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# GABA as an Inhibitory Neurotransmitter in Human Cerebral Cortex

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## SUMMARY AND CONCLUSIONS

*1.* The possible role of γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter in the human cerebral cortex was investigated with the use of intracellular recordings from neocortical slices maintained in vitro.

*2.* Electrical stimulation of afferents to presumed pyramidal cells resulted in an initial excitatory postsynaptic potential (EPSP) followed by fast and slow inhibitory postsynaptic potentials (IPSPs). The early IPSP had an average reversal potential of −68 mV, was associated with a mean 67-nS increase in membrane conductance, was reduced by the $GABA_A$ antagonist bicuculline, was sensitive to the intracellular injection of $Cl^-$, and was mimicked by the $GABA_A$ agonist muscimol.

*3.* The late IPSP, in contrast, had an average reversal potential of −95 mV, was associated with a mean 12-nS increase in membrane conductance, was reduced by the $GABA_B$ antagonist phaclofen, and was mimicked by the $GABA_B$ agonist baclofen.

*4.* Block of the early IPSP by bicuculline or picrotoxin led to the generation of paroxysmal epileptiform activity, which could be further enhanced by reduction of the late IPSP.

*5.* These data strongly support the hypothesis that GABA is a major inhibitory neurotransmitter in the human cerebral cortex and that GABAergic IPSPs play an important role in controlling the excitability and responsiveness of cortical neurons.

## INTRODUCTION

γ-Aminobutyric acid (GABA) has been proposed to be a major inhibitory neurotransmitter in the mammalian cerebral cortex (see Krnjević 1974, 1987). There is abundant evidence in favor of this hypothesis, including the presence of specialized neurons that synthesize, contain, and release GABA (Iversen et al. 1971; Ottersen and Storm-Mathisen 1984; Ribak 1978); physiological actions of GABA that mimic those of electrical activation of local circuit interneurons (Avoli 1986; Connors et al. 1988; Satou et al. 1982; Scharfman and Sarvey 1986; Tseng and Haberly 1988); and block of intrinsic inhibitory postsynaptic potentials (IPSPs) by specific GABA antagonists (Connors et al. 1988; Dutar and Nicoll 1988; Krnjević 1974).

GABA has also been proposed to be a major inhibitory substance in the human cerebral cortex, although very few investigations have been performed to test this hypothesis. Neurons that are immunocytochemically positive for the presence of GABA have been reported in the human temporal cortex (Schiffman et al. 1988). Indeed, many of the cell types that are known to be GABAergic in the rodent and nonhuman primate cerebral cortex are also present in the human, such as the axoaxonic or chandelier cell (De Carlos et al. 1987; Kisvarday et al. 1986; Marin-Padilla 1987; Seldon 1981) and the basket cell (Marin-Padilla 1969, 1972, 1974; Seldon 1981). Furthermore, GABA antagonists are potent convulsants in humans; this implies the presence and fundamental importance of GABAergic processes in regulating neuronal processing in the human CNS.

The lack of knowledge concerning the actions of GABA in the human cerebral cortex has directly resulted from the technical problems associated with a study of this kind. Application of the in vitro slice technique to cortical tissue removed during neurosurgery for the treatment of neurological disorders largely overcomes these problems. Through the use of intracellular recording and slice techniques, I have shown that the electrophysiological consequences of activation of $GABA_A$ receptors is similar in man to that previously shown in rodent and represents a large increase in membrane chloride conductance. In addition, it is demonstrated that the late IPSP is mediated in part by the activation of $GABA_B$ receptors and probably represents the activation of an inwardly rectifying potassium current. Pharmacologic block of $GABA_A$ receptors results in epileptiform bursts, the amplitude and duration of which can be further modified by the block of $GABA_B$ receptors.

## MATERIALS AND METHODS

The methods used for the preparation of human neocortical slices and for obtaining intracellular recordings were similar to those previously described for rodent neocortex (Connors et al. 1988; McCormick et al. 1985). Human neocortical tissue used in the present study was a small portion of that which is normally removed for the treatment of intractable epilepsy. Most often (19/23 cases) the tissue was obtained from the anterior portions of the temporal lobe, although samples from frontal ($n$ = 2) and occipital ($n$ = 2) regions were also used. All patients suffered from recurring seizures, which were found through electroencephalography and electrocortography to originate in a circumscribed region of the neocortex or hippocampus (i.e., the focus). In most cases the neocortical tissue was obtained from the anterior-lateral portions of the temporal lobe, in which the epileptogenic focus was localized to more mesial structures (e.g., hippocampus).

The present study is not an attempt to investigate the primary deficits underlying the generation of epileptiform activity in these patients, but rather it is aimed at investigating the actions of the putative neurotransmitter GABA in as normal of a sample of human cerebral cortex as can be obtained. The investigation of a possible deficit in GABAergic inhibition, long proposed as a mechanism underlying some types of epilepsy, will require a much more intensive study that makes use of both focal, perifocal, and nonfocal tissue in a double-blind study.

Neocortical tissue was removed en bloc by the neurosurgeon (Dennis Spencer) and a small portion (~3 mm thick) of the excised tissue was placed in cold (5°C) bathing medium. After tissue was transported to the laboratory (delay of 5–10 min), 400-μm slices were prepared on a vibratome (Lancer Corporation). Slices were maintained in an interface-type recording

chamber at a temperature of 36°C (range 35–37°C). The bathing medium contained (in mM) 124 NaCl; 2.5 KCl; 2 $MgSO_4$; 1.25 $NaH_2PO_4$; 26 $NaHCO_3$; 2 $CaCl_2$; and 10 dextrose.

All agonists were applied by the use of the pressure-pulse technique. Brief pulses of pressure (~50-ms pulse at 40 psi: 280 kPa) were used to eject ~5–20 pl of agonist-containing slice solution from a fine broken micropipette. Application of muscimol and baclofen to the surface of the slice was found to generate robust responses, whereas, in the case of GABA, it was necessary to lower the micropipette into the slice to obtain adequate responses. In some experiments, the GABA antagonists bicuculline and phaclofen were applied to the exposed surface of the slice, near the entry point of the intracellular recording electrode. Phaclofen was obtained from Tocris Neuramin (Essex, U.K.), bicuculline from Pierce Chemical, and baclofen from Ciba Geigy. All other agents were obtained from Sigma.

Intracellular recording microelectrodes were formed on a Flaming and Brown P-80/PC puller, filled with 4 M KAc, and had a final resistance of 25–50 MΩ. Bridge balance was continuously monitored. Only neurons that had a steady resting membrane potential of at least −65 mV and that exhibited the ability to discharge repetitively to the intracellular injection of a steady current pulse were included for analysis.

Discontinuous single-electrode voltage clamp was performed with the use of an Axoclamp-2A amplifier (Axon Instruments) interfaced with an IBM AT computer operating the PClamp software program (Axon Instruments). A separate oscilloscope was used to monitor headstage output continuously to ensure against "false clamp." Sample frequencies were typically between 4 and 5.5 kHz and in no case were lower than 3 kHz. Amplifier gain ranged from 0.5 to 1.0 nA/mV. Current-versus-voltage (*I-V*) relationships were obtained by injecting a hyperpolarizing ramp from −50 to between −90 and −120 mV over a period of 10 s and measuring the amount of current required to do so. Two to six such *I-V* relationships were averaged pre- and postdrug application to minimize noise.

All tissue samples were allowed to recover in the interface chamber for 2–3 h before recording. After recovery, the health of the neurons within the tissue was examined by slowly passing a multiunit extracellular electrode (<1 MΩ resistance; Frederick Haer) through the slice and observing the resulting neuronal injury discharge. Healthy tissue possessed a high density of "units" that discharged with action potentials of relatively short duration and large amplitude. In contrast, regions of poor quality possessed either a low density of units or, more importantly, the action-potential discharges were broad and possessed a preponderance of low frequencies. This qualitative assessment of the tissue was essential. Human cortical slices were found to vary greatly in health both between slices and within a single slice. Spontaneous neuronal activity was examined by placing the recording electrode on the surface of the slice (which did not generate injury activity) or by waiting for recovery from lowering the electrode into the slice.

Epi-illumination of neocortical slices readily revealed the relationship of the various recording, stimulation, and drug-applying microelectrodes and the different lamina of the cortex. Cells were generally recorded in layers II–III, although a few neurons were recorded in layer V.

Electrical stimulation was achieved through application of a brief (100-μs; 0.5–1.0 mA) constant-current pulse through a concentric stimulating electrode. Current intensity and polarity were adjusted to yield maximal amplitude IPSPs with the minimum amount of current.

## RESULTS

Extracellular unit recordings revealed that many human neocortical tissue samples contained a large number of electrophysiologically intact neurons. In general, there was little or no spontaneous neuronal activity, although, in 4 out of 23 cases, there appeared regions of spontaneous field potentials (Fig. 1*B*) that were associated with the synchronous action-potential discharge of numerous neuronal elements (not shown).

Stable intracellular recordings (recording period from 1 to 6 h) were obtained from 37 neurons in tissue obtained from 23 patients. These cells had an average resting membrane potential of −79 ± 8 (SD) mV, action-potential amplitude of 98 ± 10 mV, and apparent input resistance of 36 ± 9 MΩ. The large majority (81%) of these neurons were recorded in the anterior region of the temporal cortex, with the remaining cells arising from both occipital and frontal cortical regions. There were no gross differences in the electrophysiological or GABAergic response properties of neurons in different cortical regions; therefore the data were combined for this study. The electrophysiological properties of neurons studied here were typical for pyramidal cells in rodent cerebral cortex (McCormick et al. 1985), and therefore it is assumed that the data presented is typical for this type of human cortical neuron, although this fact awaits investigation with intracellular injection of dyes.

Electrical stimulation of neuronal elements at the layer VI–white matter border directly below the recorded neuron resulted in a stereotypical sequence of postsynaptic potentials (PSPs). The initial response was a fast excitatory postsynaptic potential (EPSP) that was typically followed by two phases of IPSP (Figs. 1*C*, 2, 3, 5*A*, and 7*C*). This sequence of PSPs was seen in individual neurons even if the stimulation was applied between the pia and the recording site, or laterally to the neuron (not shown).

The early IPSP had a latency-to-peak of 28 ms, was associated with a 67-nS increase in membrane conductance, and had a reversal potential of −68 mV (Table 1). In contrast, the late IPSP was considerably slower, with a latency-to-peak of 135 ms. The late IPSP was also associated with a smaller increase in membrane conductance (12 nS) and had a more negative reversal potential (−95 mV) (Table 1; Figs. 1, *A* and *C* and 5*A*).

In rodent cerebral cortex and hippocampus, these two phases of IPSPs are thought to be because of the synaptic release of GABA from local interneurons. The release of GABA activates two pharmacologically distinct receptors: $GABA_A$ and $GABA_B$ (Bowery et al. 1982, 1987). Activation of $GABA_A$ receptors results in an increase in membrane chloride conductance, whereas activation of $GABA_B$ receptors results in an increase in potassium conductance (Avoli 1986; Connors et al. 1988; Dutar and Nicoll 1988; Gähwiler and Brown 1985; Krnjevic 1987; Newberry and Nicoll 1985). By measuring the reversal potential, sensitivity to pharmacologic antagonists, similarity to the responses generated by specific GABAergic agonists, and sensitivity to intracellular injection of $Cl^-$, I sought to test the hypothesis that a similar sequence of events may underlie the two components of IPSPs in human cerebral cortical neurons.

### *Reversal potentials*

In four cases, extracellular recordings revealed the presence of spontaneous, regular, synchronous-burst dis-

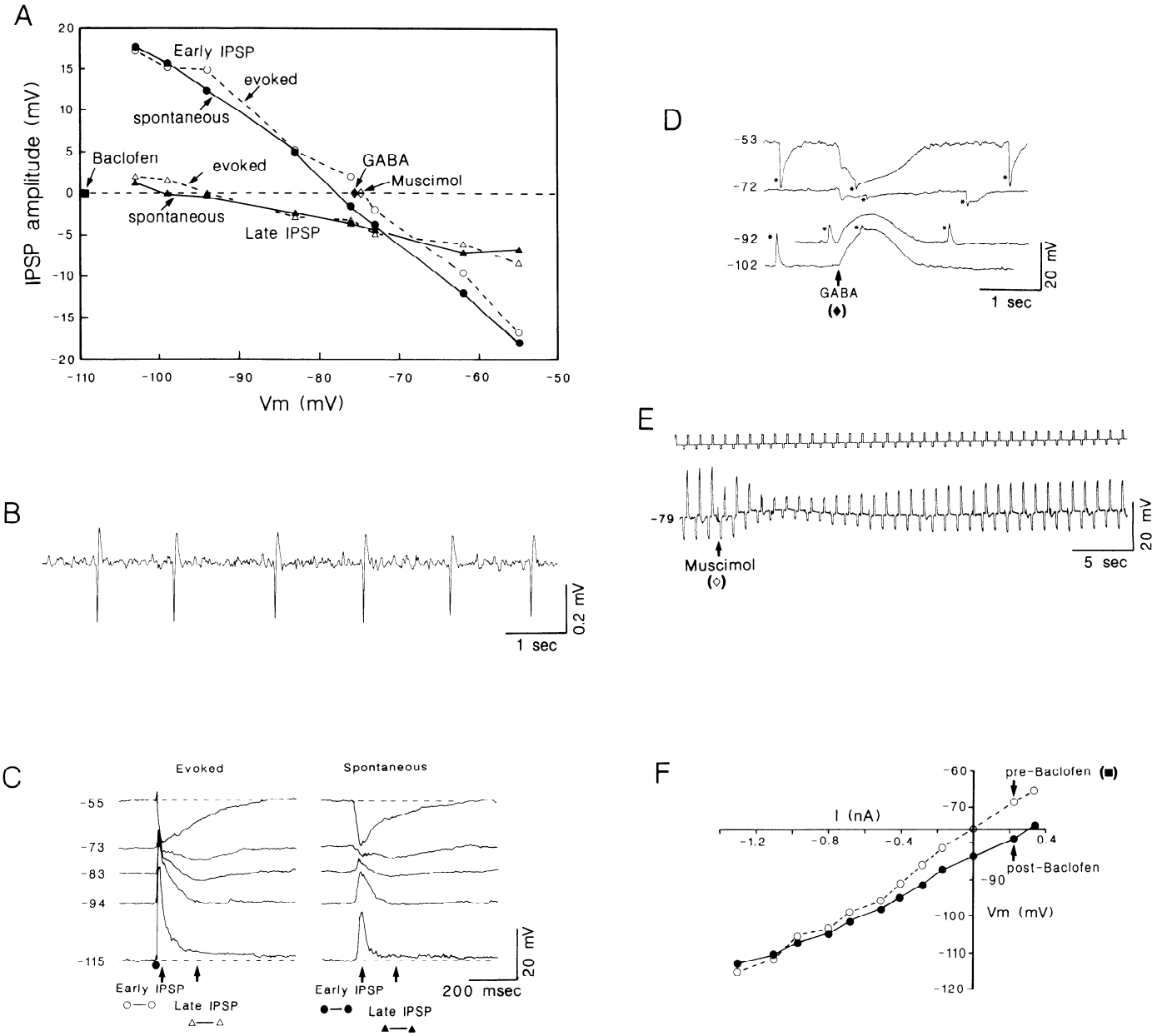


FIG. 1. Reversal potentials of IPSPs and GABA responses in human cerebral cortical neurons. *A*: graphic illustration of the data in *C–F*. Reversal potential of both evoked and spontaneous IPSPs was investigated. Spontaneous (●) and evoked (○) early IPSPs (*C*) reversed at approximately −75 mV, which is identical to the reversal potential of the response to GABA (♦, *D*) and the $GABA_A$ agonist muscimol (◇, *E*). Spontaneous (▲) and evoked (△) late IPSP reversed at approximately −95 mV, which is more positive than the response to the $GABA_B$ agonist baclofen (■, *F*). In this slice, extracellular field-potential recordings revealed spontaneous and localized "spike-wave" discharges occurring regularly at ~0.75 Hz (*B*). Large, spontaneous IPSPs occurred during these field "spikes" in this neuron (*C* and *D*, *). All data are from the same layer II–III cell, which had a resting membrane potential of −79 mV. *B*: trace was low-pass filtered at 20 Hz to illustrate field-potential activity. *E*: reversal potential for muscimol response was obtained by injecting both a depolarizing and hyperpolarizing current pulse (*top*). *F*: reversal potential for baclofen response was obtained by injecting a family of current pulses before and after application of baclofen and constructing *I-V* plots. Data in this and all subsequent figures were obtained from neurons located in the anterior portions of the temporal lobe.

charges from localized regions of the cortex, as mentioned above (Fig. 1*B*). Intracellular recordings from neurons within these regions revealed a mixture of EPSPs and IPSPs occurring in the same regular manner as the extracellularly recorded bursts (Fig. 1*D*), as has been reported previously (Schwartzkroin and Knowles 1984). In many neurons, these spontaneous events took the form of pure IPSPs that possessed both an early and a late component (Fig. 1*C*, spontaneous; and *D*, *). The reversal potential of these spontaneous IPSPs was nearly identical to those evoked by electrical stimulation (Fig. 1, *A* and *C*). Furthermore, the reversal potential of the early component of the IPSP was nearly identical to that of the response generated by the local application of GABA (−71 ± 6 mV; 1 mM; Fig. 1*D*) or the $GABA_A$ agonist muscimol (100 μM; −69 ± 6 mV; Figs. 1, *A* and *E*, and 6, *C* and *D*). The evoked and spontaneous late IPSPs, on the other hand, had a reversal potential (approximately −97 mV) that was similar to that of the

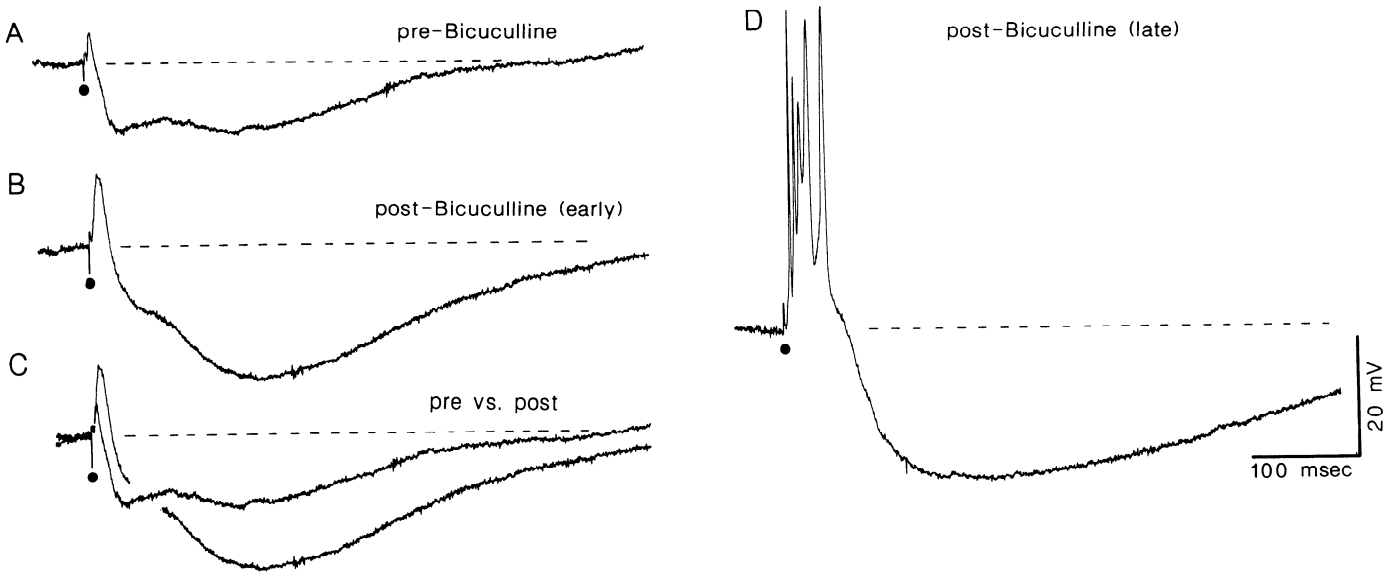


FIG. 2. Application of the $GABA_A$ antagonist bicuculline reduces the early IPSP. *A*: normal PSP sequence evoked by electrical stimulation of afferent fibers. Single shock stimuli (•) give rise to an EPSP followed by 2 phases of IPSP. *B*: local application of bicuculline (25 $\mu$M in micropipette) quickly (1 min) results in a potentiation of the EPSP and late IPSP and a diminution of the early IPSP. *C*: traces in *A* and *B* are overlaid for comparison. *D*: 10 min after bicuculline application, afferent stimulation results in a burst discharge and a prolonged after-hyperpolarization. All data obtained from a layer II–III cell. Membrane potential held at −63 mV; resting membrane potential was −79 mV.

response generated by application of the $GABA_B$ agonist baclofen (100 $\mu$M; −104 ± 6 mV; Fig. 1, *A, C,* and *F*). These data suggest that the evoked or spontaneous release of GABA may result in the activation of both $GABA_A$ and $GABA_B$ receptors. To test this hypothesis further, selective antagonists of these two receptors were employed.

### *$GABA_A$ and $GABA_B$ antagonists*

Local application of the $GABA_A$ antagonist bicuculline (25 $\mu$M in micropipette) to the surface of the slice near (50–100 $\mu$m) the recorded cell resulted in a steady reduction in the amplitude of the early IPSP (compare Fig. 2, *A* and *B*). This reduction in the early IPSP was associated with a substantial increase in the amplitude of the EPSP and the late IPSP ($n$ = 3; Fig. 2, *A–C*). Over time, the EPSP evoked by stimulation gradually became large enough to evoke a number of action potentials in what is commonly referred to as a paroxysmal depolarization shift (PDS) (see Prince and Connors 1986). The PDS was followed by a prolonged afterhyperpolarization (AHP) that lasted many seconds (Figs. 2*D* and 4*D*). These effects of bicuculline were fully reversible (not shown).

Phaclofen, an analogue of the $GABA_B$ agonist baclofen, is known to antagonize selectively the actions of GABA at postsynaptic $GABA_B$ receptors, although only at high con-

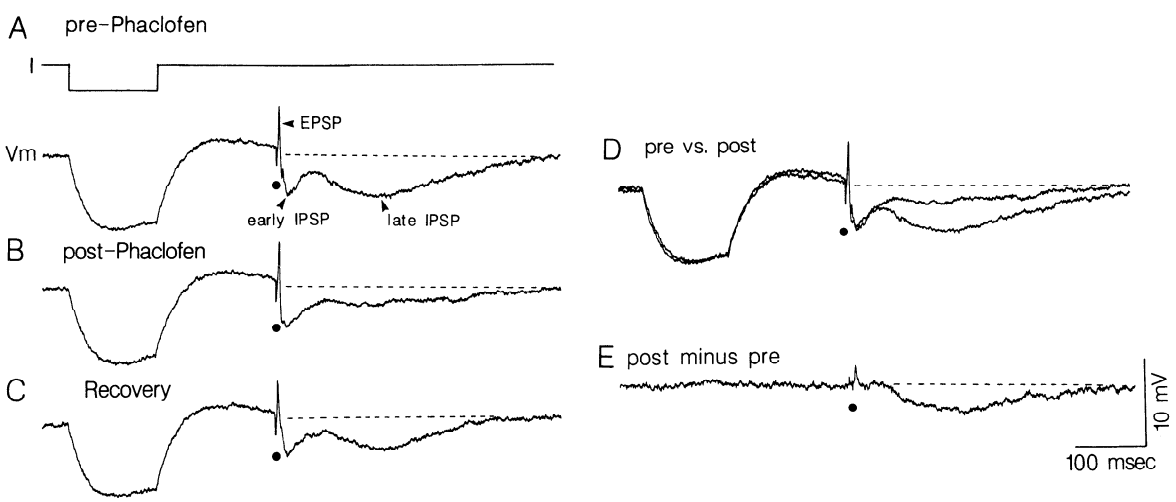


FIG. 3. $GABA_B$ antagonist phaclofen specifically reduces the late IPSP. *A*: typical PSP sequence evoked by electrical stimulation of afferents (•). Electrical stimulation was preceded by a conductance test pulse (*top trace*). *B*: local application of phaclofen (5 mM in micropipette) selectively and reversibly (*C*) reduces the late IPSP. *D*: traces in *A* and *B* are overlaid for comparison. *E*: the difference between traces in *A* and *B* illustrates the time course of the phaclofen-sensitive component of the late IPSP. Data obtained from a layer II–III cell. Membrane potential held at −58 mV. Resting membrane potential was −72 mV.

TABLE 1. *Characteristics of early and late IPSPs*

| | Early IPSP | Late IPSP |
|---|---|---|
| IPSP characteristics | | |
| Latency to peak, ms | 28 ± 2 (6) | 135 ± 24 (6) |
| Increase in conductance, nS | 67 ± 13 | 12 ± 9 |
| Reversal potential, mV | −68 ± 6 | −95 + 4 |
| Agonists | | |
| GABA reversal potential, mV | −71 ± 6 (4) | |
| Muscimol reversal potential, mV | −69 ± 6 (7) | |
| Baclofen reversal potential, mV | | −104 ± 6 (9) |
| Antagonists | | |
| Bicuculline ($GABA_A$) | Blocked | Increased |
| Phaclofen ($GABA_B$) | No effect | Blocked |

Values are means ± SD; number of neurons in parentheses. IPSP, inhibitory postsynaptic potential; GABA, γ-aminobutyric acid.

centrations (Dutar and Nicoll 1988; Kerr et al. 1987). Local application of phaclofen (5 mM in micropipette) selectively reduced the amplitude of the late IPSP in human cortical neurons (Fig. 3; $n = 4$) without affecting the resting membrane potential, apparent input resistance, or the amplitude of the EPSP or early IPSP. Similarly, phaclofen substantially reduced the outward current evoked by baclofen, but not that evoked by adenosine (not shown), both of which activate the same potassium current in cortical pyramidal cells (McCormick and Williamson 1989). Together, these data support the hypothesis that the early IPSP is mediated by the activation of $GABA_A$ receptors, whereas the late IPSP is mediated, at least in part, by the activation of $GABA_B$ receptors.

The observation that the late IPSP can become larger in the presence of bicuculline, along with the known massive discharge of presumed GABAergic interneurons during a PDS (McCormick et al. 1985), indicates that activation of $GABA_B$ receptors may contribute to the termination of epileptiform burst discharges in cortical pyramidal cells. To test this hypothesis, the effect of phaclofen on bicuculline-induced bursts and the burst-AHP was examined.

Local application of phaclofen (5 mM in micropipette) caused a substantial lengthening of the burst discharge

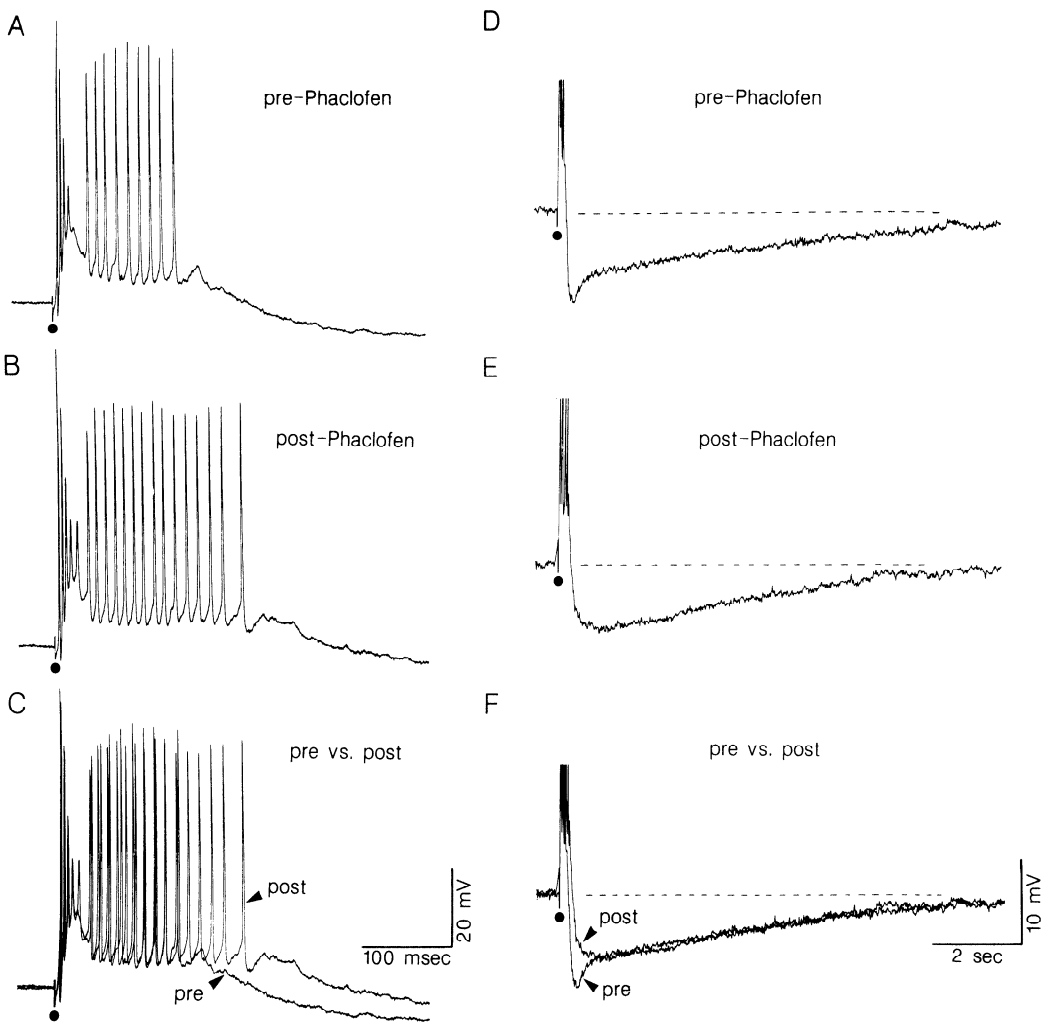


FIG. 4. Effect of phaclofen on paroxysmal burst discharges. Electrical stimulation of afferents in the presence of bicuculline (8 μM) results in the generation of a paroxysmal burst discharge (*A*) followed by a prolonged afterhyperpolarization (*D*). Local application of phaclofen (5 mM in micropipette) prolonged the burst discharge (*B*) and blocked the initial component of the burst afterhyperpolarization (*E*). Comparison of pre vs. post reveals that phaclofen enhances the later, but not the early, components of the burst discharge (*C*) and blocks the early, but not the slow, components of the burst afterhyperpolarization (*F*). Membrane potential was −56 mV; resting membrane potential was −70 mV.

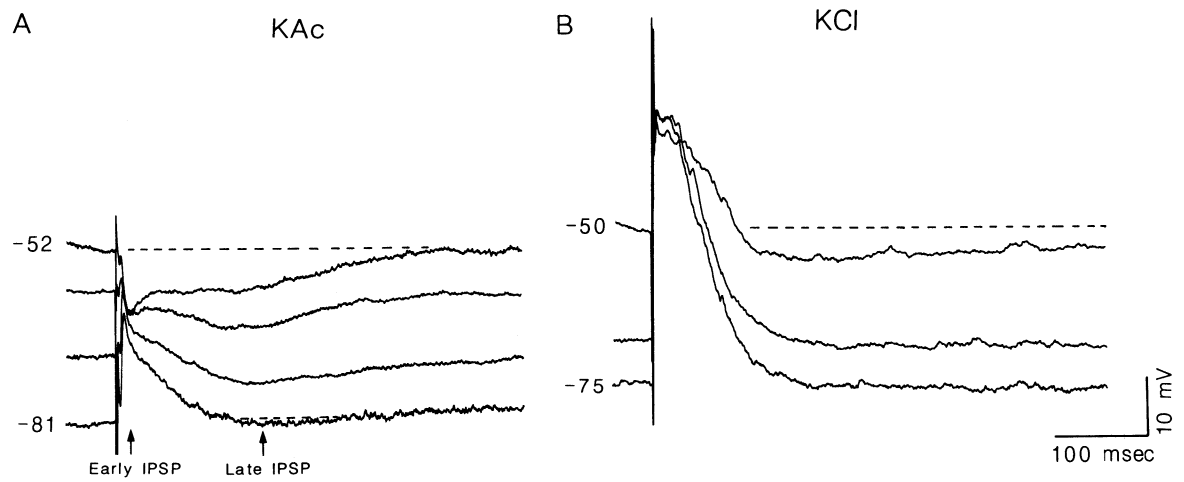


FIG. 5. Effect of intracellular injection of $Cl^-$ on evoked IPSPs. *A*: normal PSP sequence evoked by electrical stimulation of afferents in a neuron recorded with a potassium acetate (KAc)-filled microelectrode. An initial EPSP is followed by both an early IPSP and a late IPSP. Early IPSP reverses at about −75 mV. *B*: afferent stimulation in a neuron recorded from the same region as the cell in *A*, except with a microelectrode filled with potassium chloride (KCl), results in a large and prolonged depolarization followed by a late hyperpolarization.

evoked in the presence of the GABA antagonist picrotoxin (10 $\mu$M) and reduced the initial component of the burst-AHP ($n = 3$; Fig. 4). The early components of the PDS were unaffected by application of phaclofen, with an increase in spike firing frequency becoming apparent only after ~45 ms, in other words, at about the same delay in time at which the late IPSP becomes apparent (compare Figs. 4, *A* and *B*, and 3*A*). The major effect of phaclofen on the burst-AHP was a reduction in the initial, relatively short-duration component (Fig. 4, *D–F*), whereas the more prolonged portions were unaffected. This result implies that the activation of $GABA_B$ receptors contributes significantly to the early portions of the PDS AHP.

### *Intracellular $Cl^-$-injection*

In many neuronal systems, the activation of $GABA_A$ receptors results in a selective increase in $Cl^-$ conductance,

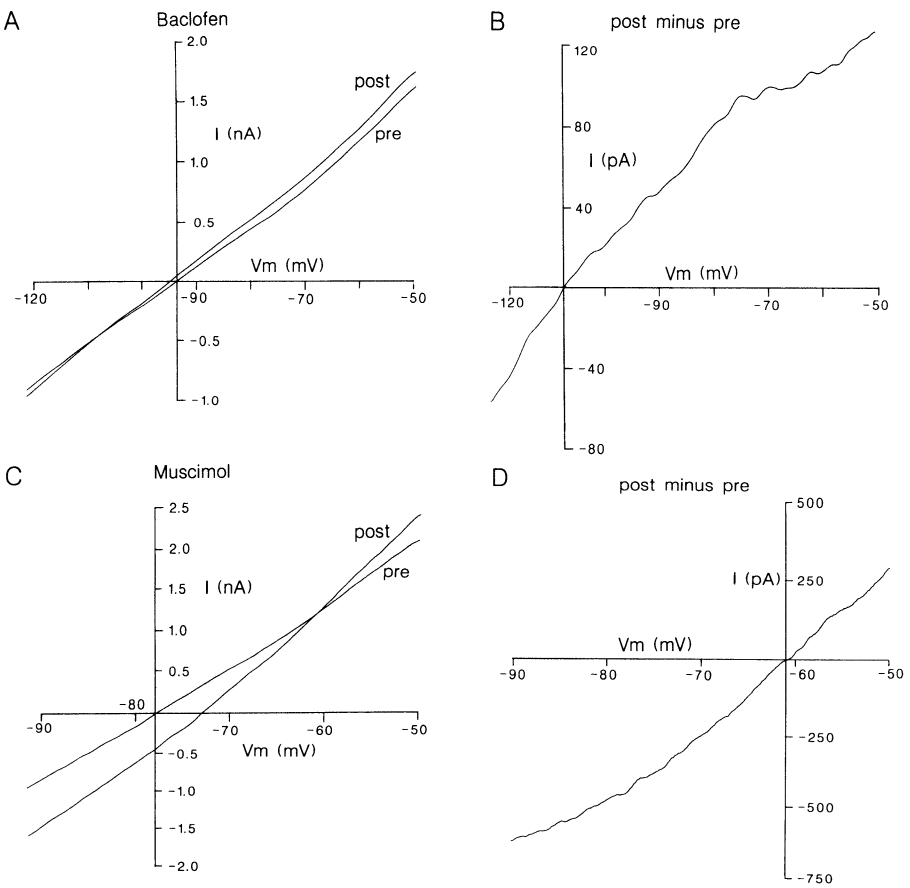


FIG. 6. Current vs. voltage (*I-V*) relationship for human cortical neurons before and after application of the $GABA_B$ agonist baclofen and the $GABA_A$ agonist muscimol. *A*: application of baclofen results in an increase in membrane conductance and a reversal potential of −108 mV. *B*: difference curve (postbaclofen *I-V* minus pre) illustrates the relationship between baclofen-induced current and membrane potential. *C*: muscimol causes an increase in membrane conductance with a reversal potential of −62 mV. *D*: relationship of muscimol-induced current and membrane potential. Data in *A*, *B* and *C*, *D* obtained from 2 separate layer II–III neurons.

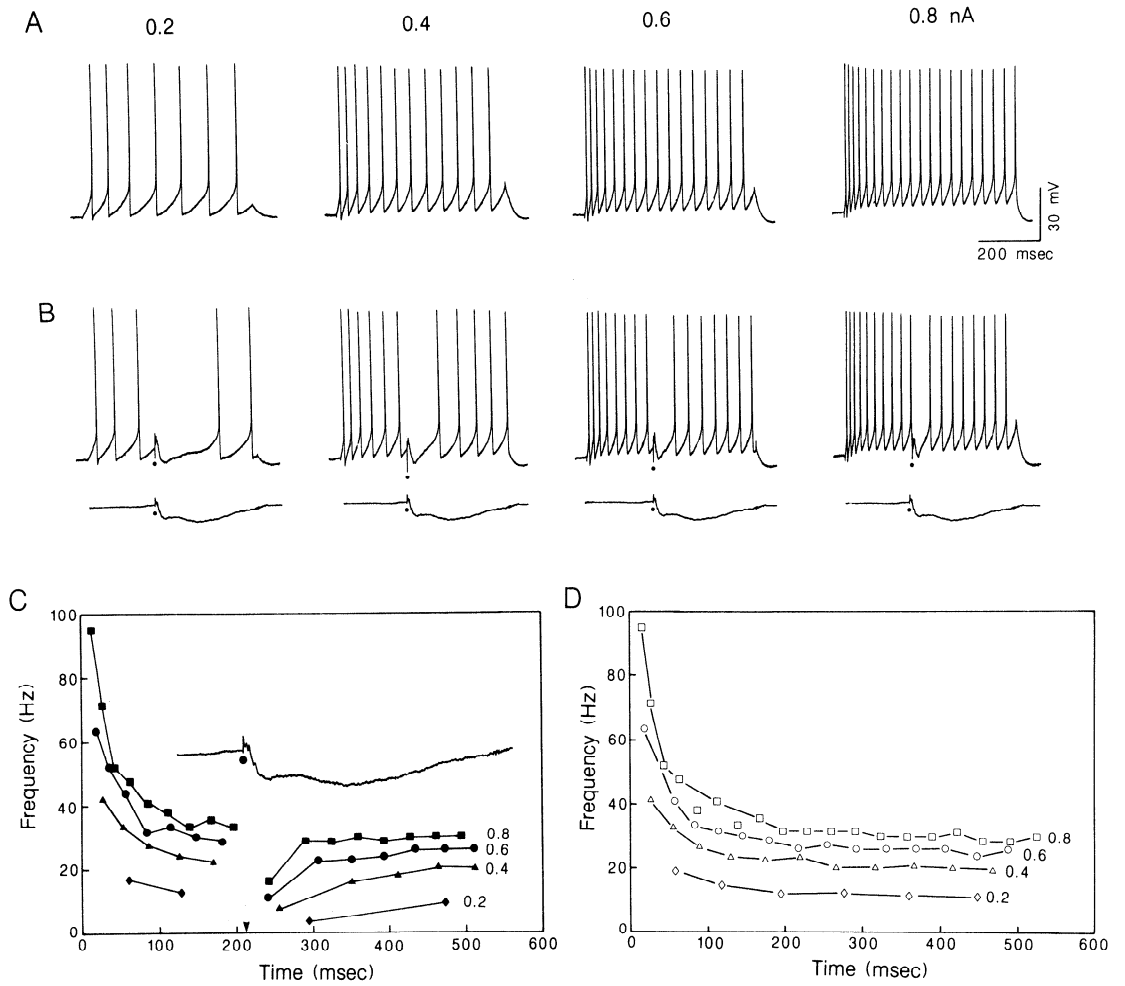


FIG. 7. Effect of early and late IPSPs on steady-state discharge of a cortical neuron. *A*: response of the neuron to intracellular injection of 0.2-, 0.4-, 0.6-, and 0.8-nA amplitude, 500-ms-duration current pulses. *B*: afferent stimulation during the steady-state portions of the neuronal discharge illustrates the inhibitory properties of the early and late IPSPs. The PSP sequence recorded in this neuron at a membrane potential just subthreshold for action-potential generation is shown *below each trace* to illustrate the time course of the 2 phases of IPSP. *C* and *D*: graphic illustration of the instantaneous firing frequency (hertz) of the neuron in *A* and *B* with (*C*) and without (*D*) afferent stimulation. *Inset in C*: time course of the PSP sequence in relation to the time base of the graphic presentation. Downward arrowhead indicates point of electrical stimulation in time, which is also indicated by a dot in the illustration of the PSP sequence. At all current intensities, the early IPSP stopped neuronal firing. This point is emphasized by not connecting the data points immediately before and after activation of the PSPs. All data obtained from a layer II–III cell with a resting membrane potential of −77 mV. Cell was tonically depolarized to −65 mV with the intracellular injection of current throughout this experiment.

whereas activation of $GABA_B$ receptors causes an increase in a potassium current (Avoli 1986; Connors et al. 1988; Gahwiler and Brown 1985; Krnjevic 1987; Newberry and Nicoll 1985). To examine this possibility in human cortical neurons, cells were recorded with microelectrodes containing 4 M KAc (Fig. 5*A*) or 3 M KCl (Fig. 5*B*). The injection of $Cl^-$ into neurons is expected to shift the reversal potential for $Cl^-$-mediated events to much more positive membrane potentials, whereas intracellular injection of $Ac^-$ will have no such effect, because acetate ions do not pass through GABA-activated channels. Electrical stimulation of afferents to neurons recorded with KCl electrodes ($n$ = 3) resulted in large depolarizations, which could evoke action-potential discharge (compare Fig. 5, *B* and *A*). These large, depolarizing potentials were followed by a hyperpolarization that was similar in time to peak as the normal late IPSP but that was more prolonged in duration (compare Fig. 5, *A* and *B*). The prolonged duration of this hyperpolarizing potential may indicate the activation of additional currents, such as a calcium-sensitive potassium current, by the preceding burst of activity. Electrical stimulation of afferents to neurons recorded with KAc-filled microelectrodes (in the same region as those injected with $Cl^-$) resulted in the typical EPSP-IPSP sequence (Fig. 5*A*). These data indicate that the large depolarizations seen in neurons recorded with KCl electrodes were particular to them and did not represent a generalized paroxysmal discharge.

### *Voltage dependence of baclofen and muscimol-induced currents*

The $Cl^-$ and $K^+$ currents associated with activation of $GABA_A$ and $GABA_B$ receptors are known to rectify (Ashwood et al. 1987; Barker and Harrison 1988; Gähwiler and Brown 1985; Weiss 1988). The possibility that similar rec-

tification may be exhibited by GABA responses in human cortical pyramidal neurons was examined through the use of the single-electrode voltage-clamp technique. Ramp *I-V* relationships were generated under single-electrode voltage clamp by steadily hyperpolarizing the neurons from −50 to approximately −90 to −120 mV over a period of 10 s and measuring the amount of current required to do so. Local application of baclofen resulted in an increase in membrane conductance represented by an increase in slope of the *I-V* relation (Fig. 6*A*). The *I-V* relationships pre- and post-application of baclofen crossed at approximately −108 mV (−104 ± 6, mean ± SD; $n = 9$). Subtracting the post- from the pre-*I-V* relationship resulted in a plot of the amplitude of baclofen-induced current versus membrane potential (Fig. 6*B*). The baclofen-induced current was relatively linear with a small amount of inward rectification appearing at membrane potentials positive to approximately −70 mV (Fig. 6*B*).

Application of muscimol resulted in an increase in slope of the *I-V* relationship, indicating an increase in membrane conductance (Fig. 6*C*). The *I-V* relationships pre- and postmuscimol application were found to cross at approximately −62 mV (−69 ± 6 mV, mean ± SD; $n = 7$). A plot of the muscimol-induced current versus membrane potential revealed that this current was relatively linear, except at hyperpolarized membrane potentials, where outward rectification was evident (Fig. 6*D*). The reversal potential of the muscimol response (−69 mV) is consistent with an increase in $Cl^-$ conductance, whereas the reversal potential of the baclofen response (−104 mV) is consistent with an increase in $K^+$ conductance.

### *Effect of early and late IPSPs on neuronal firing*

The physiological consequences of the early and late IPSPs on the excitability of postsynaptic neurons was investigated in one of two manners: either through activation of the PSPs during current-induced neuronal discharge (Fig. 7), or through the intracellular injection of a short-duration (50 ms) pulse to mimic an EPSP at varying times in the PSP sequence (not shown). Both methods gave essentially identical results and therefore were combined.

The early IPSP, when fully activated, is capable of ceasing all neuronal activity at all firing frequencies tested (Fig. 7). However, the duration of the pause in neuronal activity decreased as the intensity of the depolarizing current activating the cell was increased (Fig. 7). Even with large depolarizing pulses (Fig. 7*B*, 0.8 nA), the early IPSP was still effective in briefly inhibiting action-potential output. In contrast, the late IPSP completely inhibited action-potential discharge only when the neuron was firing slowly (Fig. 7*B*, 0.2 nA). As the intensity of depolarization steadily increased, the effectiveness of the late IPSP decreased, until there appeared to be little, if any, alteration in firing frequency (compare Fig. 7, *B* with *A* and 7, *C* with *D* at 0.8 nA).

These results emphasize the physiological difference between these two phases of IPSP. Whereas the early IPSP is a potent inhibitory influence on neuronal firing, the late IPSP is considerably weaker and affects predominantly the response of the cell to small-to-moderate-amplitude depolarizing inputs.

## DISCUSSION

Together with previous results, the present study gives strong support to the hypothesis that GABA is a major inhibitory neurotransmitter in the human cerebral cortex. The positive identification of a substance as a neurotransmitter requires the satisfaction of a number of criteria, including the presence of cellular mechanisms for the synthesis, storage, and specific release of the agent at synaptic specializations (see Werman 1966). Furthermore, mechanisms should exist by which the synaptic action of the substance is terminated. Artificial application of the substance should mimic the physiological actions of its release in situ. Finally, pharmacologic agents that are known to block specifically the actions resulting from artificial application of the suspected transmitter should also block those resulting from endogenous release of the substance (see Werman 1966).

Human cerebral cortex not only contains significant amounts of GABA, but also the major enzyme in its synthesis [glutamic acid decarboxylase (GAD)] as well as an enzyme (GABA-transaminase: GABA-T) important for its degradation (see Lloyd et al. 1986). The human cerebral cortex is also known to contain a moderately high density of GABA receptors (Walker et al. 1984). The postsynaptic actions of GABA reported here mimic those of the fast IPSP in that both cause a large increase in membrane conductance and possess identical equilibrium potentials (Fig. 1). Furthermore, the fast IPSP is also mimicked by application of the specific $GABA_A$ agonist muscimol and blocked by the specific $GABA_A$ antagonist bicuculline (Figs. 1 and 2).

Although the late IPSP was not mimicked by application of GABA to the region of the soma, it was mimicked by the application of the $GABA_B$ agonist baclofen. The apparent lack of $GABA_B$-mediated responses to GABA may result from the masking of this response by the large increase in $Cl^-$ conductance associated with stimulation of $GABA_A$ receptors. Indeed, other investigators have found that large hyperpolarizations mimicking those of the late IPSP can be evoked by GABA in the presence of bicuculline (Connors et al. 1988). This result remains to be tested adequately in human cortical neurons. However, specific and substantial reduction of the late IPSP by phaclofen confirms the hypothesis that this PSP may be due largely, if not entirely, to the activation of $GABA_B$ receptors by the release of GABA.

### *Evolution of cortical GABAergic inhibition*

The physiological mechanisms underlying IPSPs in the cerebral cortex have now been studied in detail in a number of species, including turtle (Kriegstein and Connors 1986), rat (Avoli 1986; Connors et al. 1988; Howe et al. 1987; Scharfman and Sarvey 1986; Tseng and Haberly 1988), rabbit (Satou et al. 1982), cat (Connors et al. 1988), and humans (present study). The remarkable similarity of IPSPs seen across these species suggests that their ionic mechanisms may have evolved in a common ancestor (e.g., they are homologous). However, it is also possible that they arose and evolved separately in separate branches of vertebrate evolution. In either case, the similarity in, and widespread nature of, GABAergic IPSPs in the cerebral cortex from turtle to man implies that these synaptic potentials

perform a basic function that is essential for efficient synaptic processing in a number of different circumstances.

*Functional comparison of fast and slow IPSP*

Because of the physiological differences in the fast and slow IPSPs, they may play different roles in the regulation of cortical neuronal activity and synaptic processing. The short latency to onset, shorter duration, and potentially powerful inhibitory influence of the early IPSP makes it useful for participating in relatively high-frequency synaptic processing. In this manner, the fast IPSP is the counterpart of the fast EPSP.

Block of the early IPSP with bicuculline quickly leads to an increase in the amplitude and duration of the initial EPSP. Although it is possible that this effect represents an increase in polysynaptic EPSPs, or a reduction in presynaptic inhibition, the most parsimonious explanation is that the very early stages of EPSP enhancement represent an increase in the amplitude of the monosynaptic EPSP. If this hypothesis is true, then the duration and amplitude of the EPSP activated by incoming afferents may be under the direct control of intrinsic inhibitory elements (e.g., Fig. 2). Thus changes in the excitability of the inhibitory element may result in large changes in the amplitude and time course of these EPSPs and, therefore, the neuronal response generated by them.

An extreme example of the powerful control that the fast IPSP exerts over the response of cortical neurons to activation of afferents is seen after substantial block of this IPSP and takes the form of the PDS (Figs. 2 and 4) (Prince and Connors 1986). PDSs have as their underlying driving force a giant EPSP that derives from both extra- and intracortical neuronal elements. Reduction of the fast IPSP allows the initial EPSP to become large enough in amplitude and long enough in duration to activate neuronal spike activity from intracortical excitatory cells (e.g., spiny stellate and pyramidal cells). The local recurrent axon collaterals of these cells then serve as a source of rapid dispersion of this excitatory signal. If enough of the fast IPSP is reduced, these recurrent excitatory connections are left largely unchecked (for at least a couple of hundred milliseconds) and result in a massive and near-synchronous firing of all neurons in the cortex (see Prince and Connors 1986). Termination of the PDS is achieved through activation of a variety of potassium currents (Alger et al. 1980, 1988).

Of particular interest to the termination of the PDS is the observation that the massive discharge among pyramidal cells results in an even more dramatic high-frequency discharge of intracortical interneurons (McCormick et al. 1985). The large release of GABA that is expected to occur from such a discharge may participate in the containment and termination of the PDS by increasing the activation of both $GABA_A$ and $GABA_B$ receptors (Fig. 4). Therefore, even if the late IPSP did not play a primary role in preventing the occurrence of PDSs, it could make an important contribution to its termination.

*Increase in late hyperpolarization by bicuculline*

The ability of the $GABA_A$ antagonist bicuculline to increase the late hyperpolarizing potential activated by synaptic stimulation may have a number of causes. First, some types of intracortical GABAergic neurons are known to receive GABAergic terminals, presumably from other GABAergic interneurons (see Jones and Hendry 1984; McCormick et al. 1985). Therefore the local application of bicuculline may not only result in disinhibition of the postsynaptic pyramidal cell, but also disinhibition of the postsynaptic nonpyramidal cell, resulting in an increase in release of GABA.

Another distinct possibility is that the early and late IPSPs overlap considerably in time. In this case, block of the early IPSP may result in the late IPSP being much more effective in bringing the membrane potential close to the equilibrium potential for $K^+$. Estimates of the time course of the late IPSP with phaclofen (see Fig. 3) support the hypothesis that there is substantial overlap of the two IPSPs, although the exact degree of this is not yet known.

The final factor is the possibility that the increase in late IPSP after application of bicuculline is because of the activation of an additional potassium current (e.g., $I_C$ or $I_{AHP}$) because of the increase in the preceding EPSP. In this situation, increasing the amplitude of the synaptic barrage on the postsynaptic neuron may result in a substantial increase in the entry of $Ca^{2+}$ into the neuron, which then can activate $I_C$ and $I_{AHP}$ (Alger et al. 1980, 1988). Although these potassium currents almost certainly play an important role in the AHP seen after a large PDS, their contribution to the increase in the late hyperpolarizing potential seen immediately after application of bicuculline is questionable. For instance, the increase in amplitude of the late hyperpolarizing potential by bicuculline does not require the generation of action potentials or even a "giant" EPSP (see Fig. 2, *A–C*). Similarly, increases in the late hyperpolarizing potential after the application of bicuculline has been seen in relay neurons of the dorsal division of the lateral geniculate nucleus. These relay neurons give off few, if any, recurrent collaterals within the nucleus and, therefore, cannot support the generation of PDSs, as in the cerebral cortex (Crunelli et al. 1988). Clearly, further experiments are required to explain completely the bicuculline-induced increase in the late hyperpolarizing potential.

Changes in the prevalence or mechanisms of GABAergic inhibition in the CNS have been proposed to underlie a number of neurological deficits and disorders, especially those associated with epilepsy (see Ribak 1987). Such alterations may take the form of decreased postsynaptic actions of GABA, GABA release, or numbers of GABAergic terminals or neurons. The results of the present report suggest that reductions in $GABA_A$-mediated inhibition in the human cerebral cortex may be very potent in leading to the generation of epileptiform activity, whereas decreases in $GABA_B$-mediated inhibition would be less so. Investigation of a possible selective reduction in one or the other type of inhibition in patients suffering from chronic epilepsy will require more extensive experiments on tissue obtained from regions both near and far from the cortical locus of electrographic seizure initiation.

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