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BARBITURATE REDUCTION OF CALCIUM-DEPENDENT ACTION POTENTIALS: CORRELATION WITH ANESTHETIC ACTION

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SUMMARY

Calcium-dependent action potentials were recorded from mouse spinal cord neurons in primary dissociated cell culture following addition of the potassium channel blockers tetraethylammonium ion and 3-aminopyridine. The pharmacologically active barbiturates, pentobarbital and phenobarbital, but not the pharmacologically inactive barbiturate, barbituric acid, produced reversible, dose-dependent reduction of action potential duration at sedative-hypnotic and anesthetic concentrations. Pentobarbital reduced action potential duration at concentrations from 25 to 600 μM (50% reduction at 170 μM) while phenobarbital reduced action potential duration at concentrations from 100 to 5000 μM (50% reduction at 900 μM). The barbiturate concentrations which reduced calcium-dependent action potential duration in this study correlate with reduction of neurotransmitter release from other neuronal preparations and with reduction of calcium uptake by synaptosomes. The results suggest that barbiturates may produce anesthesia in part by reduction of *presynaptic* calcium entry and consequent reduction of neurotransmitter release in addition to *postsynaptic* increase of membrane chloride ion conductance. Barbiturate anticonvulsant actions are probably due to *postsynaptic* augmentation of GABA-mediated inhibition and depression of excitatory synaptic transmission. The major difference between anticonvulsant (phenobarbital) and anesthetic (pentobarbital) barbiturates was the dose-dependency of these actions. Phenobarbital produced postsynaptic modulation of neurotransmitter responses at low concentrations and decreased calcium-dependent action potential duration and increased chloride ion

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conductance at high concentrations. In contrast, pentobarbital produced all actions at low concentrations. Thus for phenobarbital there would be a large therapeutic index for anticonvulsant activity compared to anesthetic activity but for pentobarbital there would be a small therapeutic index.

INTRODUCTION

Barbiturates are used clinically as anticonvulsants, sedative-hypnotics and anesthetics[17,41]. Long-acting barbiturates such as phenobarbital and mephobarbital are useful as chronic anticonvulsants since they have little sedative action at therapeutic serum concentrations. Intermediate and short acting barbiturates such as pentobarbital, thiopental or amobarbital are efffective anticonvulsants but cannot be administered chronically due to their sedative side effects; therefore, they are used primarily as sedative-hypnotics or anesthetics. What are the mechanisms of barbiturate anticonvulsant, sedative-hypnotic and anesthetic action, and why do barbiturates differ in their clinical usefulness as anticonvulsants and anesthetics?

Barbiturates have both synaptic and non-synaptic (membrane) actions in the central nervous system. Barbiturates act: (1) *postsynaptically* to increase GABA-mediated inhibition[2,12,26–28,31,32,37,42,48,49], to reduce glutamate[2,26–28,32,45,49], aspartate[32] and acetylcholine[1,34] mediated excitation, and to directly activate GABA-receptors thus increasing membrane chloride conductance[26,28,32,49]; (2) *presynaptically* to block release of neurotransmitters[6,15,25,29,44,53,54,56], including GABA[7,9,10,18,55], glutamate[10,38,55], aspartate[38,55,57], acetylcholine[20,24,29,46,47,55] and norepinephrine[18], and (3) directly or *non-synaptically*[51] on neuronal membranes to reduce sodium and potassium conductances[3,30,50].

Since modulation of amino acid responses by the anticonvulsant barbiturate phenobarbital has been demonstrated at concentrations that are present in brain when anticonvulsant serum levels are therapeutic[49], it is likely that barbiturates have anticonvulsant action by postsynaptic enhancement of GABAergic inhibitory and reduction of excitatory synaptic transmission[26–28].

Barbiturates may produce anesthesia by a combination of pre- and postsynaptic mechanisms. First, direct increase of postsynaptic chloride conductance by the anesthetic barbiturate pentobarbital was produced at high anesthetic barbiturate concentrations[49]. Chloride conductance increase would produce membrane hyperpolarization and thus postsynaptic inhibition. Second, since release of neurotransmitter is calcium-dependent[11,14], requiring entry of calcium through voltage-dependent presynaptic calcium channels[21,22] and since barbiturates have been shown to block the uptake of calcium by presynaptic terminals[4,5,35], it has been suggested that barbiturates reduce transmitter release by antagonizing calcium entry into presynaptic terminals[4,5,35]. Essential to this hypothesis of anesthetic action is the demonstration that barbiturates reduce the voltage-dependent inward calcium current in presynaptic terminals at anesthetic barbiturate concentrations. While it is not feasible to record from presynaptic terminals of mammalian neurons, calcium-dependent action poten-

tials can be recorded from somata of mouse spinal cord neurons in cell culture if membrane potassium conductance is reduced[19]. Therefore, we have studied the actions of barbiturates on somatic calcium-dependent action potentials under the assumption that calcium conductance of neuronal somata and synaptic terminals are similar.

In the present study, we have shown that pentobarbital and phenobarbital, but not barbituric acid, reduced calcium-dependent action potential duration at sedative-hypnotic and at anesthetic drug concentrations. Thus barbiturate anesthesia may be due in part to: (1) blockade of *presynaptic* calcium entry and consequent reduction of neurotransmitter release, as well as to (2) direct *postsynaptic* enhancement of chloride conductance and consequent membrane hyperpolarization.

METHODS

Primary dissociated cell culture

Dissociated neuronal cell cultures were prepared from dissected spinal cords and attached dorsal root ganglia from 12–13.5-day-old fetal mice as described previously[43]. Following trypsinization and mechanical dissociation by trituration, the cells were plated on collagen-coated 35 mm culture dishes. The cultures were maintained in a growth medium containing 80% minimal essential medium, 10% fetal calf serum and 10% heat-activated horse serum at a pH of 7.3–7.4 and osmolarity 340 mOsm. Between days 6 and 8, uridine and 5′-fluoro-2′-deoxyuridine were added to suppress growth of non-neuronal cells. The growth medium was changed twice a week. The cultures were incubated at 35–37 °C in an atmosphere enriched with 10% carbon dioxide for 4–6 weeks prior to electrophysiological recording.

Intracellular recording

Neurons were visualized on the stage of an inverted phase contrast microscope modified to maintain the culture plate at 35–37 °C. Intracellular recordings were made from large multipolar spinal cord neurons (> 20 μm diameter) using omega dot glass microelectrodes filled with 4 M potassium acetate (KAc) (25–50 MΩ) connected to a unity gain high impedance amplifier and conventional bridge circuit (WPI M 707) to permit simultaneous measurement of membrane potential and injection of current through a single microelectrode. Data were recorded on a 6-channel Brush recorder and photographed from the screen of a Tektronix storage oscilloscope (Grass camera mounted with Bioelectric Reflexor).

Solutions

All recordings were made in balanced salt solutions (total volume 2.5 ml) after removal of growth medium. Heavy parafin oil was applied to the surface of the bathing solution to retard evaporation, unless the bathing solution was to be changed during the course of the experiment. The basic solution (referred to as control bathing solution) was adjusted to pH 7.30–7.40 and osmolarity 305–325 mOsm. It consisted of (in mM): NaCl 137.5; KCl 5.3; $CaCl_2$ 5.0; glucose 5.6; $MgCl_2$ 0.8; and Tris-HCl 13.

When tetraethylammonium (TEA) (25 mM) and 3-aminopyridine (3-AP) (5 mM) were added to the bathing solution, the sodium chloride concentration was lowered to 115 mM to keep the solution osmolarity constant. Tetrodotoxin (TTX) was added from a 1 mM stock solution directly to the bathing solutions, final concentration was either 1 or 3 μM prior to addition to the culture plate.

Stock solutions (100 mM) of the barbiturates (sodium salts) were prepared in control solution containing TEA and 3-AP on the day of the experiment. Aliquots of these stock solutions were removed and added to the bathing solutions to make final test concentrations (30–2000 μM). The pH was measured and adjusted to between 7.30 and 7.40 when necessary. Barbiturates were stable in aqueous solution for the length of the experiments (6–8 h).

Superfusion

Known concentrations of barbiturates were applied to individual neurons during intracellular recordings by the technique of superfusion. The bathing solution in the culture dish was completely exchanged by test solutions made up of bathing solution plus barbiturates at physiological pH, constant osmolarity and constant temperature (35–37 °C). Solutions were delivered to and removed from the 35 mm culture dish using a peristaltic pump adjusted to a rate of 0.5–1 ml/min. Exchange was considered complete only after 3 times the original bathing solution volume was superfused through the culture dish. In many cases 10 or more 'complete' exchanges were performed while recording intracellularly from a single spinal cord neuron.

Miniperfusion

Barbiturates could also be applied to the neuronal surface during intracellular recording by the technique of miniperfusion. A microelectrode whose tip was manually broken to a diameter of 2–10 μm was filled with test solution. Test solution consisted of bathing solution with barbiturates. The open end of each miniperfusion pipette was connected to a pressure regulator by tight fitting polyethylene tubing. Pressure pulse durations were regulated by a voltage-activated 3-way valve. Closure of the valve switched the miniperfusion pipette pressure from atmospheric to that selected on the pressure regulator (0.5–2.0 pounds per square inch (psi)). Test solutions were applied to the recorded neuron by positioning the miniperfusion pipette 10–50 μm from the neuronal surface following generation of a control action potential and removing it 1–3 s before the next stimulated action potential. Small hyperpolarizing artifacts ($<$ 5 mV) were occasionally produced by miniperfusion, but these could be minimized by using small diameter miniperfusion pipettes, low miniperfusion pressure, more distant placement of the electrode and removal of the electrode prior to stimulation. No change in membrane conductance was seen during the hyperpolarizing artifact. The miniperfusion pipettes (usually 3) and recording microelectrodes were held by Leitz micromanipulators. To decrease leakage of barbiturate into the bathing medium, the tips of the miniperfusion electrodes were kept in the oil phase between drug application trials. They were lowered into the aqueous phase only during the interstimulus interval when drug application was desired.

Percentage shortening of calcium-dependent action potential duration

Action potential durations were measured at half maximal action potential amplitude. Percentage shortening of action potential durations was determined, and the percentage shortening of duration after application of barbiturate was compared to the control duration before drug application.

RESULTS

Sodium- and calcium-dependent action potentials

In all experiments, recordings were obtained from spinal cord neurons. As previously reported[19], action potentials recorded from the somata of spinal cord neurons in control bathing solution were brief in duration (0.6 ms) (Fig. 1A). The rising phase of the action potential required sodium ions and was blocked by the sodium conductance blocker TTX. Addition of TTX, 25 mM TEA and 5 mM 3-AP to the bathing solution increased the average input resistance from 22.0 $\pm$ 4.4 MΩ ($\pm$ standard error of the mean (S.E.M.)) (10 neurons) to 57.4 $\pm$ 11.6 MΩ (8 neurons) and in about 50% of the neurons long-duration action potentials which were calcium-dependent could be elicited by depolarizing stimuli (Fig. 1B). Calcium-dependent action potentials could be elicited in a higher percentage of spinal cord neurons if the concentration of TEA was increased above 50 mM; however, at these concentrations the neurons deteriorated morphologically. The duration of the calcium-dependent action potentials in different neurons varied from 10 to 400 ms.

Phenobarbital and pentobarbital reduced calcium-dependent action potential duration

Application of phenobarbital and pentobarbital, but not barbituric acid, shortened calcium-dependent action potentials. Action potentials were elicited by intracellular depolarizing stimuli every 30 s (Fig. 2A, B). Control action potentials in this and subsequent figures were denoted by '1' (Fig. 2A, B); after superfusion of 3 ml of phenobarbital (2000 μM) or pentobarbital (300 μM), the action potential was

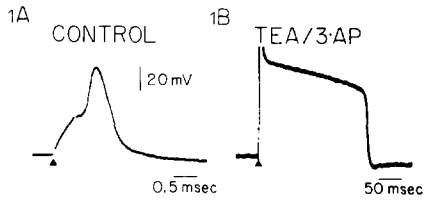

Fig. 1. Action potentials elicited from spinal cord neurons in control bathing solution (A) and bathing solution containing tetraethylammonium (TEA) and 3-aminopyridine (3-AP) (B). Action potentials were elicited from resting membrane potential (RMP) after brief depolarizing stimuli; stimulus onset in this and subsequent figures is denoted by filled triangles. The action potentials were recorded intracellularly in different bathing solutions: A was recorded in control bathing solution as described in Methods, and B in control bathing solution containing 25 mM TEA, 5 mM 3-AP and 3 μM TTX. Sodium chloride was reduced to 115 mM to maintain isosmolarity. Stimulus pulse duration and RMP were: A, 0.4 ms, —62 mV; B, 10 ms, —56 mV. This and subsequent figures were retouched to remove grid markings, and to fill in the rising phase of the action potentials when reproduced faintly.

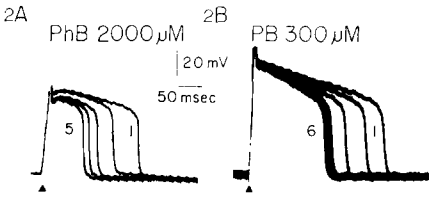

Fig. 2. Phenobarbital (PhB) and pentobarbital (PB) shortened calcium-dependent action potentials in spinal cord neurons. Superimposed calcium-dependent action potentials prior to (1) and after superfusion of 2000 μM PhB (A) and 300 μM PB (B). The action potentials in A and B were elicited with 10 ms depolarizing pulses every 30 ms. 5 and 6 are the fith and sixth action potentials elicited after superfusion of PhB or PB. 1, 5 and 6 are to the left of their respective action potentials. RMP were: A, —52 mV and B, —50 mV. The bathing solution was control bathing solution with 25 mM TEA, 5 mM 3-AP and 3 μM TTX.

shortened by 58% (Fig. 2A) and 45% (Fig. 2B). In addition to shortening of action potential duration, the plateau amplitude of the action potential decreased.

Shortening of action potential duration was dose-dependent. Superfusion of phenobarbital at 3 different concentrations (500, 1000 and 2000 μM) onto the same neuron produced progressively greater percentage shortening of the action potential duration (Fig. 3A_1, A_2, A_3). A similar reduction of action potential duration was seen with superfusion of pentobarbital onto another neuron at 3 different concentrations (100, 200 and 300 μM) (Fig. 3B_1, B_2, B_3). The number '2' (Fig. 3) denotes the action potential elicited after twice the initial volume of bathing solution was exchanged. After drug application, 5 times the volume of control solution was superfused through the culture. 'Control' action potential durations recorded before and after barbiturate

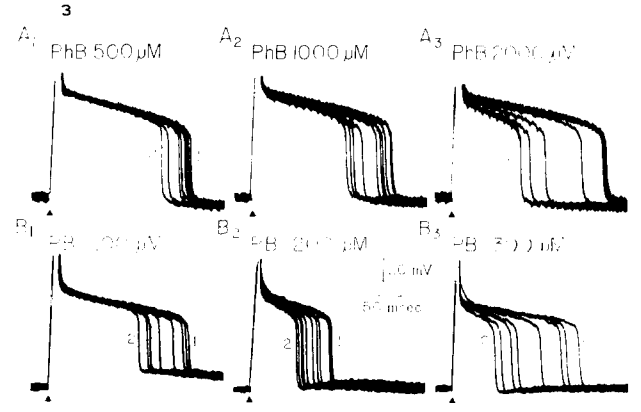

Fig. 3. Phenobarbital (PhB) and pentobarbital (PB) shortening of calcium-dependent action potentials was dose-dependent. PhB and PB were applied to spinal cord neurons by superfusion. Superimposed calcium-dependent action potentials stimulated with 10 ms depolarizing pulses every 30 s prior to (1) and after (2) superfusion of 500 (A_1), 1000 (A_2) or 2000 μM (A_3) PhB, and 100 (B_1), 200 (B_2) or 300 μM (B_3) PB. 1 and 2 are to the right and left of their action potentials, respectively. A_1, A_2 and A_3 were recorded from the same neuron (RMP —58 mV); B_1, B_2 and B_3 were recorded from the same neuron (RMP —55 mV). The bathing solutions were control bathing solution with 25 mM TEA, 3 μM TTX in both A_1, A_2, A_3 and B_1, B_2, B_3 and 5 mM 3-AP only in A_1, A_2, A_3.

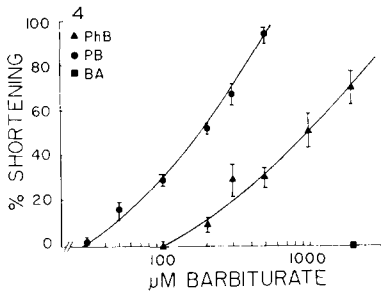

Fig. 4. Phenobarbital (PhB) and pentobarbital (PB) shortening of calcium-dependent action potentials was dose-dependent. PhB (filled triangles), PB (filled circles) and barbituric acid (BA) (filled square) were applied to spinal cord neurons by superfusion. Action potential duration was determined at half maximal amplitude. Percentage shortening is the percentage decrement in duration of the action potential after application of barbiturate compared to the control duration. This percentage shortening was plotted as a function of barbiturate concentration. Different doses of PhB or PB were applied onto the same neuron. Each new dose of barbiturate was superfused only after the neuron had been returned to bathing solution without barbiturate and the action potential duration returned to within 20% of its previous control value. BA was superfused twice during experiments of either PB or PhB. The filled triangles or circles were averages of shortening tested on 4–11 neurons. The bars above and below these averages were their standard errors of the mean (S.E.M.). The bathing solution contained control solution plus 25 mM TEA, 5 mM 3-AP and 3 μM TTX.

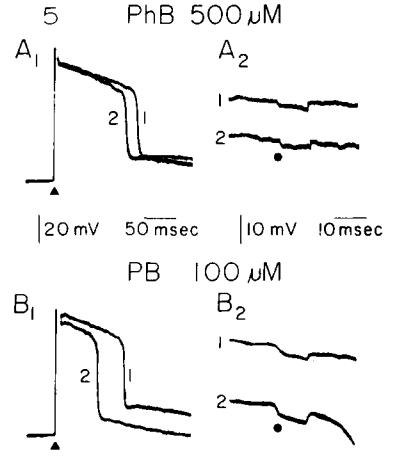

Fig. 5. Phenobarbital (PhB) and pentobarbital (PB) decreased conductance during the plateau of calcium-dependent action potentials. Superimposed calcium-dependent action potentials were elicited by 5 ms depolarizing stimuli every 30 s. Control action potentials (labeled '1' in A_1, A_2, B_1 and B_2) were elicited before application of 500 μM PhB (A_1 and A_2) and 100 μM PB (B_1 and B_2). 2 denotes the action potential elicited 6 s after a 4 s pressure pulse ejected either PhB or PB. A_2 and B_2 are high gain records of the action potential plateau before (1) or after (2) barbiturate application. 1 and 2 are separated by displacing the oscilloscope trace. A constant current pulse was superimposed on the plateau at a time indicated by the filled circles (A_2 and B_2). All records (A_1, A_2, B_1 and B_2) were obtained from the same spinal cord neuron. RMP was —57 mV. The bathing solution was control bathing solution with 25 mM TEA, 5 mM 3-AP and 1 μM TTX.

exposure differed by less than 20% in most experiments. Using superfusion, complete dose–response curves could be generated on single neurons (Fig. 4) with 6 different concentrations of barbiturate separated by 6 washings with drug-free medium. Each point on the dose–response curve was the average percentage shortening at each dose of barbiturate (Fig. 4). Pentobarbital was more potent than phenobarbital in producing action potential shortening. Pentobarbital shortened calcium-dependent action potentials over a 25–600 μM range with a 50% shortening at about 170 μM. Phenobarbital reduced calcium action potential duration over a 100–5000 μM range with a 50% shortening at about 900 μM. Barbituric acid (2 mM) did not reduce calcium-dependent action potential duration (Fig. 4; filled square).

Phenobarbital and pentobarbital decreased action potential plateau conductance

Miniperfusion of either phenobarbital (500 μM) or pentobarbital (100 μM) onto spinal cord neurons shortened the action potential duration ('2' in Fig. $5A_1$, B_1). Shortening of the action potential could have been produced by a decrease in calcium-conductance and/or by an increase in potassium or chloride conductance. To determine membrane conductance during action potential plateau, short constant current pulses (10 ms in duration) were superimposed on calcium-dependent action potentials before ('1' in Fig. $5A_2$, B_2) and after ('2' in Fig. $5A_2$, B_2) miniperfusion of 500 μM phenobarbital (Fig. $5A_2$) and 100 μM pentobarbital (Fig. $5B_2$). The potential produced in response to constant current pulses increased in amplitude after application of 500 μM phenobarbital ('2' Fig. $5A_2$) and 100 μM pentobarbital ('2' Fig. $5B_2$) compared to control ('1' in Fig. $5A_2$, B_2), indicating decreased conductance.

DISCUSSION

Barbiturates and calcium-dependent action potentials

We have demonstrated that the pharmacologically active barbiturates phenobarbital and pentobarbital, but not the pharmacologically inactive barbiturate, barbituric acid, decreased the duration of calcium-dependent action potentials in mouse spinal cord neurons in cell culture. Action potential duration is determined by the relative magnitude of depolarizing inward current (in this case calcium current) and hyperpolarizing outward currents (potassium and chloride currents). Action potential shortening could have been produced, therefore, by a reduction of calcium conductance or by an enhancement of either potassium or chloride conductances. Several lines of evidence suggest that barbiturates reduce calcium conductance. At anesthetic concentrations, pentobarbital reduced the calcium influx induced by potassium depolarization of mouse brain synaptosomes[5,13,23,35] and rat sympathetic ganglia[4] and by preganglionic stimulation in rat sympathetic ganglia[4] (Table I). Pentobarbital also reduced the maximum rate of rise ($\dot{V}_{max}$) of calcium dependent action potentials in the R2 neuron of *Aplysia* abdominal ganglia[16]. Since the recordings were made in sodium-free medium, $\dot{V}_{max}$ was a measure of an early inward calcium current. However, pentobarbital and phenobarbital have also been shown to increase membrane chloride conductance of frog spinal motoneurons and dorsal root af-

TABEL I

One-half maximal or average effective concentrations (range of effective concentrations)

Plasma barbiturate concentrations were calculated assuming full distribution in total body water (70% body weight) and brain barbiturate concentrations were calculated assuming a brain to plasma ratio of 0.75.

	Pentobarbital (μM)	*Phenobarbital (μM)*
GABA augmentation[49]	50 (10–400)	75 (20–500)
Anticonvulsant action[40]	75 (50–100)	90 (60–120)
Reduced calcium-dependent action potential duration*	170 (25–600)	900 (100–5000)
Reduction of synaptically evoked spontaneous activity[49]	250 (100–500)	1250 (500–2500)
Reduced release of ACh from the vagal innervation of the heart[24]	170 (50–500)	—
Reduced release of ACh from midbrain slices[47]	297 (50–1000)	1440 (200–2000)
Reduction of Ca^{2+} uptake of synaptosomes[5]	450 (50–1600)	—
Increased membrane chloride conductance[49]	(100–500)	(500–4000)
Neurotoxic or sedative action[40]	75 (50–100)	270 (180–360)
Anesthetic action[44]	187 (150–225)	(>500)

* Data from the present study.

ferents[32,33] and mouse spinal cord neurons in cell culture[26,28,49] at high anesthetic concentrations. The direct barbiturate action was blocked by the GABA-antagonists picrotoxin, bicuculline and penicillin and thus was probably due to an interaction with GABA receptors. Since barbiturates did not displace [^{3}H]GABA from its binding sites on brain membranes[36], it is unlikely that barbiturates bind to the active GABA recognition site but rather they probably bind to a coupling site between the GABA-binding site and the chloride channel. Finally, barbiturates have been shown to increase a slowly developing voltage-dependent potassium conductance in *Aplysia* neurons at anesthetic concentrations[8]. In the present experiments, pentobarbital and phenobarbital increased the amplitude of short, small hyperpolarizing constant current pulses applied during the action potential plateau. Since the hyperpolarizations were small, their amplitudes were proportional to the membrane conductance during the plateau. An increase in the amplitude of the pulses suggested that net membrane conductance was reduced, consistent with a primary barbiturate action on calcium conductance. This does not exclude the possibility that barbiturates reduced calcium-dependent action potential duration by increasing potassium and/or chloride conductance. Direct measurement of calcium, chloride and potassium currents using the voltage clamp technique would be required to determine which ionic conductance is involved in action potential shortening.

Barbiturates may reduce release of neurotransmitter by blocking presynaptic calcium entry

Pentobarbital reduced the amplitude of the monosynaptic L7 ventral root reflex

responses evoked by stimulation of the triceps surae nerve[56]. Quantal analysis of excitatory postsynaptic potentials recorded in motoneurons which were evoked by stimulation of single group Ia afferent fibers demonstrated that pentobarbital reduced the presynaptic release of neurotransmitter without altering the postsynaptic response to the neurotransmitter. Pentobarbital reduced the quantal content 24.7% at a subanesthetic intravenous dose (10 mg/kg). Assuming uniform distribution in total body water (70% of total body weight) and a brain to plasma ratio for PB of 0.75, the barbiturate concentration in spinal cord would have been about 45 μM. In the present study, pentobarbital reduced the calcium-dependent action potential by about 10% at 45 μM.

Furthermore, pentobarbital reduced acetylcholine release from vagal terminals in chicken heart evoked by field stimulation[24] and from rat cortical, striatal, hippocampal, midbrain and pons-medulla slices evoked by potassium-induced depolarization[46,47,55] at concentrations ranging from 50 to 500 or 1000 μM (Table I) and reduced release of GABA[7,9,10,18], glutamate[10,38], aspartate[38,57] and norepinephrine[18] from cortical synaptosomes at concentrations above 100 μM. Phenobarbital reduced acetylcholine release from rat midbrain slices[47] over a higher concentration range (200–2000 μM) (Table I) but did not significantly reduce release of GABA, aspartate or glutamate from rat cortex at 1 mM[10]. In mouse spinal cord neurons in cell culture, pentobarbital reduced synaptically driven spontaneous activity at concentrations of 100–500 μM (50% of cells having no spontaneous activity at 250 μM) (Table I)[49]. Phenobarbital also reduced synaptically driven spontaneous activity but at the higher concentrations of 500–2500 μM (50% of cells having no spontaneous activity at 1250 μM) (Table I)[49]. Calcium uptake by rat brain synaptosomes was reduced by pentobarbital[5] over a concentration range (50–1600 μM (Table I) similar to that reducing transmitter release, while phenobarbital produced only a small (15%) decrease in calcium uptake at 900 μM[5].

In the present study, calcium action potential duration was reduced by pentobarbital over the same concentration range (25–600 μM) (Table I) effective in reducing neurotransmitter release and synaptosomal calcium uptake. Phenobarbital also reduced calcium-dependent action potential duration but over a higher concentration range (100–5000 μM) (Table I), similar to that necessary for reduction of neurotransmitter release. Thus, it is likely that barbiturates reduce release of neurotransmitters from presynaptic terminals by reducing presynaptic calcium entry.

Mechanism of barbiturate anticonvulsant and anesthetic actions

Barbiturates have multiple actions on neurons but these actions are produced at different barbiturate concentrations. For a specific barbiturate action to have significance for its clinical mechanism of action, it must occur at clinically relevant concentrations. Both phenobarbital and pentobarbital have anticonvulsant actions, but only phenobarbital is used in ambulatory patients due to the undesirable sedative side effects of pentobarbital. Plasma phenobarbital levels of 43–129 μM (10–30 μg/ml) are within the therapeutic range in humans, but brain to plasma phenobarbital ratios are about 75%[52]. Thus free phenobarbital levels in the central nervous system should

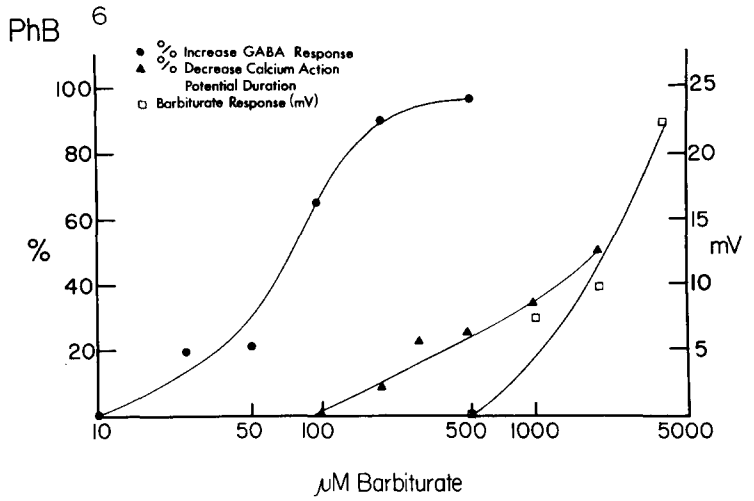

Fig. 6. Dose-dependency of phenobarbital actions on GABA responses, calcium-dependent action potentials and membrane chloride ion conductance. Phenobarbital: (1) augmented GABA responses produced iontophoretically (filled circles); (2) reduced calcium-dependent action potential duration (filled triangles), and (3) increased membrane chloride ion conductance in mouse spinal cord neurons in cell culture. Data on GABA augmentation and reduction of chloride ion conductance are from Schulz and Macdonald[49].

be about 30–90 μM. Similarly, phenobarbital had anticonvulsant action in rats against pentylenetetrazol (PTZ) and maximal electroshock (MES) seizures from about 14–28 mg/kg when administered intraperitoneally[40]. Brain phenobarbital concentrations should have been about 60–120 μM. Phenobarbital augmented GABA responses on spinal cord neurons in cell culture over a 20–500 μM range[49] (Fig. 6; Table I) and reduced glutamate responses at similar concentrations[27].

Sedative or neurotoxic actions of phenobarbital in rats were produced from 40–80 mg/kg when administered intraperitoneally[40]. Brain phenobarbital levels should have been about 180–360 μM corresponding to reduction of calcium-dependent action potential duration of about 10–25 % (Fig. 6; Table I). Phenobarbital produced anesthesia at brain concentrations greater than 500 μM[7] corresponding to a reduction of calcium-dependent action potential duration of about 35 % (Fig. 6; Table I). Phenobarbital also increased chloride conductance at concentrations greater than 500 μM[49] (Fig. 6; Table I).

Pentobarbital had anticonvulsant actions in rats against PTZ and MES seizures from 12–24 mg/kg when administered intraperitoneally[40]. Brain pentobarbital levels should have been about 50–100 μM (Table I). Pentobarbital augmented GABA responses over a similar concentration range (10–400 μM)[49]. Pentobarbital produced neurotoxic (sedative) actions at similar brain concentrations (50–100 μM)[40] and produced anesthesia at slightly higher concentrations (150–225 μM)[44]. Pentobarbital reduced calcium action potential duration 15–30 % at neurotoxic (sedative) concentrations and 40–60 % at anesthetic concentrations (Fig. 7; Table I). Pentobarbital also increased chloride ion concentration at anesthetic concentrations ($>$ 150 μM) (Fig. 7; Table I)[49]. Direct or non-synaptic actions of barbiturates are not likely to be involved

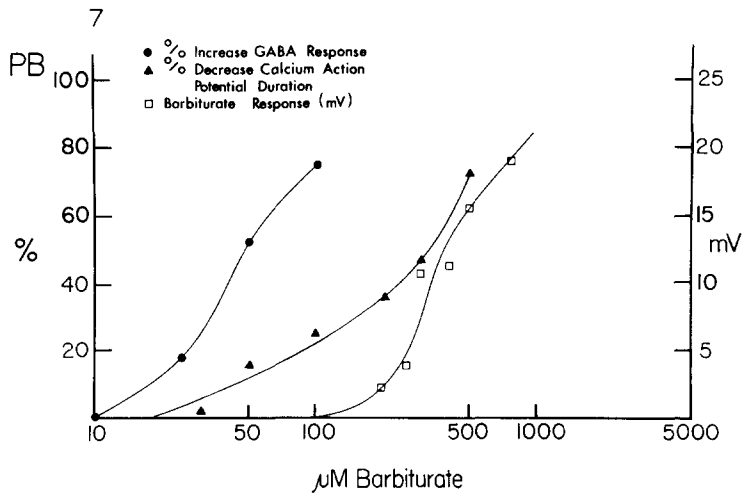

Fig. 7. Dose-dependency of pentobarbital actions on GABA responses, calcium-dependent action potentials and membrane chloride ion conductance. Pentobarbital: (1) augmented GABA-responses produced iontophoretically (filled circles); (2) reduced calcium-dependent action potential duration (filled triangles), and (3) increased membrane chloride ion conductance in mouse spinal cord neurons in cell culture. Data on GABA augmentation and reduction of chloride ion conductance are from Schulz and Macdonald[49].

in anticonvulsant, sedative-hypnotic or anesthetic actions since voltage-dependent sodium and potassium conductances were reduced only at high barbiturate concentrations ($> 500\ \mu M$)[3,30,50].

Thus postsynaptic augmentation of GABA-mediated inhibition and antagonism of excitatory synaptic transmission occurred at anticonvulsant barbiturate concentrations. Neurotoxic (sedative) actions occurred at barbiturate concentrations which reduced calcium-dependent action potential duration 10–30%. Anesthesia occurred at barbiturate concentrations which reduced calcium-dependent action potential duration greater than 35% and directly increased postsynaptic chloride conductance. Barbiturate anticonvulsant action was therefore correlated with postsynaptic modulation of amino acid responses. Neurotoxic (sedative) actions were correlated with addition of some presynaptic reduction of transmitter release to postsynaptic amino acid response modulation. Anesthesia was correlated with an increase in inhibition of transmitter release and direct postsynaptic increase in chloride ion conductance in addition to the postsynaptic amino acid response modulation. These results also suggest that anticonvulsant and anesthetic barbiturates differ only in the dose dependency of their actions. Phenobarbital is useful as an anticonvulsant in ambulatory patient because there is a good therapeutic index between amino acid modulation (anticonvulsant action) and reduction of transmitter release and increase of chloride ion conductance (sedative and anesthetic actions). Pentobarbital is useful only for sedation and anesthesia because the dose-dependency of these actions are overlapping and there is a poor therapeutic index.

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