

CHAPTER

18

GABA

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INTRODUCTION

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). Roberts and Awapara discovered it in 1950. Electrophysiological studies between 1950 and 1965 suggested a role for GABA as a neurotransmitter in the mammalian CNS. Since then, GABA has met the five classical criteria for assignment as a neurotransmitter: it is present in the nerve terminal, it is released from electrically stimulated neurons, there is a mechanism for terminating the action of the released neurotransmitter, its application to target neurons mimics the action of inhibitory nerve stimulation, and specific receptors exist (Olsen & Betz, 2006; Martin & Olsen, 2000).

In view of the ubiquitous nature of GABA in the CNS, it is perhaps not too surprising that its functional significance should be far reaching. A growing body of evidence suggests a role for

altered GABAergic function in neurological and psychiatric disorders of humans, primarily related to hyperexcitability. These include developmental malfunctions, mental retardation, and epilepsy; sleep disorders; drug dependence, especially alcoholism; sensorimotor processing, including various types of learning as well as schizophrenia; and motor coordination disorders like tardive dyskinesia, Huntington's disease, and Parkinson's disease. Pharmacological manipulation of GABAergic transmission, e.g., with benzodiazepines, is an effective approach for the treatment of anxiety (Olsen, 2001; Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Rudolph et al., 2001). In addition, it has been demonstrated that the nervous system depressant actions of the major clinical general anesthetics, both intravenous and volatile, as well as alcohol, very likely result from an enhancement of inhibitory synaptic transmission mediated by GABA_A receptors (Jurd et al., 2003; Yamakura et al., 2001; Li et al., 2006; Wallner et al., 2006).

GABA SYNTHESIS, RELEASE AND UPTAKE

GABA is formed *in vivo* by a metabolic pathway referred to as the GABA shunt

The GABA shunt is a closed-loop process with the dual purpose of producing and conserving the supply of GABA. GABA is present in high concentrations (millimolar) in many brain regions. These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions. This is in accord with the powerful, abundant and specific actions of GABAergic neurons in these regions. Glucose is the principal precursor for GABA production *in vivo*, although pyruvate and other amino acids also can act as precursors. The first step in the GABA shunt is the transamination of α -ketoglutarate, formed from glucose metabolism in the Krebs cycle by GABA α -ketoglutarate transaminase (GABA-T), into L-glutamic acid (Olsen & Betz, 2006; Martin & Olsen, 2000) (Fig. 18-1). Glutamic acid decarboxylase (GAD) catalyzes the decarboxylation of L-glutamic acid to form GABA. GAD appears to be expressed only in cells that use GABA as a neurotransmitter. GAD, localized with antibodies or mRNA hybridization probes, serves as an excellent marker for GABAergic neurons in the CNS. Two related but different genes for GAD have been cloned, suggesting independent regulation and properties for the two forms of GAD: GAD₆₅ and GAD₆₇ (Martin & Olsen, 2000). GAD₆₅ is localized to nerve endings and synthesis of GABA for secretory synaptic vesicles; mouse knockouts for this isozyme show seizure susceptibility and impaired experience-modulated plasticity (Hensch et al., 1998).

The newly synthesized GABA is then packaged into synaptic vesicles by aid of a vesicular transporter. These proteins, with 10 membrane-spanning regions, are involved in active transport of GABA from the cytosol into secretory vesicles inside the cell, a mechanism energized by an ATP-dependent proton electrochemical gradient, independent of Na⁺ (Martin & Olsen, 2000; Gasnier, 2000) (see also Ch. 3).

GABA release into the synaptic cleft is stimulated by depolarization of presynaptic neurons. GABA diffuses across the cleft to the target receptors on the postsynaptic surface. The action of GABA at the synapse is terminated by reuptake into both presynaptic nerve terminals and surrounding glial cells (Chen et al., 2004; Madsen et al., 2009). The plasmalemma transport systems mediating reuptake of GABA are both temperature and ion dependent. These transporter molecules are capable of bidirectional neurotransmitter transport. They have an absolute requirement for extracellular Na⁺ ions and an additional dependence on Cl⁻ ions (see also Ch. 3). The ability of the reuptake system to transport GABA against a concentration gradient has been demonstrated using synaptosomes. Under normal physiological conditions, the ratio of internal to external GABA is about 200. The driving force for this reuptake process is supplied by the movement of Na⁺ down its concentration gradient (Martin & Olsen, 2000; Chen et al., 2004). GABA taken back up into nerve terminals is available for reutilization, but GABA in glia, as in neurons, is metabolized to succinic semialdehyde by GABA: α -ketoglutarate transaminase (GABA-T) which in the same reaction converts

α -ketoglutarate to glutamate. But, GABA cannot be resynthesized from glutamate in this compartment since glia lack GAD. Ultimately, GABA can be recovered from this source by a circuitous route involving the Krebs cycle (Olsen & Betz, 2006); succinic semialdehyde formed from GABA in glia enters the Krebs cycle leading to the formation of glutamine, which is transferred back to the neuron, where glutamine is converted by glutaminase to glutamate and the latter is decarboxylated to GABA (see Ch. 17).

The family of GABA plasmalemma transporters is a set of 80-kDa glycoproteins with 12 transmembrane regions; they have no sequence homology with GABA receptors, but helped to establish the superfamily of sodium-dependent neurotransmitter transporters. Pharmacological and kinetic studies have suggested a variety of subtypes, and four separate but related entities have been demonstrated by molecular cloning (Chen et al., 2004; Madsen et al., 2009). This has led to rapid developments in understanding the localization, pharmacological specificity, structure-function and mechanism of GABA transport across the cell membrane.

GABA is metabolized by GABA-T to form succinic semialdehyde. To conserve the available supply of GABA, this transamination generally occurs when the initial parent compound, α -ketoglutarate, is present to accept the amino group removed from GABA, reforming glutamic acid (see also Ch. 17). Therefore, a molecule of GABA can be metabolized only if a molecule of precursor is formed. Succinic semialdehyde can be oxidized by succinic semialdehyde dehydrogenase (SSADH) into succinic acid and can then re-enter the Krebs cycle, completing the loop (Fig. 18-1 (Olsen & Betz, 2006)).

GAD₆₇ appears to be associated with synthesis of non-vesicular GABA; GABA in this pool can be released from cells by reversal of the plasma membrane transport process (Martin & Olsen, 2000). Furthermore, expression of GAD and some GABA receptor subunits has been demonstrated in some non-neuronal tissues, indicating the likely function of GABA outside of the CNS. A mouse knockout of GAD₆₇ shows normal brain activity but cleft palate (Martin & Olsen, 2000).

GABA RECEPTOR PHYSIOLOGY AND PHARMACOLOGY

GABA receptors have been identified electrophysiologically and pharmacologically in all regions of the brain

Because GABA is widely distributed and utilized throughout the CNS, early GABAergic drugs had very generalized effects on CNS function. The development of more selective agents has led to the identification of at least two distinct classes of GABA receptor, GABA_A and GABA_B. They differ in their pharmacological, electrophysiological, and biochemical properties. Like the receptors for acetylcholine and glutamate, receptors for GABA employ both ligand-gated ion channels and G protein-coupled receptors (GPCR, discussed in Ch. 12). Electrophysiological studies of the GABA_A-receptor complex indicate that it mediates an increase in membrane conductance with an equilibrium

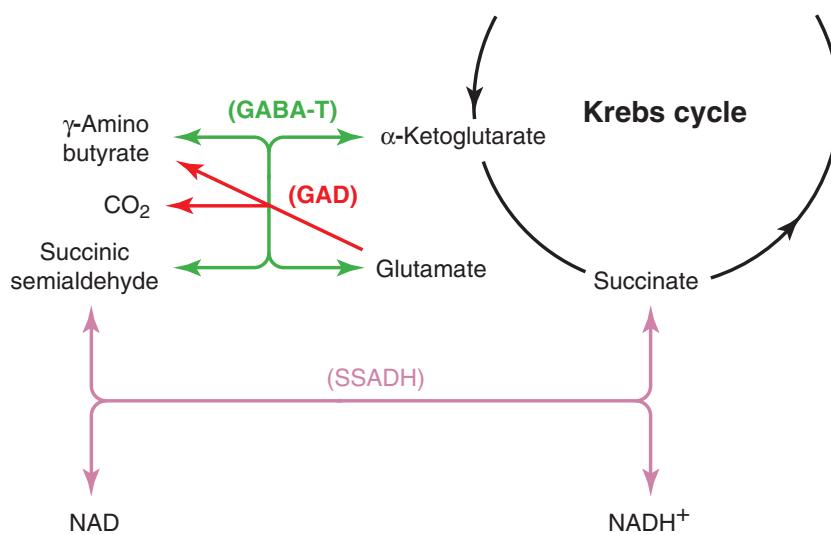


FIGURE 18-1 Reactions of the GABA shunt that are responsible for the synthesis, conservation, and metabolism of GABA. GABA-T, GABA α -ketoglutarate transaminase; GAD, glutamic acid decarboxylase; SSADH, succinic semialdehyde dehydrogenase.

potential (E_{GABA}) near the resting level of -70mV . This conductance increase often is accompanied by a membrane hyperpolarization, resulting in an increase in the firing threshold and, consequently, a reduction in the probability of action potential initiation, causing neuronal inhibition. This reduction in membrane resistance is accomplished by the GABA-dependent facilitation of Cl^- ion influx through the channel, which is an integral part of the pentameric GABA_A receptor (Olsen & MacDonald, 2002). Reduction in excitatory potentials also can result from shunting Cl^- currents mediated by GABA_A receptors with or without hyperpolarization. Some GABA_A receptors are located on nerve terminals where their activation inhibits release of neurotransmitter, be it excitatory or inhibitory (Olsen & Betz, 2006; Martin & Olsen, 2000). On the other hand, increased Cl^- permeability can depolarize the target cell under some conditions of high intracellular Cl^- . This in turn potentially can excite the cell to fire or to activate Ca^{2+} entry via voltage-gated channels and has been proposed as a physiologically relevant event, especially in embryonic neurons (Olsen 2001; Olsen & MacDonald, 2002; Ben-Ari et al., 2007; Blaesse et al., 2009) (see also Ch. 4). Thus the E_{GABA} is a physiologically important entity, which turns out to depend on the levels and activity of various Cl^- pumps in the cell (Ben-Ari et al., 2007). Furthermore, in addition to the well-known age-dependence of E_{GABA} , it can be altered by plastic changes in Cl^- pumps under pathological conditions including epilepsy (Blaesse et al., 2009).

Electrophysiological data suggest that there are two GABA recognition sites per GABA_A receptor complex (Olsen & MacDonald, 2002). An increase in the concentration of GABA results in an increase in the mean channel open time due to opening of doubly liganded receptor forms, which exhibit open states of long duration. It has been demonstrated that the increase in the ionic permeability of the GABA_A receptor complex is transient in the continuing presence of agonist (Olsen & MacDonald, 2002). This phenomenon is known as desensitization and is rapidly reversible. The molecular mechanism of desensitization is not understood, and various hypotheses remain under investigation.

STRUCTURE AND FUNCTION OF GABA RECEPTORS

GABA_B receptors are coupled to G proteins and a variety of effectors

GABA_B receptors were identified by their insensitivity to the GABA_A antagonist bicuculline and certain GABA_A -specific agonists (Martin & Olsen, 2000; Olsen, 2001; Barnard et al., 1998; Bowery et al., 2002). The GABA analog (–) baclofen (β -(4-chloro-phenyl)- γ -aminobutyric acid) was found to be a potent and selective GABA_B agonist. GABA_B receptors (Bowery et al., 2002; Pinard et al., 2010) are members of the Group III G protein-coupled receptors (Ch. 12), with structural similarity to the metabotropic glutamate receptors.

GABA_B receptors can mediate both postsynaptic and presynaptic inhibition through coupling to a variety of effector systems (Bowery et al., 2002; Pinard et al., 2010). Some GABA_B receptors are coupled to activation of certain K^+ channels, producing slow inhibitory synaptic currents. Other GABA_B receptors can decrease Ca^{2+} conductance, regulate inositol trisphosphate production (Ch. 23), and/or inhibit cAMP production (Ch. 22). Presynaptic inhibition may occur as a result of GABA_B receptors on nerve terminals causing a decrease in the influx of Ca^{2+} , thereby reducing the release of neurotransmitters (Ch. 12). These responses may have a net excitatory effect when inhibiting GABA nerve ending release (auto-receptors), or may inhibit the release of other neurotransmitters including glutamate, having a net inhibitory effect.

GABA_B receptors are heterodimers

Two GABA_B receptor subunits have been cloned, R1 and R2. Neither of these appears to express functional receptors on its own, but both are active when co-expressed, suggesting that a dimer is trafficked to the cell surface and forms an active complex. Evidence shows that the R1 subunit contains the GABA binding site, while the R2 subunit interacts with the G protein (Bowery et al., 2002). In addition, a set of four

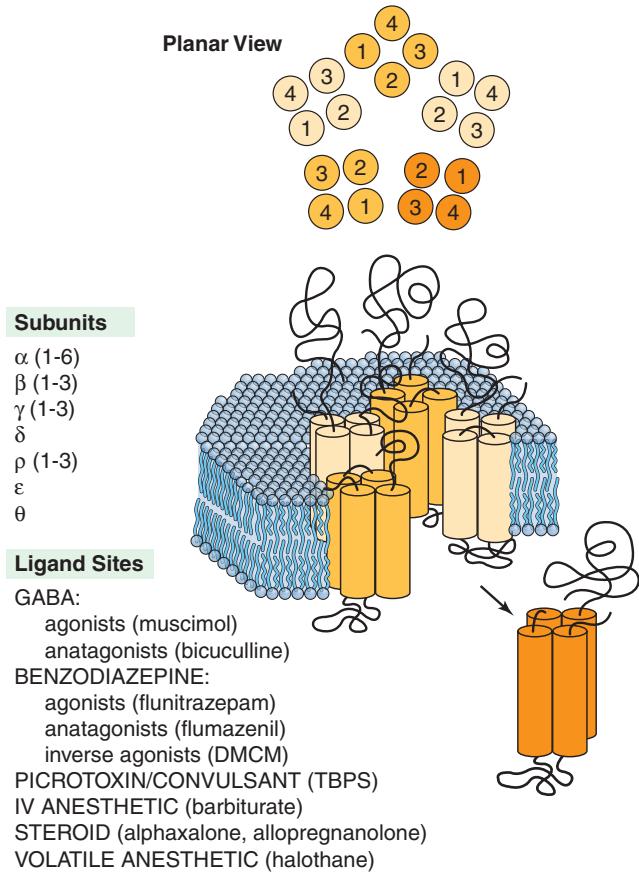


FIGURE 18-2 Schematic of GABA_A receptors. The protein is shown as a pseudo-symmetric membrane-spanning ion channel protein made of five homologous subunits, each of which has four membrane-spanning regions, as shown in the pull-out subunit. The view from outside the cell (planar view) shows the arrangement around the central core, the chloride ion channel. Also indicated are the subunit families that can be utilized in composing each receptor, and the ligand binding sites present on the receptor.

associated subunits have been identified which account for heterogeneity in GABA_B receptor localization, functional coupling, and pharmacology (Pinard et al., 2010).

GABA_A receptors are chloride channels and members of a superfamily of ligand-gated ion channel receptors

The GABA_A receptor is a pseudo-symmetric pentamer spanning the membrane and forming a chloride ion channel (Fig. 18-2). The binding of GABA or of structural analogues with agonist activity opens the chloride ion channel (Olsen, 2001; Barnard et al., 1998; Olsen & MacDonald, 2002). GABA_A receptors have structural and functional homology with certain other Cys-loop containing ligand-gated ion channel receptors, and their subunits have sequence homology, thus defining a gene superfamily. This family includes

the nicotinic acetylcholine receptor (Ch. 13), the strychnine-sensitive glycine receptor (see Box Ch12), and the serotonergic 5-HT₃ receptor (Ch. 15). The superfamily is also sometimes called the 'Cys-loop' family, to distinguish it from the ionotropic excitatory amino acid receptors or the 'pentameric ligand-gated ion channel' family (Barnard et al., 1998; Olsen & Sieghart, 2008).

Each GABA_A receptor subunit contains four putative α -helical membrane-spanning domains (M1-M4) with a predominantly hydrophobic character (Fig. 18-2). One or more membrane-spanning regions from each subunit, principally M2, form the walls of the channel pore (see also Ch. 13 for the nicotinic cholinergic receptor). The sequences of these transmembrane segments are highly conserved between the subunits of the GABA_A receptor as well as between members of the gene superfamily. The region between M3 and M4 contains a long, variable intracellular domain (Fig. 18-3). This contributes to the subtype specificity and may participate in intracellular regulatory mechanisms such as phosphorylation and interaction with other cellular constituents (Martin & Olsen, 2000; Olsen, 2001; Barnard et al., 1998; Olsen & MacDonald, 2002).

A family of pentameric GABA_A-receptor protein subtypes exists; these vary in their localization, and in virtually every property

The GABA_A receptor was first cloned using partial protein sequence, and verification of these cDNAs as GABA-receptor subunits was made by expression in *Xenopus* oocytes of GABA-activated channels (Martin & Olsen, 2000; Olsen, 2001; Barnard et al., 1998; Olsen & MacDonald, 2002). According to current understanding of the molecular structure of the GABA_A receptor-ionophore complex, it is a heteropentameric glycoprotein of about 275 kDa composed of combinations of at least 19 different but closely related polypeptide gene products (Fig. 18-4 (Simon et al., 2004)). The subunits are all ~55 kDa and have 20 to 30% sequence identity between classes and about 70% identity within a class: $\alpha 1 - 6$, $\beta 1 - 3$, $\gamma 1 - 3$, δ , ϵ , θ , π , and $\rho 1-3$ (Fig. 18-2 (Simon et al., 2004)). The mammalian ϵ and θ subunits probably correspond to $\gamma 4$ and $\beta 4$ identified in birds. This suggests that the genes probably evolved from a common ancestral sequence; the evolutionary relationships are shown in the dendrogram of Fig. 18-4 (Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Simon et al., 2004). In addition, splice variants exist for several of the subunits.

Differential distribution of GABA_A-receptor-subunit mRNAs and polypeptides in brain is consistent with data indicating regional variations in physiological function, pharmacology and biochemistry. It seems clear that different combinations with differing pharmacologic and ion channel properties are expressed in different neuronal populations, and even different membrane locations in a given cell. The subunit composition of native isoforms has been deduced by a combination of techniques: determining which polypeptides are present in a given cell, finding which ones can be isolated together as an oligomer by using subunit-specific antibodies, and analyzing which pharmacological properties can be reconstituted from recombinant subunits of known combinations (Olsen & Sieghart, 2008). Table 18-1

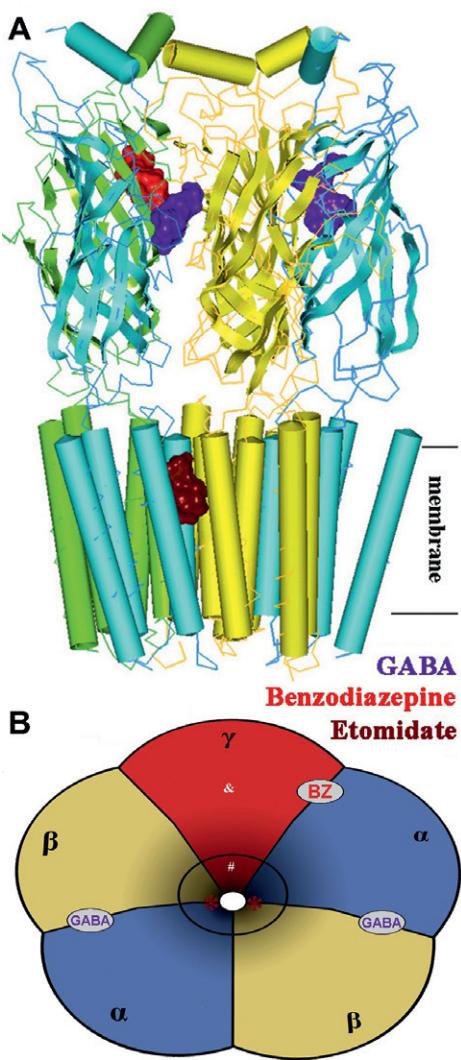


FIGURE 18-3 A. Views of the GABA_AR homology model (β_3 , cyan; α_1 , yellow; γ_2 , green) from perspectives parallel to the membrane surface. Pockets in the vicinity of the etomidate (maroon), GABA (purple), and benzodiazepine (red) binding sites are shown as Connolly surfaces. Modified from Li et al. (2006)(10). B. 3D-view 'Donut' model of GABA_A receptor heteropentamer with central pore and ligand binding sites at subunit interfaces. The 5 subunits ($-\beta - \gamma - \alpha - \beta - \alpha -$) and 5 subunit interfaces are shown, each subunit having an extracellular domain (&) and a transmembrane domain (#). GABA and benzodiazepine (BZ) sites are indicated at $\beta - \alpha$ and $\alpha - \gamma$ subunit interfaces, respectively. The etomidate sites (*) are located in the transmembrane domain at $\beta - \alpha$ subunit interfaces, the same subunit interface as the GABA binding site in the extracellular domain, some 50 Å away.

summarizes data on the most abundant isoforms identified, their localization and their unique pharmacological properties (Martin & Olsen, 2000; Olsen 2001; Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Olsen & MacDonald, 2002). The GABA_A ρ subunits are expressed primarily, if not exclusively, in the retina, where they appear to form Cl⁻ channels, possibly homomers, with novel pharmacology.

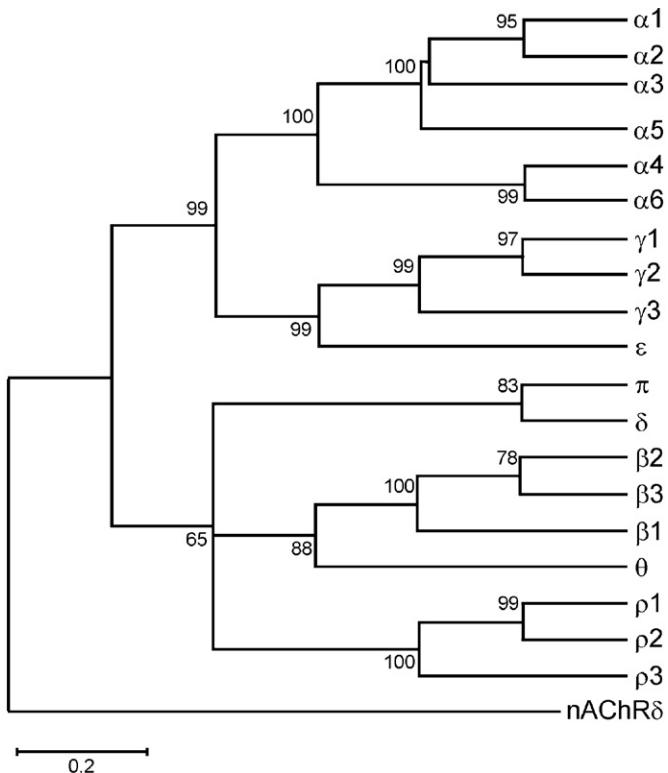


FIGURE 18-4 Dendrogram of the known 19 genes for human GABA_A receptors (Simon et al., 2004). The distances along each line are proportional to the degree of sequence identity between the different homologous subunits. The Greek letters signify subunit families of high (>70%) identity, with different Greek letter subunit families showing homology but lower sequence identity. The distances reflect the evolutionary times required to generate sufficient sequence divergence.

Pharmacological responses to GABA that are insensitive to both bicuculline and baclofen (non-A, non-B) are attributed by some authors to receptors termed GABA_C (Olsen & Sieghart, 2008; Abdel-Halim et al., 2008). Some, but not all, of these responses are produced by the ρ subunit, which is also insensitive to the usual GABA_A modulatory drugs like benzodiazepines and anesthetics (Martin & Olsen, 2000; Barnard et al., 1998; Olsen & Sieghart, 2008; Olsen & MacDonald, 2002; Abdel-Halim et al., 2008). However, the Nomenclature Committee of the International Union of Pharmacology has recommended against the nomenclature of GABA_C receptors, ruling that they are a subtype of GABA_A receptors (Barnard et al., 1998; Olsen & Sieghart, 2008).

Proteins associated with the intracellular loop of GABA_A receptor subunits participate in the trafficking of receptors from Golgi membranes to the plasmalemma, the concentrating of receptors at synaptic sites, and the production of changes in subcellular localization and density associated with plasticity (Hensch et al., 1998; Fritschy & Brunig, 2003). Such changes in plasticity include endocytotic removal of receptors from the plasmalemma following long-term ligand exposure (Moss & Smart, 2001) (see also Ch. 7). Proper direction of receptors to

TABLE 18-1 GABA_A Receptor Native Oligomer List (Olsen & Sieghart, 2008)

A. Identified		
α1β2γ2	α4βγ2	α6βγ2
α2βγ2	α4βδ	α6βδ
α3βγ2	α5βγ2	ρ
B. Existence with high probability		
α1β3γ2	α5β3γ2	αβ1γ/δ
α1βδ	α6β2δ	αβ
α4β2δ	α6β3δ	α1α6βγ/δ
α4β3δ		
C. Tentative		
ρ1	αβγ1	αβε
ρ2	αβγ3	αβπ
ρ3	αβθ	αxαγβγ2

specific cell surface loci and specific synapses requires the γ2 subunit and the microtubule linker-protein, GABARAP, which is, however, not a synaptic anchoring protein. The protein gephyrin, isolated with glycine receptors (Kneussel & Betz, 2000), co-localizes with synaptic GABA_A receptors and appears to have an anchoring function (Marchionni et al., 2009). The construction of synapses and remodeling during experience is currently an intensive area of research. Regulation of GABA_A receptor function depends critically on receptor subtype localization and trafficking in addition to protein levels determined by gene expression. Some subunits have been observed to be more ‘plastic’ than others. For example, in several cases where GABA_A receptors are down-regulated by overuse, they are rapidly replaced by α4 subunit-containing pentamers, which cells appear capable of synthesizing rapidly in times of need, plus the ability to traffic them to the locations where there is a deficit of inhibition (Olsen & Spigelman, 2011).

Other subunits may substitute for γ2 in the hetero-oligomeric pentamers, e.g., the δ subunit forms receptors that are excluded from synapses. Extrasynaptic receptors appear to play a functional role in mediating tonic inhibition and responding to spill-over of synaptically released transmitter, or even to ambient extracellular levels, depending on the location. They also appear to be the major target of action of certain modulatory drugs (Wallner et al., 2006; Mody & Pearce, 2004) (see below).

Phosphorylation sites for one or more kinases are present on virtually all of the subunits. Phosphorylation of the β subunits by cAMP-dependent protein kinase (PKA) and phosphorylation of β and γ subunits by protein kinase C and tyrosine kinase have been reported (Martin & Olsen, 2000; Olsen & MacDonald, 2002; Moss & Smart, 2001). Current studies are directed toward an understanding of the functional consequences of phosphorylation of GABA_A receptors for both acute and more prolonged time frames (see also Chaps. 25 and 26).

The GABA_A receptor is the major molecular target for the action of many drugs in the brain

The GABA_A receptor-chloride ion channel complex includes five major types of ligand-binding domains (Figs. 18-2; 18-3). Two copies of the GABA-binding site are present at α/β subunit interfaces. One of the most useful agonists is the compound muscimol, a naturally occurring GABA analog isolated from the psychoactive mushroom *Amanita muscaria*. It is a potent and specific agonist at GABA_A receptors and has been a valuable tool for pharmacological and radioligand-binding studies. Other GABA agonists include isoguvacine, 4,5,6,7-tetrahydroisoxazolo-[5,4-c] pyridin-3-ol (THIP), 3-aminopropane-sulfonate, and imidazole-acetic acid. The classical GABA_A-receptor antagonist is the convulsant bicuculline, which reduces current by decreasing the opening frequency and mean open time of the channel (Martin & Olsen, 2000; Olsen & MacDonald, 2002). It is likely that bicuculline produces its antagonistic effects on GABA_A receptor currents by competing with GABA for its binding sites. On the other hand, the convulsant picrotoxin binds to separate sites that block the chloride channel (Martin & Olsen, 2000; Barnard et al., 1998; Olsen & Sieghart, 2008).

The GABA-gated chloride ion channel is modulated by several classes of drugs that bind to allosteric sites on the receptor complex: the benzodiazepines, barbiturates and related intravenous general anesthetics like etomidate and propofol, as well as anesthetic steroids and endogenous neurosteroids. Further, it appears that some types of GABA_A receptors are directly enhanced by ethanol and volatile general anesthetics (Olsen et al., 2008; Jurd et al., 2003; Yamakura et al., 2001; Wallner et al., 2006; Mody & Pearce, 2004; Smith, 2003).

Benzodiazepine receptor-binding sites co-purify with the GABA-binding sites, including co-immunoprecipitation with antibodies that were developed to recognize the protein containing the GABA-binding site (Martin & Olsen, 2000; Olsen, 2001; Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Rudolph et al., 2001; Olsen & MacDonald, 2002). This indicates that the benzodiazepine receptor is an integral part of the GABA_A receptor-Cl⁻ channel complex. Benzodiazepine agonists represent the currently most useful group of agents in the general class of depressant drugs, which also includes barbiturates, that show anticonvulsant, anxiolytic and sedative-hypnotic activity (see Chs. 40 and 57). Well-known examples include diazepam and chlordiazepoxide, which often are prescribed for their anti-anxiety effects (Olsen 2001; Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Rudolph et al., 2001). The mechanism of action of benzodiazepine agonists is to enhance GABAergic transmission. From electrophysiological studies, it is known that these benzodiazepines increase the frequency of channel opening in response to GABA, thus accounting for their pharmacological and therapeutic actions (Martin & Olsen, 2000; Barnard et al., 1998; Olsen & Sieghart, 2008; Olsen & MacDonald, 2002). In addition, the benzodiazepine site is coupled allosterically to the binding sites for the other modulatory ligands, like barbiturate, steroid, anesthetic and picrotoxin (Martin & Olsen, 2000). GABA_A receptors show heterogeneity with respect to certain benzodiazepine-site ligands (Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Rudolph et al., 2001). A wide variety of non-benzodiazepines, such as the

RECEPTOR ENDOCYTOSIS IN TREATMENT OF REFRACTORY STATUS EPILEPTICUS

George J. Siegel

Status epilepticus (SE) is a state of continuous seizures in a patient for more than 20 minutes. This condition of continuing seizures is a neurologic emergency since it can lead to serious neurologic sequelae or death. Intravenous administration of rapid-acting benzodiazepines (BZD) is usually successful in stopping SE. However, in a significant number of such patients seen in emergency rooms, these seizures become refractory to benzodiazepines. There are a number of reasons why status epilepticus may be refractory to pharmacologic treatment. One of the primary neurophysiologic mechanisms that, when functioning normally, limits seizures, consists of inhibitory neurotransmission due to GABA liganding to its postsynaptic receptors (GABA_AR) to produce inhibitory postsynaptic currents by opening Cl^- channels (see text). That is why a number of potent antiseizure medicines in use, benzodiazepines in particular, are those that potentiate GABAergic inhibitory transmission (see Ch. 40).

Experimental studies have demonstrated refractoriness to benzodiazepines at GABAergic synapses after 10–20 min of seizure activity and that seizures become self-sustaining even after blockade of the original stimulus. Glutamatergic excitatory synapses (see Ch. 17), in contrast, do not become refractory to blockade of NMDA receptors by ketamine or MK801 even after an hour of seizure activity (Mazarati et al., 2010).

Studies of mouse hippocampal slices *in vitro* have shown that prolonged stimulation of GABA receptors leads to a marked decrease in the miniature inhibitory postsynaptic currents (mIPSC) and an increase in mEPSC, thus implying decrease in functional GABA receptors and increase in functional glutamate receptors (Naylor, 2010). The majority of BZD-sensitive GABA receptors in brain are composed of $\alpha,\beta,\gamma 2$ subunits (see text). Synapses are particularly highly enriched in $\gamma 2$ and these are responsible for phasic inhibition, the type that interrupts seizures. GABA receptors composed of α,β , and δ subunits are extrasynaptic and mediate tonic inhibition. These are insensitive to BZD. SE reduces selectively the proportion of GABA_AR with the $\alpha,\beta,\gamma 2$ subunits that are expressed at the plasmalemma surface, but not the total in the cell. This implies that the trafficking of these particular GABA_A receptors to the plasmalemma is altered by the SE. The reduction is found accompanied by SE-dependent reduction in PKC-stimulated phosphorylation of the Ser408/9 residues in the $\beta 3$ subunit. This phosphorylation site lies within the AP2-binding motif, which binding is critical

to clathrin-dependent endocytosis of the subunit (Goodkin et al., 2008; Terunuma et al., 2008). The mechanism by which SE leads to inhibition of GABA_AR phosphorylation may involve Ca^{2+} -calmodulin-dependent calcineurin phosphatase activity, which is increased by SE. The activation of the phosphatase by SE is accompanied by decreased phosphorylation of GABA_AR $\beta 2/3$ while FK506, a potent inhibitor of the phosphatase, suppresses the calcineurin activity and reverses the SE-induced dephosphorylation of GABA_AR $\beta 2/3$ (Wang et al., 2009). Of course, modifications of local Ca^{2+} concentrations either through release from ER or membrane channels may be involved in regulating the calcineurin activity.

From these investigations, it follows that discovery of means to pharmacologically control calcineurin phosphatase activity or AP2 binding/clathrin-induced endocytosis of GABA_AR may help in treating refractory epilepsy (see also Jadeep Kapur in Noebels et al., 2011).

References

- Goodkin, H. P., Joshi, S., Mtchedlishvili, Z., Brar, J., & Kapur, J. (2008). Subunit-specific trafficking of GABA(A) receptors during status epilepticus. *Journal of Neuroscience*, 28(10), 2527–2538.
- Mazarati, A. M., & Wasterlain, C. G. (1999). N-methyl-D-aspartate receptor antagonists abolish the maintenance phase of self-sustaining status epilepticus in rat. *Neuroscience Letters*, 265(3), 187–190.
- Naylor, D. E. (2010). Glutamate and GABA in the balance: convergent pathways sustain seizures during status epilepticus. *Epilepsia*, 51(Suppl. 3), 106–109.
- Noebels, J. L., Avoli, M., Rogawski, M. A., Olsen, R. W., & Delgado-Escueta, A. V. (Eds.). (2011). *Jasper's basic mechanisms of the epilepsies* (4th ed.). New York : Oxford University Press. (In press).
- Terunuma, M., Xu, J., Vithlani, M., Sieghart, W., Kittler, J., Pangalos, M., et al. (2008). Deficits in phosphorylation of GABA(A) receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *Journal of Neuroscience*, 28(2), 376–384.
- Wang, A., Chi, Z., Wang, S., Wang, S., & Sun, Q. (2009). Calcineurin-mediated GABA(A) receptor dephosphorylation in rats after kainic acid-induced status epilepticus. *Seizure*, 18(7), 519–523.

β -carbolines, cyclopyrrolones and imidazopyridines, also bind to the benzodiazepine site.

Barbiturates comprise another class of drugs commonly used therapeutically for anesthesia and control of epilepsy (Ch. 40). Phenobarbital and pentobarbital are two of the most commonly used barbiturates. Phenobarbital has been used to treat patients with epilepsy since 1912. Pentobarbital is also an anticonvulsant, but it is sedative at the effective concentration. This differential selectivity for different barbiturates suggests heterogeneity of drug targets, either GABA

and non-GABA mechanisms, or subtypes of GABA_A receptors. Barbiturates at pharmacological concentrations allosterically increase binding of benzodiazepines and GABA to their respective binding sites (Martin & Olsen, 2000). Measurements of mean channel open times show that barbiturates act by increasing the proportion of channels opening to the longest open state (9 ms) while reducing the proportion opening to the shorter open states (1 and 3 ms), resulting in an overall increase in mean channel open time and Cl^- flux (Olsen & MacDonald, 2002).

Channel blockers, such as the convulsant compound picrotoxin, cause a decrease in mean channel open time. Picrotoxin works by preferentially shifting opening channels to the briefest open state (1 ms). Thus, both picrotoxin and barbiturates appear to act on the gating process of the GABA_A receptor channel, but their effects on the open states are opposite to each other. Experimental convulsants like pentylenetetrazole and the cage convulsant t-butyl bicyclophosphorothionate (TBPS) act in a manner similar to picrotoxin, preventing Cl⁻ channel permeability. The antibiotic penicillin is a channel blocker with a net negative charge. It blocks the channel by interacting with the positively charged amino acid residues within the channel pore, consequently occluding Cl⁻ passage through the channel (Martin & Olsen, 2000; Olsen & MacDonald, 2002).

There have been numerous studies on the role of GABA_A receptors in anesthesia. A considerable amount of evidence has been compiled to suggest that general anesthetics, including barbiturates, volatile gases, steroids and alcohols, enhance GABA-mediated Cl⁻ conductance at anesthetic concentrations. A proper assessment of this phenomenon requires not only a behavioral assay of anesthesia but also *in vitro* models for the study of receptor function. In this regard, not only electrophysiological methods but also neurochemical measurements of Cl⁻ flux and ligand binding have been useful. For example, a strong positive correlation exists between anesthetic potencies and the stimulation of GABA-mediated Cl⁻ uptake. This is seen with barbiturates and anesthetics in other chemical classes (Martin & Olsen, 2000; Olsen, 2001; Rudolph et al., 2001; Jurd et al., 2003; Yamakura et al., 2001).

Comparison of ligand-gated ion channels that vary in sensitivity to anesthetic modulation, using the chimera and site-directed mutagenesis approach, has identified amino acids in the membrane-spanning domains that are critical for anesthetic sensitivity (Jurd et al., 2003; Yamakura et al., 2001). Residues binding the intravenous anesthetic etomidate were identified in the transmembrane domain by photoaffinity labeling and sequencing (Li et al., 2006). Ethanol augmentation of GABA_A receptor function, measured either by electrophysiological techniques or agonist-mediated Cl⁻ flux, has been reported (Yamakura et al., 2001; Wallner et al., 2006). The similarity between the actions of ethanol and sedative drugs such as benzodiazepines and barbiturates that enhance GABA action suggests that ethanol may exert some of its effects by enhancing the function of GABA_A receptors. Ethanol potentiation of GABA_A receptor function appears to be dependent upon the cell type tested and the method of assay. This suggests that the ethanol interaction may be an indirect action, but more likely is specific for certain receptor subtypes (Martin & Olsen, 2000; Olsen & Sieghart, 2008; Jurd et al., 2003; Yamakura et al., 2001; Wallner et al., 2006; Mody & Pearce, 2004; Smith, 2003).

Neurosteroids, which may be physiological endogenous modulators of brain activity, enhance GABA_A receptor function

This enhancement by steroids involves direct action on the membrane receptor protein rather than through the classical genomic mechanism mediated by soluble

high-affinity cytoplasmic steroid hormone receptors (see Ch. 55). Chemically reduced analogs of the hormones progesterone and corticosterone derivatives administered to animals and humans exert sedative-hypnotic and anti-anxiety effects. This led to the development of a synthetic steroid anesthetic, alphaxalone. These neuroactive steroids are potent modulators of GABA_A-receptor function *in vitro* (Olsen & Sieghart, 2008; Jurd et al., 2003; Wallner et al., 2006; Olsen & MacDonald, 2002; Mody & Pearce, 2004; Smith, 2003). Neurosteroids can be produced in the brain endogenously and may influence CNS function under certain physiological or pathological conditions. Numerous observations suggest that neurosteroids physiologically affect the CNS: these include the rapid behavioral effects of administered steroids; diurnal and estrous cycle effects on behavior; gender-specific pharmacology, especially of GABAergic drugs; and the development of withdrawal symptoms following cessation of chronically administered steroids. Neuroactive steroids have effects similar to those of barbiturates in that they enhance agonist binding to the GABA site and allosterically modulate benzodiazepine and t-butyl bicyclophosphorothionate (TBPS) binding (Martin & Olsen, 2000; Olsen, 2001; Olsen & MacDonald, 2002; Smith 2003). Also, like barbiturates, high concentrations of neurosteroids directly activate the GABA_A receptor Cl⁻ channel. These observations led to the hypothesis that the neurosteroid binding site may be similar to the barbiturate site, but the sites of action for the two classes of drugs are not identical (Martin & Olsen, 2000; Li et al., 2006; Smith 2003).

The three-dimensional structures of ligand-gated ion channel receptors are being modeled successfully

Recent remarkable progress in structural definition of the nicotinic acetylcholine receptor, both extracellular domain, and the membrane-spanning domains (see Chapter 13), are beginning, in turn, to allow modeling of the GABA_A receptor. This will be helpful in suggesting mechanisms whereby GABA binding can gate the chloride ion channel and the action of modulatory ligands. Photoaffinity labeling and site-directed mutagenesis of the GABA_A receptors suggest that the binding sites for benzodiazepines are localized at the interface of the α and γ subunits and that those for GABA ligands are located at the interface between the α and β subunits (Olsen & Sieghart, 2008; Yamakura et al., 2001; Olsen & MacDonald, 2002). The binding pockets for each class of ligand appear to be formed from several loops of amino acids (Fig. 18-3). These models are consistent with studies on recombinant GABA_A receptors expressed in heterologous cells. Such studies show that the nature of the α and β subunits determines the pharmacological specificity at the GABA and benzodiazepine sites and that the γ subunits are necessary for sensitivity to benzodiazepines and insensitivity to Zn²⁺ inhibition (Olsen, 2001; Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Rudolph et al., 2001; Jurd et al., 2003; Yamakura et al., 2001; Olsen & MacDonald, 2002). GABA_A receptors come in five flavors with respect to benzodiazepines. Combinations including the $\alpha 1$ subunit have a high affinity for certain "type

1-selective" benzodiazepine site ligands, while those with the $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits have moderate affinity. The $\alpha 5$ subunit has a unique specificity to bind most benzodiazepine ligands but not the sedative drug zolpidem (Olsen, 2001; Barnard et al., 1998; Whiting, 2006; Olsen & Sieghart, 2008; Rudolph et al., 2001; Jurd et al., 2003; Yamakura et al., 2001). Some GABA_A receptors apparently lack benzodiazepine binding sites altogether or have a novel pharmacological profile at this site. Subunit combinations containing the $\alpha 4$ or $\alpha 6$ subunit with a γ subunit bind benzodiazepine inverse agonists but not agonists and are moderately sensitive to Zn²⁺ and neurosteroids. Combinations containing $\alpha 4$ or $\alpha 6$ with a δ subunit instead of a γ subunit do not bind traditional benzodiazepine-site ligands, but participate in tonic inhibitory currents that are sensitive to a subset of benzodiazepine ligands, and are relatively more sensitive to neurosteroids, other general anesthetics, ethanol, and Zn²⁺ (Olsen & Sieghart, 2008; Rudolph et al., 2001; Jurd et al., 2003; Yamakura et al., 2001; Wallner et al., 2006; Mody & Pearce, 2004; Smith, 2003). This implicates the extrasynaptic GABA_A receptors in the action of many allosteric modulators such as endogenous neurosteroids, general anesthetics, and alcohol.

Mouse genetics reveal important functions for GABA_A receptor subtypes

The use of mouse genetics has given tremendous insights into the functions of various subtypes of GABA_A receptors. Gene targeting (knockouts) for several subunits reveal important phenotypic traits resulting from the loss of the receptor isoform. Most striking has been the relatively benign nature of phenotypes in mice lacking major GABA_A receptor subunits, including $\alpha 1$ and $\beta 2$, as well as $\alpha 6$. The $\gamma 2$ and $\beta 3$ knockouts are neonatally lethal, indicating important functions. The $\alpha 5$ and δ knockout phenotypes are subtler. Mice lacking the δ become insensitive to neurosteroids, while mice lacking $\alpha 5$ show improved performance in hippocampus-dependent spatial memory acquisition (Martin & Olsen, 2000; Whiting, 2006; Rudolph et al., 2001) (see also Ch. 56).

Further, the removal of benzodiazepine sensitivity in a selective α subunit in a mouse using the gene knock-in technique has established that the $\alpha 1$ subunit plays a major role in the sedative and amnestic effects of benzodiazepines, part of the anticonvulsant effects, and little of the anxiolytic effects; the latter effects are more importantly mediated by the $\alpha 2$ subunit (Rudolph et al., 2001). The β subunit selectivity for the drugs lorazepam (an anxiolytic) and etomidate (an anesthetic) allowed determination that a single residue in the M2 domain could account for this selectivity ($\beta 2 = \beta 3 > \beta 1$). When a mouse knock-in selectively removed the etomidate sensitivity of the $\beta 2$ subunit, the animals showed reduced sensitivity to sedative effects of etomidate, but no reduction of the true anesthetic effects. In contrast, mutation of the $\beta 3$ subunit to negate etomidate sensitivity of that subunit alone resulted in a mouse with no sensitivity to the anesthesia produced by etomidate. This proved that the GABA receptor is the target of at least this one anesthetic (etomidate), and furthermore that the specific locations in the brain of $\beta 3$ subunits are important for anesthetic action, while the corresponding $\beta 2$ subunits,

even when insensitive to this drug, did not produce a loss of anesthetic action in the organism (Olsen & Sieghart, 2008; Jurd et al., 2003).

GABA IS THE MAJOR RAPIDLY ACTING INHIBITORY NEUROTRANSMITTER IN BRAIN

The neurotransmitter GABA uses both metabotropic G-protein-coupled GABA_B receptors, and, especially, ionotropic ligand-gated chloride channel GABA_A receptors with structural homology to the other pentameric, cys-loop ligand-gated ion channel receptor superfamily. A high degree of diversity of isoforms has been exhibited (19 subunit genes and two to three dozen oligomeric subtypes) in GABA_A receptors. The data suggest a varied pharmacology and physiology associated with differing isoform combinations. An understanding of the nature of these combinations should assist in the development of a new series of therapeutic agents that interact with GABA_A receptors in a more specific manner than currently available drugs. Furthermore, a detailed understanding of the functional domains of the proteins may aid in rational drug design. Gene targeting studies and analyses of existing mutant mice have revealed important roles for GABA receptors in nervous system function and development. Finally, plastic changes in subunit composition have been documented as a result of environmental experiences, giving new clues to understanding mechanisms of learning; disease states, such as epilepsy; and drug dependence, such as alcoholism (Martin & Olsen, 2000; Li et al., 2006; Fritschy & Brunig, 2003; Olsen & Spigelman, 2011).

References

- Abdel-Halim, H., Hanrahan, J. R., Hibbs, D. E., et al. (2008). A molecular basis for agonist and antagonist actions at GABA(C) receptors. *Chemical Biology & Drug Design*, 71, 306–327.
- Barnard, E. A., Skolnick, P., Olsen, R. W., et al. (1998). Subtypes of GABA_A receptors: Classification on the basis of subunit structure and receptor function. *International Union Pharmacological XV. Pharmacological Reviews*, 50, 291–313.
- Ben-Ari, Y., Gaiarsa, J. L., Tyliz, R., & Khazipov, R. (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiological Reviews*, 87, 1215–1284.
- Blaesse, P., Airaksinen, M. S., Rivera, C., & Kaila, K. (2009). Cation-chloride co-transporters and neuronal function. *Neuron*, 61, 820–838.
- Bowery, N. G., Bettler, B., Froestl, W., et al. (2002). Mammalian γ -aminobutyric acidB receptors: Structure and function. *International Union Pharmacological XXXIII Pharmacological Reviews*, 54, 247–264.
- Chen, N. -H., Reith, M. E. A., & Quick, M. W. (2004). Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. *Pflugers Archiv: European Journal of Physiology*, 447, 519–531.
- Fritschy, J. -M., & Brunig, I. (2003). Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacology & Therapeutics*, 98, 299–323.
- Gasnier, B. (2000). The loading of neurotransmitters into synaptic vesicles. *Biochimie*, 82, 327–337.

- Hensch, T. K., Fagiolini, M., Mataga, N., et al. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science*, 282, 1504–1508.
- Jurd, R., Arras, M., Lambert, S., et al. (2003). General anesthetic actions *in vivo* strongly attenuated by a point mutation in the GABA_A receptor $\beta 3$ subunit. *The FASEB Journal*, 17, 250–252.
- Kneussel, M., & Betz, H. (2000). Clustering of inhibitory neurotransmitter receptors at developing postsynaptic sites: the membrane activation model. *Trends in Neurosciences*, 23, 429–435.
- Li, G.-D., Chiara, D. C., Sawyer, G. W., et al. (2006). Identification of a GABA_A receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. *Journal of Neuroscience*, 26, 11599–11605.
- Madsen, K. K., Clausen, R. P., Larsson, O. M., et al. (2009). Synaptic and extrasynaptic GABA transporters as targets for anti-epileptic drugs. *Journal of Neurochemistry*, 109(Suppl. 1), 139–144.
- Mody, I., & Pearce, R. A. (2004). Diversity of inhibitory neurotransmission through GABA(A) receptors. *Trends in Neurosciences*, 27, 569–575.
- Moss, S. J., & Smart, T. G. (2001). Constructing inhibitory synapses. *Nature Reviews Neuroscience*, 2, 240–250.
- Marchionni, I., Kasap, Z., Mozzrymas, J. W., et al. (2009). New insights on the role of gephyrin in regulating both phasic and tonic GABAergic inhibition in rat hippocampal neurons in culture. *Neuroscience*, 164, 55–562.
- Martin, D. L., & Olsen, R. W. (Eds.). (2000). *GABA in the nervous system: The view at 50 years*. Philadelphia: Lippincott, Williams & Wilkins.
- Olsen, R. W., & Macdonald, R. L. (2002). GABA_A receptor complex: Structure and function. In J. Ejeberg, A. Schousboe & P. Krosgaard-Larsen (Eds.), *Glutamate and GABA receptors and transporters: structure, function, and pharmacology* (pp. 202–235). London: Routledge.
- Olsen, R. W., & Betz, H. (2006). GABA and glycine. In G. J. Siegel, R. W. Albers, S. T. Brady & D. L. Price (Eds.), *Basic neurochemistry* (7th ed.). Boston: Elsevier Academic Press.
- Olsen, R. W. (2001). GABA. In K. L. Davis, D. Charney, J. T. Coyle & C. Nemeroff (Eds.), *Neuropsychopharmacology: Fifth generation of progress* (pp. 159–168). Philadelphia: American College of Neuropsychopharmacology, Lippincott, Williams & Wilkins.
- Olsen, R. W., & Sieghart, W. (2008). International union of pharmacology LXX. Subtypes of γ -aminobutyric acid_A receptors: Classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacological Reviews*, 60, 243–260.
- Olsen, R. W., & Spigelman, I. (2011). GABA_A receptor plasticity in alcohol withdrawal. In J. L. Noebels, M. Avoli, M. A. Rogawski, R. W. Olsen & A. V. Delgado-Escueta (Eds.), *Jasper's Basic Mechanisms of the Epilepsies* (4th ed.). New York: Oxford University Press.
- Pinard, A., Seddik, R., & Bettler, B. (2010). GABA_B receptors: Physiological functions and mechanisms of diversity. *Advances in Pharmacology (San Diego, Calif.)*, 58, 231–255.
- Rudolph, U., Crestani, F., & Möhler, H. (2001). GABA_A receptor subtypes: Dissecting their pharmacological functions. *Trends in Pharmacological Sciences*, 22, 188–194.
- Simon, J., Wakimoto, H., Fujita, N., et al. (2004). Analysis of the set of GABA_A receptor genes in the human genome. *The Journal of Biological Chemistry*, 279, 41422–41435.
- Smith, S. S. (Ed.). (2003). *Neurosteroid effects in the central nervous system: The role of the GABA_A receptor*. Boca Raton, FL: CRC Press.
- Wallner, M., Hanchar, H. J., & Olsen, R. W. (2006). Low dose acute alcohol effects on GABA_A receptor subtypes. *Pharmacology & Therapeutics*, 112, 513–528.
- Whiting, P. J. (2006). GABA-A receptors: A viable target for novel anxiolytics? *Current Opinion in Pharmacology*, 6, 24–29.
- Yamakura, Y., Bertaccini, E., Trudell, J. R., & Harris, R. A. (2001). Anesthetics and ion channels: Molecular models and sites of action. *Annual Review of Pharmacology and Toxicology*, 41, 23–51.