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Facilitation of Recurrent Inhibition in Rat Hippocampus by Barbiturate and Related Nonbarbiturate Depressant Drugs[1]

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ABSTRACT

The effects of anticonvulsant, anesthetic and convulsant barbiturates and of related depressant drugs were characterized on excitatory and inhibitory synaptic transmission in slices of rat hippocampus. The duration of recurrent GABAergic inhibition was increased by all of the drugs tested, including the convulsant barbiturate 5-ethyl-5-[1,3-dimethylbutyl]barbituric acid, anesthetic barbiturates such as pentobarbital and nonbarbiturate anesthetics such as (+)-etomidate. Several barbiturates, including phenobarbital and (+)-mephobarbital facilitated inhibition, but the maximal responses to these agents were significantly less than with pentobarbital. In general, there was a good correspondence between the potencies of these drugs in facilitating inhibition and their previously reported abilities to regulate binding at the γ-aminobutyric acid/benzodiazepine/barbiturate receptor complex. In addition to facilitating recurrent GABAergic inhibition, at successively higher doses most of these drugs induced direct depression of the population spike response, field excitatory postsynaptic potential and presynaptic fiber spike. 5-Ethyl-5-[1,3-dimethylbutyl]barbituric acid, (+)-mephobarbital and pentobarbital facilitated excitatory synaptic transmission at the Schaffer collateral/commissural synapses on the CA1 pyramidal neurons at low doses, but caused depression at higher doses. The net effects observed with each drug tested (facilitation/depression of excitatory transmission, enhancement of GABAergic inhibition) correlated well with the behavioral effects of these agents *in vivo*.

Barbiturate drugs have a multitude of actions in intact animals including anticonvulsant, sedative, anxiolytic, anesthetic and in some cases convulsant effects. The particular responses that are observed depend upon both the specific barbiturate as well as the concentration of drug reaching the central nervous system. In electrophysiological terms the actions of the barbiturates are no less complex; these drugs have been reported 1) to potentiate the actions of GABA in concentrations that have no direct action by themselves, 2) to antagonize the actions of GABA antagonists such as picrotoxin in concentrations that do not potentiate responses to GABA, 3) to mimic the effects of GABA, 4) to antagonize excitatory responses to amino acids such as glutamate, 5) to exert local anesthetic-like actions on cellular excitability and 6) to directly depolarize neurons in a picrotoxin- and bicuculline-insensitive fashion [see Willow and Johnston (1983) for review]. These actions are further complicated by the subdivision of barbiturates into "anticonvulsant," "anesthetic" and "convulsant" drug classes. Most barbiturates have multiple actions, and the differences between them are primarily quantitative rather than qualitative.

In biochemical studies, perhaps the most striking action of the barbiturates is their ability to allosterically regulate GABA and BZ binding and to bind to a putative picrotoxin-sensitive barbiturate binding site associated with the GABA-BZ receptor complex. However, the ability of drugs to regulate GABA-BZ binding does not necessarily predict their actions *in vivo*; anesthetic barbiturates such as pentobarbital are relatively potent modulators of GABA-BZ binding, but the convulsant barbiturate DMBB (Downes *et al.*, 1970) shares this action as well (Olsen and Snowman, 1982). On the other hand, anticonvulsant barbiturates such as phenobarbital have little effect upon GABA-BZ binding. At the cellular level, it has been equally difficult to relate biochemical actions to specific physiological responses, because in many studies only a single response is characterized, or alternatively the effects of a single drug are characterized on several different kinds of responses.

In the present experiments, we have tried to resolve these difficulties by characterizing the actions of barbiturate drugs in a preparation that contains a known barbiturate-sensitive GABAergic pathway, *viz.*, the recurrent inhibitory circuit to the CA1 pyramidal neurons of the rat. Previous studies have

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ABBREVIATIONS: GABA, γ-aminobutyric acid; DMBB, 5-ethyl-5-[1,3-dimethylbutyl]barbituric acid; BZ, benzodiazepine; IPI, interpulse interval; fEPSP, field excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; MPPB, N'-methyl-5-phenyl-5-propylbarbituric acid.

shown that recurrent inhibition in the hippocampus is potently affected by anesthetic barbiturates such as pentobarbital. Nicoll *et al.*, (1975) originally demonstrated that barbiturates prolong the duration of GABAergic inhibition in intact animals, and a variety of studies using both intra- and extracellular recording techniques have confirmed these original observations both *in situ* and in *in vitro* brain slices as well (Wolf and Haas, 1977; Alger and Nicoll, 19821a; Roth *et al.*, 1983; Gage and Robertson, 1985). We have recently demonstrated that such actions are shared by etomidate, a nonbarbiturate drug that has pharmacological actions similar to pentobarbital (Proctor *et al.*, 1986; Ashton and Wauquier, 1985). Pentobarbital has also been reported to facilitate a depolarizing potential elicited by *synaptic* activation of the CA1 pyramids (Alger and Nicoll, 1982a), but we have not observed such a response after purely antidromic activation of these neurons (Proctor *et al.*, 1986). Thus the *in vitro* hippocampal slice provides an appropriate test system in which to characterize the actions of drugs that facilitate transmission at GABAergic synapses in the central nervous system and to compare these actions to the previously reported ability of these agents to modulate GABA and BZ binding to brain membranes. Finally, we have characterized not only the facilitation of GABAergic inhibition, but also the other physiological responses to barbiturates that can be observed in this preparation.

Methods

Male Sprague-Dawley rats weighing between 120 and 200 g obtained from Sasco (Omaha, NE) were decapitated, and the hippocampus was dissected free of surrounding tissue. Coronal sections taken from the middle portion of the hippocampi were prepared, as described previously (Mueller *et al.*, 1981; Dunwiddie and Lynch, 1978). Slices were cut at 400 μm on a Sorvall tissue chopper and immediately placed in ice-cold medium consisting of (millimolar): NaCl, 124; KCl, 4.9; KH_2PO_4, 1.2; $MgSO_4$, 2.4; $CaCl_2$, 2.5; $NaHCO_3$, 25.6; and glucose, 10 (pH 7.5); that was pregassed with 95% O_2 and 5% CO_2. Slices were transferred within 5 min to a recording chamber maintained at 34.5°C. The slice was not superfused with medium during the pretreatment recovery period, and the level of the medium was maintained level with or just below the upper surface of the slice. Slices were normally maintained without perfusion until they were to be tested, at which time a constant flow of fresh oxygenated preheated medium was initiated at a rate of 2 ml/min.

Electrophysiological recordings were made with 2- to 3-megohm glass microelectrodes filled with 5 M NaCl, placed in the cell layer under visual guidance. Twisted nichrome wire stimulation electrodes were placed in the stratum radiatum near the border of CA1-CA2 (synaptically evoked population spike response) or on the alveus near the CA1-subicular border (antidromic stimulation). Monophasic 0.1-msec pulses of 6 to 30 V were delivered to the synaptic pathway at 1-min intervals, and the voltage was adjusted to evoke a 3-mV population spike. In the case of barbiturates that directly affected the population spike amplitude (*e.g.*, DMBB and pentobarbital), the stimulation voltage was changed during the course of drug perfusion so that the amplitude of the control spike remained at 3 mV. The stimulation protocol involved either single synaptically evoked responses (control response) or paired stimuli consisting of antidromic stimulation of the axons of the pyramidal neurons to activate the recurrent GABAergic inhibitory pathway, followed by stimulation of the Schaffer/commissural synaptic input to determine the degree of inhibition of the population of CA1 pyramidal neurons (test response). The amplitude of the antidromic stimulus was adjusted so that the test response was initially inhibited by 50% at a 40-msec IPI. Slices that could not meet this criterion were not tested further. The interval between the antidromic stimulation and the test response was then systematically varied, and the percent inhibition of the population spike (I) was determined for a range of IPIs. Plots of $\log_{10}$(IPI) *vs.* $\log_{10}((100-I)/I)$ were usually quite linear (correlation coefficients between 0.80 and 0.99), and the intercept with the abscissa, which corresponds to the IPI for which the population spike response is inhibited by 50% (IPI_{50}), was estimated by fitting a line to the data using a least-squares criterion. The increase in the IPI_{50} during perfusion with drug was used as the measure of drug action. This measure is particularly sensitive to changes in the *duration* of the recurrent inhibition, which has previously been shown to be markedly affected by barbiturates such as pentobarbital (Nicoll *et al.*, 1975; Proctor *et al.*, 1986).

All drugs were made up in deionized water at 100 to 1000 times the desired final concentration, then added to the flow of perfusion fluid with a calibrated Sage model 355 syringe pump. Pentobarbital, phenobarbital and mephobarbital were obtained commercially (Sigma Chemical Co., St. Louis, MO), (+)- and (−)-etomidate from Janssen Pharmaceutica (Beerse, Belgium), etazolate from E.R. Squibb and Sons, Inc. (Princeton, NJ), DMBB from Eli Lilly & Co. (Indianapolis, IN) the isomers of mephobarbital and MPPB from Dr. J. Knabe.

In comparisons between drugs and in dose-response curves, *N* refers to the number of different slices that were tested with a given drug at a fixed concentration. Although several slices from the same preparation were often tested with the same drug, care was taken that each drug was tested in slices from at least three different animals. When multiple doses were tested on the same slice, the same dose was not tested more than once.

Individual slices were considered to have shown a significant response to a drug when the mean amplitude during drug perfusion was more than 2 S.D. different from the amplitude of the response during a 10-min predrug control period. The significance of changes in the IPI_{50} (again, for single slices) was assessed by determining the 95% confidence limits for the intercept with the *x*-axis, which is the IPI_{50} (Tallarida and Jacob, 1979); if the IPI_{50} for the control condition was not contained within the 95% confidence limits for the treatment condition, the change was considered to be statistically significant with $P < .05$. The statistical significance of the correlation coefficient was assessed according to Meredith (1967).

Results

Direct actions. The primary intent of the present experiments was to characterize the actions of barbiturate and related drugs upon recurrent GABAergic inhibition. Nonetheless, it was clear from the outset that most of these drugs directly affected the amplitude of evoked synaptic responses. For this reason, an initial series of experiments was conducted to characterize the effects of some of these drugs upon transmission at the Schaffer and commissural fiber afferents to the CA1 region.

The most common direct effect of perfusion with barbiturate drugs was a depression in the amplitude of evoked synaptic responses. The presynaptic fiber spike, which reflects activity in the presynaptic nerve fibers and terminals, the fEPSP, which is the extracellular manifestation of synaptic currents in the postsynaptic cell, and the population spike response, which results from the summated firing of the target neurons, were all reduced in magnitude by these depressant agents (fig. 1). These types of effects were seen with all of the drugs subsequently tested (see below), provided that high enough concentrations of drug were used. Dose-response curves for pentobarbital and phenobarbital are illustrated in figure 2. Marked changes in the amplitude as well as in the latency of all of these responses were observed, with pentobarbital being somewhat more potent than phenobarbital. We have previously shown that the decreased amplitude of the fiber spike, the increase in latency and similar changes in the antidromically activated

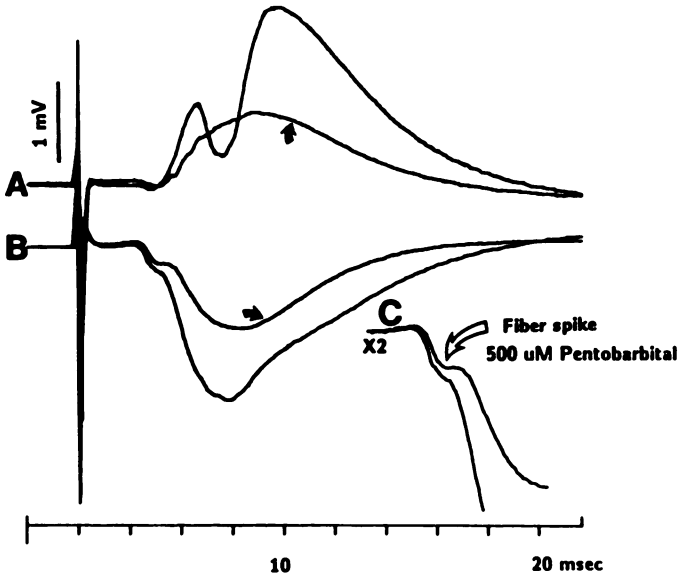


Fig. 1. Local anesthetic actions of pentobarbital. The direct depressant effect of pentobarbital is illustrated on the evoked response to Schaffer collateral and commissural stimulation. The responses were recorded in the cell layer (A) and in stratum radiatum (B). The inset (C) shows the same records as in B at higher gain. The responses were recorded either before or after 10 min of perfusion with 500 μM pentobarbital (solid arrows). The presynaptic fiber spike (open arrow), which represents the compound action potential generated by activation of presynaptic fibers, was also depressed in amplitude by pentobarbital. Time calibration for all records is at the bottom, voltage calibration is 1 mV for the full-size records and 0.5 mV for the inset. In this and in all subsequent records, positive polarity is up, and the deflection at 2 msec delay is the stimulus artifact corresponding to the electrical stimulation of the presynaptic fibers.

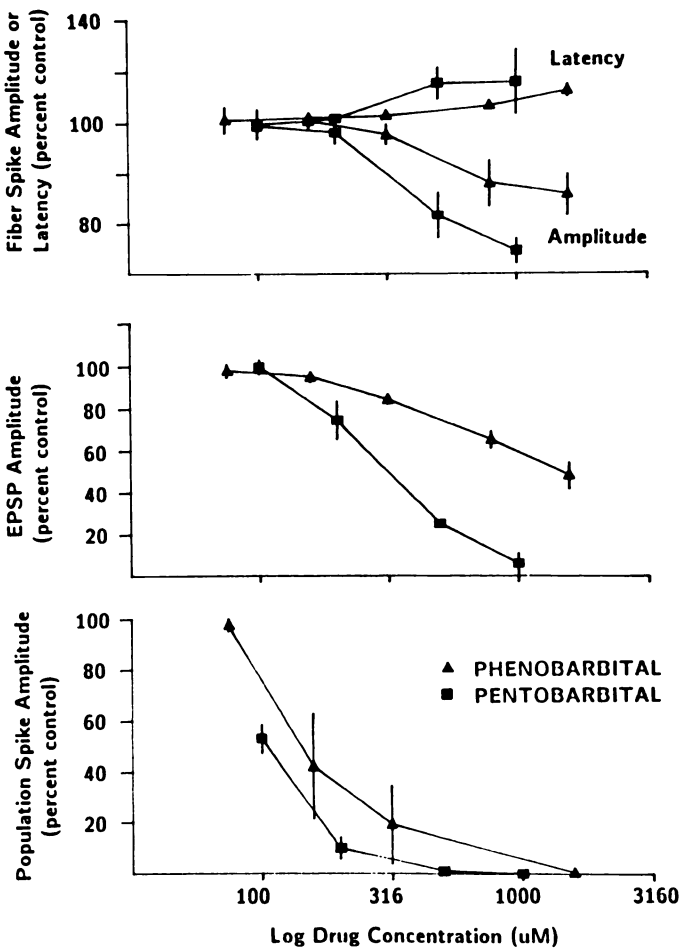


Fig. 2. Log dose-response curves for the direct depressant effect of pentobarbital and phenobarbital. Dose-response curves for the depressant actions of pentobarbital and phenobarbital on evoked synaptic responses are illustrated. Both drugs increased the latency to the peak of the presynaptic fiber spike and depressed its amplitude (top) and depressed the amplitude of the fEPSP (middle) and population spike response (bottom). The effect of drug is presented as a percentage of the predrug control response, and each point represents the mean ± S.E.M. for at least four slices. As with other local anesthetic drugs (*e.g.*, cocaine, lidocaine), the relative sensitivity of these responses in terms of percent change was population spike > fEPSP > presynaptic fiber spike. EC_{50} values were calculated from Hill plots of the raw data as described by Tallarida and Jacob (1979); pentobarbital was significantly more potent than was phenobarbital in depressing the fiber spike, fEPSP and population spike amplitudes, in that there was no overlap between the 95% confidence limits for the EC_{50} values.

population spike (not shown) are all characteristic of local anesthetic drugs (Yasuda *et al.*, 1984; Scholfield and Harvey, 1975). It was not possible to determine whether synaptic transmission *per se* was affected by the barbiturates, because the drug-induced shift in both the latency and magnitude of the fiber spike made it impossible to measure the magnitude of the fiber spike accurately, and hence to characterize shifts in the fiber spike/fEPSP ratio. For similar reasons, it was not possible to use input-output curves to determine whether the barbiturates directly depressed the population spike response or whether the changes in this parameter were secondary to decreases in the fEPSP response. However, the marked decline in the population spike response with no apparent change in the fEPSP (*e.g.*, fig. 2, 100 μM pentobarbital) suggested that these drugs could directly reduce excitability of the pyramidal neurons without affecting either excitatory synaptic transmission or the excitability of the afferent fibers.

In addition to the depressant effects of these drugs, excitatory actions were observed as well. An example is shown in figure 3A, where a low dose of pentobarbital induced a 2.2-fold increase in the magnitude of the population spike response; this effect was reversed at a concentration of 100 μM, and the population spike was abolished at 500 μM. With pentobarbital, the excitatory effect was not always observed, and when it did occur, it was at low drug concentrations, or occurred early during drug perfusion and was transient. With pentobarbital, the most consistent excitations occurred at a concentration of 75 μM, with four of six slices showing such increases in the population spike response. The convulsant barbiturate DMBB had excitatory effects on nearly every slice tested, and it proved easier to study these actions using this drug. Figure 3B illustrates the effects of DMBB on both the fEPSP and population spike responses to constant voltage stimulation. Perfusion with DMBB increased the magnitude of the population spike response and the fEPSP response, but not the presynaptic fiber spike (not illustrated). These findings suggest that the increased spike was secondary to increased synaptic excitation of the pyramidal neurons; because of the lack of change in the presynaptic fiber spike, the increased synaptic response cannot be the result of the activation of a greater number of presynaptic fibers. Table 1 summarizes the direct actions of all of the barbiturate and nonbarbiturate drugs tested in terms of effects on the amplitude of the population spike response.

Effects upon recurrent inhibition. We have previously

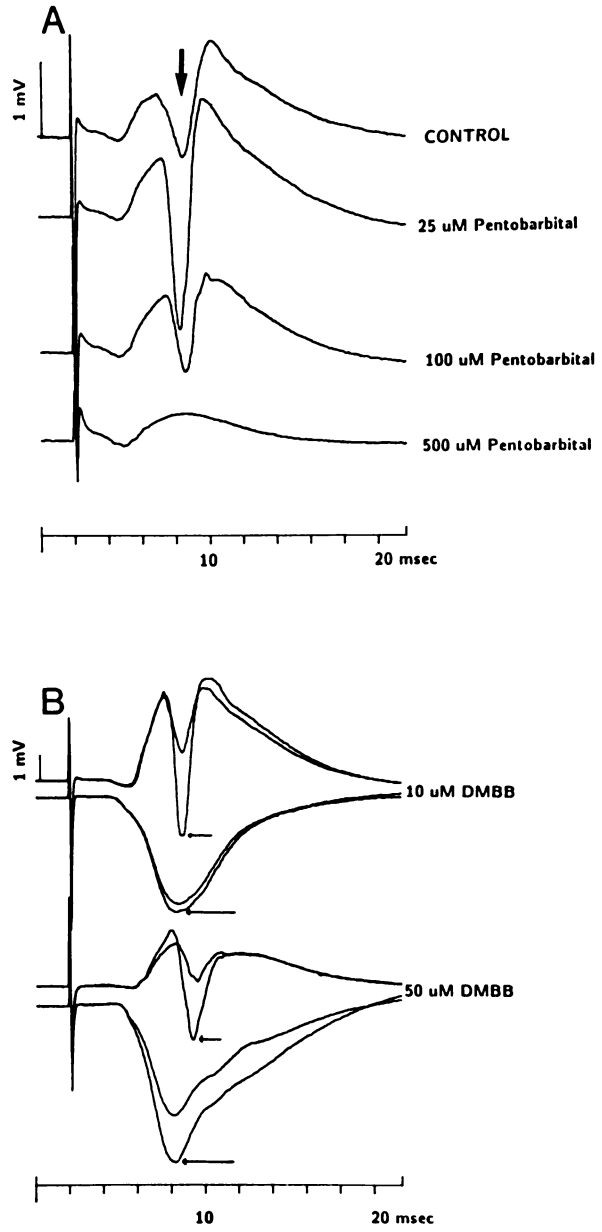


Fig. 3. Direct excitatory/inhibitory actions of barbiturates. In A, the biphasic direct actions of pentobarbital are shown; 25 μM pentobarbital significantly increased the amplitude of the population spike response (solid arrow). A 100 μM concentration of pentobarbital depressed the amplitude back to control levels, and 500 μM completely abolished the population spike response, but not the inverted fEPSP recorded in the cell layer. B illustrates responses of two different slices to perfusion with DMBB. Both showed excitatory responses, with increases in both the population spike responses as well as in the fEPSP. The upper records of each set are derived from the cell layer, the lower from stratum radiatum, and the control and drug responses are superimposed in each case. The evoked responses in the presence of drug are denoted by arrows. Calibration bar represents 1 mV for all records.

shown that antidromic stimulation of the alveus initiates a biphasic inhibitory potential in the CA1 pyramidal neurons (recorded intracellularly) and that barbiturates increase the duration of the initial bicuculline-sensitive chloride-dependent GABAergic component of the IPSP (Proctor *et al.*, 1986). In the present study we have used extracellular recording techniques to characterize this effect for a number of depressant drugs. The protocol used to measure the duration of recurrent

TABLE 1

Direct effects of depressant drugs upon population spike amplitude

Direct effects on population spike amplitude are characterized as frequent (observed >50% of the time; ++), occasional (10–50%, +), or not observed (N.O.) The approximate threshold concentrations required to elicit these direct effects are given as well. A slice was considered to have responded when the mean amplitude of the response during drug was more than 2 S.D. different from the mean control amplitude. In the case of drugs showing excitatory responses, a biphasic excitatory/inhibitory response to high drug concentrations was usually observed.

Drug	Inhibitory Responses	Excitatory Responses
DMBB	+ (250 μM)	++ (10 μM)
(+)-Mephobarbital	+ (250 μM)	++ (100 μM)
(+)-MPPB	+ (250 μM)	++ (100 μM)
Pentobarbital	++ (25–50 μM)	++ (25 μM)
(−)-Mephobarbital	++ (50 μM)	+ (50 μM)
(±)-Mephobarbital	+ (50–100 μM)	+ (50–75 μM)
(−)-MPPB	+ (250 μM)	+ (250 μM)
Etazolate	++ (5–10 μM)	N.O.
(+)-Etomidate	++ (10 μM)	N.O.
Phenobarbital	++ (100 μM)	N.O.
(−)-Etomidate	++ (100 μM)	N.O.

inhibition is illustrated in figure 4. Varying the interval between antidromic stimulation of the alveus and subsequent stimulation of the Schaffer and commissural fibers results in differing degrees of inhibition of the test response. When a numerical transformation of the percent inhibition is plotted *vs.* the log interpulse interval (see "Methods"), a nearly linear function is obtained, and the intercept with the abscissa can be used to estimate the IPI corresponding to a 50% inhibition of the test response (IPI_{50}).

When slices were tested at a fixed antidromic-synaptic interval, perfusion with low concentrations of pentobarbital led to increased inhibition of the test response with little or no change in the amplitude of the control response (fig. 5). Because barbiturates have frequently been reported to have a GABA-mimetic effect at higher concentrations (as opposed to facilitating the effects of GABA), we contrasted the response to pentobarbital with the response to GABA itself. At a concentration that did not affect the population spike amplitude (100 μM), GABA had no effect on the inhibition of a test response; a higher concentration that directly depressed the population spike (500 μM) also did not increase recurrent inhibition (fig. 6). The only significant effect of GABA upon the IPI_{50} was a *decrease* in the duration of inhibition that was sometimes observed at high GABA concentrations with long periods of exposure to the transmitter. Thus, the increase in the duration of inhibition is a response that is characteristic of pentobarbital, but not of GABA itself.

Dose-response relationships for pentobarbital and other depressant drugs were obtained using the protocol outlined in fig. 4. Figure 7 illustrates recurrent inhibition in a single slice perfused with 25, 50 and 75 μM pentobarbital in succession; each increment in the concentration of pentobarbital induced a rightward shift in the line, indicating an increase in the duration of inhibition, and, in most cases, an increase in the slope as well. Qualitatively similar shifts in IPI_{50} curves were observed with all of the active drugs tested. Figure 8 illustrates dose-response curves for the drug-induced shift in the IPI_{50} curves. By and large, all of the drugs tested appeared to fall into two categories, being either full agonists (able to increase the IPI_{50} by at least a factor of 5, with a relatively steep slope for the dose-response curve), or weak agonists with shallow dose-response relationships (phenobarbital, the isomers of MPPB and (+)-mephobarbital, not shown).

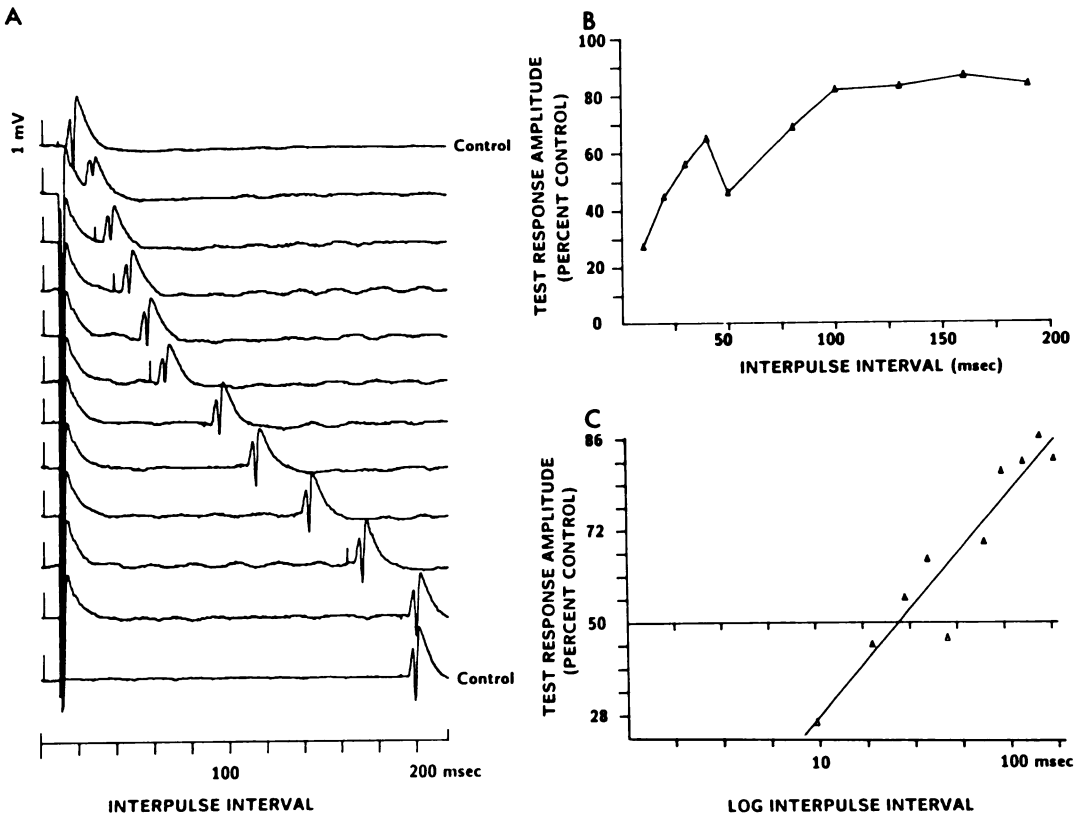


Fig. 4. Determination of IPI_{50} values. A illustrates single evoked test responses with varying delays between antidromic stimulation of the alveus and synaptic stimulation of the CA1 pyramidal neurons. The antidromic responses (which occur at approximately 10 msec after the beginning of the record) are not clearly seen in this example because at this gain they are superimposed. Records marked "Control" are those in which alvear (antidromic) stimulation was not given. Interpulse intervals were 10, 20, 30, 40, 50, 80, 100, 130, 160 and 190 msec. Depression was greatest at the shorter intervals. In B, the data from A are illustrated with the amplitude of the test response at each interval expressed as a percentage of the average of the two control responses. The break in the curve at 50 msec was occasionally observed and is characteristic of intracellular recordings of the recurrent IPSP as well (Proctor *et al.*, 1986). In C, these same data are graphed as the $\log_{10}$ (IPI) *vs.* $\log_{10}((100-I)/I)$, and the points all fall relatively close to a straight line fit by a least-squares criterion; correlation coefficient was 0.95. The intercept of this line was the *x*-axis (in this case, at 27.7 msec) corresponds to the interval at which one would expect to observe a 50% inhibition of the test response (IPI_{50}).

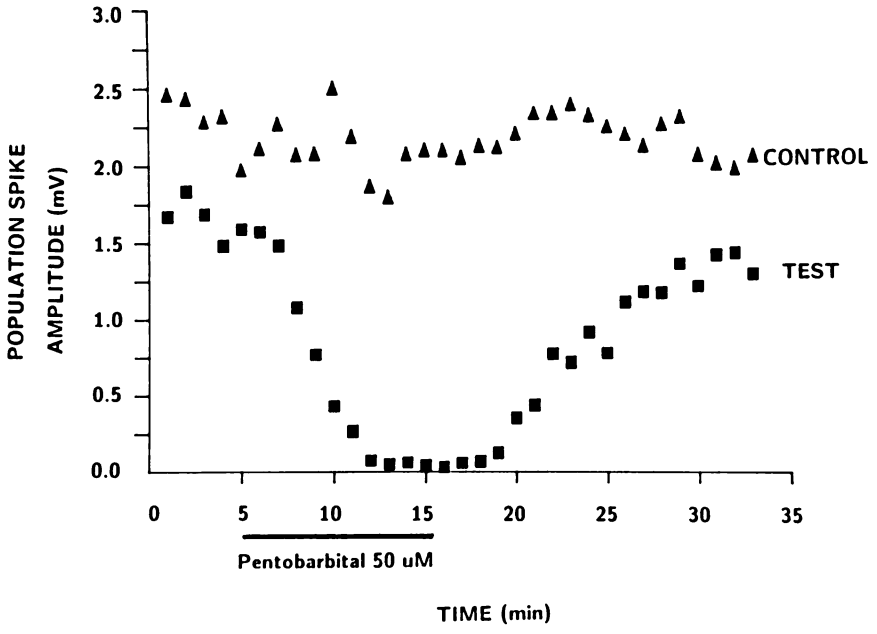


Fig. 5. Selective effect of pentobarbital on recurrent inhibition. Control and test responses elicited with a 100-msec IPI were evoked alternatively at 30-sec intervals before, during and after perfusion with 50 μM pentobarbital. In this slice, 50 μM pentobarbital had no significant effect upon the amplitude of single synaptically evoked population spike responses (control). The test response, which was depressed by recurrent inhibition by approximately 25% prior to pentobarbital perfusion, was completely suppressed at the 100-msec IPI during perfusion, but recovered to near base-line levels after drug washout.

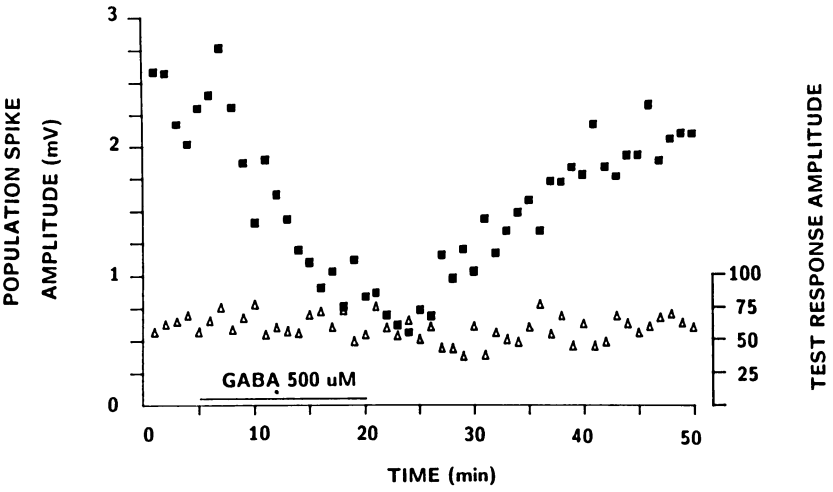


Fig. 6. Direct and indirect actions of GABA. Slices were tested with the same protocol as in the legend to figure 5, except that the IPI was 40 msec instead of 100 msec. Note that the amplitude of the test response (△) is plotted as a percentage of the preceding control response (right scale) instead of in millivolts. Although GABA depressed the control response by 80%, the test response expressed as a percentage of control was unaffected; in absolute terms, the test response was also depressed by 80%.

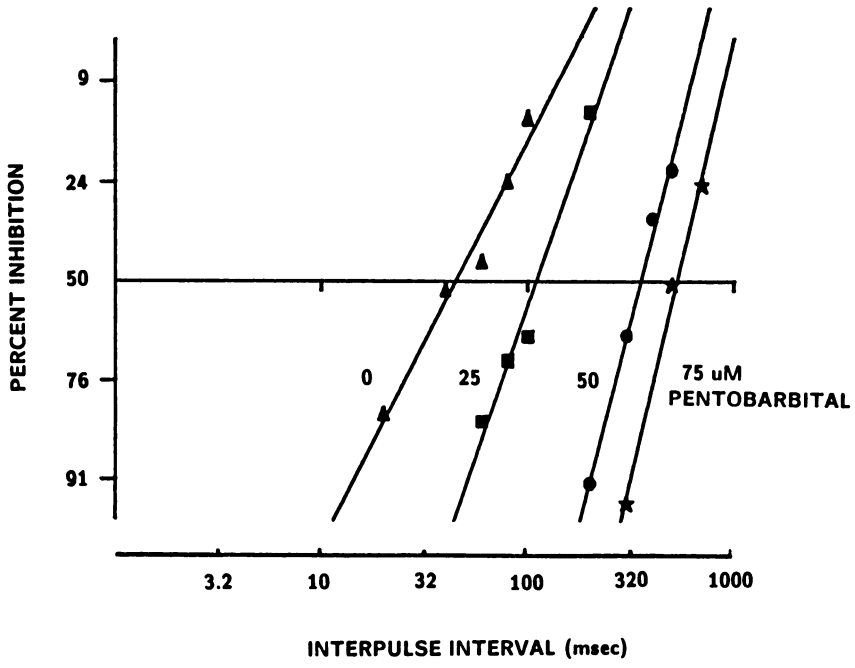


Fig. 7. Effect of pentobarbital upon recurrent inhibition. The duration of recurrent inhibition in a single slice was tested and analyzed as in the legend to figure 4C under control conditions (0) and during perfusion with 25, 50 and 75 μM pentobarbital. Lines were fit to the points by a least-squares criterion. Each increase in pentobarbital concentration shifted the IPI_{50} curves to the right. The magnitude in the shift in the x-intercept of the least-squares line was subsequently used as a measure of the drug response (see fig. 8).

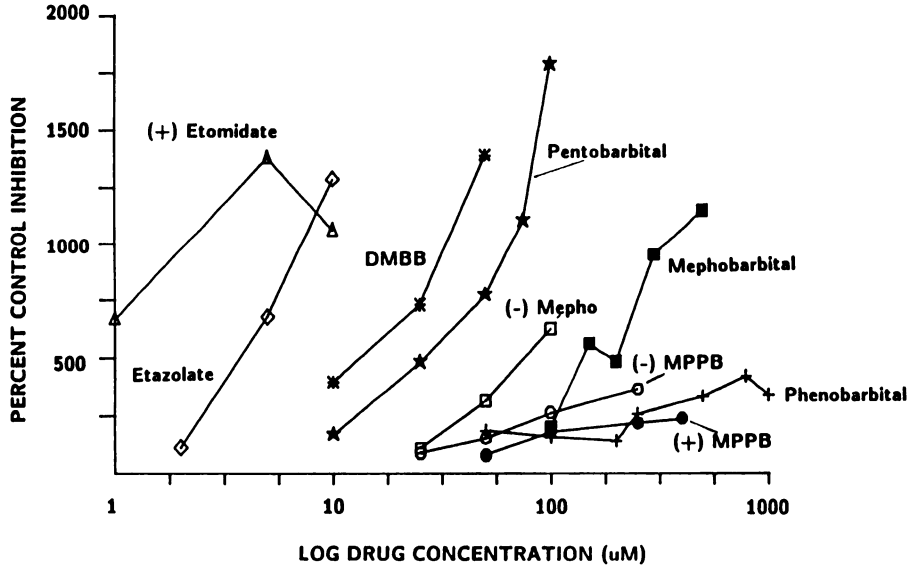


Fig. 8. Log dose-response curves for effects upon recurrent inhibition. Slices were tested with drugs as in the legend to figure 7, and the IPI_{50} in the presence of drug was expressed as a percentage of the control IPI_{50}. The mean shifts in the IPI_{50} for groups of slices treated in this manner are shown for a variety of depressant drugs (each point represents the mean from at least three slices tested at that concentration of drug). Dose-response curves for (−)-etomidate, (+)-mephobarbital and S.E.M. bars were omitted for clarity.

Determining quantitative estimates of the potency of these agents was somewhat difficult for several reasons. First, at higher concentrations of most drugs the direct effects interfered substantially with synaptic responses, making it impossible to determine an IPI_{50} value. For example, at a concentration of 500 μM pentobarbital, a 3-mV control response could not be evoked from most slices regardless of stimulation voltage. For this reason, a "maximal" drug response often could not be determined. In addition, at higher concentrations many of the dose-response curves began to decline, perhaps because of difficulties in evoking the control response. Finally, the maximal shift in the IPI_{50} curve for a given slice was not a fixed value, but depended upon a variety of factors such as the viability of the slice, the amplitude of the control response and the amplitude of the antidromic stimulus. Despite these difficulties, in order to gain some type of qualitative measurement of drug potency, we used the dose-response data derived from all of the slices tested with a given drug to calculate the concentration required to increase the IPI_{50} interval 5-fold ($EC_{5\times}$ value). These results are summarized in table 2.

In terms of absolute potencies, the nonbarbiturate depressants etazolate and (+)-etomidate were the most potent, followed by the so-called convulsant barbiturate DMBB, pentobarbital and (−)-mephobarbital. At the other end of the range, concentrations of (+)-MPPB and phenobarbital sufficient to induce a 5-fold shift in inhibition could not be attained; these could only be estimated by extrapolation. However, it should be emphasized that even though these compounds appeared to be very inactive, this was more a reflection of weak maximal activity rather than low potency. For example, even a concentration of 50 μM phenobarbital produced a significant 3-fold increase in the IPI_{50} in one slice tested; what distinguished phenobarbital from drugs such as pentobarbital was that even at high concentrations, these drugs were not very efficacious. Out of 25 slices tested with phenobarbital at concentrations between 50 to 1000 μM, *none* showed greater than 7-fold shifts in the IPI_{50}. By comparison, 14 of 29 slices tested with pentobarbital at concentrations between 10 to 100 μM showed *greater* than 7-fold shifts. The ability to elicit weak but significant shifts in the IPI_{50} value was observed with phenobarbital, (+)-mephobarbital and the isomers of MPPB.

The (+) and (−) forms of etomidate have been reported to differ by over 1 order of magnitude in terms of their ability to regulate GABA and BZ binding (Ashton *et al.*, 1981; Olsen *et al.*, 1986), and a comparable difference was observed in terms of their ability to facilitate GABAergic inhibition. Figure 9 illustrates IPI_{50} curves for the same brain slice tested with a 10 μM concentration of both (+)- and (−)-etomidate. This concentration of the (+)-isomer induced about a 10-fold shift in the IPI_{50} value, whereas there was an approximately 3-fold shift with (−)-etomidate at this concentration. The calculated $EC_{5\times}$ values support this difference in potency, with there being a 100-fold difference between the isomers (table 2). In comparison with the effects of the active barbiturates tested in these studies, benzodiazepines were only weakly active. As seen in figure 10, flurazepam at a relatively high concentration (10 μM) induced a significant but quantitatively small shift in the IPI_{50} compared to the same concentration of (+)-etomidate.

In binding experiments, phenobarbital has been reported to *antagonize* the ability of pentobarbital to allosterically regulate binding of ligands to BZ receptors (Leeb-Lundberg and Olsen, 1982). To test for such an antagonistic interaction, slices were treated according to several different protocols. Groups of slices were pretreated with phenobarbital (500 μM) or control medium for 1 hr (without electrophysiological testing) and then tested for pentobarbital-induced changes in the IPI_{50}. At a concentration of 50 μM pentobarbital, there was no significant difference in the magnitude of the response to pentobarbital (IPI_{50} 167+/−29 msec for control, 149+/−21 msec for phenobarbital pretreated, $N = 7$, $P > .10$, Student's t test). A second group of slices were tested initially with 100 μM pentobarbital, which induces a maximal increase in the IPI_{50} (fig. 8), then 500 μM phenobarbital was added to the superfusion medium. In no case was a decrease in the IPI_{50} observed, which would have been expected had phenobarbital acted as a partial agonist. A third protocol is illustrated in figure 11; in this example, a slice was tested with 100 μM pentobarbital alone, the pentobarbital was washed out, and then it was tested with phenobarbital and pentobarbital. The effects of pentobarbital and phenobarbital were not additive, because the initial response to pentobarbital was near maximal for this slice, but phenobarbital clearly did not significantly antagonize the effects of pentobarbital on the IPI_{50}.

TABLE 2

Effects of drugs upon recurrent inhibition

The concentration of each drug required to increase the IPI_{50} curve 5-fold was calculated by plotting the $\log_{10}$ of the percent increase in the $IPI_{50}/500$ *vs.* $\log_{10}$ drug concentration. The intercept with the abscissa corresponds to the concentration at which the IPI_{50} is increased 5-fold, and 95% confidence limits were calculated by determining the 95% limits for the estimate of the intercept based upon a line fit to the data points by a least squares criterion. Values marked with ‡ were calculated by extrapolation, because it was not possible to test either drug at the appropriate concentration. The correlation coefficient and the slope pertain to the least squares line; these parameters could not be calculated for (+)-mephobarbital, because although it did significantly enhance inhibition (albeit weakly), there was an overall negative correlation between concentration and inhibition within the range tested (100–250 μM). *N* refers to the number of slices tested with each drug.

Drug	$EC_{5\times}$	95% Confidence Limits	Correlation Coefficient	Slope	*N*
	μM	μM			
(+)-Etomidate	1.3	0.31–5.6	0.66	0.33	13
Etazolate	6.6	3.8–11	0.55	1.21	11
DMBB	16	7.4–33	0.73	0.58	10
Pentobarbital	31	21–45	0.69	0.93	29
(−)-Mephobarbital	80	49–130	0.79	1.25	9
(−)-Etomidate	130	19–920	0.81	0.39	6
Mephobarbital	195	140–280	0.68	0.79	29
(−)-MPPB	490	190–1300	0.64	0.52	18
Phenobarbital	2100‡	500–9000	0.55	0.42	25
(+)-MPPB	2500‡	200–33000	0.53	0.35	16
(+)-Mephobarbital					7

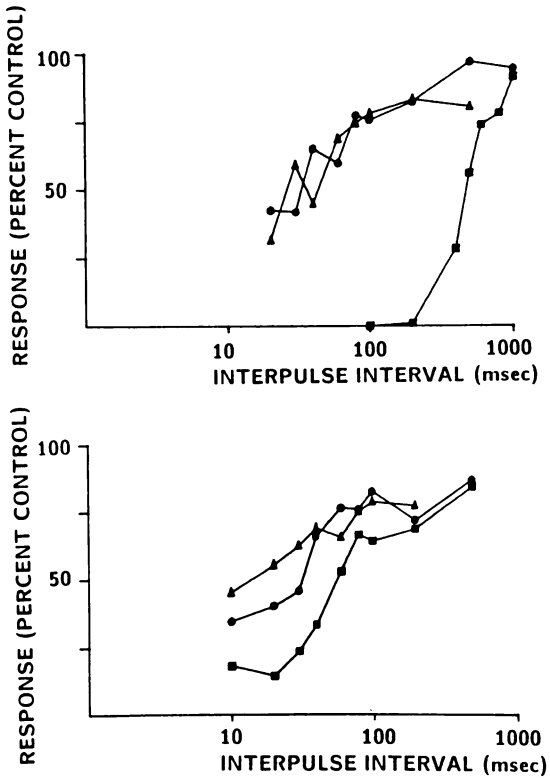
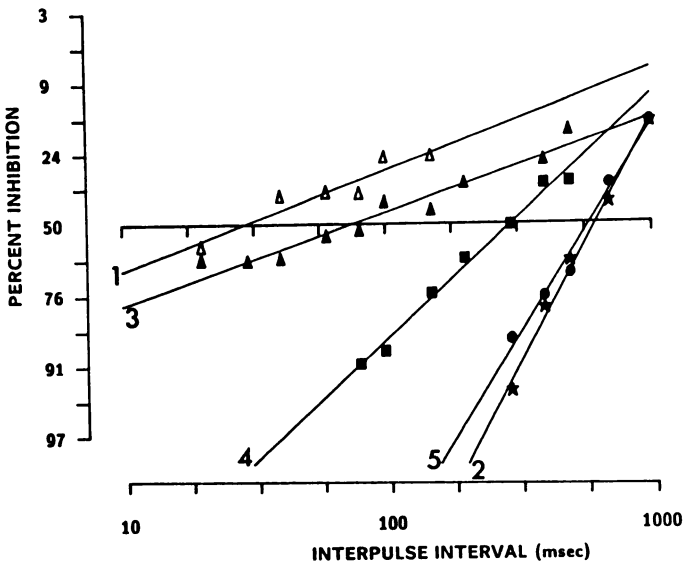


Fig. 9. Effects of (+)- and (−)-etomidate on recurrent inhibition. The effects of successive treatment with the isomers of etomidate on the same slice are illustrated. In each case, the predrug curve is denoted by ▲, the drug response by ■ and the washout by ●. Perfusion with 10 μM (+)-etomidate (top) markedly increased the duration of recurrent inhibition, and this effect was completely reversible upon washing. Subsequent testing with the same concentration of (−)-etomidate (bottom) elicited a significant but quantitatively much smaller shift in the IPI_{50} value.



	CONDITION	R	IPI_{50} (msec)
1	CONTROL	0.94	28.8
2	PENTOBARBITAL 100 uM	0.99	622 [577-671]
3	WASHOUT	0.97	75
4	PHENOBARBITAL 500 uM	0.99	302
5	PHENOBARBITAL + PENTOBARBITAL 100 uM	0.99	590 [531-655]

Fig. 11. Pentobarbital/phenobarbital interactions. A single slice was perfused successively with control medium, 100 μM pentobarbital, control medium, 500 μM phenobarbital and 500 μM phenobarbital + 100 μM pentobarbital. The legend at the bottom gives the correlation coefficients for the least-squares lines, the IPI_{50} values and the 95% confidence limits for the IPI_{50} values during the two pentobarbital responses. The responses to phenobarbital and pentobarbital were clearly not additive, but the response to pentobarbital, which is essentially a maximal drug response, was not significantly different under the two conditions.

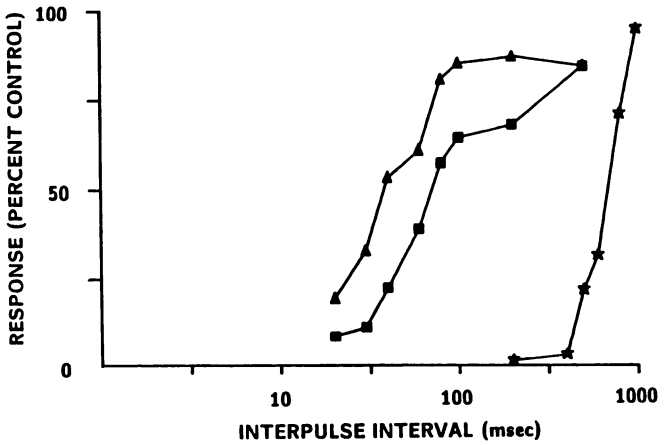


Fig. 10. Comparison of the effects of flurazepam and (+)-etomidate. Inhibition curves are shown for a single slice prior to drug perfusion (▲), during perfusion of 10 μM flurazepam (■) and during subsequent perfusion with 10 μM (+)-etomidate (★). The inhibition curve obtained during a washout period between the flurazepam and etomidate is not shown, but was comparable to the predrug base line. Although there was a significant shift induced by flurazepam (both the control and washout IPI_{50} values were outside of the 95% confidence limit range for flurazepam), such effects were quite small in magnitude, particularly in comparison with the effects of active drugs such as (+)-etomidate or pentobarbital.

Discussion

Multiple effects of barbiturates in the *in vitro* hippocampus. Previous work with barbiturate anesthetics such as pentobarbital has demonstrated at least four major types of responses. The first of these, GABA facilitation, is manifested as an increase in the duration of spontaneous and evoked chloride-dependent GABAergic IPSPs (Nicoll *et al.*, 1975; Scholfield, 1977; Alger and Nicoll, 1982b; Gage and Robertson, 1985), an increase in the duration of GABAergic inhibition of cell firing (Wolf and Haas, 1977; Tsuchiya and Fukushima, 1978), a selective facilitation of a depolarizing response to GABA (Alger and Nicoll, 1982a; Ransom and Barker, 1976), or as an increase in the amplitude and/or duration of responses to exogenous GABA (Ransom and Barker, 1976; Macdonald and Barker, 1979; Nicoll and Wojtowicz, 1980). The specific mechanism that appears to underlie many of these responses is a prolongation of the mean open time of the GABA-activated chloride channel (Study and Barker, 1981) and is reflected as well in a barbiturate-dependent facilitation of $^{36}Cl^-$ flux in hippocampal slices and in cell-free preparations from brain (Wong *et al.*, 1984a; Harris and Allan, 1985). A second major class of actions of pentobarbital is the antagonism of the excitatory effects of amino acids such as glutamate; this has been observed both with exogenous application of excitatory

agents (Nicoll and Wojtowicz, 1980; Macdonald and Barker, 1979; Richards and Smaje, 1976) and at synapses that are thought to utilize excitatory amino acids as the transmitter (Richards, 1972; Scholfield and Harvey, 1975). In higher concentrations pentobarbital has direct GABA-mimetic actions (Macdonald and Barker, 1979; Schulz and Macdonald, 1981; Nicoll and Wojtowicz, 1980; Akaike *et al.*, 1985), and a barbiturate-stimulated $^{36}Cl^-$ flux has been demonstrated as well (Schwartz *et al.*, 1984; Wong *et al.*, 1984a; Harris and Allan, 1985). Finally, pentobarbital also appears to have local anesthetic type actions (Scholfield and Harvey, 1975; however, see Richards, 1972).

In the present studies, the most frequent response to low doses of pentobarbital was an increase in the duration of recurrent GABAergic inhibition, as evidenced by increases in the IPI_{50}. Previous reports have suggested that antagonism of the effects of excitatory amino acids is as important to the depressant effects of the barbiturates as is GABA facilitation (Richards, 1972; Richards and Smaje, 1976; Barker and Ransom, 1978; Macdonald and Barker, 1979; Nicoll and Wojtowicz, 1980). In contrast, we have consistently observed that at the Schaffer collateral/commissural synapses, which use either glutamate, aspartate or possibly N-acetylaspartylglutamate as the primary transmitter (Nadler *et al.*, 1976; Cotman *et al.*, 1981; Fonnum, 1984; Bernstein *et al.*, 1985), pentobarbital and phenobarbital markedly facilitate GABAergic inhibition at concentrations that were 6 to 20 times lower than those required to affect excitatory synaptic transmission (*e.g.*, fig. 5). The barbiturate-induced depression of transmission at a synapse in olfactory cortex (Scholfield and Harvey, 1975), at which an excitatory amino acid or N-acetylaspartylglutamate is the transmitter (Ffrench-Mullen *et al.*, 1985), and the depression of the fEPSP in the present experiments show an almost identical sensitivity to pentobarbital, suggesting that excitatory amino acid synapses in general may be less sensitive than are GABAergic synapses.

It is unclear why previous studies have indicated that the thresholds for pentobarbital modulation of glutamate and GABA responses are equivalent, whereas the present experiments seem to indicate a significantly greater sensitivity of GABAergic systems. However, there are clear pharmacological differences between responses to exogenous aspartate or glutamate, and the endogenous transmitter of the lateral olfactory tract (Ffrench-Mullen *et al.*, 1985), and it is possible that synaptic receptors are substantially less sensitive to barbiturates than are receptors activated by the application of exogenous glutamate. In this context, Teichberg *et al.* (1984) have shown that quisqualate- and kainic acid-stimulated $^{22}Na^+$ fluxes were reduced by barbiturates, whereas glutamate- and aspartate-stimulated fluxes were unaffected, supporting the hypothesis that different excitatory amino acid receptors may differ in their sensitivity to the barbiturates.

The local anesthetic actions of pentobarbital, which are manifested as a decrease in the presynaptic fiber spike component of the response, are only observed with rather high concentrations of drug (threshold approximately 300 μM). The depressant effects of both pentobarbital and phenobarbital upon the presynaptic fiber volley have previously been described by Scholfield and Harvey (1975) in slices from olfactory cortex, and there is both qualitative and quantitative agreement with the results of the present investigation. The threshold for the direct inhibition of veratridine-stimulated ^{24}Na uptake into synaptosomes (approximately 300 μM; Harris and Bruno, 1985) also corresponds well with the local anesthetic effects, suggesting that this latter response may directly reflect an inhibition of the function of sodium channels.

In summary, for both phenobarbital and pentobarbital, the concentrations of drug that facilitated GABAergic inhibition were usually below those required to elicit other types of physiological changes. In terms of relative sensitivity to pentobarbital, GABA facilitation $\geq$ direct excitation of the population spike response $>$ direct depression of the population spike $>$ depression of the fEPSP $>$ depression of the presynaptic fiber spike. Responses to phenobarbital had a similar rank sensitivity, with the exception that excitatory responses were not observed.

Pharmacological differences between barbiturates. Much attention has been drawn to the differences between barbiturates: some appear to have relatively greater utility as anticonvulsants (*e.g.*, phenobarbital), others are primarily useful as anesthetics (*e.g.*, pentobarbital), and some are convulsants (DMBB). In this context, the results of the present experiments suggest that the differences between phenobarbital and pentobarbital are both quantitative and qualitative. In good agreement with previous studies, the direct depressant effects of phenobarbital appear to be very similar to those of pentobarbital, except that phenobarbital is somewhat less potent (fig. 2). However, with respect to the facilitation of recurrent inhibition, phenobarbital is not only less potent, but also there is a striking difference in the maximal responses to the two agents, with pentobarbital eliciting a considerably larger maximal response. One possibility is that phenobarbital (and related barbiturates that share this limited response, such as MPPB) are partial agonists. However, if phenobarbital were a partial agonist, it would be expected to reduce responses to pentobarbital, and this was clearly not the case. An alternative possibility is that phenobarbital (as has been previously suggested by others) does not increase the duration but only the magnitude of the recurrent IPSP. Due to the indirect method of determining the duration of inhibition used in this study, changes in the amplitude of the IPSP without changes in the decay time could result in modest increases in the IPI_{50}. However, inspection of the raw data did not suggest that pentobarbital and phenobarbital facilitated inhibition in qualitatively different ways or that phenobarbital had a selective action only at short intervals. Experiments currently in progress using intracellular recording will clearly be required to determine which of these hypotheses is correct.

Regardless of the mechanism underlying phenobarbital action, it is clear that the drugs with high efficacy in these electrophysiological experiments, such as etomidate, pentobarbital, (−)-mephobarbital, etc., are central nervous system depressants in low concentrations and induce anesthesia in high concentrations. Drugs that do not possess full activity (phenobarbital, MPPB, (+)-mephobarbital, flurazepam) also have sedative and anticonvulsant properties in low concentrations, but are relatively less effective as anesthetics (Butler, 1942). We suggest that there may be multiple behavioral manifestations of facilitation of GABAergic inhibition, and that drugs classified as "anesthetic" *vs.* "anticonvulsant" differ primarily in terms of the maximal change in inhibition that they can elicit and possibly also in the extent to which they directly depress activity *via* a GABA-mimetic action.

The basis for the convulsant action of DMBB, pentobarbital

and other barbiturates was quite apparent in the present studies and was not related to effects upon recurrent GABAergic inhibition. DMBB induced increases in the population spike and fEPSP response (fig. 3B) concurrently with *increases* in the duration of recurrent inhibition. Either an increase in transmitter release, such as has been described at the neuromuscular junction (Proctor and Weakly, 1976), or an increase in postsynaptic sensitivity to the transmitter would be consistent with increases in the fEPSP response. In general, there was a clear dissociation between the effects of these agents on recurrent inhibition and excitatory responses; some drugs, such as DMBB, were very potent in eliciting both types of response, others were relatively selective in eliciting only the GABA-enhancing (*e.g.*, etomidate, etazolate) response, while others such as (+)-MPPB had primarily excitatory effects. Pentobarbital was somewhat between these two extremes, in that it occasionally had direct excitatory actions, but these were most often observed at low concentrations or were transient in nature. These findings provide a physiological basis for why drugs such as DMBB, which would be predicted to have depressant effects based upon their biochemical properties, cause convulsions when administered to animals; the direct excitatory actions can clearly predominate over the indirect GABA facilitatory actions, despite the fact that DMBB is one of the more potent enhancers of GABAergic inhibition. The present results confirm that, as was hypothesized on the basis of behavioral studies, the depressant and excitatory responses to barbiturates represent separate pharmacological actions.

Relationship between physiology and binding. Barbiturate anesthetics such as pentobarbital have been shown to regulate the binding of ligands to the GABA-BZ-barbiturate receptor complex, enhancing the binding of GABA and BZ agonists and inhibiting the binding of GABA antagonists, benzodiazepine inverse agonists, and convulsants such as [^{35}S]*t*-butyl bicyclophosphorothionate (Ticku and Olsen, 1978; Leeb-Lundberg *et al.*, 1980; Olsen and Snowman, 1982; Olsen *et al.*, 1986). The present results demonstrate that drugs that allosterically regulate the binding of ligands to the GABA-BZ-barbiturate receptor complex in a similar fashion all have a common electrophysiological action in the hippocampus, *viz.*, they increase the duration of recurrent GABAergic inhibition, regardless of whether they are anesthetic, anticonvulsant or convulsant in intact systems. Given the multiplicity of barbiturate actions, it is probably not possible to rule out the possibility that some other physiological response *might* be linked to occupation of the barbiturate binding site. However, in terms of the absolute as well as relative concentrations of drug involved, there is a good correspondence between binding and this physiological response. For purposes of comparison, correlation coefficients for the EC_{5x} values (present experiments) *vs.* estimates of drug potency in regulating ligand binding reported in the literature (Olsen *et al.*, 1986) were calculated (table 3 and fig. 12); these correlations were all statistically significant. This relationship holds for the barbiturates as well as for the nonbarbiturate depressant agents such as etomidate and etazolate, which have also been reported to facilitate GABA-BZ binding (Ashton *et al.*, 1981; Leeb-Lundberg *et al.*, 1981; Barnes *et al.*, 1983; Wong *et al.*, 1984b), and in the case of etazolate, to facilitate GABAergic responses as well (Barnes *et al.*, 1983). The present results also correspond quite closely to the potentiation of responses to muscimol observed in the rat cuneate nucleus *in vitro* (Harrison and Simmonds, 1983),

TABLE 3

Correlations between electrophysiological potency and binding

The EC_{5x} values (Table 2) were correlated with the potencies of these same drugs in inhibiting [^{35}S]-*t*-butyl-bicyclophosphorothionate (TBPS), [^{3}H]β-carboline-3-carboxylate methyl ester (BCCM) or [^{3}H]bicuculline methochloride binding or in enhancing the binding of [^{3}H]GABA or N-[*methyl*-^{3}H]-diazepam to rat cortical membranes (Olsen *et al.*, 1986). EC_{5x} values greater than 1000 μM (which were estimates based upon extrapolation) were arbitrarily set to 2000 μM. All correlation coefficients were significant at $P < .05$ (single-tailed comparison).

Binding Assay	Correlation Coefficient	Slope	y-Intercept	No. of Drugs Tested
[^{35}S]TBPS	0.83	0.71	0.57	10
[^{3}H]GABA	0.75	0.70	0.89	7
[^{3}H]Bicuculline methochloride	0.85	0.60	0.83	10
[^{3}H]Diazepam	0.64	0.54	0.62	7
[^{3}H]BCCM	0.82	0.55	1.17	10

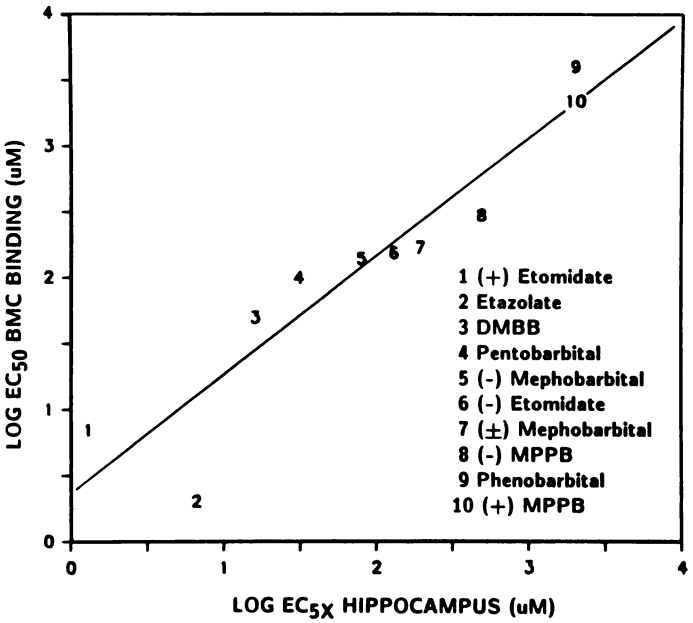


Fig. 12. Correlation between electrophysiology and binding. The correlation between the facilitation of recurrent inhibition (EC_{5x} values) *vs.* the ability to displace [^{3}H]bicuculline methochloride binding (from Olsen *et al.*, 1986) is illustrated. Each number corresponds to a single drug (listed in key), the line is the least-squares fit to the points, and the correlation coefficient is 0.85. The calculated EC_{5x} values for (+)-mephobarbital, phenobarbital and (+)-MPPB could not be determined directly, so they were arbitrarily set to 2000 μM for illustration; without these points the correlation coefficient was 0.79.

suggesting that similar receptors are involved. Finally, the different activities of *stereoisomers* in that study as well as in the present study support a specific receptor-mediated mechanism of action, rather than a more nonspecific action of these drugs on neuronal activity.

The greatest discrepancy between binding studies and the present findings concerns barbiturates such as phenobarbital, (+)-mephobarbital or (+)-MPPB, which either have no effect upon binding to GABA and benzodiazepine sites, alter the kinetics but not equilibrium binding (Olsen *et al.*, 1986) or even act as antagonists for the effects of pentobarbital (Leeb-Lundberg and Olsen, 1982). On the other hand, most electrophysiological studies suggest that pentobarbital and phenobarbital differ only in potency, with little if any difference in the maximal response or the types of effects that are observed (see above). Although we did not find that any of the drugs tested in these studies were inactive in facilitating GABA actions, a

distinction could be drawn between the magnitude of the maximal responses to drugs such as pentobarbital on the one hand, and phenobarbital, (+)-mephobarbital and the isomers of MPPB on the other. These latter agents produced significant but minor enhancement of the IPI_{50} even in very high concentrations. However, in no case did phenobarbital appear to antagonize the actions of pentobarbital.

Several factors might underlie the differences between binding studies and the present results. The conditions under which binding is determined are not identical to those in which electrophysiological responses are tested, and it is possible that the ability of phenobarbital to regulate GABA binding is lost in the *in vitro* binding assay. Alternatively, the physiological significance of changes in binding kinetics (but not equilibrium binding), such as has been reported for phenobarbitol and [^{3}H] diazepam binding (Leeb-Lundberg and Olsen, 1982), is unclear. When GABA is released synaptically, the receptor on-rate and not the equilibrium constant may be the primary determinant of the magnitude of the response. Thus, although there may be no changes in the *equilibrium* binding, more subtle changes might be reflected in altered transmission at GABAergic synapses.

In conclusion, barbiturates and related depressant drugs have a variety of actions on synaptic transmission in rat hippocampus, both facilitating and depressing excitatory synaptic transmission and enhancing the duration of recurrent GABAergic inhibition. This latter response corresponds quite well to the abilities of these drugs to facilitate or inhibit binding of ligands to the GABA-BZ-barbiturate receptor complex. However, the pharmacological actions of these analogs *in vivo* clearly reflect not only the indirect effects upon recurrent synaptic inhibition, but also the direct changes upon excitatory transmission and neuronal excitability as well.

Acknowledgments

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