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Facilitatory Action of Etomidate and Pentobarbital on Recurrent Inhibition in Rat Hippocampal Pyramidal Neurons

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Biochemical studies have shown that the non-barbiturate anesthetic etomidate can interact in a stereoselective, barbiturate-like fashion with the GABA/benzodiazepine receptor complex, enhancing both benzodiazepine and GABA binding, but its electrophysiological effects upon the mammalian CNS are largely unknown. The present investigations were designed to characterize the electrophysiological effects of etomidate on the recurrent GABAergic inhibitory pathway in the CA1 region of the rat *in vitro* hippocampal slice and to compare the actions of etomidate to those of pentobarbital. Electrical stimulation of the alveus elicited a biphasic hyperpolarizing response, consisting of an initial bicuculline-sensitive GABAergic IPSP. This was followed by a second component, termed the late hyperpolarizing potential (LHP), which is thought to reflect an increase in potassium conductance. Both pentobarbital (100 μM) and (+)-etomidate (10 μM) markedly increased the duration of the initial GABA-mediated IPSP, and frequently increased its amplitude as well. However, no significant effects of either of these drugs were observed on the LHP. Together with previous biochemical findings, our data suggest that the depressant effects of etomidate and barbiturates on the nervous system may reflect a common action upon a stereoselective receptor site intimately associated with bicuculline-sensitive GABA receptors and the chloride ion channel.

Barbiturates are widely used for their sedative, hypnotic, anticonvulsant, and anesthetic actions, and numerous behavioral, biochemical, and electrophysiological investigations have attempted to characterize the mechanisms involved in their depressant effects on the CNS. Nevertheless, the actions of barbiturates upon nervous tissue are complex, and it has been difficult to relate drug actions at the cellular level to specific effects upon behavior.

Biochemical studies have established several different actions of barbiturates upon brain tissue (for reviews, see Ho and Harris, 1981; Olsen et al., 1984; Willow and Johnston, 1983). Like other general anesthetics, high concentrations of barbiturates appear to increase membrane fluidity (Harris and Schroeder, 1982; Lee, 1976; Pang et al., 1979). In addition, lower doses of barbiturates elicit more specific effects upon the CNS, including changes in calcium binding (Mule, 1969), calcium flux (Blaustein and Ector, 1975), GABA binding (Harris and Allan, 1985; Olsen and Snowman, 1982; Willow and Johnston, 1979; Wong et al., 1984a), and modulation of transmitter release (Cutler and Dudzinski, 1975; Cutler and Young, 1979; Grewaal and Quastel, 1973; Haycock et al., 1977; Richter and Waller, 1977; Skerritt et al., 1983). Barbiturates can also increase GABA and benzodiazepine (BZ) receptor binding in a chloride-dependent manner (Asano and Ogasawara, 1981; Wong et al., 1984b) and can allosterically regulate binding of ligands to receptors that comprise the GABA receptor–chloride ionophore complex (Leeb-Lundberg and Olsen, 1982; Leeb-Lundberg et al., 1980; Wong et al., 1984b). Although all of these diverse actions may contribute to the overall effects of the barbiturates on the nervous system, the specific interactions with the GABA/BZ/barbiturate receptor complex appear most likely to underlie the depressant effects that occur at low doses of these agents (Olsen, 1982).

Barbiturates also have been reported to have a variety of electrophysiological actions on the nervous system, including potentiation of the actions of GABA, antagonism of the effects of excitatory amino acids, reversal of the actions of GABA antagonists such as picrotoxin at barbiturate concentrations that do not potentiate responses to GABA; in higher concentrations, barbiturates have GABA-mimetic and local anesthetic-like actions (Barker, 1975; Macdonald and Barker, 1979; Schulz and Macdonald, 1981; for reviews, see Nicoll, 1979; Willow and Johnston, 1983). In the hippocampus, GABA facilitation is apparent not only as an increase in the amplitude of GABAergic IPSPs, but also as a prolongation of these inhibitory potentials (Alger and Nicoll, 1982b; Gage and Robertson, 1985; Nicoll et al., 1975; Roth et al., 1983). The mechanism underlying these effects appears likely to be the prolongation of the mean channel lifetime for GABA-activated chloride channels (Study and Barker, 1981). Pentobarbital also has been reported to facilitate a depolarizing potential elicited by *synaptic* activation of the CA1 pyramids (Alger and Nicoll, 1982a); however, unlike most responses to GABA, this response does not reflect a pure chloride conductance but may involve other ions as well. These findings suggest that, in addition to the effects of barbiturates upon the GABA/BZ/barbiturate receptor/chloride channel complex, there may be other actions upon GABA receptors that are coupled to channels with somewhat less ionic selectivity. To complicate matters further, responses to antidromic stimulation of the CA1 pyramidal neurons clearly consist of 2 components (present results), but previous studies of hippocampal recurrent inhibition have not determined whether barbiturates affect one or both components of this response (Alger and Nicoll, 1982a; Nicoll et al., 1975).

Because of the multiplicity of both biochemical and electrophysiological responses to barbiturates, attempts to link specific biochemical actions of these drugs (such as the allosteric regulation of ligand binding) to responses at either the electrophysiological or behavioral level have been problematic. The primary purpose of the present investigation was to compare 2 structurally different drugs that share the ability to produce general anesthesia and that both facilitate the binding of GABA

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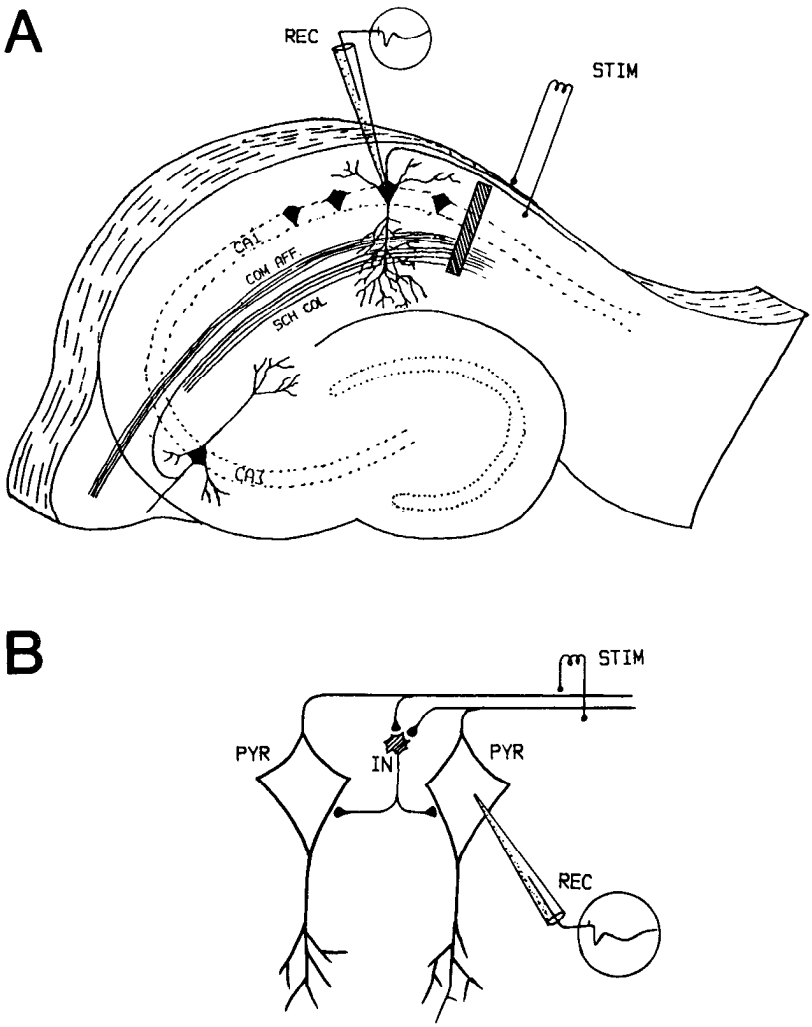

Figure 1. *A*, Diagram of a rat hippocampal slice. CA1 pyramidal neuron is shown impaled by an intracellular recording microelectrode (*REC*) and antidromically stimulated by a bipolar electrode (*STIM*) placed in the outer portion of the alveus. The *hatched bar* indicates the cut made to eliminate excitatory synaptic activation of the pyramidal neurons due to fibers passing through the strata oriens and radiatum. Also shown are the synaptic inputs to the apical dendrites via ipsilateral Shaffer collaterals (*SCH COL*) and contralateral commissural afferents (*COM AFF*) from CA3 axons. *B*, Schematic diagram of the recurrent inhibitory pathway. Activation of a pyramidal neuron (*PYR*) can synaptically activate an inhibitory interneuron (*IN*) or basket cell that forms inhibitory GABAergic synapses on pyramidal neurons throughout the CA1 cell layer. This pathway can be selectively activated by stimulating pyramidal cell axons in the alveus (*STIM*).

and BZs to brain membranes. We have examined the electrophysiological effects of pentobarbital, a prototypic short-acting barbiturate anesthetic, and etomidate, a non-barbiturate anesthetic with similar pharmacological actions. Etomidate has been reported to facilitate BZ binding in a stereoselective manner and to enhance GABA binding (Ashton et al., 1981; Thyagarajan et al., 1983; Willow, 1981; for review, see Olsen et al., 1984). The (+)-enantiomer of etomatidate is approximately 10–100× more potent than the (−)-enantiomer and about 10× more potent than pentobarbital. Etomidate has been reported to have a GABA-mimetic action on nerve terminals and motoneurons in the spinal cord of the frog and superior cervical ganglion of the rat (Evans and Hill, 1977, 1978), and there is a recent report that it can facilitate paired-pulse inhibition in hippocampus (Ashton and Wauquier, 1985), an effect that would be consistent with a facilitation of GABAergic inhibition. In the present studies, we have characterized the electrophysiological actions of both pentobarbital and etomidate on intracellularly recorded recurrent inhibitory potentials (IPSPs) of rat hippocampal CA1 pyramidal neurons *in vitro*. Our findings suggest that both etomidate and pentobarbital act similarly to enhance the GABAergic recurrent IPSP, while having no significant effect on the slower late hyperpolarizing potential (LHP).

Materials and Methods

Preparation

Rat hippocampal slices were prepared as described previously (Proctor and Dunwiddie, 1983). Male Sprague-Dawley rats (150–200 g) were decapitated and the brains quickly removed and placed in 5°C artificial CSF medium (124 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1 mM KH_2PO_4, 25.7 mM $NaHCO_3$, and 10 mM *d*-glucose, equilibrated with humidified 95% O_2/5% CO_2). The hippocampus was dissected out and cut into 400-μm-thick slices with a Sorvall tissue slicer, then placed in a recording chamber maintained at 32.5°C while suspended on nylon netting at a medium/gas interface and allowed to recover for 1 hr. Prior to intracellular recording, slices were submerged and superfused at 2 ml/min with artificial CSF.

Individual CA1 pyramidal neurons were impaled with glass microelectrodes filled with 2.5 M potassium acetate, potassium chloride, or cesium acetate having tip resistances of 50–80 MΩ. In the present experiments, recordings were made from 46 cells in 35 slices. The recurrent GABAergic inhibitory pathway (Fig. 1*B*) was activated by stimulating the outer edge of the alveus with a wire stimulating electrode as shown in Figure 1*A*. In order to minimize orthodromic synaptic stimulation due to current spread to fibers in the stratum oriens, a cut was made in each slice through the stratum oriens, the pyramidal cell body layer, and the stratum radiatum between the stimulating electrode and the CA1 pyramidal cell recording site.

Stimulation of the recurrent inhibitory pathway usually evoked clearly biphasic responses: an initial GABAergic IPSP followed by a second component consisting of the LHP. This was the case in nearly all slices, even following lesion of afferent fibers in the stratum oriens and part of the alveus (see also Alger and Nicoll, 1982a). Although the circuitry involved in the LHP is unknown, it is certainly possible that this component could reflect activation of monosynaptic afferents to interneurons, despite the measures taken to prevent this. In order to block the LHP, recordings were made with cesium acetate-filled microelectrodes. To block the initial GABAergic component, bicuculline methiodide (10 μM) was added to the perfusate so that the effects of pentobarbital and etomidate on the LHP alone could be analysed.

Drugs

Pentobarbital (Sigma), (+)- and (−)-stereoisomers of etomidate (Dr. D. Ashton, Janssen Pharmaceutica), and bicuculline methiodide (BMI; Research Biochemicals, Inc.) were dissolved in warmed degassed distilled water at 100 times the desired chamber concentration. All drugs were delivered to the recording chamber by injection into the artificial CSF perfusate. Unless otherwise stated, "etomidate" refers to the (+)-enantiomer of the drug.

Data analysis

Individual intracellular recordings following antidromic stimulation of pyramidal neurons and/or intracellular current pulse injection were stored on a multichannel FM analog tape recorder and subsequently digitized. Ten to 50 sweeps for each drug condition (before, during, and after washout) were averaged and used for comparisons. Various parameters of the averaged responses were measured (maximum rate of rise, time to peak, maximum amplitude, and duration of the response; see Fig. 5), and the mean effect of drug treatment was calculated as a percentage of the control values (before and after washout of the drug) ± the standard error of the mean. If responses did not recover to control values following extended drug washout (>30 min), data from that cell were not included in the analyses. Significance of drug treatment compared to control values was determined by paired 2-tailed Student's t test. Membrane potentials were continuously monitored on a chart recorder.

Results

Typical responses to stimulation of the alveus in CA1 hippocampal pyramidal neurons are shown in Figure 2*A*. The initial hyperpolarizing component (small arrow) has properties that are consistent with what has previously been reported to be the hyperpolarizing, chloride-dependent response to recurrent activation of GABAergic neurons in this region (Alger and Nicoll, 1982a, b; Dingledine and Langmoen, 1980; Newberry and Nicoll, 1984). This initial component was observed in every cell tested, and could be inverted to a depolarizing response when cells were impaled with chloride-containing electrodes or injected with hyperpolarizing current (Fig. 2*A*). In addition, this component of the response was blocked when slices were perfused with low concentrations of BMI (cf. Fig. 2*B*) but was unaffected by intracellular cesium (Fig. 2*C*).

The second component of the response appeared to be the LHP that is frequently observed in pyramidal cells following synaptic activation (Allen et al., 1977; Dingledine and Langmoen, 1980; Nicoll and Alger, 1981; Thalmann, 1984; Thalmann and Ayala, 1982) and that has been hypothesized to be due to the activation of a bicuculline-insensitive $GABA_B$ receptor (Alger and Nicoll, 1982a; Newberry and Nicoll, 1984; Nicoll and Alger, 1981). This second component was present initially in 37 out of 46 cells tested. It remained hyperpolarizing in cells impaled with chloride-containing electrodes, could not be readily reversed by the injection of hyperpolarizing current, and was relatively insensitive to perfusion of the slices with BMI (Fig. 2*B*). On the other hand, this potential usually disappeared in cells impaled with cesium-containing electrodes (Fig. 2*C*). The depolarizing GABA response that has been previously described (Alger and Nicoll, 1982a) was not normally observed because we employed only antidromic stimulation to activate the inhibitory circuitry.

Perfusion of slices with 100 μM pentobarbital most frequently increased the amplitude of the IPSP (16 of 19 cells), and the distinct break between the 2 components of the response was usually lost (Fig. 3*A*). Etomidate, 10 μM (Fig. 3*B*), had a very similar action upon the inhibitory responses to antidromic stimulation. Again, an apparent facilitation (16 of 20 cells) was observed, with the response appearing mono- rather than biphasic following drug treatment. Etomidate was more potent in this respect (threshold between 1–5 μM) than was pentobarbital (threshold between 10–30 μM). The peak amplitude of the IPSP response was increased by 36 ± 10% by 100 μM pentobarbital and by 60 ± 17% by 10 μM etomidate (mean ± SEM, n = 19 and 18 cells, respectively). The effects of both agents could be readily reversed by perfusion with control medium (Fig. 3).

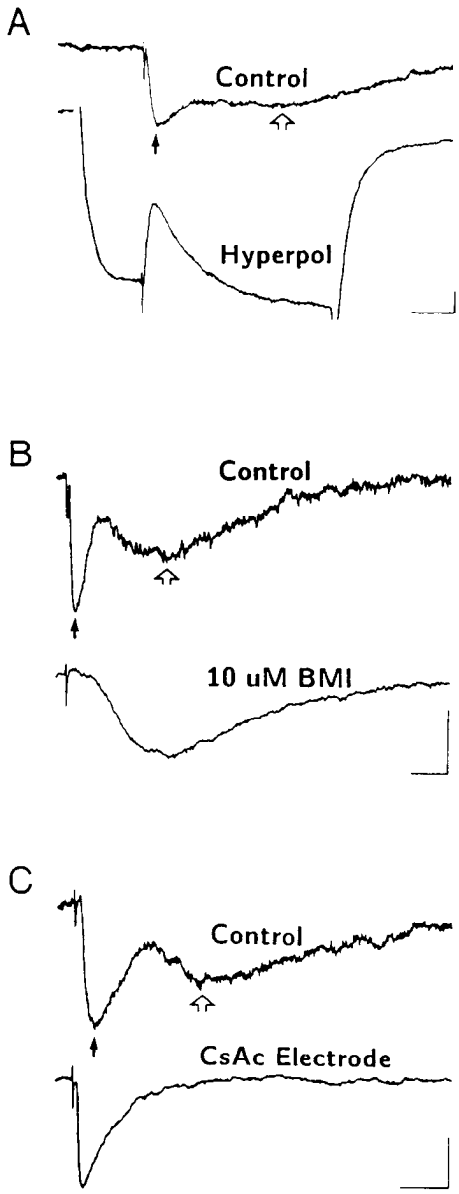

Figure 2. Effect of hyperpolarizing current, bicuculline, and cesium on recurrent IPSPs. Panels *A–C* are responses from different cells following antidromic stimulation of the alveus. The early hyperpolarizing component (peak indicated by *solid arrows*) following the stimulus artifact is the GABAergic IPSP; the slower hyperpolarizing potential (*open arrows*) is the LHP component. The *lower sweep in each pair* is the result of experimental treatment. *A*, Cell was hyperpolarized beyond the reversal potential for the GABAergic IPSP, so that the response became depolarizing, whereas the LHP did not reverse. *B*, Treatment with 10 μM BMI blocked the GABA IPSP without affecting the LHP. *C*, Cell impaled for 20–30 min with a cesium acetate-filled microelectrode showed a loss of the LHP without any change in the GABA IPSP. Calibration bars, 50 msec and 1 mV. Each sweep is an average of 15–50 responses. In most cases, more records were averaged from the treated condition, which resulted in a lower noise level in the averaged records.

Because of the biphasic nature of the recurrent inhibitory response, it was not possible to determine from these initial experiments whether the drugs were increasing the amplitude of the GABAergic IPSP and/or the LHP, or were changing the time course of one or both of these hyperpolarizing potentials. For this reason, we used several approaches to isolate the 2 components of the response. In some experiments, we intro-

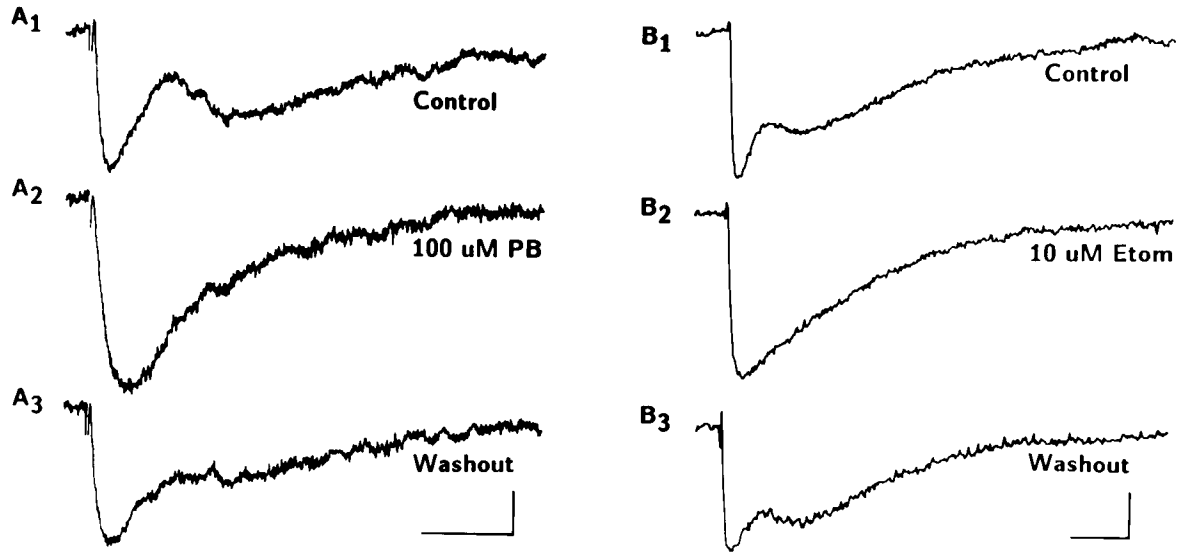

Figure 3. Effects of pentobarbital and etomidate on recurrent inhibition. *A,* Antidromic responses before (A_1), during (A_2), and after washout (A_3) of 100 μM pentobarbital. *B,* Control response (B_1), the effect of 10 μM etomidate (B_2), and washout (B_3) on a different pyramidal neuron. Calibration bars, 50 msec and 1 mV.

duced cesium into the cells using cesium acetate-filled microelectrodes. As shown in Fig. 2*C*, this effectively eliminated the LHP component. Under these conditions, both pentobarbital (Fig. 4*A*) and etomidate (Fig. 4*B*) clearly increased the duration of the GABAergic IPSP elicited by alvear stimulation. In a few cells (9 of 46), inhibitory responses were recorded with K^+-acetate electrodes that did not appear to have a secondary component; in these cells, the duration of the IPSP was also markedly enhanced by pentobarbital and etomidate. In cells impaled with chloride-containing electrodes, the initial component of the IPSP reversed, whereas the LHP did not; in these cells, the duration of the depolarizing response to antidromic stimulation was markedly prolonged (Fig. 4*C*). Finally, the increase in duration of the IPSPs could also be readily observed when the initial component of the response but not the LHP was inverted by passing hyperpolarizing current pulses through the electrode (Fig. 4*D*).

As shown in Figure 5, the response to 10 μM etomidate was both qualitatively as well as quantitatively almost identical to the effect of 100 μM pentobarbital. Both drugs induced significant increases in the peak amplitude of the IPSP and slowed the rate at which it declined. At concentrations that markedly

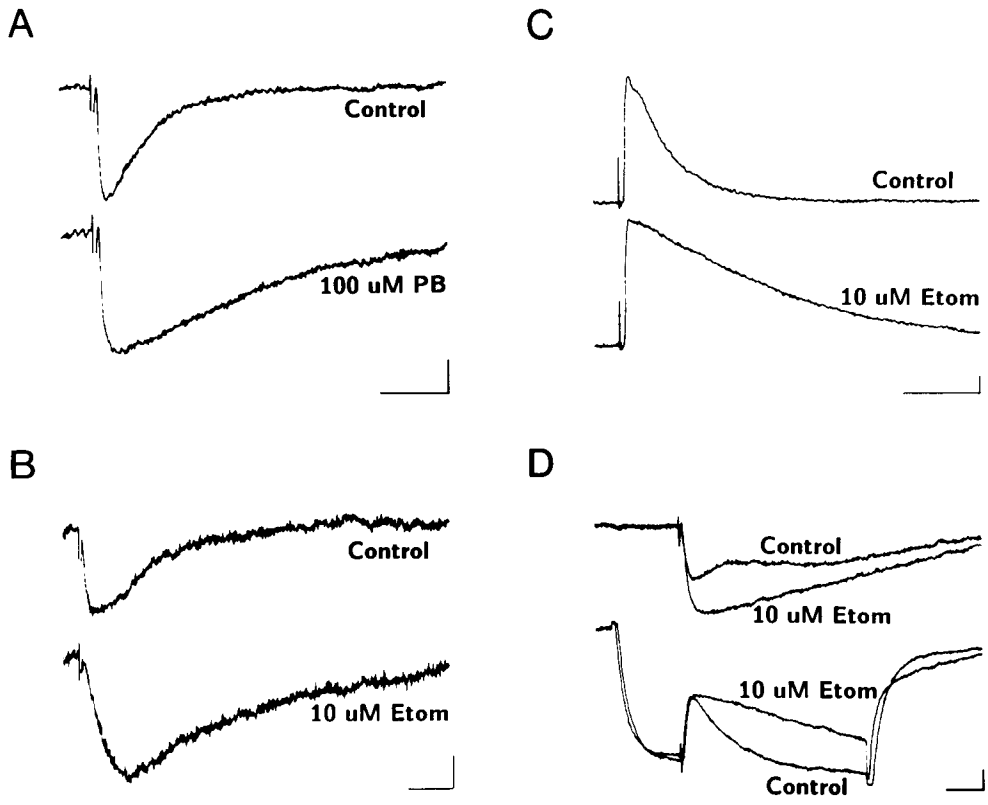

Figure 4. Drug effects on the GABAergic IPSP. *A* and *B,* Responses from 2 cells impaled with cesium acetate recording microelectrodes before and after 100 μM pentobarbital (*A*) or 10 μM etomidate (*B*). Note that only the fast GABAergic component of the IPSP is observed under these conditions since the cesium has blocked the K^+-dependent LHP. *C,* Averaged responses to antidromic stimulation using a KCl intracellular microelectrode, and the effect of perfusion with 10 μM etomidate. Movement of chloride into the cell from the microelectrode inverts the hyperpolarizing IPSP into a depolarizing response. *D,* Recurrent inhibitory responses are shown at the resting membrane potential (*upper*) and during a hyperpolarizing current pulse (*lower*); control and drug responses are superimposed. Hyperpolarization inverts the GABAergic IPSP but not the LHP, and 10 μM etomidate prolongs the duration of the reversed IPSP. Calibration bars, 50 msec and 1 mV.

affected the GABAergic IPSP, there were no significant changes in the resting membrane potential or input impedance, and the latency to peak, peak amplitude, and rate of decay of the LHP were similarly unaffected. By comparison, the (−)-enantiomer of etomidate was relatively inactive (Fig. 6*A*); 10 μM (+)-etomidate increased the time for 63% decay of the GABAergic IPSP by 429 ± 121% (n = 6), while the (−)-isomer had little or no effect (increase of 10 ± 5%, n = 9). At a concentration of 100 μM, (−)-etomidate increased the GABAergic IPSP amplitude and duration in a fashion similar to that observed with 10 μM (+)-etomidate or 100 μM pentobarbital (Fig. 6*C*).

In order to determine if the LHP was affected by pentobarbital or etomidate, hippocampal slices were perfused with BMI (10 μM), which blocked the GABAergic IPSP but not the LHP (Fig. 7). Some cells showed an increase in the LHP during BMI treatment, suggesting that the conductance underlying the GABAergic IPSP might shunt the LHP. Subsequent perfusion with either pentobarbital (Fig. 7*A*) or etomidate (Fig. 7*B*) did not significantly increase the amplitude or duration of the LHP component of the response (3 of 3 cells tested with etomidate; 5 of 5 cells tested with pentobarbital). In a few cases, the LHP had a slightly earlier onset following etomidate or pentobarbital treatment (cf. Fig. $7B_2$ and $7B_3$), perhaps as the result of the facilitation of a residual GABAergic IPSP not blocked by this relatively low concentration of BMI. However, the duration of the LHP at half-maximal amplitude was not significantly affected by either etomidate (257 ± 8 vs. 262 ± 12 msec; n = 3) or pentobarbital (295 ± 31 vs. 311 ± 24 msec; n = 5).

When recordings are made from pyramidal neurons with chloride-containing electrodes, evoked GABAergic IPSPs are inverted, and spontaneous depolarizing potentials appear that are thought to represent endogenously occurring IPSPs. Nicoll et al. (1975) reported that the time courses of these spontaneous IPSPs were prolonged by pentobarbital. Therefore, we characterized the effects of etomidate upon these spontaneous IPSPs using KCl microelectrodes. Changes in the duration and frequency of these spontaneous IPSPs were monitored before, during, and after perfusion with 10 μM etomidate and 100 μM pentobarbital (Fig. 8). Etomidate and pentobarbital both increased the duration and decreased the frequency of the spontaneous IPSPs, findings consistent with the effects on the evoked GABAergic IPSP.

Discussion

The present experiments have shown that recurrent inhibitory responses from hippocampal pyramidal cells are usually rather long (300–1000 msec) and frequently biphasic. These responses appear to reflect the sequential activation of 2 separate conductances, an initial Cl^- component followed by a K^+-dependent potential of much longer duration. Attempts to reduce the second component by sectioning the pyramidal cell layer and the stratum oriens proximal to the antidromic stimulation electrode were largely unsuccessful, except in isolated cases. Although the biphasic nature of this response is apparent in previous reports (e.g., Alger and Nicoll, 1982a, Fig. 1; Alger and Nicoll, 1982b, Fig. 10), these studies did not determine which component of the response was affected by barbiturates. By utilizing a selective blockade of either the GABA receptors by bicuculline methiodide, or the potassium conductance underlying the LHP by intracellular cesium, it is possible to isolate these 2 aspects of the response to recurrent stimulation of the pyramidal cells pharmacologically. The present results demonstrate that both pentobarbital and etomidate have a selective effect upon the GABAergic IPSP, with no significant action upon the K^+-dependent LHP. Facilitation of an occult depolarizing response by either drug, such as was reported by Alger and Nicoll (1982a, b) for pentobarbital following *orthodromic* synaptic stimulation of the CA1 pyramidal cells, was rarely observed in the cells

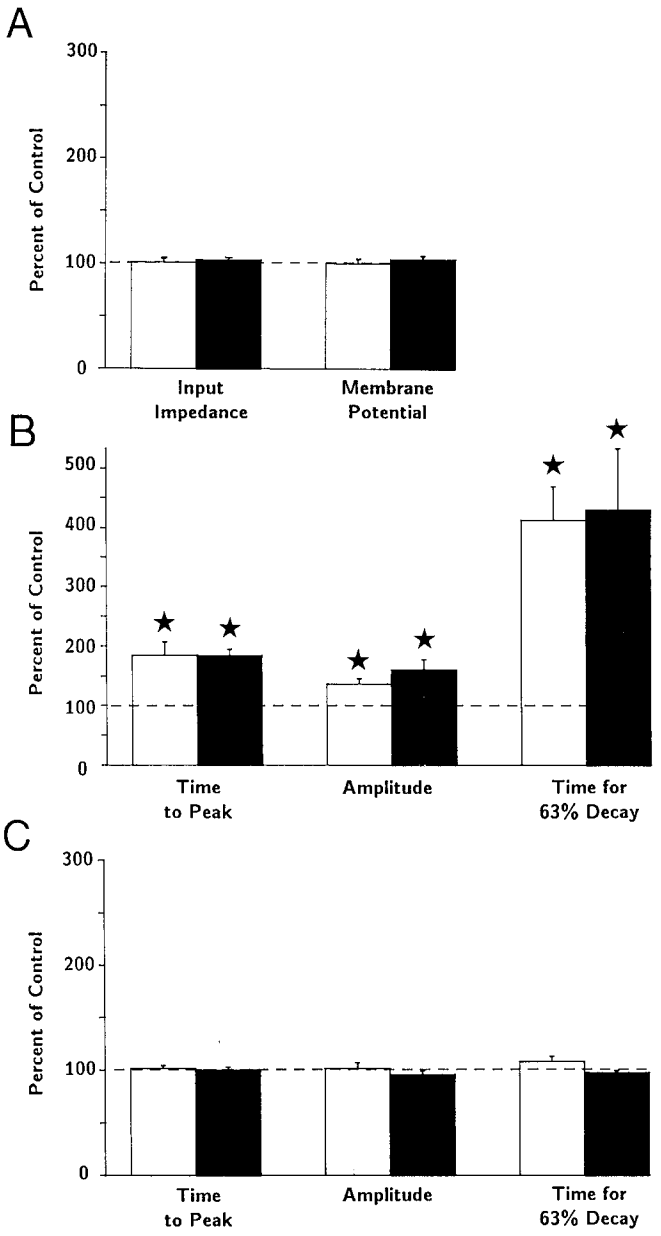

Figure 5. Comparison of pentobarbital and etomidate. Effects of treatment with 100 μM pentobarbital (*open bars*) or 10 μM (+)-etomidate (*solid bars*) are shown on passive membrane characteristics (*A*), recurrent GABAergic IPSPs (*B*), and on LHP responses (*C*). Each bar indicates the response expressed as a mean percentage of the control value; *vertical lines* denote the SEM. *A*, Effects of these drugs upon baseline parameters. Mean control values for cells treated with pentobarbital and etomidate, respectively, were −61 ± 3 and −65 ± 4 mV for the resting membrane potential, and 18.8 ± 2.7 and 24.2 ± 3.6 MΩ for the input impedance. *B*, Effects upon the GABAergic IPSPs. Baselines are as follows: 14.3 ± 1.8 and 16.3 ± 1.2 msec for latency to peak, 2.8 ± 0.4 and 2.5 ± 0.3 mV for the peak amplitude, and 30.0 ± 3.4 and 53.8 ± 11.7 msec for the time to decay from the peak to 37% of the maximum value (duration). *C*, Effects of pentobarbital and etomidate on the LHP. Baseline values for these responses were 177 ± 14 and 150 ± 7 msec for latency to peak, 2.72 ± 0.59 and 3.00 ± 0.36 mV for the peak amplitude, and 241 ± 19 and 201 ± 8 msec for the time to decay from the peak to 37% of the maximum value (duration). Each mean was determined from 12–22 cells (membrane properties), 9–25 cells (GABAergic IPSP), or from 5 (pentobarbital) or 3 cells (etomidate) for the LHP (see text). * = $p < 0.005$, 2-tailed paired t test (each response compared to control).

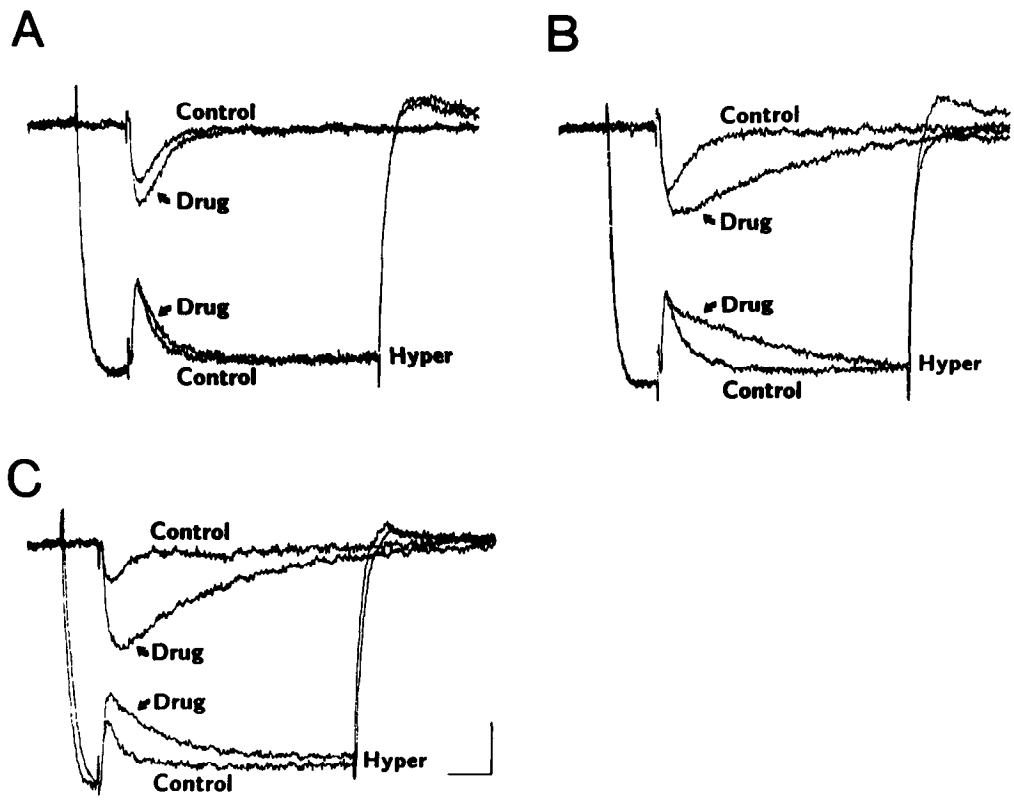

Figure 6. Effect of the stereoisomers of etomidate on the GABAergic IPSP. Records in *A* and *B* are from the same cell and show averaged responses at the resting membrane potential (*upper traces*) and during a hyperpolarizing current pulse (*Hyper*). *A*, IPSP before and during 10 μM (−)-etomidate treatment, which resulted in only a small increase in amplitude. Following washout, the slice was then perfused with 10 μM (+)-etomidate (*B*). An increase in amplitude and a large increase in the IPSP duration are readily apparent. *C*, Averaged responses before and during perfusion of 100 μM (−)-etomidate in another pyramidal neuron. This larger dose of the (−)-stereoisomer produced significant increases in amplitude and duration. Calibration bars, 50 msec and 2 mV.

tested. Thus, it would appear that the LHP evoked by antidromic stimulation is unrelated to the putative feed-forward GABA responses characterized by these investigators.

The present results correspond well with previous biochemical studies, which have demonstrated that pentobarbital and etomidate can both increase GABA and BZ binding in brain membranes (Willow, 1981; Wong et al., 1984b). Moreover, the stereoselectivity exhibited by etomidate, with the (+)-enantiomer being more potent than the (−)-enantiomer, is again consistent with the effects of these agents upon GABA and BZ binding. Unlike some of the optically active barbiturates (e.g., pentobarbital, methyl-5-phenyl-5-propyl barbituric acid), the less active isomer of etomidate had no excitatory effects in the hippocampus at any concentration tested (1–100 μM). The essentially identical effects of pentobarbital and etomidate on responses to antidromic stimulation suggest that the modulation of binding of endogenous GABA to receptors located on the CA1 pyramidal neurons may be a common mechanism of action

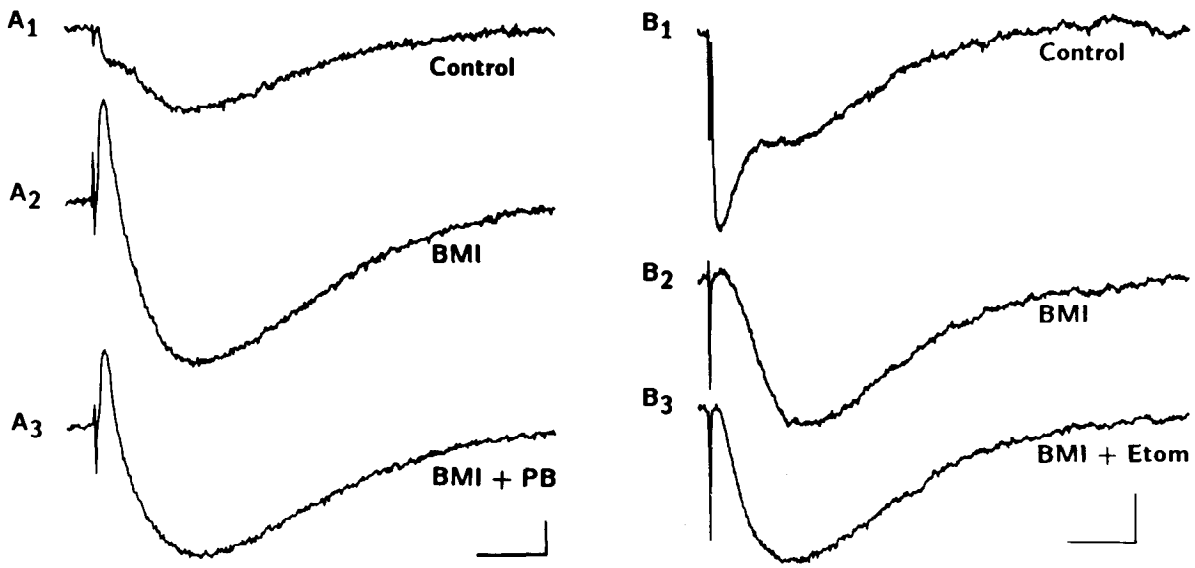

Figure 7. Lack of effect of pentobarbital or etomidate on the LHP. A_1 and B_1, Control responses to antidromic stimulation; perfusion with 10 μM BMI effectively eliminates the initial GABAergic hyperpolarizing response (BMI), and in *A* uncovered an underlying depolarizing response as well. The addition of 100 μM pentobarbital (A_3) or 10 μM (+)-etomidate (B_3) did not result in a further change of the amplitude or decay time of the LHP component. The apparent decrease in the latency of and broadening of the peak in B_3 probably represents an increase in a residual GABAergic component that was not completely blocked by BMI. Calibration bars, 100 msec and 1 mV.

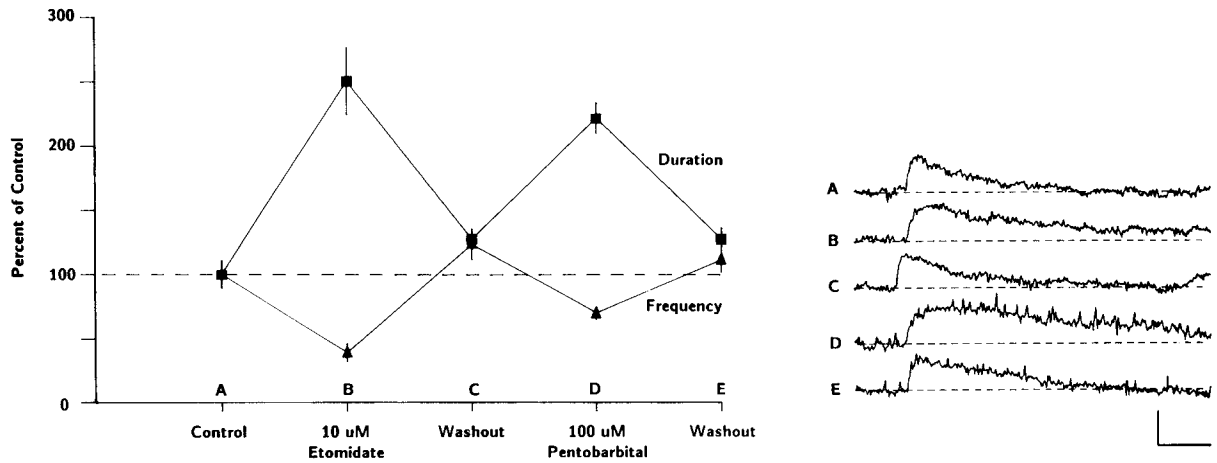

Figure 8. Effect of etomidate and pentobarbital on spontaneous IPSPs. Spontaneous IPSPs were recorded from a pyramidal neuron impaled with a KCl microelectrode. Duration was determined as the time between the peak of the depolarization and when the response declined to 37% of its peak value (*square symbols*), and each point represents the mean ± SEM percent of control for 25–50 individual events. Frequency determinations are indicated by *triangles* and represent the mean ± SEM (percentage of control) rate determined from 10–17 10 sec epochs during each condition. The mean control values are 57 ± 6 msec and 33 ± 3.8 spontaneous responses/min for the duration and frequency, respectively. At *right,* sample records of inverted IPSPs are shown for each condition. The increase in duration of the spontaneous IPSP is evident in the presence of either drug. All records shown are single sweeps. Calibration bars, 25 msec and 0.5 mV.

for these 2 drugs. Other electrophysiological studies have indicated that the manifestation of this interaction with GABA binding is likely to be a prolongation of the time during which the chloride channel remains open (Study and Barker, 1981). This conclusion is consistent with our findings, in that there was no change in rate of rise of the recurrent IPSP and relatively small changes in IPSP amplitude, but a markedly enhanced duration. The increased amplitude and somewhat prolonged latency to peak for the IPSP in the presence of pentobarbital or etomidate may be the result of greater synchrony in the response; if the channels remain open for a longer period of time, then it is less likely that some will have closed before others have opened. Our general conclusion, that the GABA-mediated IPSP is selectively enhanced by these drugs while the inhibitory K^+-dependent LHP remains unchanged, is consistent with the recent observation that barbiturates can enhance the fast GABA-stimulated chloride flux into brain microsacs (Harris and Allan, 1985). In those studies, the effects of barbiturates were quite selective, in that they did not appear to affect the flux of ions through calcium, sodium, or potassium channels.

Although etomidate does not have the characteristic barbiturate structure, the similarity between the biochemical and functional effects of these drugs suggests that they act at a common stereoselective site on brain membranes that appears to be intimately associated with the GABA receptor and Cl^- ion channel. The interactions of etomidate and pentobarbital with a receptor coupled to the putative GABA/BZ/chloride channel complex may be responsible for the depressant effects of these drugs upon the CNS.

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