potential to determine the reversal potential of IPSP<sub>A</sub> ( $-72.5$  mV) and IPSP<sub>B</sub> ( $-89.4$  mV). RP indicates resting potential ( $-80.3$  mV). Note that  $E_{IPSP_A}$  is more positive than resting potential. (C) Ionic basis of the reversal potential of GABA<sub>A</sub> receptor-mediated IPSPs. Scheme depicting the neuron which is equipped with a K<sup>+</sup>-Cl<sup>-</sup> cotransporter extruding Cl<sup>-</sup> and with an acid extrusion mechanism (an Na<sup>+</sup>-H<sup>+</sup> exchanger or its equivalent) which maintains an outwardly directed HCO<sub>3</sub><sup>-</sup> electrochemical gradient. The current across the GABA<sub>A</sub> receptor channel is carried by an influx of Cl<sup>-</sup> and by an efflux of HCO<sub>3</sub><sup>-</sup>. (D) Dependence on pH<sub>i</sub> of  $E_{IPSP_A}$  calculated on the basis of the Goldman-Hodgkin-Katz voltage equation (Eqn. 1) and plotted assuming a HCO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> permeability ratio of 0.2 and that  $E_{Cl^-} = -85$  mV. Note that  $E_{IPSP_A}$  equals  $E_{Cl^-}$  only if the HCO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> permeability ratio = 0. It is evident that, even if  $E_{Cl^-}$  stays constant, a rise in pH<sub>i</sub> will lead to a depolarizing shift of  $E_{IPSP_A}$  while a fall in pH<sub>i</sub> has the opposite effect. (Reproduced by permission from Kaila *et al.*, 1993, slightly modified.)

$IPSP_A$  by the preceding EPSP had little influence on the measured values of  $E_{IPSP_A}$ . In order to study whether  $IPSP_A$  generation involves a current component carried by  $HCO_3^-$ , the sensitivity of  $E_{IPSP_A}$  to experimental manoeuvres known to alter  $pH_i$  and, hence,  $[HCO_3^-]_i$ , was examined at a constant extracellular  $Cl^-$  concentration. Application of 20 mM trimethylamine (TriMA), a membrane-permeant weak base which is expected to produce a rise in  $pH_i$  (Eisner *et al.*, 1989; Szatkowski and Thomas, 1989), was found, indeed, to produce a reversible positive shift in  $E_{IPSP_A}$  of up to +9.0 mV. In some experiments, the shift in reversal potential was associated with a change in the polarity of  $IPSP_A$  from hyperpolarizing to depolarizing. In a completely opposite manner, application of 20 mM lactate (a membrane-permeant weak acid) which is known to induce an intracellular acid load (Sharp and Thomas, 1981; Roos and Boron, 1981; Szatkowski and Thomas, 1989) produced a hyperpolarizing shift in  $E_{IPSP_A}$ , and, in some experiments, changed the polarity of  $IPSP_A$  from depolarizing to hyperpolarizing. The shifts in  $E_{IPSP_A}$  were not associated with a synaptic conductance change. This is in agreement with observations indicating that  $GABA_A$  receptor channels are insensitive to changes in  $pH_i$  (Pasternack *et al.*, 1992a).

All the above results obtained on neocortical neurons are fully compatible with the assumption that  $HCO_3^-$  ions contribute significantly to the generation of  $IPSP_A$  (Fig. 10C,D). This implies that even at a constant  $E_{Cl^-}$  more negative than the resting potential, a hyperpolarizing  $IPSP_A$  can be converted to a depolarizing one simply by a sufficiently large rise in  $pH_i$  which leads to an increase in  $[HCO_3^-]_i$  (cf. Fig. 6). Conversely, an initially depolarizing  $IPSP_A$  can become hyperpolarizing upon an intracellular acidosis. Using the Goldman-Hodgkin-Katz (GHK) equation (Eqn. 1 above) with reasonable values for the intracellular  $Cl^-$  and  $HCO_3^-$  concentrations, the observations suggested that, under control conditions,  $E_{IPSP_A}$  in rat neocortical neurons is 10–20 mV more positive than  $E_{Cl^-}$ . In Fig. 10D,  $E_{Cl^-}$  is assumed to be –85 mV. It is evident from the GHK equation that a lower  $[Cl^-]$ , yielding a more negative value of  $E_{Cl^-}$  would lead to an even larger deviation of  $E_{IPSP_A}$  from  $E_{Cl^-}$ .

#### 4.3.3. Neonatal neurons

During early developmental stages in various parts of the vertebrate CNS, GABA has a strongly depolarizing effect mediated by  $GABA_A$  receptor channels (e.g. Bixby and Spitzer, 1982; Swann *et al.*, 1989; Luhmann and Prince, 1991; Ben-Ari *et al.*, 1989; Cherubini *et al.*, 1990) and it acts mainly as an excitatory transmitter (Cherubini *et al.*, 1991). In pyramidal cells of area CA3 in rat neonatal hippocampal slices, spontaneously occurring giant depolarizing potentials (GDPs), apparently mediated by  $GABA_A$  receptors, can be observed. The GDPs disappear at the end of the first postnatal week, at a time when the reversal potential of  $GABA_A$  current shifts to a hyperpolarizing level (Ben-Ari *et al.*, 1989; see also Obata *et al.*, 1978; Janigro and Schwartzkroin, 1988).

The ionic basis of the  $GABA_A$  receptor-mediated

depolarizing actions is not known, but an obvious explanation could be, of course, that it is due to a high intracellular  $Cl^-$  concentration generated by active uptake of chloride (Janigro and Schwartzkroin, 1988; Cherubini *et al.*, 1991). There is evidence from experiments on neocortical slices involving intracellular  $Cl^-$  loading that extrusion of chloride is relatively inefficient in immature neurons (Luhmann and Prince, 1991). Again, however, the additional possibility has to be taken into account that currents carried by  $HCO_3^-$  play a role in the generation of depolarizing  $GABA_A$  responses. In this context, an interesting observation made on fetal and neonatal neurons in the rat spinal cord is that the  $Cl^-$ -sensitive depolarizations induced by GABA were much larger than those evoked by glycine, despite the fact that both amino acids induced a similar increase in membrane conductance (Wu *et al.*, 1992). This difference is hard to explain if both types of receptors were permeable to a single ionic species ( $Cl^-$ ) only. As recognized by the authors, a significantly higher relative permeability of  $HCO_3^-$  vs.  $Cl^-$  of the  $GABA_A$  receptor channels would readily account for the more potent depolarizing action of GABA (Wu *et al.*, 1992; cf. Bormann *et al.*, 1987). However, in contrast to the above observations, experiments on neonatal hippocampal neurons have yielded virtually identical values for the reversal potentials of the depolarizing responses evoked by GABA and glycine (Ito and Cherubini, 1991).

Taken together, the information presently available on the ionic basis of the developmental changes in  $GABA_A$  receptor function is rather scanty and much more work needs to be done in order to identify the mechanisms that generate and maintain the gradients of the relevant anions. There is not, either, any information available on the question whether  $GABA_A$  receptor channel types which are expressed during early developmental stages differ from those of mature neurons with respect to their ion selectivity.

#### 4.3.4. Sympathetic and sensory neurons

The phenomenon of presynaptic inhibition mediated by axo-axonal synapses was discovered by Dudel and Kuffler (1961) in work on the crayfish neuromuscular synapse and subsequent studies demonstrated that the inhibitory effect was mediated by a GABA-induced anion conductance (Takeuchi and Takeuchi, 1966a). Work on the vertebrate spinal cord showed that GABAergic synapses are responsible for presynaptic inhibition at primary afferent terminals (Eccles, 1964a) with at least part of the effect being mediated by  $GABA_A$  receptors (Nicoll and Alger, 1979; Sivilotti and Nistri, 1991).

Extracellular recordings of dorsal root potentials strongly suggested that presynaptic inhibition was linked to a depolarization of the terminals of primary afferents (Eccles, 1964a). Recent intraterminal recordings from sensory afferents (and from interneurons) in the lamprey spinal cord have, indeed, directly demonstrated a depolarizing action mediated by  $GABA_A$  receptors (Alford *et al.*, 1991; for work on invertebrates, see e.g. Cattaert *et al.*, 1992). Due to the unfeasibility of making direct intracellular recordings from the afferent terminals in the spinal cord of

amphibians and mammals, a large number of electrophysiological studies have, instead, been conducted on the somata of the sensory neurons which are known to possess both GABA<sub>A</sub> and GABA<sub>B</sub> receptors.

It has been known for a long time that GABA exerts a potent Cl<sup>-</sup>-dependent depolarizing action in cell bodies of both sympathetic and sensory neurons of vertebrates and that this effect is blocked by GABA<sub>A</sub> antagonists such as picrotoxin and bicuculline (De Groat, 1970, 1972; Nishi *et al.*, 1974; Feltz and Rasmussen, 1974; Adams and Brown, 1975; Davidson and Simpson, 1976). While compounds known to interfere with Cl<sup>-</sup> transport have sometimes yielded variable and conflicting results (e.g. Wojtowicz and Nicoll, 1982; Gallagher *et al.*, 1983), more recent studies carried out using intracellular Cl<sup>-</sup>-selective microelectrodes and blockers of chloride transport have shown that in rat sympathetic neurons (Ballanyi and Grawe, 1985) and in frog sensory neurons (Alvarez-Leefmans *et al.*, 1988), the depolarizing effect of GABA can be explained on the basis of an outwardly directed electrochemical gradient for Cl<sup>-</sup>, maintained by active uptake of chloride on Na<sup>+</sup>-K<sup>+</sup>-Cl<sup>-</sup> cotransport. In rat sympathetic neurons, Cl<sup>-</sup> uptake was blocked by furosemide (0.5 mM) but not by DIDS (0.2 mM) and in frog neurons, inhibition was achieved using bumetanide (10 μM) or, again, fairly high concentrations of furosemide (0.5–1.0 mM).

It is readily evident from Eqn. 1 (Section 3.3) that the influence of a HCO<sub>3</sub><sup>-</sup>-mediated current component on  $E_{GABA-A}$  becomes small at high values of [Cl<sup>-</sup>]. In addition, a depolarization promoted by a Cl<sup>-</sup> current will diminish the HCO<sub>3</sub><sup>-</sup> driving force. Hence, it is not likely that HCO<sub>3</sub><sup>-</sup> currents gated by GABA<sub>A</sub> receptors play a crucial role in the depolarizing actions of GABA that are typical for sensory and sympathetic neurons.

#### 4.3.5. Glial cells

Both cultured and native glial cells are known to express GABA<sub>A</sub> receptor channels with basic properties similar to those of their neuronal counterparts (Kettenmann *et al.*, 1987; Bormann and Kettenmann, 1988; Backus *et al.*, 1988; Malchow *et al.*, 1989; Berger *et al.*, 1992; MacVicar *et al.*, 1989; von Blankenfeld and Kettenmann, 1991). In mammalian astrocytes, oligodendrocytes and glioblasts, GABA produces a depolarization which is due to activation of GABA<sub>A</sub> receptor channels, with no detectable contribution from a plasmalemmal electrogenic uptake mechanism (Gilbert *et al.*, 1984; Kettenmann *et al.*, 1984; Berger *et al.*, 1992). However, a considerable GABA uptake current has been identified in skate retinal Müller cells (Malchow and Ripps, 1990).

The depolarizing action of GABA in glial cells is mainly attributable to the high level of intracellular Cl<sup>-</sup>, maintained by active uptake of Cl<sup>-</sup> on Na<sup>+</sup>-K<sup>+</sup>-Cl<sup>-</sup> cotransport and, in astrocytes, by Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange as well (Hoppe and Kettenmann, 1989a,b; Kimelberg, 1990; Kettenmann, 1990; but see Bührle and Sonnhof, 1983; Ballanyi *et al.*, 1987). This interpretation is consistent with the decrease in intracellular Cl<sup>-</sup> that is linked to the

GABA-induced depolarization (Hoppe and Kettenmann, 1989b).

As is the case with sensory and sympathetic neurons, the high intracellular Cl<sup>-</sup> level in glial cells will make the influence on membrane potential of a possible HCO<sub>3</sub><sup>-</sup> component of GABA<sub>A</sub>-mediated currents rather small. Nevertheless, channel-mediated net fluxes of HCO<sub>3</sub><sup>-</sup> can give rise to GABA-induced pH shifts (Kaila *et al.*, 1991).

The functional role of GABA<sub>A</sub> receptors in glial cells is not known. It has been suggested that they might be involved in the buffering of extracellular chloride at synaptic clefts (Bormann and Kettenmann, 1988; MacVicar *et al.*, 1989; von Blankenfeld and Kettenmann, 1991). However, it is questionable whether an extracellular anion which is present at a concentration in excess of 0.1 M needs buffering, especially if one recalls that net diffusion is proportional to local concentration differences, not ratios. Another possibility is that glial GABA<sub>A</sub> receptors play a role in the regulation of extracellular K<sup>+</sup>, providing a pathway for a counterion (i.e. Cl<sup>-</sup>) which would facilitate net uptake of K<sup>+</sup> (Malchow *et al.*, 1989; Barres *et al.*, 1990). It may be worth pointing out, however, that facilitation of potassium uptake by an anion conductance would compromise the capacity of glial cells in spatial buffering of K<sup>+</sup> (cf. Nicholson, 1980; Barres *et al.*, 1990). Finally, it is possible that glial GABA<sub>A</sub> receptor channels are involved in the regulation of interstitial pH within nervous tissue (Kaila *et al.*, 1991).

### 5. IONIC SHIFTS MEDIATED BY GABA<sub>A</sub> RECEPTORS

Net transmembrane movements of ions in response to the activation of GABA<sub>A</sub> receptors can arise either as a direct consequence of the operation of the anion conductance pathway, or secondarily, as a result of changes in the membrane potential of the target cells. Hence, GABA and specific GABA<sub>A</sub> receptor agonists have been shown to produce substantial extra- and intracellular shifts not only of Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup>, but also of cations such as K<sup>+</sup> and Ca<sup>2+</sup>. An increase in the extracellular K<sup>+</sup> concentration ([K<sup>+</sup>]<sub>o</sub>) is often seen in the vicinity of cells that are depolarized by GABA (Kudo and Fukuda, 1976; Ballanyi and Grawe, 1985; Bührle and Sonnhof, 1985; Jarolimek *et al.*, 1989; Müller *et al.*, 1989; Barolet and Morris, 1991) and a depolarization probably accounts also for GABA<sub>A</sub> receptor-mediated increases in [Ca<sup>2+</sup>]<sub>i</sub> which are thought to be central in the trophic actions of GABA in developing neurons (Connor *et al.*, 1987; Yuste and Katz, 1991; see also Sorimachi *et al.*, 1991; Cherubini *et al.*, 1991). However, in what follows, the main emphasis will be on the ionic shifts that directly reflect net movements of the permeant anions across the GABA<sub>A</sub> receptor channel.

GABA<sub>A</sub> receptor-mediated changes in the transmembrane anion distribution may exert an influence on nervous excitability by two kinds of mechanisms. First (as already mentioned above; Section 3.5.3), tonic activation of GABA<sub>A</sub> receptors can produce a dissipation of the IPSP driving force with a concomitant decrease in the efficacy of inhibition. In addition

to this, net movements of acid equivalents across  $\text{GABA}_A$  receptor channels give rise to changes in  $\text{pH}_i$  and  $\text{pH}_o$  which may act on  $\text{H}^+$  sensitive channels and thereby exert an influence on neuronal excitability (Section 5.2). Indeed, an increasing amount of evidence indicates that the brain cell extracellular microenvironment acts as a communication channel (cf. Nicholson, 1979, 1980) where interactions among neurons, as well as between neurons and glial cells, are mediated by various factors including ionic shifts (Heinemann *et al.*, 1990; Taira *et al.*, 1993; Ransom, 1993).

### 5.1. NET MOVEMENTS OF $\text{Cl}^-$

#### 5.1.1. Changes in intracellular $[\text{Cl}^-]$

Activation of a significant fraction of the  $\text{GABA}_A$  conductance produces a dramatic increase in the permeability of neuronal membranes to  $\text{Cl}^-$  and hence, it is not surprising that prolonged pharmacological or synaptic activation of  $\text{GABA}_A$  receptors leads to voltage shifts in  $E_{\text{GABA}-\text{A}}$  and  $E_{\text{IPSP}-\text{A}}$ , which are primarily attributable to a dissipation of the  $\text{Cl}^-$  driving force (Barker and Ransom, 1978; Wong and Watkins, 1982; Akaike *et al.*, 1987a; Huguenard and Alger, 1986; see also Dudel, 1977). In neurons with an inwardly directed  $\text{Cl}^-$  electrochemical gradient, such an effect will lead to a positive voltage shift in  $E_{\text{IPSP}-\text{A}}$ , which contributes to the use-dependent depression that is typical of GABA-mediated inhibition in various parts of the brain, including the hippocampus (Wong and Watkins, 1982; McCarron and Alger, 1985; Thompson and Gähwiler, 1989b).

A simple channel-mediated dissipation of the  $\text{Cl}^-$  gradient is probably not the only mechanism that accounts for use-dependent depolarizing shifts in  $E_{\text{GABA}-\text{A}}$  and  $E_{\text{IPSP}-\text{A}}$ . It is likely that an additional factor of importance, especially during intense activity, is an inhibition of net  $\text{Cl}^-$  extrusion on  $\text{K}^+-\text{Cl}^-$  cotransport in response to an activity-induced rise in  $[\text{K}^+]_o$  (cf. McCarron and Alger, 1985; Thompson and Gähwiler, 1989b).

In the work cited above, the activity-induced ionic shifts were deduced on the basis of shifts in currents mediated by  $\text{GABA}_A$  receptors. Direct measurements of GABA-evoked intracellular chloride shifts by aid of  $\text{Cl}^-$ -selective microelectrodes have been carried out in rat sympathetic neurons (Ballanyi and Grafe, 1985), crayfish muscle fibres and neurons (Kaila and Voipio, 1987; Kaila *et al.*, 1989, 1992b) and in cultured mouse oligodendrocytes (Hoppe and Kettenmann, 1989b).

In agreement with the high resting  $[\text{Cl}^-]_i$ , activation of the GABA-gated anion conductance leads to a depolarization and to a fall in intracellular  $\text{Cl}^-$  in sympathetic neurons and glial cells (Ballanyi and Grafe, 1985; Hoppe and Kettenmann, 1989b). In contrast to this, the depolarizing action of GABA in crayfish muscle fibres and neurons was linked to an increase in  $[\text{Cl}^-]_i$  (Kaila *et al.*, 1989, 1992b). In both crayfish preparations, this was due to a redistribution of  $\text{Cl}^-$  in response to a  $\text{Cl}^-$ -independent depolarizing current component activated by GABA (see Fig. 8). In the muscle fibre, this depolarizing current component was mediated by  $\text{GABA}_A$  receptors and car-

ried by  $\text{HCO}_3^-$ ; in the crayfish neuron (examined in the absence of  $\text{HCO}_3^-$ ), the depolarizing current was generated by sodium-dependent uptake of GABA.

The fact that a depolarizing action mediated by  $\text{GABA}_A$  receptors can result in either a net decrease in  $[\text{Cl}^-]_i$  as observed in sympathetic neurons (Ballanyi and Grafe, 1985), or in a net increase in  $[\text{Cl}^-]_i$ , as seen in crayfish muscle fibres (Kaila *et al.*, 1989) implies that it is difficult if not impossible to examine the polarity of the  $\text{Cl}^-$  driving force on the basis of  $\text{GABA}_A$  receptor-mediated extracellular  $\text{Cl}^-$  shifts (cf. Müller *et al.*, 1989). This topic will be dealt with in detail below.

#### 5.1.2. Changes in extracellular $[\text{Cl}^-]$

In view of the large difference between the concentrations of intra- and extracellular  $\text{Cl}^-$  in nervous tissue, physiologically important shifts of this anion can occur in the intracellular compartment only. However, changes in extracellular  $\text{Cl}^-$  evoked by GABA and by  $\text{GABA}_A$  agonists have been examined in an attempt to gain insight into the mechanisms underlying the hyperpolarizing and depolarizing effects mediated by  $\text{GABA}_A$  receptors in the hippocampus (Müller *et al.*, 1989; see Section 4.3.2). In this work, carried out on guinea pig hippocampal slices, microdrop application of GABA produced a bicuculline-sensitive decrease in  $[\text{Cl}^-]_o$  in stratum pyramidale in areas CA1 and CA3. When applied to the dendritic layer, GABA induced an initial decrease followed by a long-lasting increase in  $[\text{Cl}^-]_o$ . An effect of the latter kind was also observed in both the somatic and dendritic layers in the dentate gyrus. The authors conclude that these results support the view that the hyperpolarizing action mediated by  $\text{GABA}_A$  receptors in pyramidal neuron somata are due to a channel-mediated influx of  $\text{Cl}^-$  while the depolarizations seen in pyramidal dendrites and in the granular cells of the dentate gyrus are caused by an efflux of  $\text{Cl}^-$  across the  $\text{GABA}_A$  receptor channels (see Misgeld *et al.*, 1986).

However, it is obvious that a number of difficulties remain in the interpretation of the above measurements of  $[\text{Cl}^-]_o$  transients. First, even when corrected for the decrease in extracellular volume that takes place during GABA application (Müller *et al.*, 1989; see also Dietzel *et al.*, 1982), the initial action of GABA in all hippocampal regions examined was a decrease in  $[\text{Cl}^-]_o$ . Secondly, extracellular ionic shifts induced by exogenous GABA, especially an increase in  $\text{Cl}^-$ , may well contain a significant glial contribution (Hoppe and Kettenmann, 1989b). Third, as pointed out above, GABA-induced net fluxes of  $\text{Cl}^-$  do not show a simple relation to the transmembrane  $\text{Cl}^-$  driving force. In particular, it is of interest to note that the initial GABA-induced decrease in  $[\text{Cl}^-]_o$  observed by Müller and coworkers (1989) in the pyramidal cell dendritic layers and in the granule cell layer is not inconsistent with a depolarizing action of GABA. If one accepts the neuronal origin of the transient drop in  $[\text{Cl}^-]_o$ , the only assumption that has to be made is that GABA evokes a current which is the sum of a hyperpolarizing  $\text{Cl}^-$  mediated component and a powerful depolarizing component carried by another ion, such as  $\text{HCO}_3^-$  (cf. Fig. 10C,D).

### 5.2. pH SHIFTS

As pointed out earlier on, the HCO<sub>3</sub><sup>-</sup> permeability of GABA<sub>A</sub> receptor channels provides a two-way link between the control of GABA<sub>A</sub> receptor-mediated inhibition and the regulation and modulation of pH in nervous tissue. It is evident *a priori* that a channel-mediated net efflux of HCO<sub>3</sub><sup>-</sup> (which manifests itself as an inwardly directed component of current) will lead to a fall in pH<sub>i</sub> which is paralleled by an increase in pH in the extracellular aqueous phase in the vicinity of the plasma membrane. Both intra- and extracellular acid-base shifts caused by a net efflux of HCO<sub>3</sub><sup>-</sup> from cells equipped with GABA<sub>A</sub> receptors have been reported in preparations of both invertebrate and vertebrate origin. An intriguing aspect of pH shifts produced by synaptic activation of GABA<sub>A</sub> receptor channels is that, under conditions *in vivo*, they must be highly circumscribed both spatially and temporally and thus might well serve as modulatory factors acting on ligand- or voltage-gated channels which are sensitive to extra- or intracellular H<sup>+</sup> ions (e.g. Tang *et al.*, 1990; Peers and Green, 1991; Pasternack *et al.*, 1992a).

Recent work (reviewed by Chesler and Kaila, 1992) suggests that the early alkaline shifts in pH<sub>o</sub> that are evoked by synchronous activity in various brain areas (Urbanics *et al.*, 1978; Kraig *et al.*, 1983) are mainly due to a net influx into postsynaptic neurons of acid equivalents mediated by GABA<sub>A</sub> and glutamate receptor channels. However, it has become evident that despite their superficial similarity, the GABAergic and glutamatergic alkaline transients are based on two different kinds of mechanisms. While the former are most likely caused by a net efflux of HCO<sub>3</sub><sup>-</sup>, the latter may be due to an influx of H<sup>+</sup> ions. This difference in the underlying ionic mechanism is clearly evidenced by the opposite role of the enzyme carbonic anhydrase in their generation: both intracellular and extracellular pH shifts evoked by GABA are enhanced by carbonic anhydrase activity while the opposite is true for glutamatergic interstitial alkaline shifts as will be described below.

#### 5.2.1. Changes in intracellular HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup>

The first evidence indicating that activation of GABA<sub>A</sub> receptors generates a considerable intracellular acid load was gained in experiments carried out with H<sup>+</sup>-selective microelectrodes in crayfish opener muscle fibres (Kaila and Voipio, 1987). Application of a near-saturating concentration (0.5 mM) of GABA in a solution equilibrated with 5% CO<sub>2</sub> in air produced an instantaneous net efflux of HCO<sub>3</sub><sup>-</sup> of up to 10 mmol/(l cell water)/min and a subsequent steady-state fall in pH<sub>i</sub> of 0.4–0.5 units (Kaila *et al.*, 1990; see Fig. 11A). The magnitude of the drop in pH<sub>i</sub> caused by a prolonged acid load is set by the H<sup>+</sup> regulating capacity of the cell under examination. In crayfish muscle fibres, the GABA-induced acid load and the active extrusion of H<sup>+</sup> equivalents attained a steady-state level of about 2–3 mmol/(l cell water)/min during the plateau acidosis.

The conclusion that the GABA-induced intracellular acid load was caused by a channel-mediated net efflux of HCO<sub>3</sub><sup>-</sup> was based on its dependence on

CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>; on the inhibitory actions of picrotoxin and of membrane depolarization (Kaila *et al.*, 1990); and on parallel electrophysiological experiments which demonstrated the presence of substantial bicarbonate-mediated currents and conductances gated by GABA<sub>A</sub> receptors (Kaila *et al.*, 1989). Effects of GABA on pH<sub>i</sub> similar to those above were subsequently seen in experiments on the crayfish stretch-receptor neuron (Voipio *et al.*, 1991).

It is generally recognized that the capacity of an open CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> buffer to attenuate pH shifts in response to the addition of H<sup>+</sup> or OH<sup>-</sup> is strongly enhanced by the presence of the enzyme carbonic anhydrase (CA), which catalyzes the reversible hydration of CO<sub>2</sub> and hence provides a tight coupling between the concentration of H<sup>+</sup> and of the highly mobile buffer species, CO<sub>2</sub> (Maren, 1984; Dodgson, 1991). Very interestingly, however, CA activity does not always simply buffer acid-base transients but it can also play a role in the generation of pH shifts, as was found to be the case with those mediated by GABA<sub>A</sub> receptors (Kaila *et al.*, 1990). Inhibition of intracellular carbonic anhydrase activity by acetazolamide resulted in a marked slowing down of the GABA-induced acidosis in crayfish muscle fibres and neurons (Kaila *et al.*, 1990; Voipio *et al.*, 1991). This result indicated that the proximate cause of the fall in pH<sub>i</sub> was a net influx of CO<sub>2</sub> caused by a fall in the intracellular CO<sub>2</sub> concentration which, in turn, was due to the channel-mediated loss of internal HCO<sub>3</sub><sup>-</sup> (see Fig. 11E).

The fact that intracellular carbonic anhydrase activity plays a central role in promoting GABA<sub>A</sub> receptor-mediated pH<sub>i</sub> shifts has to be paid attention to when considering the action of GABA on pH<sub>i</sub> in different types of cells. A modest intracellular acid load produced by GABA<sub>A</sub> receptor activation cannot be taken as evidence for the absence of a significant HCO<sub>3</sub><sup>-</sup> permeability—such a situation may also arise from the absence of intracellular carbonic anhydrase activity. Indeed, the rather small acidosis evoked by muscimol in cultured rat astrocytes (Kaila *et al.*, 1991) might well reflect the absence of intracellular carbonic anhydrase.

At the time of writing this article, the only published work on mammalian neurons done to examine the actions of GABA on pH<sub>i</sub> has been carried out on acutely isolated pyramidal neurons obtained from area CA1 of the rat hippocampus (Pasternack *et al.*, 1993). As illustrated in Fig. 11C, application of GABA produced a considerable fall in pH<sub>i</sub> as measured using BCECF fluorescence. The GABA-induced acidosis was attenuated by acetazolamide and completely blocked by picrotoxin.

A question which is of much interest but hard to answer on the basis of work on agonist-exposed isolated neurons is, whether GABA<sub>A</sub> receptor-mediated synaptic inhibition within intact nervous tissue can lead to a significant postsynaptic intracellular acid load. That this is, indeed, the case, is in line with the observations that selective stimulation of a hippocampal monosynaptic inhibitory pathway produced an extracellular alkalosis that bore all the earmarks of a pH shift mediated by GABA<sub>A</sub> receptor channels (Kaila *et al.*, 1992a; see below).

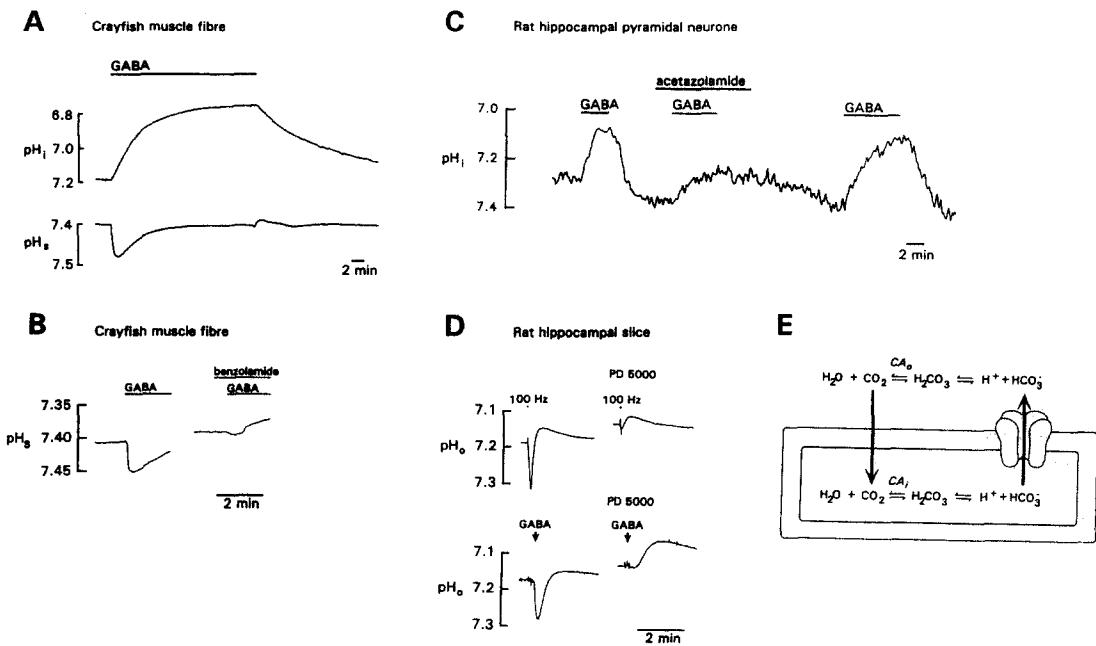


FIG. 11. Intracellular acid and extracellular alkaline pH shifts mediated by GABA<sub>A</sub> receptors in crayfish muscle fibers (A, B), in a rat hippocampal pyramidal neuron (C) and in a rat hippocampal slice (D). Responses in (A–C) were induced by bath-applied GABA while those shown in (D) were evoked by stimulation of a monosynaptic inhibitory pathway (upper trace) during pharmacological blockade of all excitatory transmission and by microdrop application of GABA (lower trace). The GABA-induced fall in pH<sub>i</sub> is attenuated (C) by the membrane-permeant carbonic anhydrase inhibitor, acetazolamide and the extracellular alkaline shifts can be blocked (B, D) by a poorly permeant (benzolamide) or impermeant (Prontosil-dextran 5000; PD 5000) inhibitor. The mechanism underlying GABA<sub>A</sub> receptor-mediated pH shifts is schematically depicted in (E): following a channel-mediated net efflux of HCO<sub>3</sub><sup>-</sup> down its electrochemical gradient, the equilibrium of the intracellular (de)hydration reaction is shifted to the right, which leads to a net influx of CO<sub>2</sub> and thereupon to a release of H<sup>+</sup> ions within the cell. On the extracellular side of the membrane, the equilibrium of the (de)hydration reaction is shifted to the left which leads to an extracellular alkaline shift. The (de)hydration of CO<sub>2</sub> is catalyzed by an intracellular and an extracellular carbonic anhydrase (CA<sub>i</sub> and CA<sub>e</sub>). The measurements in (A, B) and (D) were carried out using H<sup>+</sup>-selective microelectrodes; (C) is based on BCECF fluorescence. (A) reproduced by permission from Kaila and Voipio (1987), (B) and (E) (slightly modified) from Kaila *et al.* (1990), (C) from Pasternack *et al.* (1993) and (D) from Kaila *et al.*, (1992a).

### 5.2.2. Changes in extracellular HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup>

In addition to the evidence reviewed earlier on, the view that the intracellular acidosis caused by GABA in crayfish muscle fibers was due to a channel-mediated net efflux of HCO<sub>3</sub><sup>-</sup> ions was further supported by the finding that the initial fall in pH<sub>i</sub> was linked to a transient rise in external pH (pH<sub>e</sub>) in the vicinity of the cell membrane (Kaila *et al.*, 1990; see Fig. 11A). The transient nature of the pH<sub>e</sub> shift is easy to understand since, during plateau acidosis within the cell, the net flux of acid equivalents across the membrane must be zero. Rather interestingly, brief exposures of the crayfish muscle fibre to benzolamide, a carbonic anhydrase inhibitor which is much less permeant than acetazolamide (Saarikoski and Kaila, 1992), abolished the GABA-induced rise in pH<sub>e</sub> (Fig. 11B) with no effect on the fall in pH<sub>i</sub> (Kaila *et al.*, 1990). Taken together, the sensitivity to carbonic anhydrase inhibitors of both the intracellular (see above) and extracellular pH shift indicated that both are caused by a net influx of CO<sub>2</sub> generated in response to the efflux of HCO<sub>3</sub><sup>-</sup>. This mechanism is depicted in Fig. 11E and, as is evident from

the pertinent work (see below; and Pasternack *et al.*, 1993), it also explains the GABAergic pH shifts which take place in the mammalian hippocampus.

In view of the similar permeability properties of crayfish and vertebrate GABA<sub>A</sub> receptor channels, it is not surprising that GABA as well as specific GABA<sub>A</sub> agonists have been found to produce an extracellular alkalosis in the turtle cerebellum (Chen and Chesler, 1991), in the mammalian hippocampus (Jarolimek *et al.*, 1989; Lux *et al.*, 1970; Chen and Chesler, 1992; Kaila *et al.*, 1992a) and in the spinal cord (Sykova *et al.*, 1992). That exogenous GABA evokes an extracellular alkalosis in the vertebrate CNS does not, however, provide a definite answer to the question whether similar pH transients take place during GABA<sub>A</sub> receptor-mediated synaptic inhibition. This is because the effects on pH<sub>e</sub> of exogenous agonists could well be mediated by extrasynaptic or glial GABA<sub>A</sub> receptors. This problem was settled in experiments on rat hippocampal slices (Kaila *et al.*, 1992a), which showed that, following pharmacological blockade of all excitatory transmission (see Davies *et al.*, 1990), activation of a monosynaptic

GABAergic pathway evoked an immediate extracellular alkaline shift (cf. Fig. 11D) which was potentiated by pentobarbital and blocked by picrotoxin, but not by the GABA<sub>B</sub>-receptor antagonist, 2-hydroxy saclofen.

In a manner strikingly parallel to that observed in crayfish muscle fibres, the extracellular alkaline shifts in rat hippocampal slices evoked by both monosynaptic and pharmacologic activation of GABA<sub>A</sub> receptors were blocked by the carbonic anhydrase inhibitor, benzolamide (Kaila *et al.*, 1992a; Chen and Chesler, 1992). However, unlike the situation with the crayfish preparation which permitted a simultaneous measurement of intra- and extracellular pH, the possibility that a prolonged application of benzolamide might inhibit intracellular carbonic anhydrase activity (Saarikoski and Kaila, 1992) could not be ruled out in the experiments on hippocampal slices. Hence, it was of much consequence to observe that the effects of benzolamide were mimicked by pronostil-dextran 5000 (Fig. 11D), an impermeant macromolecular carbonic anhydrase inhibitor (Kaila *et al.*, 1992a; cf. Geers *et al.*, 1985). In addition to demonstrating a role for extracellular carbonic anhydrase activity in the modulation of activity-induced extracellular pH shifts in the brain, these results provided the first strong evidence to suggest the very presence of a carbonic anhydrase isozyme in the interstitium of mammalian brain tissue.

A mechanistic scheme that explains the intra- and extracellular pH changes mediated by GABA<sub>A</sub> receptors in crayfish muscle fibres and in the rat hippocampus is shown in Fig. 11E. As already stated above, the fact that both pH shifts are inhibited by carbonic anhydrase blockers indicates that their proximate cause is the net transmembrane influx of CO<sub>2</sub> which, in turn, is driven by the channel-mediated efflux of HCO<sub>3</sub><sup>-</sup> (Kaila *et al.*, 1990, 1992).

In sharp contrast to the findings above, GABA has been observed to produce a slight fall in extracellular pH in isolated rat cervical ganglia (Ballanyi and Grafe, 1985). On purely thermodynamic grounds, it is obvious that such an effect cannot be directly due to a channel-mediated flux of acid-base equivalents. One possibility is that the extracellular acid shift might have been caused by the rather large GABA-induced increase in [K<sup>+</sup>] (cf. Fig. 7 in Ballanyi and Grafe, 1985). There is, in fact, much evidence that glial cells secrete acid in response to an elevated [K<sup>+</sup>] (Chesler, 1990; Ransom, 1993). It is not, either, known whether extracellular carbonic anhydrase is present or absent in sympathetic ganglia and the absence of a GABA-induced extracellular alkalosis would not be surprising in the latter case.

The demonstration that synaptic activation of GABA<sub>A</sub> receptors in the hippocampus leads to detectable extracellular pH shifts (Kaila *et al.*, 1992a) implies that substantial pH<sub>o</sub> transients must take place in the vicinity of the postsynaptic receptors during nervous activity. GABA<sub>A</sub> receptors are known to be sensitive to extracellular pH (Section 3.6.2) and an interesting question that emerges here is whether

localized pH shifts exert a modulatory action during neuronal inhibition. While this question has not yet been experimentally addressed, recent evidence (Taira *et al.*, 1993) suggests that synaptically generated H<sup>+</sup> transients play a modulatory role in excitatory transmission. This type of intrinsic proton modulation was found to be mainly attributable to the H<sup>+</sup> sensitivity of NMDA receptors (cf. Tang *et al.*, 1990; Traynelis and Cull-Candy, 1991). An intriguing property of both GABA<sub>A</sub> and NMDA receptors is that both are apparently capable of generating acid-base shifts and, in addition to this, show a marked sensitivity to external pH.

**Acknowledgements**—The original research work of the author has been supported by the Academy of Finland and by the Sigrid Juselius Foundation. I wish to thank M. Pasternack, T. Taira and J. Voipio for comments on the manuscript.

## REFERENCES\*

- ADAMS, P. R. and BROWN, D. A. (1975) Actions of  $\gamma$ -aminobutyric acid on sympathetic ganglion cells. *J. Physiol., Lond.* **250**, 85–120.
- AICKIN, C. C., DEISZ, R. A. and LUX, H. D. (1982) Ammonium action on post-synaptic inhibition in crayfish neurones: implications for the mechanism of chloride extrusion. *J. Physiol., Lond.* **329**, 319–339.
- AKAIKE, N., INOUE, M. and KRISHNAL, O. A. (1986) "Concentration-clamp" study of  $\gamma$ -aminobutyric-acid-induced chloride current kinetics in frog sensory neurons. *J. Physiol., Lond.* **379**, 171–185.
- AKAIKE, N., INOMATA, N. and TOKUTOMI, N. (1987a) Contribution of chloride shifts to the fade of  $\gamma$ -aminobutyric acid-gated currents in frog dorsal root ganglion cells. *J. Physiol., Lond.* **391**, 219–234.
- AKAIKE, N., YAKUSHIJI, T., TOKUTOMI, N. and CARPENTER, D. O. (1987b) Multiple mechanisms of antagonism of  $\gamma$ -aminobutyric acid (GABA) responses. *Cell. molec. Neurobiol.* **7**, 97–103.
- AKAIKE, N., INOMATA, N. and YAKUSHIJI, T. (1989a) Differential effects of extra- and intracellular anions on GABA-activated currents in bullfrog sensory neurons. *J. Neurophysiol.* **62**, 1388–1399.
- AKAIKE, N., OYAMA, Y. and YAKUSHIJI, T. (1989b) Influences of external Ca<sup>2+</sup> on the GABA-induced chloride current and the efficacy of diazepam in internally perfused frog sensory neurons. *Brain Res.* **504**, 293–296.
- ALFORD, S., CHRISTENSON, J. and GRILLNER, S. (1991) Presynaptic GABA<sub>A</sub> and GABA<sub>B</sub> receptor-mediated phasic modulation in axons of spinal motor interneurons. *Eur. J. Neurosci.* **3**, 107–117.
- ALGER, B. E. (1984) Hippocampus: electrophysiological studies of epileptiform activity *in vitro*. In: *Brain Slices*, pp. 155–199. Ed. R. Dingledine. Plenum, New York.
- ALGER, B. E. (1991) Gating of GABAergic inhibition in hippocampal pyramidal cells. *Ann. N. Y. Acad. Sci.* **627**, 249–263.
- ALGER, B. E. and NICOLL, R. A. (1979) GABA-mediated biphasic inhibitory responses in hippocampus. *Nature* **281**, 315–317.
- ALGER, B. E. and NICOLL, R. A. (1982a) Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied *in vitro*. *J. Physiol., Lond.* **328**, 105–123.
- ALGER, B. E. and NICOLL, R. A. (1982b) Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. *J. Physiol., Lond.* **328**, 125–141.
- ALGER, B. E. and NICOLL, R. A. (1983) Ammonia does not selectively block IPSPs in rat hippocampal pyramidal cells. *J. Neurophysiol.* **49**, 1381–1391.
- ALHO, H., COSTA, E., FERRERO, P., FUJIMOTO, M., COSENZA-MURPHY, D. and GUIDOTTI, A. (1985) Diazepam-binding inhibitor: A neuropeptide located in selected neuronal populations of rat brain. *Science* **239**, 179–182.
- ALTAMIRANO, A. A. and RUSSELL, J. M. (1987) Coupled Na/K/Cl efflux. "Reverse" unidirectional fluxes in squid giant axons. *J. gen. Physiol.* **89**, 669–686.
- ALVAREZ-LEEFMANS, F. J. (1990) Intracellular Cl<sup>-</sup> regulation and synaptic inhibition in vertebrate and invertebrate neurons. In:

\*The survey of literature for this article was completed in November, 1992.

- Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 109–158. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- ALVAREZ-LEEFMANS, F. J., GAMÍNO, S. M., GIRALDEZ, F. and NOGUERÓN, I. (1988) Intracellular chloride regulation in amphibian dorsal root ganglion neurones studied with ion-selective microelectrodes. *J. Physiol., Lond.* **406**, 225–246.
- ALVAREZ-LEEFMANS, F. J., GIRALDEZ, F. and RUSSELL, J. M. (1990) Methods for measuring chloride transport across nerve, muscle and glial cells. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 3–66. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- AMMANN, D. (1986) *Ion-selective Microelectrodes*. Springer: Berlin.
- ANDERSEN, P. (1990) Synaptic integration in hippocampal CA1 pyramids. *Prog. Brain Res.* **83**, 215–222.
- ANDERSEN, P., DINGLEDINE, R., GJERSTAD, L., LANGMOEN, I. A. and MOSFELDT LAURSEN, A. (1980) Two different responses of hippocampal pyramidal cells to application of  $\gamma$ -amino butyric acid. *J. Physiol., Lond.* **305**, 279–296.
- ANDERSON, C. R. and STEVENS, C. F. (1973) Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol., Lond.* **235**, 665–691.
- ARAKI, T., ITO, M. and OSCARSSON, O. (1961) Anion permeability of the synaptic and non-synaptic motoneurone membrane. *J. Physiol., Lond.* **159**, 410–435.
- ARAM, J. A. and LODGE, D. (1987) Epileptiform activity induced by alkalosis in rat neocortical slices: block by antagonists of *N*-methyl-D-aspartate. *Neurosci. Lett.* **83**, 345–350.
- ARONSON, P. S. (1985) Kinetic properties of the plasma membrane  $\text{Na}^+ - \text{H}^+$  exchanger. *A. Rev. Physiol.* **47**, 545–560.
- ASCHER, P., KUNZE, D. and NEILD, T. O. (1976) Chloride distribution in *Aplysia* neurones. *J. Physiol., Lond.* **256**, 441–464.
- ASHWOOD, T. J., COLLINGRIDGE, G. L., HERRON, C. E. and WHEAL, H. V. (1987) Voltage-clamp analysis of somatic  $\gamma$ -aminobutyric acid responses in adult rat hippocampal CA1 neurones *in vitro*. *J. Physiol., Lond.* **384**, 27–37.
- ASSAF, S. Y. and CHUNG, S. H. (1984) Release of endogenous  $\text{Zn}^{2+}$  from brain tissue during activity. *Nature* **308**, 734–736.
- ASTION, M. L. and ORKAND, R. K. (1988) Electrogenic  $\text{Na}^+/\text{HCO}_3^-$  cotransport in neuroglia. *Glia* **1**, 355–357.
- ATWOOD, H. L. (1976) Organization and synaptic physiology of crustacean neuromuscular systems. *Prog. Neurobiol.* **7**, 291–391.
- AVOLI, M. and PERREAULT, P. (1987) A GABAergic depolarizing potential in the hippocampus disclosed by the convulsant 4-aminopyridine. *Brain Res.* **400**, 191–195.
- AVOLI, M., PIERREAU, P., OLIVIER, A. and VILLEMEURE, J. G. (1988) 4-Aminopyridine induces a long-lasting depolarizing GABAergic potential in human neocortical and hippocampal neurons maintained *in vitro*. *Neurosci. Lett.* **94**, 327–332.
- AWAPARA, J., LANDUA, A. J., FUERST, R. and SEALE, B. (1950) Free  $\gamma$ -aminobutyric acid in brain. *J. biol. Chem.* **187**, 35–39.
- BACKUS, K. H., KETTENMANN, H. and SCHACHNER, M. (1988) Effect of benzodiazepines and pentylenetetrazole on the GABA-induced depolarization in cultured astrocytes. *Glia* **1**, 132–140.
- BALLANYI, K. and GRAFE, P. (1985) An intracellular analysis of  $\gamma$ -aminobutyric-associated ion movements in rat sympathetic neurones. *J. Physiol., Lond.* **365**, 41–58.
- BALLANYI, K., GRAFE, P. and TEN BRUGGENCATE, G. (1987) Ion activities and potassium uptake mechanisms of glial cells in guinea-pig olfactory cortex slices. *J. Physiol., Lond.* **382**, 159–174.
- BARKER, J. L. and HARRISON, N. L. (1988) Outward rectification of inhibitory postsynaptic currents in cultured rat hippocampal neurones. *J. Physiol., Lond.* **403**, 41–55.
- BARKER, J. L. and MATHERS, D. A. (1981) GABA analogs activate channels of different duration on cultured mouse spinal neurones. *Science* **212**, 358–361.
- BARKER, J. L. and RANSOM, B. R. (1978) Amino acid pharmacology of mammalian central neurones grown in tissue culture. *J. Physiol., Lond.* **280**, 331–354.
- BARKER, J. L., McBURNEY, R. N. and MACDONALD, J. F. (1982) Fluctuation analysis of neutral amino acid responses in cultured mouse spinal neurones. *J. Physiol., Lond.* **322**, 365–387.
- BARKER, J. L., McBURNEY, R. M. and MATHERS, D. A. (1983) Convulsant-induced depression of amino acid responses in cultured mouse spinal neurones studied under voltage clamp. *Br. J. Pharmac.* **80**, 619–629.
- BARNARD, E. A., DARLISON, M. G. and SEEBURG, P. (1987) Molecular biology of the GABA<sub>A</sub> receptor: the receptor/channel superfamily. *Trends Neurosci.* **10**, 502–509.
- BAROLET, A. W. and MORRIS, M. E. (1991) Changes in extracellular  $\text{K}^+$  evoked by GABA, THIP and baclofen in the guinea-pig hippocampal slice. *Expl. Brain Res.* **84**, 591–598.
- BARRES, B. A. (1991) New roles for glia. *J. Neurosci.* **11**, 3685–3694.
- BARRES, B. A., CHUN, L. L. Y. and COREY, D. P. (1990) Ion channels invertebrate glia. *A. Rev. Neurosci.* **13**, 441–474.
- BAZEMORE, A. W., ELLIOTT, K. A. C. and FLOREY, E. (1957) Isolation of factor I. *J. Neurochem.* **1**, 334–339.
- BEHRENDS, J. C., MARUYAMA, T., TOKUTOMI, N. and AKAIKE, N. (1988)  $\text{Ca}^{2+}$  mediated suppression of the GABA-response through modulation of chloride channel gating in frog sensory neurones. *Neurosci. Lett.* **86**, 311–316.
- BEN-ARI, Y., CHERUBINI, E., CORRADETTI, R. and GAIARSA, J.-L. (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol., Lond.* **416**, 303–325.
- BEN-ARI, Y., ROVIRA, C., GAIARSA, J. L., CORRADETTI, R., ROBAIN, O. and CHERUBINI, E. (1990) GABAergic mechanisms in the CA3 hippocampal region during early postnatal life. *Prog. Brain Res.* **83**, 313–321.
- BERGER, T., WALZ, W., SCHNITZER, J. and KETTENMANN, H. (1992) GABA- and glutamate-activated currents in glial cells of the mouse corpus callosum slice. *J. Neurosci. Res.* **31**, 21–27.
- BISCOE, T. J. and DUCHEN, M. R. (1985) The anion selectivity of GABA-mediated post-synaptic potentials in mouse hippocampal cells. *Q. Jl. exp. Physiol.* **70**, 305–312.
- BIXBY, J. L. and SPITZER, N. C. (1982) The appearance and development of chemosensitivity in Rohon–Beard neurones of the *Xenopus* spinal cord. *J. Physiol., Lond.* **330**, 513–536.
- BLANTON, M. G., LOTURCO, J. J. and KRIEGSTEIN, A. R. (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J. Neurosci. Meth.* **30**, 203–210.
- BLATT, M. R. and SLAYMAN, C. L. (1983) KCl leakage from microelectrodes and its impact on the membrane parameters of a nonexcitable cell. *J. membr. Biol.* **72**, 223–234.
- BLATZ, A. L. (1991) Properties of single fast chloride channels from rat cerebral cortex neurons. *J. Physiol., Lond.* **441**, 1–21.
- BLOOM, F. E. and IVERSEN, L. L. (1971) Localizing <sup>3</sup>H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. *Nature* **229**, 628–630.
- BOISTEL, J. and FATT, P. (1958) Membrane permeability change during inhibitory transmitter action in crustacean muscle. *J. Physiol., Lond.* **144**, 176–191.
- BORMANN, J. (1988a) Patch-clamp analysis of GABA- and glycine-gated chloride channels. *Adv. Biochem. Psychopharmac.* **45**, 47–60.
- BORMANN, J. (1988b) Electrophysiology of GABA<sub>A</sub> and GABA<sub>B</sub> receptor subtypes. *Trends Neurosci.* **11**, 112–116.
- BORMANN, J. and CLAPHAM, D. E. (1985)  $\gamma$ -Aminobutyric acid receptor channels in adrenal chromaffin cells: a patch-clamp study. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2168–2172.
- BORMANN, J. and KETTENMANN, H. (1988) Patch-clamp study of gamma-aminobutyric acid receptor  $\text{Cl}^-$  channels in cultured astrocytes. *Proc. natn. Acad. Sci. U.S.A.* **85**, 9336–9340.
- BORMANN, J., HAMILL, O. P. and SAKMANN, B. (1987) Mechanism of anion permeation through channels gated by glycine and  $\gamma$ -aminobutyric acid in mouse cultured spinal neurones. *J. Physiol., Lond.* **385**, 243–286.
- BORON, W. F. and BOULPAEP, E. L. (1983) Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral  $\text{HCO}_3^-$  transport. *J. gen. Physiol.* **81**, 53–94.
- BOWERY, N. (1989) GABA<sub>B</sub> receptors and their significance in mammalian pharmacology. *Trends Pharmac. Sci.* **10**, 401–407.
- BOWERY, N. G., HILL, D. R., HUDSON, A. L., DOBLE, A., MIDDLEMISS, D. N., SHAW, J. and TURNBULL, M. (1980) (–) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283**, 92–94.
- BRAZIER, M. A. B. (1959) The historical development of neurophysiology. In: *Handbook of Physiology, Section 1, Neurophysiology*, Vol. I, pp. 1–58. Ed. H. W. MAGOUN. American Physiological Society.
- BROWN, D. A. and SCHOLFIELD, C. N. (1979) Depolarization of neurones in the isolated olfactory cortex of the guinea-pig by  $\gamma$ -aminobutyric acid. *Br. J. Pharmac.* **65**, 339–345.
- BROWNING, M. D., BUREAU, M., DUDEK, E. M. and OLSEN, R. W. (1990) Protein kinase C and cAMP-dependent protein kinase

- phosphorylate the  $\beta$  subunit of the purified  $\gamma$ -aminobutyric acid receptor. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1315–1318.
- BUCKLER, K. J. and VAUGHAN-JONES, R. D. (1990) Application of a new pH-sensitive fluoroprobe (carboxy-SNARF-1) for intracellular pH measurement in small, isolated cells. *Pflügers Arch.* **417**, 234–239.
- BÜHRLE, C. P. and SONNHOF, U. (1983) Intracellular ion activities and equilibrium potentials in motoneurones and glia cells of the frog spinal cord. *Pflügers Arch.* **396**, 144–153.
- BÜHRLE, C. P. and SONNHOF, U. (1985) The ionic mechanism of postsynaptic inhibition in motoneurones of the frog spinal cord. *Neuroscience* **14**, 581–592.
- CAMMER, W. (1991) Carbonic anhydrase in myelin and glial cells in the mammalian central nervous system. In: *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics*, pp. 325–332. Eds. S. J. DODGSON, R. E. TASHIAN, G. GROS and N. D. CARTER. Plenum: New York.
- CATTAERT, D., EL MANIRA, A. and CLARAC, F. (1992) Direct evidence for presynaptic inhibitory mechanisms in crayfish sensory afferents. *J. Neurophysiol.* **67**, 610–624.
- CHAO, A. C., WIDDICOMBE, J. H. and VERKMAN, A. S. (1990) Chloride conductive and cotransport mechanisms in cultures of canine tracheal epithelial cells measured by an entrapped fluorescent indicator. *J. membr. Biol.* **113**, 193–202.
- CHEN, J. C. T. and CHESLER, M. (1991) Extracellular alkalinization evoked by GABA and its relationship to activity-dependent pH shifts in turtle cerebellum. *J. Physiol., Lond.* **442**, 431–446.
- CHEN, J. C. T. and CHESLER, M. (1992) Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. *J. Neurophysiol.* **67**, 29–36.
- CHEN, Q. X., STELZER, A., KAY, A. R. and WONG, R. K. (1990) GABA<sub>A</sub> receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. *J. Physiol., Lond.* **420**, 207–221.
- CHERUBINI, E., ROVIRA, C., GAIARSA, J. L., CORRADETTI, R. and BEN-ARI, Y. (1990) GABA mediated excitation in immature rat CA3 hippocampal neurons. *Int. J. dev. Neurosci.* **8**, 481–490.
- CHERUBINI, E., GAIARSA, J. L. and BEN-ARI, Y. (1991) GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci.* **14**, 515–519.
- CHESLER, M. (1986) Regulation of intracellular pH in reticulospinal neurones of the lamprey, *Petromyzon marinus*. *J. Physiol., Lond.* **381**, 241–261.
- CHESLER, M. (1990) The regulation and modulation of pH in the nervous system. *Prog. Neurobiol.* **34**, 401–427.
- CHESLER, M. and KAILA, K. (1992) Modulation of pH by neuronal activity. *Trends Neurosci.* **15**, 396–402.
- CHESLER, M. and KRAIG, R. P. (1989) Intracellular pH transients of mammalian astrocytes. *J. Neurosci.* **9**, 2011–2019.
- CHESNOY-MARCHAIS, D. (1990) Hyperpolarization-activated chloride channels in *Aplysia* neurons. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 367–382. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- CLARK, J. D. and LIMBIRD, L. E. (1991) Na<sup>+</sup>/H<sup>+</sup>-exchanger subtypes: A predictive review. *Am. J. Physiol.* **261**, C945–C953.
- COHEN, B. N., FAIN, G. L. and FAIN, M. J. (1989) GABA and glycine channels in isolated ganglion cells from the goldfish retina. *J. Physiol., Lond.* **418**, 53–82.
- COLEMAN, P. A. and MILLER, R. F. (1989) Measurement of passive membrane parameters with whole-cell recording from neurons in the intact amphibian retina. *J. Neurophysiol.* **61**, 218–230.
- COLLINGRIDGE, G. L., GAGE, P. W. and ROBERTSON, B. (1984) Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. *J. Physiol., Lond.* **356**, 551–564.
- COLLINGRIDGE, G., DAVIES, C. and DAVIES, S. (1988) Actions of APV and CNQX on synaptic responses in rat hippocampal slices. *Neurol. Neurobiol.* **46**, 171–178.
- COLQUHOUN, D. and SAKMANN, B. (1981) Fluctuations in the micro-second time range of the current through single acetylcholine receptor ion channels. *Nature* **294**, 464–466.
- CONNOR, J. A., TSENG, H. and HOCKBERGER, P. E. (1987) Depolarization- and transmitter-induced changes in intracellular Ca<sup>2+</sup> of rat cerebellar granule cells in explant cultures. *J. Neurosci.* **7**, 1384–1400.
- CONNOR, J. A., WADMAN, W. J., HOCKBERGER, P. E. and WONG, R. K. S. (1988) Sustained dendritic gradients of Ca<sup>2+</sup> induced by excitatory amino acids in CA1 hippocampal neurons. *Science* **240**, 649–653.
- CONNORS, B. W. (1992) GABA<sub>A</sub>- and GABA<sub>B</sub>-mediated processes in visual cortex. *Prog. Brain Res.* **90**, 335–348.
- CONNORS, B. W., GUTNICK, M. J. and PRINCE, D. A. (1982) Electrophysiological properties of neocortical neurons *in vitro*. *J. Neurophysiol.* **48**, 1302–1320.
- CONNORS, B. W., MALENKA, R. C. and SILVA, L. R. (1988) Two inhibitory postsynaptic potentials and GABA<sub>A</sub> and GABA<sub>B</sub> receptor-mediated responses in neocortex of rat and cat. *J. Physiol., Lond.* **406**, 443–468.
- COOMBS, J. S., ECCLES, J. C. and FATT, P. (1955) The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol., Lond.* **130**, 326–373.
- COSTA, E., BERKOVICH, A., WAMBEBE, C. and GUIDOTTI, A. (1988) Terminology for ligands of the allosteric modulatory center of GABA-operated Cl<sup>-</sup> channels. *Adv. Biochem. Psychopharmac.* **45**, 367–374.
- CRUNELLI, V., HABY, M., JASSIK-GERSCHENFELD, D., LERESCHE, N. and PIRCHIO, M. (1988) Cl<sup>-</sup> and K<sup>+</sup>-dependent inhibitory post-synaptic potentials evoked by interneurones of the rat lateral geniculate nucleus. *J. Physiol., Lond.* **399**, 153–176.
- CULL-CANDY, S. G. and USOWICZ, M. M. (1989) Whole-cell current noise produced by excitatory and inhibitory amino acids in large cerebellar neurones of the rat. *J. Physiol., Lond.* **415**, 533–553.
- CURTIS, D. R. and GYNTHIER, B. D. (1987) Divalent cations reduce depolarization of primary afferent terminations by GABA. *Brain Res.* **422**, 192–195.
- CURTIS, D. R., PHILLIS, J. W. and WATKINS, J. C. (1959) The depression of spinal neurones by  $\gamma$ -amino-N-butyric acid and  $\beta$ -alanine. *J. Physiol., Lond.* **146**, 185–203.
- CURTIS, D. R., DUGGAN, A. W., FELIX, D., JOHNSTON, G. A. R. and MCLENNAN, H. (1971) Antagonism between bicuculline and GABA in the cat brain. *Brain Res.* **33**, 57–73.
- CUTTING, R. C., LU, L., O'HARA, B. F., KASCH, L. M., MONTROSE-RAFIZADEH, C., DONOVAN, D. M., SHIMADA, S., ANTONARAKIS, A., GUGGINO, W. B., UHL, G. R. and KAZAZIAN, H. H. (1991) Cloning of the  $\gamma$ -aminobutyric acid (GABA)  $\rho_1$  cDNA: A GABA receptor subunit highly expressed in the retina. *Proc. natn. Acad. Sci. U.S.A.* **88**, 2673–2677.
- DARLISON, M. G. (1992) Invertebrate GABA and glutamate receptors: molecular biology reveals predictable structures but some unusual pharmacologies. *Trends Neurosci.* **15**, 469–474.
- DAVIDSON, N. and SIMPSON, H. K. L. (1976) Concerning the ionic basis of presynaptic inhibition. *Experientia* **32**, 348–349.
- DAVIES, C. H., DAVIES, S. N. and COLLINGRIDGE, G. L. (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J. Physiol., Lond.* **424**, 513–531.
- DAVIES, C. H., STARKEY, S. J., POZZA, M. F. and COLLINGRIDGE, G. L. (1991) GABA<sub>B</sub> autoreceptors regulate the induction of LTP. *Nature* **349**, 609–611.
- DE GROAT, W. C. (1970) The actions of  $\gamma$ -aminobutyric acid and related amino acids on mammalian autonomic ganglia. *J. Pharmac. exp. Ther.* **172**, 384–396.
- DE GROAT, W. C. (1972) GABA-depolarization of a sensory ganglion: Antagonism by picrotoxin and bicuculline. *Brain Res.* **38**, 429–432.
- DEISZ, R. A. and DOSE, M. (1983) Comparison of GABA analogs at the crayfish stretch receptor neurone. *Brain Res. Bull.* **11**, 283–288.
- DEISZ, R. A. and LUX, H. D. (1982) The role of intracellular chloride in hyperpolarizing post-synaptic inhibition of crayfish stretch receptor neurones. *J. Physiol., Lond.* **326**, 123–138.
- DEISZ, R. A. and PRINCE, D. A. (1989) Frequency-dependent depression of inhibition in guinea-pig neocortex *in vitro* by GABA<sub>B</sub> receptor feed-back on GABA release. *J. Physiol., Lond.* **412**, 513–541.
- DEITMER, J. W. (1991) Electrogenic sodium-dependent bicarbonate secretion by glial cells of the leech central nervous system. *J. gen. Physiol.* **98**, 637–655.
- DEITMER, J. W. and SCHLUETE, W. R. (1987) The regulation of intracellular pH by identified glial cells and neurones in the central nervous system of the leech. *J. Physiol., Lond.* **388**, 261–283.
- DEITMER, J. W. and SCHLUETE, W. R. (1989) An inwardly directed electrogenic sodium-bicarbonate co-transport in leech glial cells. *J. Physiol., Lond.* **411**, 179–194.

- DEL CASTILLO, J. and KATZ, B. (1957) Interaction at endplate receptors between different choline derivatives. *Proc. R. Soc. Lond. B* **146**, 369–381.
- DEMENEIX, B. A., TALEB, O., LOEFFLER, J. P. and FELTZ, P. (1986) GABA<sub>A</sub> and GABA<sub>B</sub> receptors on porcine pars intermedia cells in primary culture: functional role in modulating peptide release. *Neuroscience* **17**, 1275–1285.
- DIAMOND, J. M. and WRIGHT, E. M. (1969) Biological membranes: The physical basis of ion and nonelectrolyte selectivity. *A. Rev. Physiol.* **31**, 581–646.
- DICHTER, M. A. (1989) Cellular mechanisms of epilepsy and potential new treatment strategies. *Epilepsia* **30** (Suppl. 1), S3–12.
- DIETZEL, I., HEINEMANN, U., HOFMEIER, G. and LUX, H. D. (1982) Stimulus-induced changes in extracellular Na<sup>+</sup> and Cl<sup>-</sup> concentration in relation to changes in the size of the extracellular space. *Expl. Brain. Res.* **46**, 73–84.
- DINGLEDINE, R. (1984a) *Brain Slices*. Plenum: New York.
- DINGLEDINE, R. (1984b) Hippocampus: Synaptic pharmacology. In: *Brain Slices*, pp. 87–112. Ed. R. DINGLEDINE. Plenum: New York.
- DINGLEDINE, R., BOLAND, L. M., CHAMBERLIN, N. L., KAWASAKI, K., KLECKNER, N. W., TRAYNELIS, S. F. and VERDOORN, T. A. (1988) Amino acid receptors and uptake systems in the mammalian central nervous system. *CRC crit. Rev. Neurobiol.* **4**, 1–96.
- DJØRUP, A., JAHNSEN, H. and MOSFELDT LAURSEN, A. (1981) The dendrit response to GABA in CA1 of the hippocampal slice. *Brain Res.* **219**, 196–201.
- DODGSON, S. J. (1991) The carbonic anhydrases: overview of their importance in cellular physiology and in molecular genetics. In: *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics*, pp. 3–14. Eds. S. J. DODGSON, R. E. TASHIAN, G. GROS and N. D. CARTER. Plenum, New York.
- DOLPHIN, A. C. (1990) G protein modulation of calcium currents in neurons. *A. Rev. Physiol.* **52**, 243–255.
- DRAGUHN, A., VERDORN, T. A., EWERT, M., SEEBURG, P. H. and SAKMANN, B. (1990) Functional and molecular distinction between recombinant rat GABA<sub>A</sub>-receptor subtypes by Zn<sup>2+</sup>. *Neuron* **5**, 781–788.
- DREIFUSS, J. J., KELLY, J. S. and KRNIJEVIĆ, K. (1969) Cortical inhibition and  $\gamma$ -aminobutyric acid. *Expl. Brain. Res.* **9**, 137–154.
- DREW, C. A., JOHNSTON, G. A. R. and WEATHERBY, R. P. (1984) Bicuculline-insensitive GABA receptors: studies on the binding of (–) baclofen to rat cerebellar membranes. *Neurosci. Lett.* **52**, 317–321.
- DROZ, B. and KAZIMIERCZAK, J. (1987) Carbonic anhydrase in primary sensory neurons and dorsal root ganglia. *Comp. Biochem. Physiol.* **88B**, 713–717.
- DUCHEN, M. R. (1989) GABA<sub>A</sub> responses of dissociated mouse neurones are attenuated by metabolic blockade. *J. Physiol., Lond.* **415**, 48P.
- DUCHEN, M. R., VALDEOLMILLOS, M., O'NEILL, S. C. and EISNER, D. A. (1990) Effects of metabolic blockade on the regulation of intracellular calcium in dissociated mouse sensory neurones. *J. Physiol., Lond.* **424**, 411–426.
- DUDEL, J. (1977) Voltage dependence of amplitude and time course of inhibitory synaptic current in crayfish muscle. *Pflügers Arch.* **371**, 167–174.
- DUDEL, J. (1978) Relaxation after a voltage step of inhibitory synaptic current elicited by nerve stimulation (crayfish neuromuscular junction). *Pflügers Arch.* **376**, 151–157.
- DUDEL, J. and HATT, H. (1976) Four types of GABA receptors in crayfish leg muscles characterized by desensitization and specific antagonist. *Pflügers Arch.* **364**, 217–222.
- DUDEL, J. and KUFFLER, S. W. (1961) Presynaptic inhibition at the crayfish neuromuscular junction. *J. Physiol., Lond.* **155**, 543–562.
- DUDEL, J., FINGER, W. and STETTMEIER, H. (1977) GABA induced membrane current noise and the time course of the inhibitory synaptic current in crayfish muscle. *Neurosci. Lett.* **6**, 203–208.
- DUDEL, J., FINGER, W. and STETTMEIER, H. (1980) Inhibitory synaptic channels activated by  $\gamma$ -aminobutyric acid (GABA) in crayfish muscle. *Pflügers Arch.* **387**, 143–151.
- DUDEL, J., FRANKE, C., HATT, H., RAMSEY, R. L. and USHERWOOD, P. N. R. (1988) Rapid activation and desensitization by glutamate of excitatory, cation-selective channels in locust muscle. *Neurosci. Lett.* **88**, 33–38.
- DWYER, T. M., ADAMS, D. J. and HILLE, B. (1980) The permeability of the endplate channel to organic cations in frog muscle. *J. gen. Physiol.* **75**, 469–492.
- ECCLES, J. C. (1964a) Presynaptic inhibition in the spinal cord. *Prog. Brain Res.* **12**, 65–91.
- ECCLES, J. C. (1964b) *The Physiology of Synapses*. Springer: Berlin.
- ECCLES, J. C., ECCLES, R. M. and ITO, M. (1964a) Effects of intracellular potassium and sodium injections on the inhibitory postsynaptic potential. *Proc. R. Soc. Lond. B* **160**, 181–196.
- ECCLES, J. C., ECCLES, R. M. and ITO, M. (1964b) Effects produced on inhibitory postsynaptic potentials by the coupled injections of cations and anions into motoneurones. *Proc. R. Soc. Lond. B* **160**, 197–210.
- ECCLES, J. C., NICOLL, R. A., OSHIMA, T. and RUBIA, F. J. (1977) The anionic permeability of the inhibitory postsynaptic membrane of hippocampal pyramidal cells. *Proc. R. Soc. Lond. B* **198**, 345–361.
- EDWARDS, C. (1982) The selectivity of ion channels in nerve and muscle. *Neuroscience* **6**, 1335–1366.
- EDWARDS, F. A., KONNERTH, A., SAKMANN, B. and TAKAHASHI, T. (1989) A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Arch.* **414**, 600–612.
- EDWARDS, F. A., KONNERTH, A., SAKMANN, B. and BUSCH, C. (1990) Quantal analysis of inhibitory synaptic transmission in the dentate gyrus of rat hippocampal slices: a patch-clamp study. *J. Physiol., Lond.* **430**, 213–249.
- EISENMAN, G. (1962) Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2**, 259–323.
- EISENMAN, G. and HORN, R. (1983) Ionic selectivity revisited: The role of kinetic and equilibrium processes in ion permeation through channels. *J. membr. Biol.* **76**, 197–225.
- EISNER, D. A., KENNING, N. A., O'NEILL, S. C., POCOCK, G., RICHARDS, C. D. and VALDEOLMILLOS, M. (1989) A novel method for absolute calibration of intracellular pH indicators. *Pflügers Arch.* **413**, 553–558.
- EL-BEHEIRY, H. and PUUL, E. (1990) Effects of hypomagnesia on transmitter actions in neocortical slices. *Br. J. Pharmac.* **101**, 1006–1010.
- ENDRES, W., BALLANYI, K., SERVE, G. and GRAFE, P. (1986) Excitatory amino acids and intracellular pH in motoneurons of the isolated frog spinal cord. *Neurosci. Lett.* **72**, 54–58.
- ERDÖ, S. L. and WOLFF, J. R. (1990)  $\gamma$ -Aminobutyric acid outside the mammalian brain. *J. Neurochem.* **54**, 363–372.
- FABER, D. S. and KORN, H. (1980) Single-shot channel activation accounts for duration of inhibitory postsynaptic potentials in a central neuron. *Science* **208**, 612–615.
- FAGG, G. E. and FOSTER, A. C. (1983) Amino acid neurotransmitters and their pathways in the mammalian central nervous system. *Neuroscience* **9**, 701–719.
- FAN, P., LAVOIE, J., LÉ, N. L. O., SZERB, J. C. and BUTTERWORTH, R. F. (1990) Neurochemical and electrophysiological studies on the inhibitory effect of ammonium ions on synaptic transmission in slices of rat hippocampus: evidence for a postsynaptic action. *Neuroscience* **37**, 327–334.
- FELTZ, P. and RASMINSKY, M. (1974) A model for the mode of action of GABA on primary afferent terminals: depolarizing effects of GABA applied iontophoretically to neurones of mammalian dorsal root ganglia. *Neuropharmacology* **13**, 553–563.
- FLOREY, E. (1954) An inhibitory and an excitatory factor of mammalian central nervous system and their action on a single sensory neuron. *Arch. int. Physiol.* **62**, 33–53.
- FOSTER, M. (1880) *A Text Book of Physiology*. Macmillan: New York.
- FRANCIOLINI, F. and NONNER, W. (1987) Anion and cation permeability of a chloride channel in rat hippocampal neurons. *J. gen. Physiol.* **90**, 453–478.
- FRANCIOLINI, F. and PETRIS, A. (1990) Chloride channels of biological membranes. *Biochim. biophys. Acta* **1031**, 247–259.
- FRANKE, C., HATT, H. and DUDEL, J. (1986) The inhibitory chloride channel activated by glutamate as well as  $\gamma$ -amino-butyric acid (GABA). *J. comp. Physiol.* **159**, 591–609.
- FRANKE, C., HATT, H. and DUDEL, J. (1987) Liquid filament switch for ultra-fast exchanges of solutions at excised patches of synaptic membrane of crayfish muscle. *Neurosci. Lett.* **77**, 199–204.
- FREDERICKSON, C. J. and DANSCHER, G. (1990) Zinc-containing neurons in hippocampus and related CNS structures. *Prog. Brain Res.* **83**, 71–84.
- FRELIN, C., VIGNE, P., LADOUX, A. and LAZDUNSKI, M. (1988) The

- regulation of the intracellular pH in cells from vertebrates. *Eur. J. Biochem.* **174**, 3–14.
- FROSCH, M., LIPTON, S. and DICHTER, M. (1992) Desensitization of GABA-activated currents and channels in cultured cortical neurons. *J. Neurosci.* **12**, 3042–3053.
- FUJIWARA, N., HIGASHI, H., SHIMOJI, K. and YOSHIMURA, M. (1987) Effects of hypoxia on rat hippocampal neurones *in vitro*. *J. Physiol., Lond.* **384**, 131–151.
- GAGE, P. W. (1992) Activation and modulation of neuronal K<sup>+</sup> channels by GABA. *Trends Neurosci.* **15**, 46–51.
- GÄHWILER, B. (1981) Organotypic monolayer cultures of nervous tissue. *J. Neurosci. Meth.* **4**, 329–342.
- GAILLARD, S. and DUPONT, J. L. (1990) Ionic control of intracellular pH in rat cerebellar Purkinje cells maintained in culture. *J. Physiol., Lond.* **425**, 71–83.
- GALLAGHER, J. P., HIGASHI, H. and NISHI, S. (1978) Characterization and ionic basis of GABA-induced depolarizations recorded *in vitro* from cat primary afferent neurones. *J. Physiol., Lond.* **275**, 263–282.
- GALLAGHER, J. P., NAKAMURA, J. and SHINNICK-GALLAGHER, P. (1983) The effects of temperature, pH and Cl-pump inhibitors on GABA response recorded from cat dorsal root ganglia. *Brain Res.* **267**, 249–259.
- GALLER, S. and MOSER, H. (1986) The ionic mechanism of intracellular pH regulation in crayfish muscle fibres. *J. Physiol., Lond.* **374**, 137–151.
- GALZI, J., DEVILLERS-THIERY, A., HUSSY, N., BERTRAND, S., CHANGEUX, J. and BERTRAND, D. (1992) Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature* **359**, 500–505.
- GANZ, M. B., BOYARSKY, G., STERZEL, R. B. and BORON, W. F. (1989) Arginine vasopressin enhances pH regulation in the presence of HCO<sub>3</sub><sup>-</sup> by stimulating three acid-base transport systems. *Nature* **337**, 648–651.
- GASIC, G. P. and HOLLMANN, M. (1992) Molecular neurobiology of glutamate receptors. *A. Rev. Physiol.* **54**, 507–536.
- GEERS, C., GROS, G. and GÄRTNER, A. (1985) Extracellular carbonic anhydrase of skeletal muscle associated with the sarcolemma. *J. appl. Physiol.* **59**, 548–558.
- GERSCHENFELD, H. M. (1973) Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.* **53**, 1–119.
- GILBERT, P., KETTENMANN, H. and SCHACHNER, M. (1984)  $\gamma$ -Aminobutyric acid directly depolarizes cultured oligodendrocytes. *J. Neurosci.* **4**, 561–569.
- GOLAN, H., BARKAI, E. and GROSSMAN, Y. (1991) High CO<sub>2</sub>-bicarbonate buffer modifies GABAergic inhibitory effect at the crayfish neuromuscular synapse. *Brain Res.* **567**, 149–152.
- GRUOL, D. L., BARKER, J. L., HUANG, L.-Y. M., MACDONALD, J. F. and SMITH, T. G. (1980) Hydrogen ions have multiple effects on the excitability of cultured mammalian neurons. *Brain Res.* **183**, 247–252.
- GUTHRIE, P. B., SEGAL, M. and KATER, S. B. (1991) Independent regulation of calcium revealed by imaging dendritic spines. *Nature* **354**, 76–80.
- GYENES, M., FARRANT, M. and FARB, D. H. (1988) "Run-down" of gamma-aminobutyric acid<sub>A</sub> receptor function during whole-cell recording: a possible role for phosphorylation. *Molec. Pharmac.* **34**, 719–723.
- HAEFELY, W. (1984) Actions and interactions of benzodiazepine agonists and antagonists at GABAergic synapses. In: *Actions and Interactions of GABA and Benzodiazepines*, pp. 263–285. Ed. N. G. BOWERY. Raven: New York.
- HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. and SIGWORTH, F. J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391**, 85–100.
- HANSEN, A. J. (1985) Effect of anoxia on ion distribution in the brain. *Physiol. Rev.* **65**, 101–148.
- HARRISON, N. L. and LAMBERT, N. A. (1989) Modification of GABA<sub>A</sub> receptor function by an analog of cyclic AMP. *Neurosci. Lett.* **105**, 137–142.
- HARVEY, R. J., VREUGDENHIL, E., ZAMAN, S. H., BHANDAL, N. S., USHERWOOD, P. N. R., BARNARD, E. A. and DARLISON, M. G. (1991) Sequence of a functional invertebrate GABA<sub>A</sub> receptor subunit which can form a chimeric receptor with a vertebrate  $\alpha$  subunit. *EMBO J.* **10**, 3239–3245.
- HAYASHI, T. and NAGAI, K. (1956) Action of  $\omega$ -amino acids on the motor cortex of higher animals, especially  $\gamma$ -amino- $\beta$ -oxy-butyric acid as the real inhibitory principle in brain. In: *20th International Physiology Congress, Brussels (Abstr.)*, p. 410.
- HEINEMANN, U., STABEL, J. and RAUSCHE, G. (1990) Activity-dependent ionic changes and neuronal plasticity in rat hippocampus. *Prog. Brain Res.* **83**, 197–214.
- HERB, A., WISDEN, W., LÜDDENS, H., PUJA, G., VICINI, S. and SEEBURG, P. H. (1992) The third  $\gamma$  subunit of the  $\gamma$ -aminobutyric acid type A receptor family. *Proc. natn. Acad. Sci. U.S.A.* **89**, 1433–1437.
- HIGASHI, H., TANAKA, E. and NISHI, S. (1991) Synaptic responses of guinea pig cingulate cortical neurons *in vitro*. *J. Neurophysiol.* **65**, 822–833.
- HILL, D. R. and BOWERY, N. G. (1981) <sup>3</sup>H-baclofen and <sup>3</sup>H-GABA bind to bicuculline-insensitive GABA<sub>B</sub> sites in rat brain. *Nature* **290**, 149–152.
- HILLE, B. (1992) *Ion Channels of Excitable Membranes*, 2nd edn. Sinauer Associates Inc.: Sunderland, MA.
- HOFFMANN, E. K. and SIMONSEN, L. O. (1989) Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69**, 315–382.
- HOPPE, D. and KETTENMANN, H. (1989a) Carrier-mediated Cl<sup>-</sup> transport in cultured mouse oligodendrocytes. *J. Neurosci. Res.* **23**, 467–475.
- HOPPE, D. and KETTENMANN, H. (1989b) GABA triggers a Cl<sup>-</sup> efflux from cultured mouse oligodendrocytes. *Neurosci. Lett.* **97**, 334–339.
- HORN, R. and MARTY, A. (1988) Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J. gen. Physiol.* **92**, 145–159.
- HOWE, J. R., SUTOR, B. and ZIEGLGÄNSBERGER, W. (1987) Baclofen reduces post-synaptic potentials of rat cortical neurones by an action other than its hyperpolarizing action. *J. Physiol., Lond.* **384**, 539–569.
- HUGUENARD, J. R. and ALGER, B. E. (1986) Whole-cell voltage-clamp study of the fading of GABA-activated currents in acutely dissociated hippocampal neurons. *J. Neurophysiol.* **56**, 1–18.
- ILES, J. F. and JACK, J. J. B. (1980) Ammonia: Assessment of its action on postsynaptic inhibition as a cause of convulsions. *Brain* **103**, 555–578.
- INAGAKI, C., ODA, W., KONDO, K. and KUSUMI, M. (1987) Histochemical demonstration of Cl<sup>-</sup> ATPase in rat spinal motoneurons. *Brain Res.* **419**, 375–378.
- INOMATA, N., OOMURA, Y., AKAIKE, N. and EDWARDS, C. (1986) The anion selectivity of the  $\gamma$ -aminobutyric acid controlled chloride channel in the perfused spinal ganglion cell of frog. *Neurosci. Res.* **3**, 371–383.
- INOMATA, N., ISHIHARA, T. and AKAIKE, N. (1988) Effects of diuretics on GABA-gated chloride current in frog isolated sensory neurones. *Br. J. Pharmac.* **93**, 679–683.
- INOUE, I. (1985) Voltage-dependent chloride conductance of the squid axon membrane and its blockade by disulphonic stilbene derivatives. *J. gen. Physiol.* **85**, 519–537.
- INOUE, M., OOMURA, Y., YAKUSHII, T. and AKAIKE, N. (1986) Intracellular calcium ions decrease the affinity of the GABA receptor. *Nature* **324**, 156–158.
- INOUE, M., HARA, M., ZENG, X. T., HIROSE, T., OHNISHI, S., YASUKURA, T., URIU, T., OMORI, K., MINATO, A. and INAGAKI, C. (1991) An ATP-driven Cl<sup>-</sup> pump regulates Cl<sup>-</sup> concentrations in rat hippocampal neurons. *Neurosci. Lett.* **134**, 75–78.
- ITO, M. (1984) *The Cerebellum and Neural Control*. Raven: New York.
- ITO, M. and YOSHIDA, M. (1966) The origin of cellular-induced inhibition of Deiters neurones. I. Monosynaptic initiation of the inhibitory postsynaptic potentials. *Expl Brain Res.* **2**, 330–349.
- ITO, M., KOSTYUK, P. G. and OSHIMA, T. (1962) Further study on anion permeability of inhibitory post-synaptic membrane of cat motoneurones. *J. Physiol.* **164**, 150–156.
- ITO, S. and CHERUBINI, E. (1991) Strychnine-sensitive glycine responses of neonatal rat hippocampal neurones. *J. Physiol., Lond.* **440**, 67–83.
- JANIGRO, D. and SCHWARTZKROIN, P. A. (1988) Effects of GABA and baclofen on pyramidal cells in the developing rabbit hippocampus: an *in vitro* study. *Devil Brain Res.* **41**, 171–184.
- JAROLIMEK, W., MISGELD, U. and LUX, H. D. (1989) Activity depen-

- dent alkaline and acid transients in guinea pig hippocampal slices. *Brain Res.* **505**, 225–232.
- JEAN, T., FRELIN, C., VIGNE, P., BARBRY, P. and LAZDUNSKI, M. (1985) Biochemical properties of the  $\text{Na}^+/\text{H}^+$  exchange system in rat brain synaptosomes. *J. biol. Chem.* **260**, 9678–9684.
- JEAN, T., FRELIN, C., VIGNE, P. and LAZDUNSKI, M. (1986) The  $\text{Na}^+/\text{H}^+$  exchange system in glial cell lines. Properties and activation by an hyperosmotic shock. *Eur. J. Biochem.* **160**, 211–219.
- JOHNSTON, D. and BROWN, T. H. (1984) Biophysics and microphysiology of synaptic transmission in hippocampus. In: *Brain Slices*, pp. 51–86. Ed. R. DINGLEDINE. Plenum: New York.
- KAILA, K. and VOIPIO, J. (1987) Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. *Nature* **330**, 163–165.
- KAILA, K. and VOIPIO, J. (1990) GABA-activated bicarbonate conductance: Influence on  $E_{\text{GABA}}$  and on postsynaptic pH regulation. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 331–353. Eds. F. J. ALVAREZ-LEEFMANS and J. M. RUSSELL. Plenum: New York.
- KAILA, K., PASTERNAK, M., SAARIKOSKI, J. and VOIPIO, J. (1989) Influence of GABA-gated bicarbonate conductance on membrane potential, current and intracellular chloride in crayfish muscle fibres. *J. Physiol., Lond.* **416**, 161–181.
- KAILA, K., SAARIKOSKI, J. and VOIPIO, J. (1990) Mechanism of action of GABA on intracellular pH and on surface pH in crayfish muscle fibres. *J. Physiol., Lond.* **427**, 241–260.
- KAILA, K., PANULA, P., KARHUNEN, T. and HEINONEN, E. (1991) Fall in intracellular pH mediated by  $\text{GABA}_A$  receptors in cultured rat astrocytes. *Neurosci. Lett.* **126**, 9–12.
- KAILA, K., PAALASMAA, P., TAIRA, T. and VOIPIO, J. (1992a) pH transients due to monosynaptic activation of  $\text{GABA}_A$  receptors in rat hippocampal slices. *NeuroReport* **3**, 105–108.
- KAILA, K., RYDQVIST, B., PASTERNAK, M. and VOIPIO, J. (1992b) Inward current caused by sodium-dependent uptake of GABA in the crayfish stretch-receptor neurone. *J. Physiol., Lond.* **453**, 627–645.
- KAILA, K., VOIPIO, J., PASTERNAK, M., PAALASMAA, P. and DEISZ, R. A. (1993) The role of bicarbonate in  $\text{GABA}_A$  receptor-mediated IPSPs in rat neocortical neurons. *J. Physiol., Lond.* **464**, 273–289.
- KANDEL, E. R., SPENCER, W. A. and BRINLEY, F. J. (1961) Electrophysiology of hippocampal neurons. I. Sequential invasion and synaptic organization. *J. Neurophysiol.* **24**, 225–242.
- KANO, M., REXHAUSEN, U., DREESSEN, J. and KONNERTH, A. (1992) Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. *Nature* **356**, 601–604.
- KAPUR, J. and LOTHMAN, E. W. (1989) Loss of inhibition precedes delayed spontaneous seizures in the hippocampus after tetanic electrical stimulation. *J. Neurophysiol.* **61**, 427–434.
- KATZ, B. and MILEDI, R. (1970) Membrane noise produced by acetylcholine. *Nature* **226**, 962–963.
- KATZ, B. and MILEDI, R. (1972) The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol., Lond.* **224**, 665–699.
- KATZ, B. and MILEDI, R. (1973) The characteristics of "end-plate noise" produced by different depolarizing drugs. *J. Physiol., Lond.* **230**, 707–717.
- KATZ, B. and THESLEFF, S. (1957) A study of the "desensitization" produced by acetylcholine at the motor end-plate. *J. Physiol., Lond.* **138**, 63–80.
- KAY, M. M. B., HUGHES, J., ZAGON, I. and LIN, F. (1991) Brain membrane protein band 3 performs the same functions as erythrocyte band 3. *Proc. natn. Acad. Sci. U.S.A.* **88**, 2778–2782.
- KAZIMIERCZAK, J., SOMMER, E. W., PHILIPPE, E. and DROZ, B. (1986) Carbonic anhydrase activity in primary sensory neurons. *Cell Tissue Res.* **245**, 487–495.
- KELLY, J. S., KRNEVIĆ, K., MORRIS, M. E. and YIM, G. K. W. (1969) Anionic permeability of cortical neurones. *Expl. Brain Res.* **7**, 11–31.
- KERKUT, G. A. and THOMAS, R. C. (1964) The effect of anion injection and changes in the external potassium and chloride concentration on the reversal potentials of the IPSP and acetylcholine. *Comp. Biochem. Physiol.* **11**, 199–213.
- KERKUT, G. A. and WHEAL, H. V. (1989) *Electrophysiology of Isolated Mammalian CNS Preparations*. Academic Press: London.
- KETTENMANN, H. (1990) Chloride channels and carriers in cultured glial cells. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 193–208. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- KETTENMANN, H. and SCHLUE, W. R. (1988) Intracellular pH regulation in cultured mouse oligodendrocytes. *J. Physiol., Lond.* **406**, 147–162.
- KETTENMANN, H., BACKUS, K. H. and SCHACHNER, M. (1984) Aspartate, glutamate and  $\gamma$ -aminobutyric acid directly depolarize cultured astrocytes. *Neurosci. Lett.* **52**, 25–29.
- KETTENMANN, H., BACKUS, K. H. and SCHACHNER, M. (1987)  $\gamma$ -aminobutyric acid opens Cl-channel in cultured astrocytes. *Brain Res.* **404**, 1–9.
- KEYNES, R. D. (1963) Chloride in the squid giant axon. *J. Physiol., Lond.* **169**, 690–705.
- KILLISCH, I., DOTTI, C. G., LAURIE, D. J., LUDDENS, H. and SEEBURG, P. H. (1991) Expression patterns of  $\text{GABA}_A$  receptor subtypes in developing hippocampal neurons. *Neuron* **7**, 927–936.
- KIMBERG, H. K. (1990) Chloride transport across glial membranes. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 159–191. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- KOPITO, R. R., LEE, B. S., SIMMONS, D. M., LINDSEY, A. E., MORGANS, C. W. and SCHNEIDER, K. (1989) Regulation of intracellular pH by a neuronal homolog of the erythrocyte anion exchanger. *Cell* **59**, 927–937.
- KORN, S. J., BOLDEN, A. and HORN, R. (1991) Control of action potentials and  $\text{Ca}^{2+}$  influx by the  $\text{Ca}^{2+}$ -dependent chloride current in mouse pituitary cells. *J. Physiol., Lond.* **439**, 423–437.
- KRAIG, R. P., FERREIRA-FILHO, C. S. and NICHOLSON, C. (1983) Alkaline and acid transients in cerebellar microenvironment. *J. Neurophysiol.* **49**, 831–850.
- KRAVITZ, E. A. (1963) Gamma-aminobutyric acid and other blocking compounds in crustacea. III. Their relative concentrations in separated motor and inhibitory axons. *J. Neurophysiol.* **26**, 739–751.
- KRNJEVIĆ, K. (1974) Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.* **54**, 418–540.
- KRNJEVIĆ, K. (1983) GABA-mediated inhibitory mechanisms in relation to epileptic discharges. In: *Basic Mechanisms of Neuronal Hyperexcitability*, pp. 249–280. Alan R. Liss: New York.
- KRNJEVIĆ, K. and SCHWARTZ, S. (1967) The action of  $\gamma$ -aminobutyric acid on cortical neurones. *Expl. Brain Res.* **3**, 320–336.
- KRNJEVIĆ, K., XU, Y. Z. and ZHANG, L. (1991) Anoxic block of GABAergic IPSPs. *Neurochem. Res.* **16**, 279–284.
- KROGSGAARD-LARSEN, P. (1980) Inhibitors of the GABA uptake systems. *Molec. cell. Biochem.* **31**, 105–121.
- KROGSGAARD-LARSEN, P. (1988) GABA synaptic mechanisms: Stereochemical and conformational requirements. *Med. Res. Rev.* **8**, 27–56.
- KUDO, Y. and FUKUDA, H. (1976) Alteration of extracellular  $\text{K}^+$ -activity induced by amino acids in the frog spinal cord. *Jap. J. Pharmac.* **26**, 385–387.
- KUFFLER, S. W. and EDWARDS, C. (1958) Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *J. Neurophysiol.* **21**, 589–610.
- LACAILLE, J.-C. (1991) Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurons of stratum pyramidale of the CA1 region of rat hippocampal slices *in vitro*. *J. Neurophysiol.* **66**, 1441–1454.
- LAMBERT, N. A., BORRONI, A. M., GROVER, L. M. and TEYLIER, T. J. (1991) Hyperpolarizing and depolarizing  $\text{GABA}_A$  receptor-mediated dendritic inhibition in area CA1 of the rat hippocampus. *J. Neurophysiol.* **66**, 1538–1548.
- LAMBERT, N. A., LEVITIN, M. and HARRISON, N. L. (1992) Induction of giant depolarizing potentials by zinc in area CA1 of the rat hippocampus does not result from block of  $\text{GABA}_B$  receptors. *Neurosci. Lett.* **135**, 215–218.
- LEGENDRE, P. and WESTBROOK, G. L. (1991) Noncompetitive inhibition of  $\gamma$ -aminobutyric acid<sub>A</sub> channels by Zn. *Molec. Pharmac.* **39**, 267–274.
- LEIDENHEIMER, N. J., MCQUILKIN, S. J., HAHNER, L. D., WHITING, P. and HARRIS, R. A. (1992) Activation of protein kinase C selectively inhibits the  $\gamma$ -aminobutyric acid<sub>A</sub> receptor: Role of desensitization. *Molec. Pharmac.* **41**, 1116–1123.
- LERMA, J. and MARTÍN DEL RÍO, R. (1992) Chloride transport blockers prevent *N*-methyl-D-aspartate receptor-channel complex activation. *Molec. Pharmac.* **41**, 217–222.

- LEVITAN, E. S., SCHOFIELD, P. R., BURT, D. R., RHEES, L. M., WISDEN, W., KÖHLER, M., FUJITA, N., RODRIGUEZ, H. F., STEPHENSON, A., DARLISON, M. G., BARNARD, E. A. and SEEBURG, P. H. (1988) Structural and functional basis for GABA<sub>A</sub> receptor heterogeneity. *Nature* **335**, 76–79.
- LLANO, I., MARTY, A., JOHNSON, J. W., ASCHER, P. and GÄHWILER, B. (1988) Patch-clamp recording of amino acid-activated responses in "organotypic" slice cultures. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3221–3225.
- LLANO, I., LERESCHE, N. and MARTY, A. (1991) Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* **6**, 565–574.
- LLINÁS, R., BAKER, R. and PRECHT, W. (1974) Blockage of inhibition by ammonium acetate: Action on chloride pump in cat trochlear motoneurons. *J. Neurophysiol.* **37**, 522–532.
- LLINÁS, R., SUGIMORI, M. and SILVER, R. B. (1992) Microdomains of high calcium concentration in a presynaptic terminal. *Science* **256**, 677–679.
- LLOYD, D. P. C. (1961) A study of some Twentieth Century thoughts on inhibition in the spinal cord. In: *Nervous Inhibition*, pp. 13–31. Ed. E. FLOREY. Pergamon: Oxford.
- LUHMANN, H. J. and PRINCE, D. A. (1991) Postnatal maturation of the GABAergic system in rat neocortex. *J. Neurophysiol.* **65**, 247–263.
- LUTZ, P. L. (1992) Mechanisms for anoxic survival in the vertebrate brain. *A. Rev. Physiol.* **54**, 601–618.
- LUX, H. D. (1971) Ammonium and chloride extrusion: hyperpolarizing synaptic inhibition in spinal motoneurons. *Science* **173**, 555–557.
- LUX, H. D., LORACHER, C. and NEHER, E. (1970) The action of ammonium on postsynaptic inhibition of cat spinal motoneurons. *Expl Brain Res.* **11**, 431–447.
- MACDONALD, R. L. (1989) Antiepileptic drug actions. *Epilepsia* **30** (Suppl. 1), S19–28.
- MACDONALD, R. L., ROGERS, C. J. and TWYMAN, R. E. (1989a) Kinetic properties of the GABA<sub>A</sub> receptor main conductance state of mouse spinal cord neurones in culture. *J. Physiol., Lond.* **410**, 479–499.
- MACDONALD, R. L., ROGERS, C. J. and TWYMAN, R. E. (1989b) Barbiturate regulation of kinetic properties of the GABA<sub>A</sub> receptor channel of mouse spinal neurones in culture. *J. Physiol., Lond.* **417**, 483–500.
- MACVICAR, B. A., TSE, F. W. Y., CRICHTON, S. A. and KETTENMANN, H. (1989) GABA-activated Cl<sup>-</sup> channels in astrocytes of hippocampal slices. *J. Neurosci.* **9**, 3577–3583.
- MADISON, D. V., MALENKA, R. C. and NICOLL, R. A. (1986) Phorbol esters block a voltage-sensitive chloride current in hippocampal pyramidal cells. *Nature* **321**, 695–697.
- MAGLEBY, K. L. and STEVENS, C. F. (1972) The effect of voltage on the time course of end-plate currents. *J. Physiol., Lond.* **223**, 151–171.
- MAJEWSKA, M. D., HARRISON, N. L., SCHWARTZ, R. D., BARKER, J. L. and PAUL, S. M. (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA<sub>A</sub> receptor. *Science* **232**, 1004–1007.
- MALCHOW, R. P. and RIPPS, H. (1990) Effects of  $\gamma$ -aminobutyric acid on skate retinal horizontal cells: Evidence for an electrogenic uptake mechanism. *Proc. natn. Acad. Sci. U.S.A.* **87**, 8945–8949.
- MALCHOW, R. P., QIAN, H. and RIPPS, H. (1989)  $\gamma$ -aminobutyric acid (GABA)-induced currents of skate Muller (glial) cells are mediated by neuronal-like GABA<sub>A</sub> receptors. *Proc. natn. Acad. Sci. U.S.A.* **86**, 4326–4330.
- MALHERBE, P., DRAGUHN, A., MULTHAUP, G., BEYREUTHER, K. and MÖHLER, H. (1990) GABA<sub>A</sub>-receptor expressed from rat brain  $\alpha$ - and  $\beta$ -subunit cDNAs displays potentiation by benzodiazepine receptor ligands. *Brain Res. molec. Brain Res.* **8**, 199–208.
- MARCHENKO, S. M. (1991) Mechanism of modulation of GABA-activated current by internal calcium in rat central neurons. *Brain Res.* **546**, 355–357.
- MAREN, T. H. (1984) The general physiology of reactions catalyzed by carbonic anhydrase and their inhibition by sulfonamides. *Ann. N. Y. Acad. Sci.* **429**, 568–579.
- MASON, M. J., MATTSSON, K., PASTERNACK, M., VOIPIO, J. and KAILA, K. (1990) Postsynaptic fall in intracellular pH and increase in surface pH caused by efflux of formate and acetate anions through GABA-gated channels in crayfish muscle fibres. *Neuroscience* **34**, 359–368.
- MATHERS, D. A. (1991) Activation and inactivation of the GABA<sub>A</sub> receptor: insights from comparison of native and recombinant subunit assemblies. *Can. J. Physiol. Pharmac.* **69**, 1057–1063.
- MATHERS, D. A. and BARKER, J. L. (1982) Chemically induced ion channels in nerve cell membranes. *Int. Rev. Neurobiol.* **23**, 1–34.
- MAYER, M. L. and VYKLICKY, L., Jr (1989) The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus. *J. Physiol., Lond.* **415**, 351–365.
- MAYER, M. L., OWEN, D. G. and BARKER, J. L. (1990) Calcium-dependent chloride currents in vertebrate central neurons. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 355–364. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- MAZDA, G. Y., NISTRI, A. and SIVIOTTI, L. (1990) The effect of GABA on the frog optic tectum is sensitive to ammonium and to penicillin. *Eur. J. Pharmac.* **179**, 111–118.
- MCCARREN, M. and ALGER, B. E. (1985) Use-dependent depression of IPSPs in rat hippocampal pyramidal cells *in vitro*. *J. Neurophysiol.* **53**, 557–571.
- MEINECKE, D. L. and RAKIC, P. (1990) Developmental expression of GABA and subunits of the GABA<sub>A</sub> receptor complex in an inhibitory synaptic circuit in the rat cerebellum. *Brain Res. dev. Brain Res.* **55**, 73–86.
- MERCURI, N. B., CALABRESI, P., STEFANI, A., STRATTA, F. and BERNARDI, G. (1991) GABA depolarizes neurons in the rat striatum: an *in vivo* study. *Synapse* **8**, 38–40.
- MICHELSON, H. B. and WONG, R. K. S. (1991) Excitatory synaptic responses mediated by GABA<sub>A</sub> receptors in the hippocampus. *Science* **253**, 1420–1423.
- MIERLAK, D. and FARB, D. H. (1988) Modulation of neurotransmitter receptor desensitization: Chlordiazepoxide stimulates fading of the GABA response. *J. Neurosci.* **8**, 814–820.
- MILLER, R. J. (1987) Multiple calcium channels and neuronal function. *Science* **235**, 46–52.
- MILNER, R. J. and SUTCLIFFE, J. G. (1983) Gene expression in rat brain. *Nucleic Acids Res.* **11**, 5497–5520.
- MISGELD, U., DEISZ, R. A., DODT, H. U. and LUX, H. D. (1986) The role of chloride transport in postsynaptic inhibition of hippocampal neurons. *Science* **232**, 1413–1415.
- MISTRY, D. K. and HABLITZ, J. J. (1990) Activation of subconductance states by  $\gamma$ -aminobutyric acid and its analogs in chick cerebral neurons. *Pflügers Arch.* **416**, 454–461.
- MIWA, A., UI, M. and KAWAI, N. (1990) G protein is coupled to presynaptic glutamate and GABA receptors in lobster neuromuscular synapse. *J. Neurophysiol.* **63**, 173–180.
- MOODY, W. J., Jr (1981) The ionic mechanism of intracellular pH regulation in crayfish neurones. *J. Physiol., Lond.* **316**, 293–308.
- MORROW, A. L., PACE, J. R., PURDY, R. H. and PAUL, S. M. (1990) Characterization of steroid interactions with  $\gamma$ -aminobutyric acid receptor-gated chloride ion channels: Evidence for multiple steroid recognition sites. *Molec. Pharmac.* **37**, 263–270.
- MOSER, H. (1985) Intracellular pH regulation in the sensory neurone of the stretch receptor of the crayfish (*Astacus fluviatilis*). *J. Physiol., Lond.* **362**, 23–38.
- MOSS, S. J., SMART, T. G., PORTER, N. M., NAYEEM, N., DEVINE, J., STEPHENSON, F. A., MACDONALD, R. L. and BARNARD, E. A. (1990) Cloned GABA receptors are maintained in a stable cell line: allosteric and channel properties. *Eur. J. Pharmac.* **189**, 77–88.
- MOSS, S. J., SMART, T. G., BLACKSTONE, C. D. and HUGANIR, R. L. (1992) Functional modulation of GABA<sub>A</sub> receptors by cAMP-dependent protein phosphorylation. *Science* **257**, 661–665.
- MOTOKIZAWA, F., REUBEN, J. P. and GRUNDFEST, H. (1969) Ionic permeability of the inhibitory postsynaptic membrane of lobster muscle fibres. *J. gen. Physiol.* **54**, 437–461.
- MOUGINOT, D., FELTZ, P. and SCHLICHTER, R. (1991) Modulation of GABA-gated chloride currents by intracellular Ca<sup>2+</sup> in cultured porcine melanotrophs. *J. Physiol.* **437**, 109–132.
- MÜLLER, W. and CONNOR, J. A. (1991) Dendritic spines as individual neuronal compartments for synaptic Ca<sup>2+</sup> responses. *Nature* **354**, 73–76.
- MÜLLER, W., MISGELD, U. and LUX, H. D. (1989)  $\gamma$ -Aminobutyric acid-induced ion movements in the guinea pig hippocampal slice. *Brain Res.* **484**, 184–191.
- NEILD, T. O. and THOMAS, R. C. (1974) Intracellular chloride activity and the effects of acetylcholine in snail neurones. *J. Physiol., Lond.* **242**, 453–470.
- NEUBAUER, J. A. (1991) Carbonic anhydrase and sensory function in the central nervous system. In: *The Carbonic Anhydrases: Cellular*

- Physiology and Molecular Genetics*, pp. 319–323. Eds. S. J. DODGSON, R. E. TASHIAN, G. GROS and N. D. CARTER. Plenum: New York.
- NEWBERRY, N. R. and NICOLL, R. A. (1985) Comparison of the action of baclofen with  $\gamma$ -aminobutyric acid on rat hippocampal pyramidal cells *in vitro*. *J. Physiol., Lond.* **360**, 161–185.
- NEWLAND, C. F., COLQUHOUN, D. and CULL-CANDY, S. G. (1991) Single channels activated by high concentrations of GABA in superior cervical ganglion neurones of the rat. *J. Physiol., Lond.* **432**, 203–233.
- NEWLAND, C. F. and CULL-CANDY, S. G. (1992) On the mechanism of action of picrotoxin on GABA receptor channels in dissociated sympathetic neurones of the rat. *J. Physiol., Lond.* **447**, 191–213.
- NEWMAN, E. A. and ASTION, M. L. (1991) Localization and stoichiometry of electrogenic sodium bicarbonate cotransport in retinal glial cells. *Glia* **4**, 424–428.
- NICHOLSON, C. (1979) Brain-cell microenvironment as a communication channel. In: *The Neurosciences: Fourth Study Program*, pp. 457–473. Eds. F. O. SCHMITT and F. G. WARDEN. MIT Press: Cambridge, MA.
- NICHOLSON, C. (1980) Dynamics of the brain cell microenvironment. *Neurosci. Res. Prog. Bull.* **18**, 175–322.
- NICHOLSON, C. and PHILLIPS, J. M. (1981) Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. *J. Physiol., Lond.* **321**, 225–257.
- NICHOLSON, C. and RICE, M. E. (1986) The migration of substances in the neuronal microenvironment. *Ann. N. Y. Acad. Sci.* **481**, 55–71.
- NICHOLSON, C. and RICE, M. E. (1991) Diffusion of ions and transmitters in the brain cell microenvironment. *Adv. Neurosci.* **1**, 279–294.
- NICOLL, R. A. (1978) The blockade of GABA mediated responses in the frog spinal cord by ammonium ions and furosemide. *J. Physiol., Lond.* **283**, 121–132.
- NICOLL, R. A. (1988) The coupling of neurotransmitter receptors to ion channels in the brain. *Science* **241**, 545–551.
- NICOLL, R. A. and ALGER, B. E. (1979) Presynaptic inhibition: Transmitter and ionic mechanisms. *Int. Rev. Neurobiol.* **21**, 217–258.
- NICOLL, R. A. and BARKER, J. L. (1971) The pharmacology of recurrent inhibition in the supraoptic neurosecretory system. *Brain Res.* **35**, 501–511.
- NICOLL, R. A., MALENKA, R. C. and KAUFER, J. A. (1990) Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol. Rev.* **70**, 513–565.
- NISHI, S., MINOTA, S. and KARCZMAR, A. G. (1974) Primary afferent neurones: The ionic mechanism of GABA-mediated depolarization. *Neuropharmacology* **13**, 215–219.
- NISTRI, A. and CONSTANTI, A. (1979) Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Prog. Neurobiol.* **13**, 117–235.
- NUMANN, R. E. and WONG, R. K. S. (1984) Voltage-clamp study on GABA response desensitization in single pyramidal cells dissociated from the hippocampus of adult guinea pigs. *Neurosci. Lett.* **47**, 289–294.
- OBATA, K. (1977) Biochemistry and physiology of amino acid transmitters. In: *Handbook of Physiology, Section 1: The Nervous System*, Vol. I, pp. 625–650. Ed. E. R. KANDEL. American Physiological Society: Bethesda, MD.
- OBATA, K., OIDE, M. and TANAKA, H. (1978) Excitatory and inhibitory actions of GABA and glycine on embryonic chick spinal neurons in culture. *Brain Res.* **144**, 179–184.
- OGATA, N. (1987)  $\gamma$ -aminobutyric acid (GABA) causes consistent depolarization of neurons in the guinea pig supraoptic nucleus due to an absence of GABA<sub>B</sub> recognition sites. *Brain Res.* **403**, 225–233.
- OH, D. J. and DICHTER, M. A. (1992) Desensitization of GABA-induced currents in cultured rat hippocampal neurons. *Neuroscience* **49**, 571–576.
- OLSEN, R. W. and TOBIN, A. J. (1990) Molecular biology of GABA<sub>A</sub> receptors. *FASEB J.* **4**, 1469–1480.
- ONODERA, K. and TAKEUCHI, A. (1979) An analysis of the inhibitory post-synaptic current in the voltage-clamped crayfish muscle. *J. Physiol., Lond.* **286**, 265–282.
- OTSUKA, M., IVERSEN, L. L., HALL, Z. W. and KRAVITZ, E. A. (1966) Release of  $\gamma$ -aminobutyric acid from inhibitory nerves of lobster. *Proc. natn. Acad. Sci. U.S.A.* **53**, 1110–1115.
- OZAWA, S. and TSUDA, K. (1973) Membrane permeability change during inhibitory transmitter action in crayfish stretch receptor cell. *J. Neurophysiol.* **36**, 805–816.
- PASTERNACK, M., BOUNTRA, C., VOPIO, J. and KAILA, K. (1992a) Influence of extracellular and intracellular pH on GABA-gated chloride conductance in crayfish muscle fibres. *Neuroscience* **47**, 921–929.
- PASTERNACK, M., RYDQVIST, B. and KAILA, K. (1992b) GABA-gated anion channels in intact crayfish opener muscle fibers and stretch-receptor neurones are neither activated nor desensitized by glutamate. *J. comp. Physiol.* **170**, 521–524.
- PASTERNACK, M., VOPIO, J. and KAILA, K. (1993) Intracellular carbonic anhydrase activity and its role in GABA-induced acidosis in isolated rat hippocampal pyramidal neurones. *Acta physiol. scand.* **148**, 229–231.
- PEERS, C. and GREEN, F. K. (1991) Inhibition of Ca<sup>2+</sup>-activated K<sup>+</sup> currents by intracellular acidosis in isolated type I cells of the neonatal rat carotid body. *J. Physiol., Lond.* **437**, 589–602.
- PERREAU, P. and AVOLI, M. (1991) Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. *J. Neurophysiol.* **65**, 771–785.
- PETERS, J. A., LAMBERT, J. J. and COTTRELL, G. A. (1989) An electrophysiological investigation of the characteristics and function of GABA<sub>A</sub> receptors on bovine adrenomedullary chromaffin cells. *Pflügers Arch.* **415**, 95–103.
- PINNOCK, R. D., DAVID, J. A. and SATTELLE, D. B. (1988) Ionic events following GABA receptor activation in an identified insect motor neuron. *Proc. R. Soc. Lond. B.* **232**, 457–470.
- PITLER, T. A. and ALGER, B. E. (1992) Postsynaptic spike firing reduces synaptic GABA<sub>A</sub> responses in hippocampal pyramidal cells. *J. Neurosci.* **12**, 4122–4132.
- POCOCK, G. and RICHARDS, C. D. (1992) Hydrogen ion regulation in rat cerebellar granule cells studied by single-cell fluorescence microscopy. *Eur. J. Neurosci.* **4**, 136–143.
- POLENZANI, L., WOODWARD, R. M. and MILEDI, R. (1991) Expression of mammalian  $\gamma$ -aminobutyric acid receptors with distinct pharmacology in *Xenopus* oocytes. *Proc. natn. Acad. Sci. U.S.A.* **88**, 4318–4322.
- POULTER, M., BARKER, J., O'CARROLL, A.-M., LOLAIT, S. and MAHAN, L. (1992) Differential and transient expression of GABA<sub>A</sub> receptor  $\alpha$ -subunit mRNAs in the developing rat CNS. *J. Neurosci.* **12**, 2888–2900.
- PRITCHETT, D. B., SONTHEIMER, H., GORMAN, C. M., KETTENMANN, H., SEEBURG, P. H. and SCHOFIELD, P. R. (1988) Transient expression shows ligand gating and allosteric potentiation of GABA<sub>A</sub> receptor subunits. *Science* **242**, 1306–1308.
- PUIA, G., SANTI, M., VICINI, S., PRITCHETT, D. B., PURDY, R. H., PAUL, S. M., SEEBURG, P. H. and COSTA, E. (1990) Neurosteroids act on recombinant human GABA<sub>A</sub> receptors. *Neuron* **4**, 759–765.
- RAABE, W. (1990) Effects of NH<sub>4</sub><sup>+</sup> on reflexes in cat spinal cord. *J. Neurophysiol.* **64**, 565–574.
- RAABE, W. and GUMNIT, R. J. (1975) Disinhibition in cat motor cortex by ammonia. *J. Neurophysiol.* **38**, 347–355.
- RALEY-SUSMAN, K. M., CRAGOE, E. J., JR, SAPOLSKY, R. M. and KOPITO, R. R. (1991) Regulation of intracellular pH in cultured hippocampal neurons by an amiloride-insensitive Na<sup>+</sup>/H<sup>+</sup> exchanger. *J. biol. Chem.* **266**, 2739–2745.
- RANDLE, J. C. R. and RENAUD, L. P. (1987) Actions of  $\gamma$ -aminobutyric acid on rat supraoptic nucleus neurosecretory neurones *in vitro*. *J. Physiol., Lond.* **387**, 629–647.
- RANSOM, B. (1993) Glial modulation of neural excitability mediated by extracellular pH: A hypothesis. *Prog. Brain Res.* **94**, 37–46.
- RANSOM, B. and BARKER, J. L. (1975) Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture. *Nature* **254**, 703–705.
- RANSOM, B. R. and SONTHEIMER, H. (1992) The neurophysiology of glial cells. *J. clin. Neurophys.* **9**, 224–251.
- REGEHR, W. G. and TANK, D. W. (1992) Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. *J. Neurosci.* **12**, 4202–4223.
- REGEHR, W. G., CONNOR, J. A. and TANK, D. W. (1989) Optical imaging of calcium accumulation in hippocampal pyramidal cells during synaptic activation. *Nature* **341**, 533–536.
- RICHARDS, J. G., SCHOCH, P., MÖHLER, H. and HAEEFELY, W. (1986) Benzodiazepine receptors resolved. *Experientia* **42**, 121–126.
- ROBERTS, E. (1986) GABA: The road to neurotransmitter status. In: *Benzodiazepine/GABA Receptors and Chloride Channels; Struc-*

- tural and Functional Properties, pp. 1–39. Eds. R. W. OLSEN and J. C. VENTER. Alan R. Liss: New York.
- ROBERTS, E. and FRANKEL, S. (1950)  $\gamma$ -Aminobutyric acid in brain: its formation from glutamic acid. *J. biol. Chem.* **187**, 55–63.
- ROBERTSON, B. (1989) Characteristics of GABA-activated chloride channels in mammalian dorsal root ganglion neurones. *J. Physiol., Lond.* **411**, 285–300.
- ROBINSON, R. A. and STOKES, R. H. (1959) *Electrolyte Solutions*. Butterworths: London.
- ROEPSTORFF, A. and LAMBERT, J. D. C. (1992) Comparison of the effect of the GABA uptake blockers, tiagabine and nipeptic acid, on inhibitory synaptic efficacy in hippocampal CA1 neurones. *Neurosci. Lett.* **146**, 131–134.
- ROOS, A. and BORON, W. F. (1981) Intracellular pH. *Physiol. Rev.* **61**, 296–433.
- RORSMAN, P., BERGGREN, P.-O., BOKVIST, K., ERICSON, H., MÖHLER, H., ÖSTERSON, C.-G. and SMITH, P. A. (1989) Glucose-inhibition of glucagon secretion involves activation of GABA<sub>A</sub>-receptor chloride channels. *Nature* **341**, 233–236.
- ROVIRA, C. and BEN-ARI, Y. (1991) Benzodiazepines do not potentiate GABA responses in neonatal hippocampal neurons. *Neurosci. Lett.* **130**, 157–161.
- ROVIRA, C., BEN-ARI, Y. and CHERUBINI, E. (1984) Somatic and dendritic actions of  $\gamma$ -aminobutyric acid agonists and uptake blockers in the hippocampus *in vitro*. *Neuroscience* **12**, 543–555.
- RUSSEL, J. M. and BROWN, A. M. (1972) Active transport of chloride by the giant neuron of the *Aplysia abdominal ganglion*. *J. gen. Physiol.* **60**, 499–518.
- RUSSELL, J. M. (1983) Cation-coupled chloride influx in squid axon. Role of potassium and stoichiometry of the transport process. *J. gen. Physiol.* **81**, 909–925.
- RUSSELL, J. M. and BORON, W. F. (1976) Role of chloride transport in regulation of intracellular pH. *Nature* **264**, 73–74.
- RUSSELL, J. M. and BORON, W. F. (1990) Chloride transport in the squid giant axon. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, pp. 85–107. Eds. F. J. ALVAREZ-LEEFMANS and M. RUSSELL. Plenum: New York.
- SAARIKOSKI, J. and KAILA, K. (1992) Simultaneous measurement of intracellular and extracellular carbonic anhydrase activity in intact muscle fibres. *Pflügers Arch.* **421**, 357–363.
- SAHARA, Y., ROBINSON, H. P. C., MIWA, A. and KAWAI, N. (1991) A voltage-clamp study of the effects of Joro spider toxin and zinc on excitatory synaptic transmission in CA1 pyramidal cells of the guinea pig hippocampal slice. *Neurosci. Res.* **10**, 200–210.
- SAKmann, B. (1992) Nobel Lecture. Elementary steps in synaptic transmission revealed by currents through single ion channels. *Neuron* **8**, 613–629.
- SAKmann, B., HAMILL, O. P. and BORMANN, J. (1983) Patch clamp measurements of elementary chloride currents activated by the putative inhibitory transmitters GABA and glycine in mammalian spinal neurons. *J. neural. Trans.* **18**, 83–95.
- SAKmann, B., EDWARDS, F., KONNERTH, A. and TAKAHASHI, T. (1989) Patch clamp techniques used for studying synaptic transmission in slices of mammalian brain. *Q. Jl exp. Physiol.* **74**, 1107–1118.
- SATTELLE, D. B., PINNOCK, R. D., WAFFORD, K. A. and DAVID, J. A. (1988) GABA receptors on the cell-body membrane of an identified insect motor neuron. *Proc. R. Soc. Lond. B.* **232**, 443–456.
- SCAPPATICCI, K. A., DRETCHEN, K. L., CARPENTER, D. O. and PELL-MAR, T. C. (1981) Effects of furosemide on neural mechanisms in *Aplysia*. *J. Neurobiol.* **12**, 329–341.
- SCHARFMAN, H. E. and SARVEY, J. M. (1985) Responses to  $\gamma$ -aminobutyric acid applied to cell bodies and dendrites of rat visual cortical neurons. *Brain Res.* **358**, 385–389.
- SCHLUE, W. R., DÖRNER, R., REMPE, L., RIEHL, B. (1991) Glial H<sup>+</sup> transport and control of pH. *Ann. N. Y. Acad. Sci.* **633**, 287–305.
- SCHNEGGENBURGER, R. and KONNERTH, A. (1992) GABA-mediated synaptic transmission in neuroendocrine cells: A patch-clamp study in pituitary slice preparation. *Pflügers Arch.* **421**, 364–373.
- SCHOFIELD, P. R., DARLISON, M. G., FUJITA, N., BURT, D. R., STEPHENSON, F. A., RODRIGUEZ, H., RHEE, L. M., RAMACHANDRAN, J., REALE, V., GLENVERSE, T. A., SEEBURG, P. H. and BARNARD, E. A. (1987) Sequence and functional expression of the GABA<sub>A</sub> receptor shows a ligand-gated receptor super-family. *Nature* **328**, 221–227.
- SCHWARTZKROIN, P. A. (1983) Local circuit considerations and intrinsic neuronal properties involved in hyperexcitability and cell synchronization. In: *Basic Mechanisms of Neuronal Hyperexcitability*, pp. 75–108. Alan R. Liss: New York.
- SCHWIENING, C. J. and THOMAS, R. C. (1992) Mechanism of pH regulation by locust neurones in isolated ganglia: A microelectrode study. *J. Physiol., Lond.* **447**, 693–709.
- SCOTT, R. H. and DUCE, I. R. (1986) Anion selectivity of  $\gamma$ -aminobutyric acid (GABA) and 22,23-dihydroavermectin B<sub>1a</sub> (DHAVM)-induced conductance changes on locust muscle. *Neurosci. Lett.* **68**, 197–201.
- SCOTT, R. H., MCGUIRK, S. M. and DOLPHIN, A. C. (1988) Modulation of divalent cation-activated chloride ion currents. *Br. J. Pharmac.* **94**, 653–662.
- SECHENOV, I. M. (1863) *Physiologische Studien über die Hemmungsmechanismen für die Reflexfähigkeit des Rückenmarks im Gehirne des Frosches*. Hirschwald: Berlin.
- SEGAL, M. (1983) Rat hippocampal neurones in culture: responses to electrical and chemical stimuli. *J. Neurophysiol.* **50**, 1249–1264.
- SEGAL, M. and BARKER, J. L. (1984a) Rat hippocampal neurones in culture: Properties of GABA-activated Cl<sup>-</sup> ion conductance. *J. Neurophysiol.* **51**, 500–515.
- SEGAL, M. and BARKER, J. L. (1984b) Rat hippocampal neurones in culture: voltage-clamp analysis of inhibitory synaptic connections. *J. Neurophysiol.* **52**, 469–487.
- SHARP, A. P. and THOMAS, R. C. (1981) The effects of chloride substitution on intracellular pH in crab muscle. *J. Physiol., Lond.* **312**, 71–80.
- SHERRINGTON, C. S. (1906) *The Integrative Action of the Nervous System*. Cambridge University Press.
- SHERRINGTON, C. S. (1932) Inhibition as a co-ordinative factor. In: *Les Prix Nobel en 1932*. P. A. Nordstedt and Soner: Stockholm.
- SHIMADA, S., CUTTING, G. and UHL, G. R. (1992)  $\gamma$ -Aminobutyric acid A or C receptor?  $\gamma$ -Aminobutyric acid  $\rho_1$  receptor RNA induced bicuculline-, barbiturate- and benzodiazepine-insensitive  $\gamma$ -aminobutyric acid responses in *Xenopus* oocytes. *Molec. Pharmac.* **41**, 683–687.
- SHIROYA, T., FUKUNAGA, R., AKASHI, K., SHIMADA, N., TAKAGI, Y., NISHINO, T., HARA, M. and INAGAKI, C. (1989) An ATP-driven Cl<sup>-</sup> pump in the brain. *J. biol. Chem.* **264**, 17416–17421.
- SIESJÖ, B. K. (1988) Acidosis and ischemic brain damage. *Neurochem. Path.* **9**, 31–88.
- SIESJÖ, B. K. and BENGTTSSON, F. (1989) Calcium fluxes, calcium antagonists and calcium-related pathology in brain ischemia, hypoglycemia and spreading depression: a unifying hypothesis. *J. cereb. Blood Flow Metab.* **9**, 127–140.
- SIGEL, E., BAUR, R., TRUBE, G., MÖHLER, H. and MALHERBE, P. (1990) The effect of subunit composition of rat brain GABA<sub>A</sub> receptors on channel function. *Neuron* **5**, 703–711.
- SIMMONDS, M. A. (1983) Multiple GABA receptors and associated regulatory sites. *Trends Neurosci.* **6**, 279–281.
- SIMMONDS, M. A. and TURNER, J. P. (1987) Potentiators of responses to activation of  $\gamma$ -aminobutyric acid (GABA<sub>A</sub>) receptors. *Neuropharmacology* **26**, 923–930.
- SIVIOLITTI, L. and NISTRÌ, A. (1988) Complex effects of baclofen on synaptic transmission of the frog optic tectum *in vitro*. *Neurosci. Lett.* **85**, 249–254.
- SIVIOLITTI, L. and NISTRÌ, A. (1989) Pharmacology of a novel effect of  $\gamma$ -aminobutyric acid on the frog optic tectum *in vitro*. *Eur. J. Pharmac.* **164**, 205–212.
- SIVIOLITTI, L. and NISTRÌ, A. (1991) GABA receptor mechanisms in the central nervous system. *Prog. Neurobiol.* **36**, 35–92.
- SKREDE, K. K. and WESTGAARD, R. H. (1971) The transverse hippocampal slice: A well-defined cortical structure maintained *in vitro*. *Brain Res.* **35**, 589–593.
- SMART, T. G. (1990) Uncultured lobster muscle, cultured neurones and brain slices: the neurophysiology of zinc. *J. Pharmacy Pharmac.* **42**, 377–387.
- SMART, T. G. (1992) A novel modulatory binding site for zinc on the GABA<sub>A</sub> receptor complex in cultured rat neurones. *J. Physiol., Lond.* **447**, 587–625.
- SMART, T. G. and CONSTANTI, A. (1982) A novel effect of zinc on the lobster muscle GABA receptor. *Proc. R. Soc. Lond. B* **215**, 327–341.
- SMART, T. G. and CONSTANTI, A. (1983) Pre- and postsynaptic effects of zinc on *in vitro* prepyriform neurones. *Neurosci. Lett.* **40**, 205–211.
- SMART, T. G. and CONSTANTI, A. (1990) Differential effect of zinc on

- the vertebrate GABA<sub>A</sub>-receptor complex. *Br. J. Pharmac.* **99**, 643–654.
- SMART, T. G., MOSS, S. J., XIE, X. and HUGANIR, R. L. (1991) GABA<sub>A</sub> receptors are differentially sensitive to zinc: Dependence on subunit composition. *Br. J. Pharmac.* **103**, 1837–1839.
- SMITH, S. M., ZOREC, R. and MCBURNEY, R. N. (1989) Conductance states activated by glycine and GABA in rat cultured spinal neurones. *J. membr. Biol.* **108**, 45–52.
- SOMJEN, G. G. (1979) Extracellular potassium in the mammalian central nervous system. *A. Rev. Physiol.* **41**, 159–177.
- SOMOGYI, P. (1990) Synaptic organization of GABAergic neurons and GABA<sub>A</sub> receptors in the lateral geniculate nucleus and the visual cortex. In: *Neural Mechanisms of Visual Perception*, pp. 35–62. Eds. D. M. LAM and C. D. GILBERT. Gulf Publishing: Houston.
- SORENSEN, R. L., GARRY, D. G. and BRELJE, T. C. (1991) Structural and functional considerations of GABA in islets of Langerhans. *Diabetes* **40**, 1365–1374.
- SORIMACHI, M., YAMAGAMI, K., MORITA, Y. and KURAMOTO, K. (1991) GABA inhibits the rise in cytosolic free calcium concentration in depolarized immature cerebellar Purkinje cells. *Neurosci. Lett.* **122**, 229–232.
- STALEY, K. J. and MODY, I. (1992) Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABA<sub>A</sub> receptor-mediated postsynaptic conductance. *J. Neurophysiol.* **68**, 197–212.
- STEIN, W. (1986) *Transport and Diffusion across Cell Membranes*. Academic Press: San Diego.
- STELZER, A., SLATER, N. T. and TEN BRUGGENCATE, G. (1987) Activation of NMDA receptors blocks GABAergic inhibition in an *in vitro* model of epilepsy. *Nature* **326**, 698–701.
- STELZER, A., KAY, A. R. and WONG, R. K. S. (1988) GABA<sub>A</sub>-receptor function in hippocampal cells is maintained by phosphorylation factors. *Science* **241**, 339–341.
- STEPHENSON, F. A. (1988) Understanding the GABA<sub>A</sub> receptor: a chemically gated ion channel. *Biochem. J.* **249**, 21–32.
- STUDY, R. E. and BARKER, J. L. (1981) Diazepam and (–) pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of  $\gamma$ -aminobutyric acid responses in cultured central neurons. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7180–7184.
- SWANN, J. W., BRADY, R. J. and MARTIN, D. L. (1989) Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. *Neuroscience* **28**, 551–561.
- SYKOVÁ, E. (1983) Extracellular K<sup>+</sup> accumulation in the central nervous system. *Prog. Biophys. molec. Biol.* **42**, 135–189.
- SYKOVÁ, E., JENDELOVÁ, P., SIMONOVÁ, Z. and CHVATAL, A. (1992) K<sup>+</sup> and pH homeostasis in the developing rat spinal cord is impaired by early postnatal X-irradiation. *Brain Res.* **594**, 19–30.
- SZATKOWSKI, M. S. and THOMAS, R. C. (1989) The intrinsic intracellular H<sup>+</sup> buffering power of snail neurones. *J. Physiol., Lond.* **409**, 89–101.
- TAIRA, T., SMIRNOV, S., VOIPIO, J. and KAILA, K. (1993) Intrinsic proton modulation of excitatory transmission in rat hippocampal slices. *NeuroReport* **4**, 93–96.
- TAKEUCHI, A. (1977) Junctional transmission. I. Postsynaptic mechanisms. In: *Handbook of Physiology, Section 1: The Nervous System*, Vol. I, pp. 295–327. Ed. E. R. KANDEL. American Physiological Society: Bethesda, MD.
- TAKEUCHI, A. and ONODERA, K. (1972) Effect of bicuculline on the GABA receptor of the crayfish neuromuscular junction. *Nature New Biol.* **236**, 55–56.
- TAKEUCHI, A. and TAKEUCHI, N. (1965) Localized action of  $\gamma$ -aminobutyric acid on the crayfish muscle. *J. Physiol., Lond.* **177**, 225–238.
- TAKEUCHI, A. and TAKEUCHI, N. (1966a) On the permeability of the presynaptic terminal of the crayfish neuromuscular junction during synaptic inhibition and the action of  $\gamma$ -aminobutyric acid. *J. Physiol., Lond.* **183**, 433–449.
- TAKEUCHI, A. and TAKEUCHI, N. (1966b) A study of the inhibitory action of  $\gamma$ -aminobutyric acid on neuromuscular transmission in the crayfish. *J. Physiol.* **183**, 418–432.
- TAKEUCHI, A. and TAKEUCHI, N. (1967) Anion permeability of the inhibitory post-synaptic membrane of the crayfish neuromuscular junction. *J. Physiol., Lond.* **191**, 575–590.
- TAKEUCHI, A. and TAKEUCHI, N. (1969) A study of the action of picrotoxin on the inhibitory neuromuscular junction of the crayfish. *J. Physiol., Lond.* **205**, 377–391.
- TAKEUCHI, A. and TAKEUCHI, N. (1971) Anion interaction at the inhibitory post-synaptic membrane of the crayfish neuromuscular junction. *J. Physiol., Lond.* **212**, 337–351.
- TALEB, O., TROUSIARD, J., DEMENEIX, B. A., FELTZ, P., BOSSU, J. L., DUPONT, J. L. and FELTZ, A. (1987) Spontaneous and GABA evoked chloride channels on pituitary intermediate lobe cells and their internal Ca requirements. *Pflügers Arch.* **409**, 620–631.
- TANG, C.-M., DICHTER, M. and MORAD, M. (1990) Modulation of the N-methyl-D-aspartate channel by extracellular H<sup>+</sup>. *Proc. natn. Acad. Sci. U.S.A.* **87**, 6445–6449.
- TAUCK, D. L., FROSCH, M. P. and LIPTON, S. A. (1988) Characterization of GABA- and glycine-induced currents of solitary rodent retinal ganglion cells in culture. *Neuroscience* **27**, 193–203.
- TEHRANI, M. H., HABLITZ, J. J. and BARNES, E. M., JR (1989) cAMP increases the rate of GABA<sub>A</sub> receptor desensitization in chick cortical neurons. *Synapse* **4**, 126–131.
- THALMANN, R. H., PECK, E. J. and AYALA, G. F. (1981) Biphasic response of hippocampal pyramidal neurons to GABA. *Neurosci. Lett.* **21**, 319–324.
- THOMAS, R. C. (1976) The effect of carbon dioxide on the intracellular pH and buffering power of snail neurones. *J. Physiol., Lond.* **255**, 715–735.
- THOMAS, R. C. (1977) The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *J. Physiol., Lond.* **273**, 317–338.
- THOMAS, R. C. (1978) *Ion-Sensitive Intracellular Microelectrodes*. Academic Press: London.
- THOMAS, R. C. (1984) Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. *J. Physiol., Lond.* **354**, 3P–22P.
- THOMPSON, S. M. and GÄHWILER, B. (1989a) Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus *in vitro*. *J. Neurophysiol.* **61**, 501–511.
- THOMPSON, S. M. and GÄHWILER, B. (1989b) Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide and membrane potential on E<sub>Cl</sub><sup>-</sup> in hippocampal CA3 neurons. *J. Neurophysiol.* **61**, 512–523.
- THOMPSON, S. M. and GÄHWILER, B. (1989c) Activity-dependent disinhibition. III. Desensitization and GABA<sub>B</sub> receptor-mediated presynaptic inhibition in the hippocampus *in vitro*. *J. Neurophysiol.* **61**, 524–533.
- THOMPSON, S. M. and GÄHWILER, B. (1992) Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. *J. Neurophysiol.* **67**, 1698–1701.
- THOMPSON, S. M., DEISZ, R. A. and PRINCE, D. A. (1988a) Relative contributions of passive equilibrium and active transport to the distribution of chloride in mammalian cortical neurons. *J. Neurophysiol.* **60**, 105–124.
- THOMPSON, S. M., DEISZ, R. A. and PRINCE, D. A. (1988b) Outward chloride/cation co-transport in mammalian cortical neurons. *Neurosci. Lett.* **89**, 49–54.
- THOMSON, A. M. (1988) Biphasic responses of thalamic neurons to GABA in isolated rat brain slices—II. *Neuroscience* **25**, 503–512.
- TICKU, M. K. and MEHTA, A. K. (1990)  $\gamma$ -Aminobutyric acid<sub>A</sub> receptor desensitization in mice spinal cord cultured neurons: Lack of involvement of protein kinases A and C. *Molec. Pharmac.* **38**, 719–724.
- TOLKOVSKY, A. M. and RICHARDS, C. D. (1987) Na<sup>+</sup>/H<sup>+</sup> exchange is the major mechanism of pH regulation in cultured sympathetic neurons: measurements in single cell bodies and neurites using a fluorescent pH indicator. *Neuroscience* **22**, 1093–1102.
- TRAYNELIS, S. F. and CULL-CANDY, S. G. (1991) Pharmacological properties and H<sup>+</sup> sensitivity of excitatory amino acid receptor channels in rat cerebellar granule neurones. *J. Physiol., Lond.* **433**, 727–763.
- TRUSSELL, L. O. and FISCHBACH, G. D. (1989) Glutamate receptor desensitization and its role in synaptic transmission. *Neuron* **3**, 209–218.
- TSIEN, R. Y. (1989) Fluorescent probes of cell signaling. *A. Rev. Neurosci.* **12**, 227–253.
- TWYMAN, R. E. and MACDONALD, R. L. (1992) Neurosteroid regulation of GABA<sub>A</sub> receptor single-channel kinetic properties of mouse spinal cord neurones in culture. *J. Physiol., Lond.* **456**, 215–245.
- TWYMAN, R. E., ROGERS, C. J. and MACDONALD, R. L. (1990) Intra-burst kinetic properties of the GABA<sub>A</sub> receptor main conductance

- state of mouse spinal cord neurons in culture. *J. Physiol., Lond.* **423**, 193–220.
- UNSELD, E., FISCHER, C., ROTHMUND, E. and KLOTZ, U. (1990) Occurrence of "natural" diazepam in human brain. *Biochem. Pharmac.* **39**, 210–212.
- UNWIN, N. (1989) The structure of ion channels in membranes of excitable cells. *Neuron* **3**, 665–676.
- URBANICS, R., LENIGER-FOLLERT, E. and LÜBBERS, D. W. (1978) Time course of changes of extracellular H<sup>+</sup> and K<sup>+</sup> activities during and after direct electrical stimulation of the brain cortex. *Pflügers Arch.* **378**, 47–53.
- VERDOORN, T. A., DRAGUHN, A., YMER, S., SEEBURG, P. H. and SAKMANN, B. (1990) Functional properties of recombinant rat GABA<sub>A</sub> receptors depend upon subunit composition. *Neuron* **4**, 919–928.
- VERKMAN, A. S., SELLERS, M. C., CHAO, A. C., LEUNG, T. and KETCHAM, R. (1989) Synthesis and characterization of improved chloride-sensitive fluorescent indicators for biological applications. *Anal. Biochem.* **178**, 355–361.
- VICINI, S., MIENVILLE, J. M. and COSTA, E. (1988) Chloride channel modulation through the benzodiazepine recognition site. *Adv. Biochem. Psychopharmac.* **45**, 87–94.
- VOIPIO, J. and KAILA, K. (1992) Interstitial CO<sub>2</sub> and pH transients in rat hippocampal slices measured by means of a novel CO<sub>2</sub>/H<sup>+</sup>-sensitive microelectrode based on a PVC-gelled membrane. *Pflügers Arch.* **423**, 193–201.
- VOIPIO, J., PASTERNAK, M., RYDQVIST, B. and KAILA, K. (1991) Effect of  $\gamma$ -aminobutyric acid on intracellular pH in the crayfish stretch-receptor neurone. *J. exp. Biol.* **156**, 349–361.
- VON BLANKENFELD, G. and KETTENMANN, H. (1991) Glutamate and GABA receptors in vertebrate glial cells. *Molec. Neurobiol.* **5**, 31–43.
- VON BLANKENFELD, G., YMER, S., PRITCHETT, D. B., SONTHEIMER, H., EWERT, M., SEEBURG, P. H. and KETTENMANN, H. (1990) Differential benzodiazepine pharmacology of mammalian recombinant GABA<sub>A</sub> receptors. *Neurosci. Lett.* **115**, 269–273.
- WALKER, R. J. and HOLDEN-DYE, L. (1989) Commentary on the evolution of transmitters, receptors and ion channels in invertebrates. *Comp. Biochem. Physiol.* **93A**, 25–39.
- WEISS, D. S. (1988) Membrane potential modulates the activation of GABA-gated channels. *J. Neurophysiol.* **59**, 514–527.
- WEISS, D. S. and MAGLEBY, K. L. (1989) Gating scheme for single GABA-activated Cl<sup>-</sup> channels determined from stability plots, dwell-time distributions and adjacent-interval durations. *J. Neurosci.* **9**, 1314–1324.
- WEISS, D. S., BARNES, E. M., JR and HABLITZ, J. J. (1988) Whole-cell and single-channel recordings of GABA-gated currents in cultured chick cerebral neurons. *J. Neurophysiol.* **59**, 495–513.
- WESTBROOK, G. L. and MAYER, M. L. (1987) Micromolar concentrations of Zn<sup>2+</sup> antagonize NMDA and GABA responses of hippocampal neurons. *Nature* **328**, 640–643.
- WIELAND, H. A., LÜDDENS, H. and SEEBURG, P. H. (1992) A single histidine in GABA<sub>A</sub> receptors is essential for benzodiazepine agonist binding. *J. biol. Chem.* **267**, 1426–1429.
- WIERSMA, C. A. G. (1961) Inhibitory neurons: A survey of the history of their discovery and of their occurrence. In: *Nervous Inhibition*, pp. 1–7. Ed. E. FLOREY, Pergamon: Oxford.
- WIETASCH, K. and KRAIG, R. P. (1991) Carbonic acid buffer species measured in real time with an intracellular microelectrode array. *Am. J. Physiol.* **261**, R760–R765.
- WIGSTRÖM, H. and GUSTAFSSON, B. (1983) Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. *Nature* **270**, 356–357.
- WILLIAMS, P. J., MACVICAR, B. A. and PITTMAN, Q. J. (1989) Identification of a GABA-activated chloride-mediated synaptic potential in rat pars intermedia. *Brain Res.* **483**, 130–134.
- WISDEN, W. and SEEBURG, P. H. (1992) GABA<sub>A</sub> receptor channels: From subunits to functional entities. *Curr. Opin. Neurobiol.* **2**, 263–269.
- WISDEN, W., MORRIS, B. J., DARLISON, M. G., HUNT, S. P. and BARNARD, E. A. (1988) Distinct GABA<sub>A</sub> receptor  $\alpha$  subunit mRNAs show differential patterns of expression in bovine brain. *Neuron* **1**, 937–947.
- WOJTOWICZ, J. M. and NICOLL, R. A. (1982) Selective action of piretanide on primary afferent GABA responses in the frog spinal cord. *Brain Res.* **236**, 173–181.
- WONG, R. K. S. and WATKINS, D. J. (1982) Cellular factors influencing GABA response in hippocampal pyramidal cells. *J. Neurophysiol.* **48**, 938–951.
- WOODWARD, R. M., POLENZANI, L. and MILEDI, R. (1992) Characterization of bicuculline/baclofen-insensitive  $\gamma$ -aminobutyric acid receptors expressed in *Xenopus* oocytes. I. Effects of Cl<sup>-</sup> channel inhibitors. *Molec. Pharmac.* **42**, 165–173.
- WRIGHT, D. M. (1984) Zinc: Effect and interaction with other cations in the cortex of the rat. *Brain Res.* **311**, 343–347.
- WRIGHT, E. M. and DIAMOND, J. M. (1977) Anion selectivity in biological systems. *Physiol. Rev.* **57**, 109–156.
- WU, W., ZISKIND-CONHAIM, L. and SWEET, M. A. (1992) Early development of glycine- and GABA-mediated synapses in rat spinal cord. *J. Neurosci.* **12**, 3935–3945.
- WUTTKE, W. A. and WALZ, W. (1990) Sodium- and bicarbonate-independent regulation of intracellular pH in cultured mouse astrocytes. *Neurosci. Lett.* **117**, 105–110.
- XIE, X. and SMART, T. G. (1991) A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. *Nature* **349**, 521–524.
- YAKUSHIJI, T., FUKUDA, T., OYAMA, Y. and AKAIKE, N. (1989a) Effects of benzodiazepines and non-benzodiazepine compounds on the GABA-induced response in frog isolated sensory neurons. *Br. J. Pharmac.* **98**, 735–740.
- YAKUSHIJI, T., TOKUTOMI, N. and AKAIKE, N. (1989b) Augmentation of GABA-induced chloride current in frog sensory neurones by diazepam. *Neurosci. Res.* **6**, 309–320.
- YASUI, S., ISHIZUKA, S. and AKAIKE, N. (1985) GABA activates different types of chloride-conducting receptor-ionophore complexes in a dose-dependent manner. *Brain Res.* **344**, 176–180.
- YUSTE, R. and KATZ, L. C. (1991) Control of postsynaptic Ca<sup>2+</sup> influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron* **6**, 333–344.
- ZAMAN, S. H., HARVEY, R. J., BARNARD, E. A. and DARLISON, M. G. (1992) Unusual effects of benzodiazepines and cyclodiene insecticides on an expressed invertebrate GABA<sub>A</sub> receptor. *FEBS Lett.* **307**, 351–354.
- ZUFALL, F., FRANKE, C. and HATT, H. (1988) Acetylcholine activates a chloride channel as well as glutamate and GABA. *J. comp. Physiol. A* **163**, 609–620.