

## BARBITURATE ENHANCEMENT OF GABA-MEDIATED INHIBITION AND ACTIVATION OF CHLORIDE ION CONDUCTANCE: CORRELATION WITH ANTICONVULSANT AND ANESTHETIC ACTIONS

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### SUMMARY

The anesthetic-sedative barbiturate pentobarbital (PB) and the anticonvulsant barbiturate phenobarbital (PhB) were applied to mammalian spinal cord neurons in primary dissociated cell culture to assess their effects on: (1) postsynaptic GABA-responses; (2) paroxysmal activity produced by the convulsant bicuculline; (3) resting membrane properties; and (4) spontaneous neuronal activity. The results demonstrated that: (1) anticonvulsant actions occurred at barbiturate concentrations which augmented GABA-responses; (2) anesthetic actions occurred at barbiturate concentrations which produced direct increases in chloride conductance; (3) both anticonvulsant and anesthetic actions occurred at clinically relevant concentrations; and (4) concentrations of PhB, but not PB, which produced GABA-augmentation and direct conductance changes were widely separated. These findings support the hypotheses that augmentation of GABA-mediated inhibition and possibly reduction of glutamate (GLU)-mediated excitation form the basis at least in part for barbiturate anticonvulsant action and that addition of direct increases in chloride conductance to augmentation of GABA-mediated inhibition and reduction of GLU-mediated excitation may partially underlie anesthetic-sedative barbiturate action.

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### INTRODUCTION

The barbiturates phenobarbital (PhB) and pentobarbital (PB) are used clinically as anticonvulsants, sedatives and anesthetics. While PB is an effective anticonvulsant, it is not used as such due to its undesirable sedative properties. In contrast, PhB can be administered to ambulatory patients at doses which provide substantial anticonvul-

sant action without producing sedation<sup>28</sup>. While barbiturates have been demonstrated to have multiple actions on neuronal membrane properties<sup>2,12,14,17,27</sup> and to have pre-<sup>6,8,9,18,29,30</sup> and postsynaptic<sup>13,14,17,24,29,30</sup> actions on synaptic transmission, it remains uncertain as to which of these actions are responsible for their clinical effects and why barbiturates have variable efficacy as anticonvulsants, sedatives and anesthetics. It has been suggested recently that while anticonvulsant and anesthetic barbiturates have similar pharmacological and electrophysiological actions, the dose-dependencies of these actions are substantially different<sup>12,14</sup>. Using iontophoresis of barbiturates and amino acids onto mouse spinal cord neurons in primary dissociated cell culture, it was demonstrated that anticonvulsant and anesthetic barbiturates augmented GABA-mediated postsynaptic inhibition and antagonized glutamate (GLU)-mediated postsynaptic excitation over similar iontophoretic current ranges, but that anesthetic barbiturates directly increased postsynaptic chloride conductance at much lower iontophoretic charges than anticonvulsant barbiturates. It was then suggested that anticonvulsant actions of barbiturates might be due to augmentation of GABA-mediated inhibition and possibly antagonism of GLU-mediated excitation, and that the anesthetic-sedative actions of barbiturates might be the result of addition of direct chloride-mediated increases in membrane conductance to the GABA-response augmentation and GLU-response antagonism. Anticonvulsant barbiturate concentrations producing modulation of amino acid responses and direct chloride-mediated inhibition were different, consistent with an acceptable therapeutic index between the anticonvulsant and anesthetic-sedative actions. For anesthetic barbiturates, however, these actions occurred at similar concentrations, consistent with a poor therapeutic index for anticonvulsant action. Since the above experiments were performed using iontophoresis of drugs and amino acids, two important questions remain. First, are the reported differences between anticonvulsant and anesthetic barbiturates a consequence of different transport numbers for iontophoresis of the barbiturates; and second, do these actions occur at clinically meaningful barbiturate concentrations? To approach these questions, we have investigated barbiturate actions on mouse spinal cord neurons in cell culture by applying barbiturates to the bathing medium or by local miniperfusion of individual neurons. Thus a direct comparison could be made between barbiturate action and concentration.

#### MATERIALS AND METHODS

Recordings were made from neuronal cell cultures prepared by mechanically dissociating spinal cords derived from 12.5–14-day-old mouse embryos; the details of this procedure have been described elsewhere<sup>25</sup>. A minimum of 5 weeks was allowed for maturation of the cultures prior to electrophysiological experiments. Culture dishes were placed on the modified stage of an inverted phase-contrast microscope and maintained at 35–37 °C. Large diameter (25–50  $\mu\text{m}$ ) multipolar spinal cord neurons were penetrated under direct vision using either 4 M KAc- or 3 M KCl-filled micropipettes (25–40 M $\Omega$ ). Simultaneous recording of membrane potential and injected current was achieved using a conventional bridge circuit. Data were recorded on a 6-channel Brush polygraph (2600).

Four different actions of PB and PhB were investigated: (1) changes in post-synaptic GABA-responses; (2) anticonvulsant actions on bicuculline (BICUC)-induced convulsive activity; (3) alterations in levels of spontaneous activity; and (4) direct postsynaptic effects. During electrophysiological recordings, the cultures were bathed in Dulbecco's phosphate buffered saline (DPBS) (136.75 mM NaCl, 2.68 mM KCl, 0.90 mM CaCl<sub>2</sub>, 0.49 mM MgCl<sub>2</sub>·6 H<sub>2</sub>O, 8.06 mM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 5.56 mM glucose) which maintained pH between 7.2 and 7.4. When changes in membrane potential were being recorded (as in 1 and 4 above), MgCl<sub>2</sub> was added to elevate [Mg<sup>2+</sup>] to 10 mM and abolish most spontaneous synaptic activity.

A constant current stimulator was used to iontophoretically apply GABA (0.5 M, pH 1.32) from high resistance (> 50 MΩ) glass micropipettes. The tips of GABA-containing pipettes were placed about 2 μm from the cell membrane, and responses to GABA were obtained by passing 2.5–9.0 nA current pulses of 100–500 msec. These two parameters were adjusted to obtain control GABA-responses of 5–10 mV. Because KCl-filled recording micropipettes were used, the chloride equilibrium potential was depolarized to about –20 mV and neuronal responses to GABA were depolarizing. Short current pulses were continuously injected through the recording micropipette to evaluate the effects of GABA, PB and PhB on membrane conductance.

For acute administration of barbiturates, a pressure-mediated mini-perfusion (MP) system was used. The tips of glass micropipettes were broken off, leaving an opening 5–10 μm in diameter. The micropipettes were then filled with the appropriate concentration of barbiturate, fastened to micromanipulators and attached to sections of polyethylene tubing which were connected to a pressure regulator. The duration of 0.5 to 2.0 psi pressure pulses was varied from 500 msec to 3 sec. The tips of the MP pipettes were placed in the region of the tips of GABA iontophoretic pipettes, but somewhat further away from the cell (10–15 μm). Thus, GABA was applied iontophoretically prior to and during MP of cells with varying concentrations of PB and PhB. Recordings were made using KCl-containing micropipettes and membrane potential was hyperpolarized to –60 to –90 mV.

To measure anticonvulsant action, the culture was first bathed in recording medium containing 40 μM BICUC. At this dose, BICUC has been shown to produce abnormal bursting or paroxysmal depolarizing events (PDE) in all spinal cord neurons<sup>11,15</sup>. Increasing amounts of barbiturate were added to the medium, and their effects on this abnormal activity were recorded with KAc-filled micropipettes. All experiments were completed within 2 h of preparation of the BICUC solution since it is unstable in solution<sup>19</sup>. The same paradigm was used to measure barbiturate-induced alteration in spontaneous neuronal activity, except that BICUC was not added to the recording medium.

Barbiturate actions on neuronal membrane properties were studied using MP pipettes as described above; however, no iontophoretic pipettes were present. Membrane potentials were maintained at –45 to –65 mV, and barbiturates were applied to cell surfaces by MP. Changes in membrane potential and conductance were recorded using KCl-filled micropipettes, and thus the chloride-dependent barbiturate

responses were depolarizing rather than hyperpolarizing. PB, PhB, GABA and BICUC were purchased from Sigma Chemicals.

## RESULTS

### *Barbiturates abolished BICUC-induced paroxysmal depolarizing events*

Addition of 40  $\mu\text{M}$  BICUC to the bathing medium produced PDE in 100% of neurons studied (Fig. 1 top; 0  $\mu\text{M}$ ). Both PhB and PB abolished the BICUC-induced PDE (Fig. 1 top; 100  $\mu\text{M}$ , 200  $\mu\text{M}$ ) in a dose-dependent manner over a barbiturate range of 25–500  $\mu\text{M}$  (Fig. 1 bottom). PB had a 50% effective dose ( $\text{ED}_{50}$ ) for suppression of PDE at 150  $\mu\text{M}$  and was slightly more potent than PhB whose  $\text{ED}_{50}$  was 170  $\mu\text{M}$ . As barbiturate concentration was increased, neuronal firing was altered from a

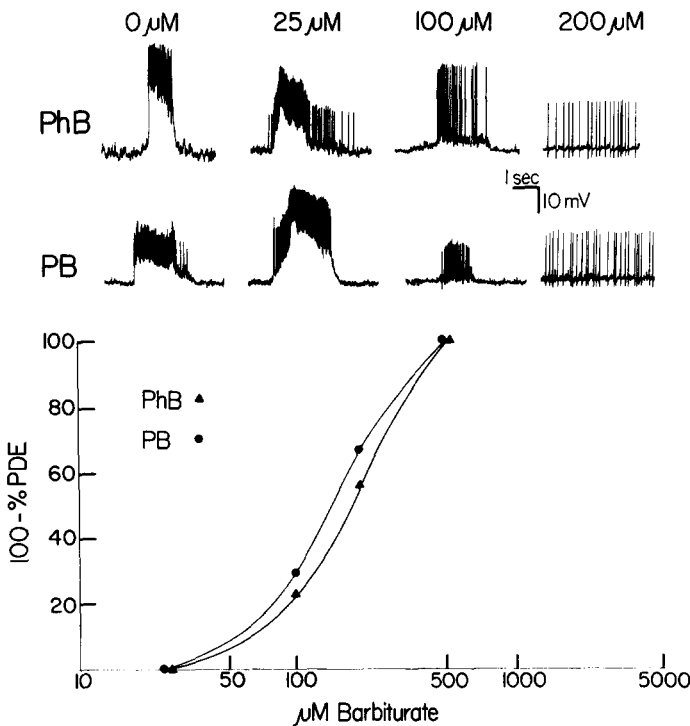


Fig. 1. PhB and PB abolished paroxysmal depolarizing events in spinal cord neurons in cell culture. When 40  $\mu\text{M}$  BICUC was added to normal bathing medium, 100% of neurons developed PDE. As increasing concentrations of barbiturates were added to the bathing medium, PDE was recorded in fewer neurons and at 500  $\mu\text{M}$  all neurons displayed normal spontaneous activity. The specimen records at the top are polygraph records of intracellular recordings made with 4 M KAc-filled micropipettes which were obtained in DPBS containing 0, 25, 100 and 200  $\mu\text{M}$  PhB and PB. Action potential amplitudes are reduced due to the limited frequency response of the recorder. The records at 0  $\mu\text{M}$  barbiturates show typical PDE and those at 200  $\mu\text{M}$  show normal spontaneous activity. Recordings were only accepted if resting membrane potential was above 40 mV, evoked action potentials were at least 40 mV and the recording was stable and maintained for more than 2 min. Each point on the graph represents from 4 to 16 recordings from single neurons with a total of 23 cells for PB and 42 cells for PhB.

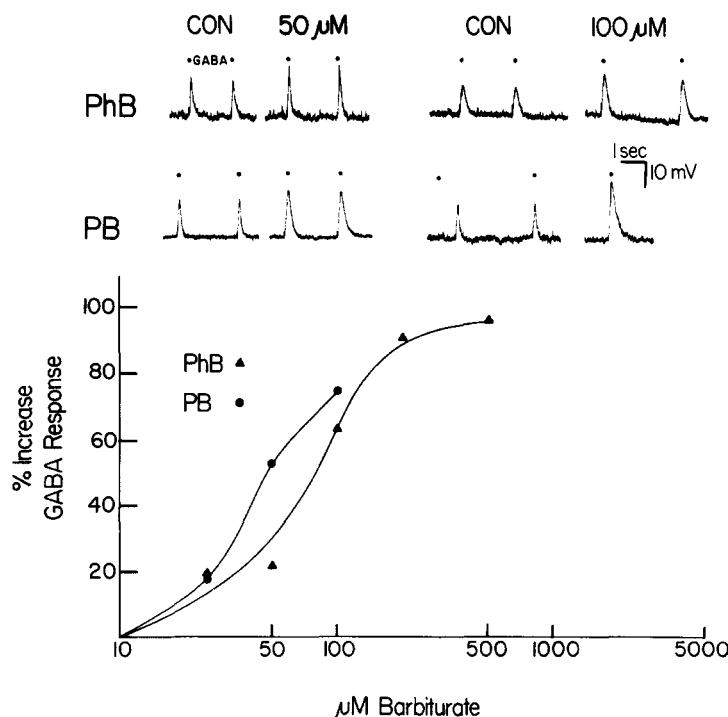


Fig. 2. PhB and PB augmented postsynaptic GABA-responses. Intracellular recordings were made using micropipettes filled with 3 M KCl in bathing medium containing 10 mM  $Mg^{++}$  to suppress spontaneous activity. GABA was applied by iontophoresis (PhB: 4, 5 nA; PB 3, 2.5 nA) for 100 msec to achieve responses of 10–15 mV. After control GABA-responses were obtained (CON), the tip of a barbiturate-containing miniperfusion pipette was positioned within 25  $\mu$ m of the tip of the GABA iontophoretic pipette. GABA-responses were then obtained while barbiturates were applied by miniperfusion. The specimen records shown at the top were representative of the augmentation of GABA-responses produced by 50 and 100  $\mu$ M PhB and PB. Each point on the plot represents the average GABA-response augmentation on 3 to 8 cells (PB 15 and PhB 33) with 2–4 trials per cell. Membrane potential was hyperpolarized to  $-60$  to  $-90$  mV to increase the separation between chloride equilibrium potential and membrane potential and thus achieve large GABA-responses at low iontophoretic currents.

fully developed paroxysmal pattern to one of periodic bursts or volleys of action potentials (Fig. 1 top). For example, at lower barbiturate concentrations action potentials were often seen on the leading edge of the depolarization and/or the amplitude of the depolarization was decreased (Fig. 1 top; 25 and 100  $\mu$ M). At higher concentrations of PB or PhB, the convulsant action of BICUC was overcome and the cells showed normal spontaneous activity (Fig. 1 top; 200  $\mu$ M). It should be noted that a range of responses was seen at intermediate barbiturate concentrations. Thus, at barbiturate concentrations between 50 and 200  $\mu$ M, there was a graded mixture of spontaneous activity from fully paroxysmal to completely non-paroxysmal.

#### *Barbiturates augmented postsynaptic GABA-responses*

Both PhB and PB augmented GABA-responses (Fig. 2 top) in a dose-dependent

fashion (Fig. 2 bottom). Augmentation occurred over barbiturate concentrations of 25–500  $\mu\text{M}$ . PB ( $\text{ED}_{50}$  of 50  $\mu\text{M}$ ) was slightly more potent than PhB ( $\text{ED}_{50}$  of 75  $\mu\text{M}$ ) (Fig. 2 bottom). Augmentation was unassociated with alterations in resting membrane properties up to 100  $\mu\text{M}$  for PB and 500  $\mu\text{M}$  for PhB. Above these concentrations, PB and PhB directly increased membrane conductance and depolarized membrane potential.

*Barbiturates suppressed spontaneous neuronal activity*

Intracellular recordings made from neurons bathed in DPBS revealed normal spontaneous activity consisting of random mixtures of action potentials and excitatory and inhibitory synaptic potentials (Fig. 3 top; con). As PB concentration was increased, no consistent alterations in spontaneous activity were recorded until the concentration was above 100  $\mu\text{M}$ . Above this concentration, spontaneous activity was reduced and in some neurons was absent (Fig. 3 top; 500  $\mu\text{M}$ ). The incidence of such quiescence or silence increased from 0 to 100% over the 100–500  $\mu\text{M}$  range (Fig. 3 bottom) with an  $\text{ED}_{50}$  of 280  $\mu\text{M}$ . As PhB concentration was increased, a similar progression from normal spontaneous activity to quiescence occurred (Fig. 3 top), but

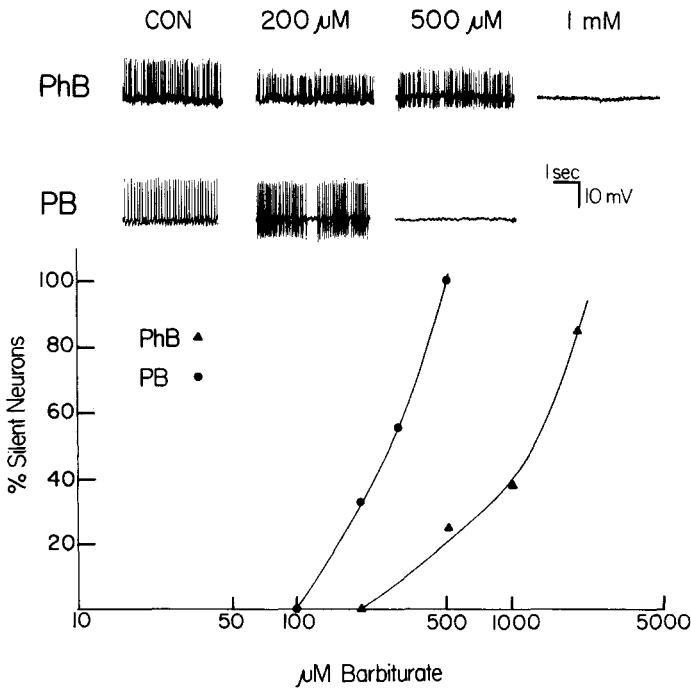


Fig. 3. PhB and PB reduced spontaneous activity. Spontaneous activity was recorded from neurons in DPBS medium using 4 M KAc-filled micropipettes and consisted of randomly occurring synaptic and action potentials (CON). As barbiturate concentration was increased, spontaneous activity was reduced and at higher concentrations suppressed (500  $\mu\text{M}$ , 1 mM). At each concentration, there was a dose-dependent increase in the percentage of cells with no spontaneous activity (bottom). In the graph, each point represents recordings from 4 to 7 different cells with a total of 22 cells for PB and 18 cells for PhB.

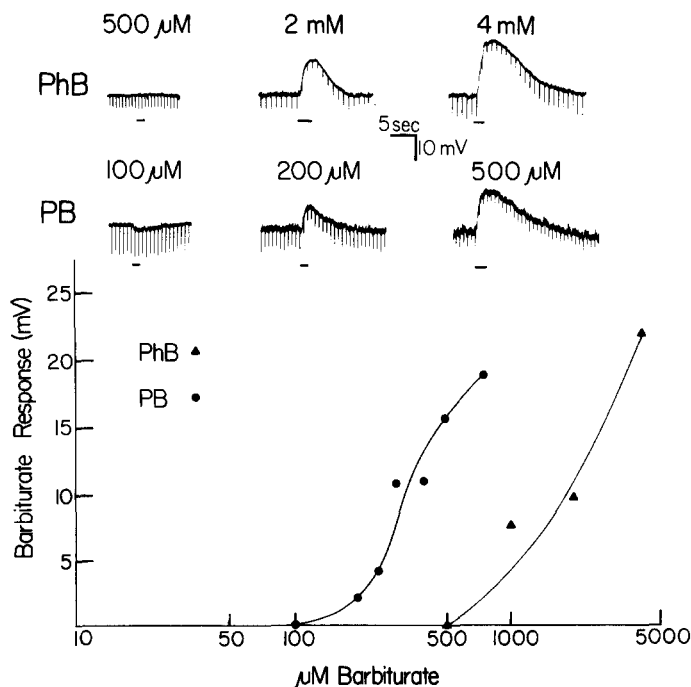


Fig. 4. PhB and PB directly altered membrane potential and conductance. Intracellular recordings were made with 3 M KCl-filled micropipettes in DPBS medium containing 10 mM  $\text{Mg}^{2+}$  and barbiturates were applied by miniperfusion. Membrane potential was hyperpolarized to  $-45$  to  $-65$  mV and brief (50 msec) hyperpolarizing constant current pulses were applied through the recording micropipette using the bridge technique. Specimen records (top) show that both barbiturates depolarized membrane potential and increased membrane conductance but higher concentrations were required for PhB than for PB. In the plot of barbiturate response vs concentrations, each point represents an average depolarization obtained from 4 to 14 cells with a total of 36 cells for PB and 35 for PhB.

the PhB concentration range that produced silence was from 500 to 4000  $\mu\text{M}$  (Fig. 3 bottom) with an  $\text{ED}_{50}$  of 1300  $\mu\text{M}$ .

#### *Barbiturates increased neuronal membrane conductance and altered membrane potential*

Barbiturates were applied to spinal cord neurons by MP and intracellular recordings were made with 3 M KCl-containing micropipettes. With concentrations up to 100  $\mu\text{M}$ , PB application did not alter membrane conductance or potential (Fig. 4 top; 100  $\mu\text{M}$ ). Above 100  $\mu\text{M}$ , however, PB increased membrane conductance and depolarized membrane potential in a dose-dependent manner (Fig. 4 top; 100, 200  $\mu\text{M}$ ). Over the range of 100–750  $\mu\text{M}$ , PB application evoked responses up to 20 mV (Fig. 4 bottom). Application of 500  $\mu\text{M}$  PhB, however, produced no direct membrane response (Fig. 4 top; 500  $\mu\text{M}$ ). Above this concentration, PhB evoked similar dose-dependent depolarization and increases in membrane conductance (Fig. 4 top; 2,4 mM). Over the concentration range of 1000–4000  $\mu\text{M}$ , PhB directly depolarized membrane potential up to 22 mV (Fig. 4 bottom). Thus, while both barbiturates directly increased membrane conductance and depolarized neurons, PB was much

PhB

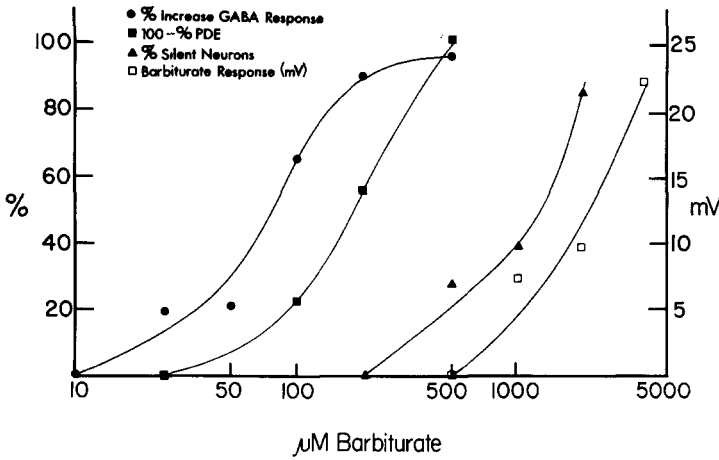


Fig. 5. Dose-dependency of PhB actions. PhB antagonized BICUC-induced PDE and augmented GABA-responses over similar concentrations (25–500 μM), but higher concentrations of PhB were required for both abolition of spontaneous activity and depolarization (500–4000 μM).

more potent than PhB with 10 mV responses being produced at 340 and 2000 μM respectively.

*Dose-dependency of PB and PhB actions was different*

As presented in the previous sections, both PB and PhB antagonized BICUC-induced PDE, produced quiescence, augmented postsynaptic GABA-responses and directly increased membrane conductance. However, these barbiturate actions did not occur at the same concentrations and there was a different dose-dependency for PhB

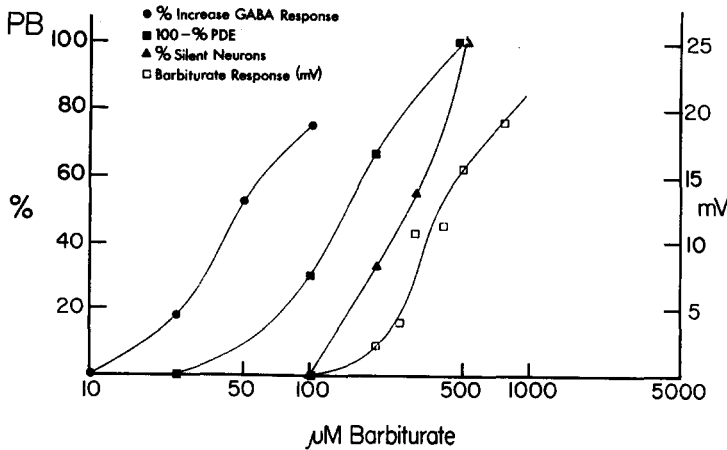


Fig. 6. Dose-dependency of PB actions. While augmentation of GABA responses and suppression of BICUC-induced PDE occurred at lower PB concentrations (25–500 μM) than abolition of spontaneous activity and depolarization (100–1000 μM), the degree of overlap was considerable (when compared with the effects of PhB on these 4 actions).



and PB. PhB antagonized BICUC-induced PDE and augmented GABA-responses over similar concentration ranges (25–200  $\mu\text{M}$ ) (Fig. 5) and also directly altered membrane properties and produced quiescence over similar concentrations (500–4000  $\mu\text{M}$ ) (Fig. 5). PB also antagonized BICUC-induced PDE and augmented GABA-responses over the same 25–200  $\mu\text{M}$  range (Fig. 6), but the direct membrane actions and production of quiescence occurred together over only a slightly higher concentration range (100–500  $\mu\text{M}$ ) (Fig. 6). Thus, augmentation of GABA-responses and anti-convulsant action were correlated and were produced over similar concentrations by both barbiturates. The direct increase in chloride conductance and quiescence were correlated for each barbiturate but occurred at much lower concentrations for PB than for PhB. Thus, while both barbiturates had similar actions, there was a difference in their dose-dependency.

## DISCUSSION

PhB and PB had similar actions in these experiments with both barbiturates: (1) abolishing convulsant-induced PDE; (2) augmenting postsynaptic GABA-responses; (3) abolishing spontaneous activity; and (4) increasing membrane chloride conductance directly. What, then, accounts for the anticonvulsant action of both barbiturates?

As a model of barbiturate anticonvulsant action, we studied the dose-dependency of barbiturate antagonism of BICUC-induced paroxysmal activity. BICUC is a convulsant compound and an antagonist of GABA-mediated postsynaptic inhibition<sup>5,11,15</sup>. Intracellular recordings obtained from cortical neurons following topical convulsant application have demonstrated abrupt and randomly occurring depolarizations termed paroxysmal depolarizing shifts which appear to be the intracellular electrophysiological events underlying interictal spikes recorded in the surface electroencephalogram of patients with epilepsy<sup>1,16,22</sup>. In neurons in primary dissociated cell culture derived from mouse spinal cord, BICUC evoked similar paroxysmal depolarizations (PDE)<sup>11,15</sup>. Furthermore, BICUC antagonized GABA-mediated postsynaptic responses<sup>11,15</sup> and displaced [<sup>3</sup>H] GABA from GABA-binding sites<sup>31</sup>. Thus, in spinal cord neurons in cell culture, BICUC is a GABA antagonist and produces paroxysmal activity. We selected a high BICUC concentration (40  $\mu\text{M}$ ) which produced PDE in 100% of neurons so that in each recording we would have a relatively unambiguous endpoint, i.e. presence or absence of PDE. Both PB and PhB abolished BICUC-induced PDE over a 25–500  $\mu\text{M}$  range with  $\text{ED}_{50}$ s of 150 and 170  $\mu\text{M}$  respectively. PB and PhB also augmented GABA-responses over similar concentrations (25–200  $\mu\text{M}$ ) with  $\text{ED}_{50}$ s of 50 and 75  $\mu\text{M}$  respectively. During clinical use of PhB, serum levels of 10–40  $\mu\text{g}/\text{ml}$  (43–172  $\mu\text{M}$ ) are generally considered to be in the therapeutic range. Since in plasma PhB is about 50% protein bound<sup>10</sup>, free-serum levels should be 5–20  $\mu\text{g}/\text{ml}$  (21.5–86  $\mu\text{M}$ ), and in steady-state, this should equilibrate with the central nervous system (CNS) and cerebrospinal fluid. Variations in protein binding in different patients could alter these concentrations somewhat, but nonetheless, CNS concentrations of 20–100  $\mu\text{M}$  cover most of the therapeutic range for PhB. The somewhat

increased range of PhB concentration needed to achieve 100% suppression of PDE was probably a consequence of the high BICUC concentration that we selected. PB had anticonvulsant activity over a similar range of concentrations but was slightly more potent, consistent with the known anticonvulsant activity of PB. Thus, PhB and PB antagonized BICUC-induced PDE and augmented GABA-responses over concentration ranges which were in the therapeutic range for PhB anticonvulsant action. In addition, both barbiturates antagonized GLU-responses at the same concentrations that augmented GABA-responses<sup>13,14</sup>. PhB and PB were equally effective in this in vitro model of anticonvulsant action and thus their anticonvulsant properties may be due at least in part to augmentation of GABA-mediated postsynaptic inhibition.

What, then, underlies barbiturate anesthetic action? PB and other anesthetic barbiturates produce dose-dependent alterations in electroencephalographic (EEG) recordings during the development of increasing stages of anesthesia<sup>4</sup>. The normal EEG is replaced by synchronous, high amplitude, slow (delta) waves during initial stages of anesthesia. As surgical anesthesia is achieved, the amplitude of the EEG decreases and a suppression pattern develops in which silent low amplitude periods are interrupted by bursts of slow wave activity. If anesthetic concentration is further increased a silent flat EEG ensues. Based on the characteristic finding of a flat EEG with deep barbiturate surgical anesthesia or overdose, we used quiescence or silence in neuronal activity as an in vitro analog of the deep anesthetic state. It is likely that barbiturate concentrations which produced total quiescence were in excess of those required to produce surgical anesthesia and thus our assessment of anesthetic action tended to bias the dose-response curve for anesthesia to somewhat higher barbiturate concentrations. Both PB and PhB decreased spontaneous activity and increased the percentage of neurons with no spontaneous activity. However, these two barbiturates were not equipotent. PB produced quiescence over the 100–500  $\mu\text{M}$  range while PhB required concentrations of 500–4000  $\mu\text{M}$ . PB and PhB also increased membrane conductance with a similar difference in potency with PB active from 100 to 750  $\mu\text{M}$  (10 mV responses at 340  $\mu\text{M}$ ) and PhB active from 1000 to 4000  $\mu\text{M}$  (10 mV responses at 2000  $\mu\text{M}$ ). For PB, surgical anesthesia is produced at concentrations (200–300  $\mu\text{M}$ <sup>26</sup>) which overlap the midportion of the PB quiescence dose-response curve. Thus, it would appear that clinically relevant concentrations of PB are associated with an in vitro correlate of anesthesia, suggesting that the model chosen may be relevant for study of anesthetic actions. For PhB, however, very high concentrations were required to produce quiescence. This is consistent with the clinical observation that large doses of PhB can be administered to patients in status epilepticus without inducing anesthesia. This anesthetic action was correlated with the direct action of PB on membrane chloride conductance. For PhB, a similar correlation was present between quiescence and direct GABA mimetic action but at much higher concentrations. These findings suggest that barbiturate anesthesia may be produced, at least in part, by a direct PB action to increase chloride conductance and thus hyperpolarize neurons and reduce activity. The difference between the clinical actions of PhB and PB may be due to the different dose-dependency of these actions. For PhB, anticonvulsant action and GABA-augmentation were produced at low concentrations (25–200  $\mu\text{M}$ ) while

anesthetic action and direct increase in chloride conductance occurred at high concentrations (500–4000  $\mu\text{M}$ ). Thus the therapeutic index for anticonvulsant action with PhB would be excellent, with a large dose separation between anticonvulsant effects and anesthetic-sedative actions. For PB, anticonvulsant and anesthetic-sedative actions would occur at comparable concentrations, and thus while PB would have both actions, its therapeutic index for anticonvulsant action would be poor. Therefore, PB would be useful as an anticonvulsant only if needed for status epilepticus and if concurrent anesthesia was acceptable. Consistent with this interpretation was the finding that both PhB and PB produced dose-dependent anticonvulsant actions in mice against pentylenetetrazol-induced and maximal electroshock seizures and that both barbiturates exerted dose-dependent neurotoxic effects as measured using a rotorod technique<sup>23</sup>. However, the 'protective index' (PI: ratio of neurotoxic to anticonvulsant  $\text{ED}_{50}$ s) was different for each barbiturate with PB having a poor PI of 0.79–0.98 and PhB a large PI of 2.71–3.41. This is similar to our finding of an anesthetic to anticonvulsant  $\text{ED}_{50}$  ratio of 1.9 for PB and 7.2 for PhB.

Barbiturates also have presynaptic actions to decrease calcium-accumulation by cortical synaptosomes<sup>3,7,21</sup> and to block calcium-dependent release of GABA, glutamate and acetylcholine<sup>6,9</sup>. However, PB decreased calcium accumulation only at concentrations of at least 300  $\mu\text{M}$ <sup>7</sup>. Release of GABA from cortical synaptosomes was not reduced by 100  $\mu\text{M}$  PB<sup>20</sup> but was decreased by 200  $\mu\text{M}$  to 1 mM PB<sup>6,8</sup>. Thus, barbiturates decreased presynaptic calcium entry and reduced neurotransmitter release at concentrations that were anesthetic but not anticonvulsant. This suggests that barbiturate anesthesia may be a consequence of a presynaptic action of barbiturates to reduce transmitter release as well as of postsynaptic actions to increase GABA-mediated inhibition, to decrease GLU-mediated excitation and to directly increase chloride conductance.

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