MOLECULAR PHARMACOLOGY, 28:269–277

Barbiturates Decrease Voltage-Dependent Calcium Conductance of Mouse Neurons in Dissociated Cell Culture

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Received July 25, 1984; Accepted June 20, 1985

SUMMARY

Barbiturates have been shown to reduce presynaptic release of neurotransmitter. It is likely that barbiturates alter transmitter release by decreasing calcium entry since barbiturates decrease calcium influx into snaptosomes and reduce the maximal rate of rise and duration of calcium-dependent action potentials. The mechanisms of barbiturate action on neuronal calcium entry have been studied using mouse dorsal root ganglion neurons in cell culture. Dorsal root ganglion neuron action potentials have a calcium-dependent component which is decreased by the barbiturates, pentobarbital (50–500 μM) and phenobarbital (500–2000 μM). Calcium-dependent action potential after hyperpolarization was also decreased by barbiturates. Intracellular injection of the potassium channel blocker, cesium, enhanced barbiturate actions. In voltage-clamp studies, barbiturates reduced inward calcium current and calcium chord conductance without altering the leak conductance which is present after all calcium conductance was blocked by application of cadmium ions (100 μM). Calcium current inactivation was accelerated by barbiturates but unaffected by cadmium. We conclude that barbiturates reduce calcium conductance by enhancing calcium channel inactivation or by producing open channel block of calcium channels.

INTRODUCTION

Barbiturates have been shown to have multiple synaptic and nonsynaptic action in the central nervous system (1–3). One of these actions is to reduce presynaptic release of neurotransmitter (4–16) and to reduce calcium entry into synaptosomes (17–20). Furthermore, barbiturates have been shown to reduce the duration of presynaptic calcium-dependent action potentials, and thus calcium entry, at the squid giant synapse at concentrations that reduce release of neurotransmitter (13). Thus, it is likely that barbiturates reduce release of neurotransmitter by blocking presynaptic calcium entry. In vertebrate and most invertebrate neurons, recordings cannot be made from presynaptic terminals. However, calcium-dependent action potentials have been recorded from cell bodies of many neurons (21–28). Several neurotransmitters and drugs have been shown to reduce somatic calcium-dependent action potentials at concentrations effective in reducing the release of neurotransmitters (29–35). Thus, actions of neurotransmitters and drugs on somatic calcium-dependent action potentials may be similar to their actions on presynaptic calcium entry. Barbiturates have been shown to reduce the maximal rate of rise of calcium-dependent action potentials of cell R_2 of the *Aplysia* abdominal ganglion (36). Barbiturates also reduced the duration of calcium-dependent action potentials of mouse neurons in dissociated cell culture (32) at concentrations that reduced spontaneous release of neurotransmitter (37) and K^+-stimulated uptake of $^{45}Ca^{2+}$ (38). Thus, it is likely that barbiturates also modify calcium entry at synaptic terminals as well as neuronal cell bodies.

Barbiturates could modify calcium-dependent action potential duration by directly blocking calcium channels. However, barbiturates could also reduce calcium-dependent action potential duration indirectly by enhancing voltage- or calcium-dependent potassium conductance which would result in less net inward current and thus less calcium entry.

To investigate the mechanism of barbiturate reduction of calcium-dependent action potentials, we have studied the action of barbiturates on calcium-dependent action potentials and inward currents of mouse DRG[1] neurons in cell culture with and without blockade of potassium channels with intracellular and extracellular replacement of potassium ion by the potassium channel blocker cesium ion. We report that barbiturates reduce voltage-

Research was supported by United States Public Health Service Grant NS 19692 and by National Science Foundation Grant BNS-8118762. M. A. W. was supported by National Institutes of Health Grant DA 5244; R. L. M. was supported by National Institutes of Health Grant RCDA-NS 00480.

[1] The abbreviations used are: DRG, dorsal root ganglion; TBS, Tris-buffered balanced saline; TEA, tetraethylammonium; Ac, acetate; TTX, tetrodotoxin.

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dependent calcium conductance of DRG neurons without altering potassium conductance.

MATERIALS AND METHODS

Cell culture. Cultures were prepared, as previously described (38), by dissecting spinal cords with attached dorsal root ganglia from 12–14-day-old fetal mice, then mechanically dissociating the tissue and plating the resultant cell suspension on 35-mm collagen-coated plates at a density of ⅛ or ¼ spinal cord/plate. Initial culture medium consisted of 80% Eagle's minimum essential medium, 10% horse serum, and 10% fetal calf serum. Approximately 4 days after initial plating, 5′-fluoro-2′-deoxyuridine and uridine were added to the cultures to inhibit the division of non-neuronal background cells after which growth medium was modified to 90% Eagle's minimum essential medium and 10% horse serum. All culture medium contained nerve growth factor at a concentration of 5 ng/ml to promote survival and growth of DRG neurons. Two- to twelve-week-old cultures were used in electrophysiology experiments.

Intracellular recording and single microelectrode voltage clamp. For intracellular recording, neurons were placed in TBS (pH 7.3, 320 mosmol) that contained in mM: NaCl, 135.0; KCl, 5.3; $MgCl_2$, 0.8; $CaCl_2$, 5.0; Tris base, 13; glucose, 5.6; and TEA, 5.0. Cultures were placed on the heated stage (35°c) of an inverted phase contrast microscope allowing neuronal impalement by a recording micropipette under visual observation. Drug actions on DRG neurons somatic calcium-dependent action potentials were determined during intracellular recording using high resistance (20–50 megaohms) micropipettes filled with 4 M KAc or 4 M CsAc. A modified bridge circuit allowed simultaneous current application and voltage recording using single micropipettes.

For voltage clamp, neurons were bathed in the same TBS or in TBS with the potassium channel blocker cesium substituted for potassium (cesium TBS). In all voltage clamp experiments, 5–10 μM TTX was added to the recording medium. Micropipettes used for voltage-clamp recordings were filled with either 3 M potassium chloride (KCl) (for recording in TBS medium) or 3 M CsCl (for recording in cesium TBS medium) and had resistances of 10–25 megaohms. Micropipette tip capacitance was reduced by coating the micropipette shank with polystyrene (Q-dope, GC Electronics), and noise was reduced by painting the pipette shaft with conductive material and driving the shield. The somata of DRG neurons were voltage clamped using a single microelectrode voltage clamp preamplifier (Dagan 8100) that switched between voltage sampling and current injection modes at 3 KHz with a 50-50 duty cycle. In several experiments an Axoclamp-2 preamplifier was used as a single microelectrode voltage clamp that switched at 6 KHz between voltage sampling and current injection modes with a 70-30 duty cycle. The accuracy of the single microelectrode voltage clamp procedure was assessed by inserting a second intracellular micropipette connected to a separate recording amplifier to independently monitor membrane potential. Depolarizing clamp steps applied to neurons bathed in cesium TBS and impaled with cesium-containing micropipettes produced inward currents that inactivated slowly. Voltage steps measured by the voltage-clamp micropipette and independent micropipette corresponded to within 10%.

Drug application by pressure ejection or diffusion. Pentobarbital (Mallincrodt), phenobarbital (Mallincrodt), barbituric acid (Sigma), and cadmium chloride (Matheson, Coleman and Bell) were dissolved in recording medium. Drugs were applied locally to neurons by pressure ejection (0.5–1.5 p.s.i.) for 1–2 sec from micropipettes with a tip diameter of 2–5 μm or, when long term application was required as during determination of current-voltage relations, by diffusion from micropipettes with a tip diameter of 15–25 μm. Micropipette tips were cracked under direct visual observation, approximately 400× magnification, to be of appropriate tip diameters.

RESULTS

Action potentials of mouse DRG neurons had sodium- and calcium-dependent components. DRG neuron action potentials were previously determined to have two components: an initial sodium-dependent component that was only partially sensitive to block by tetrodotoxin as well as a slower calcium-dependent component (28). In recording medium containing 1.0 mM calcium, DRG neuron action potentials had durations of about 2.5 msec and had a convex inflection on the repolarizing phase (Fig. 1, A_1). Cadmium, a calcium channel blocker, abolished the convex inflection leaving an action potential

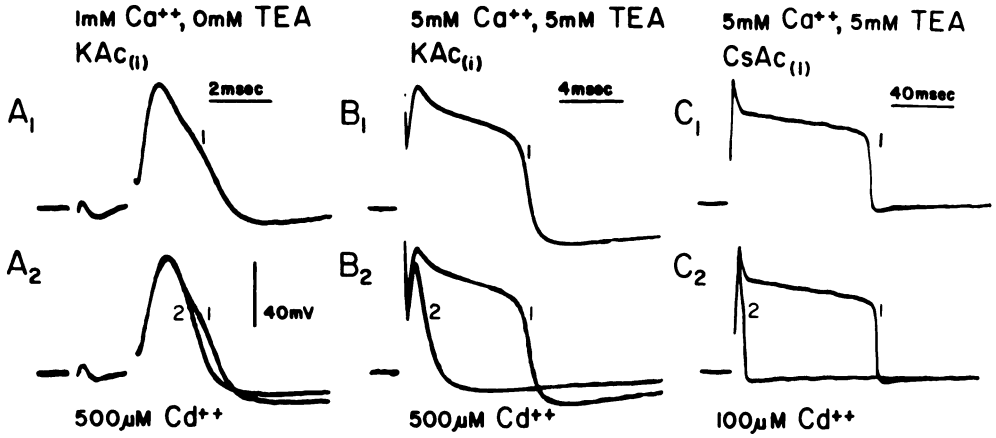

FIG. 1. *Action potentials of dorsal root ganglion neurons have sodium- and calcium-dependent components and have a potassium-dependent after-hyperpolarization*

Action potentials were evoked from resting membrane potential at 15-sec intervals by 100–500-sec duration depolarizing stimuli. The action potentials illustrated in A_1 and A_2, however, were evoked by 2-sec depolarizing stimuli while the modified Wheatstone bridge circuit was maintained in balance. Action potentials were evoked in medium containing 1 mM Ca^{2+}, and 0 mM TEA^+ (A_1, A_2) or 5 mM Ca^{2+} and 5 mM TEA^+ (B_1, B_2) during recording with KAc-filled micropipettes and in medium containing 5 mM Ca^{2+}, 5 mM TEA^+ during recording with CsAc-filled micropipettes (C_1, C_2). A_2, B_2, C_2: superimposed action potentials evoked prior to (*1*) and subsequent to (*2*) application of cadmium (Cd^{2+}), a calcium channel blocker. TEA at 5 mM partially blocked potassium conductance and augmented the calcium component of the action potential to a duration of about 10 msec (*A*, *B*). Intracellular cesium abolished substantial potassium conductance, thus abolishing the after-hyperpolarization and producing calcium-dependent action potentials with durations of 100–2000 msec (*C*).

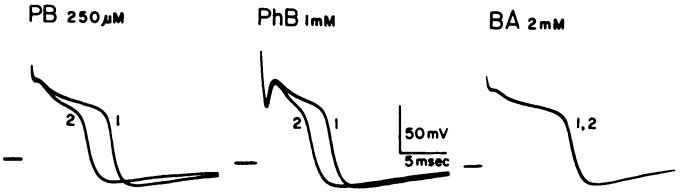

FIG. 2. *Pentobarbital and phenobarbital but not barbituric acid decreased DRG neuron calcium-dependent action potential duration*

Action potentials were evoked at a frequency of 4/min by 100-μs depolarizing current pulses. Action potentials evoked before (*1*) and after (*2*) barbiturate application were superimposed. Pentobarbital (*PB*) at 250 μM, phenobarbital (*PhB*) at 1 mM but not the inactive barbiturate, and barbituric acid (*BA*) at 2 mM decreased the calcium component of action potentials recorded in a single DRG neuron. The recording medium bathing the neurons during the experiments described in Figs. 2–8 contained 5 mM Ca^{2+}, 5 mM TEA, and 5 mM K^+. A complete description of the medium is given in the text.

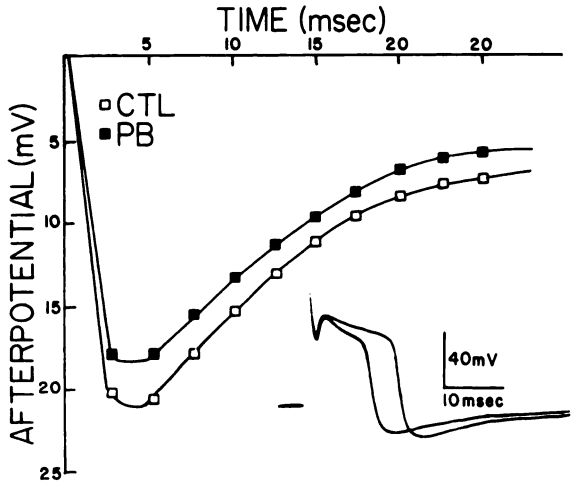

FIG. 3. *Barbiturates decreased action potential after-hyperpolarization*

The amplitude of action potential after-hyperpolarizations prior to (□) and following (■) pentobarbital application (*inset*) are plotted as a function of time. In each case, measurement of the after-hyperpolarization was begun where the repolarizing limb of the action potential intersected resting membrane potential.

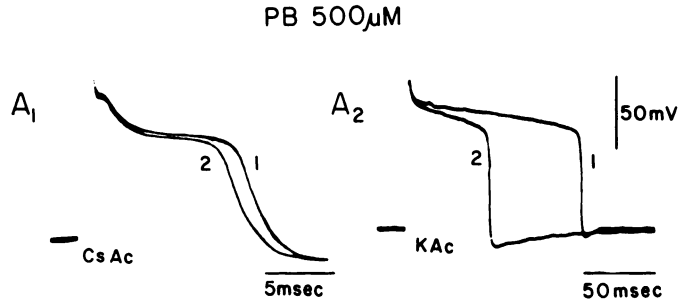

FIG. 4. *Pentobarbital decreases of calcium-dependent action potential duration were enhanced by intracellular cesium injection*

During recording with a KAc-filled micropipette, pentobarbital decreased calcium-dependent action potential duration by less than 10%. The KAc-filled micropipette was then withdrawn, and the neuron was reimpaled with a CsAc-filled micropipette. Following intracellular iontophoresis of cesium, pentobarbital reduced calcium-dependent action potential duration by approximately 55%.

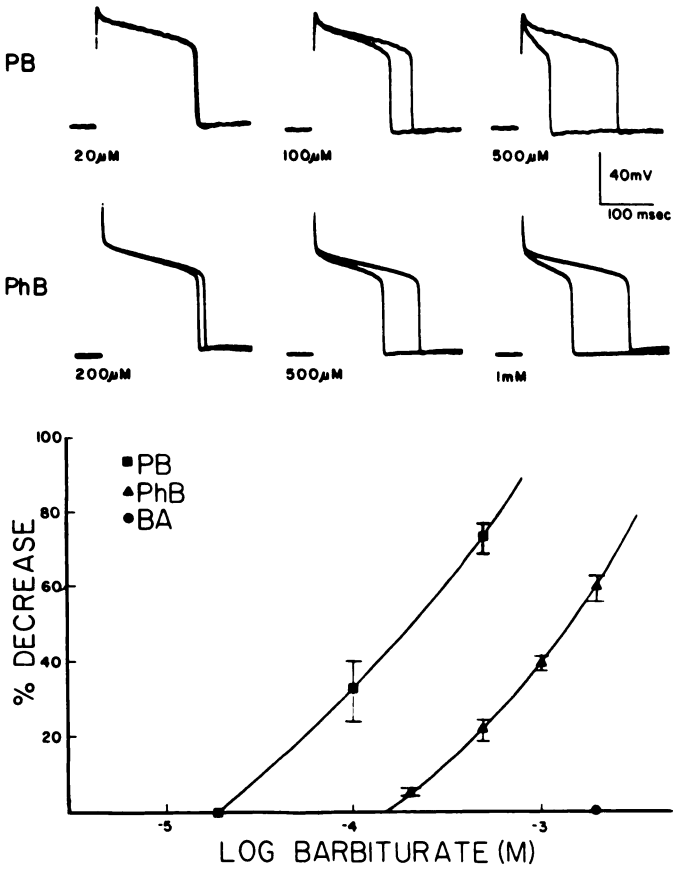

FIG. 5. *Pentobarbital and phenobarbital decreased calcium-dependent action potential duration dose-dependently but over different concentration ranges*

Semilogarithmic plot of dose-dependent actions of pentobarbital (n = 8) and phenobarbital (n = 5) on DRG neurons impaled with CsAc-filled micropipettes. Bars represent standard error of mean. Dose-dependent action of pentobarbital (*PB*) and phenobarbital (*PhB*) on single DRG neurons is shown. *BA*, barbituric acid.

with a duration of about 2.0 msec which was sodium-dependent (Fig. 1, A_2). The calcium component of action potentials was augmented to 5–25 msec by recording medium containing 5 mM calcium and 5 mM TEA (Fig. 1, B_1). The first 2.0 msec of the action potentials were dependent upon sodium with the remaining broad plateau dependent upon calcium, as indicated by the blockade of the plateau by cadmium (Fig. 1, B_2). Although 5 mM TEA augmented the calcium component of action potentials, substantial voltage- and/or calcium-dependent potassium conductance remained as indicated by the large after-hyperpolarization recorded following action potentials. Intracellular injection of cesium, with the extracellular medium containing either 5.3 mM potassium or substituting cesium for potassium, decreased membrane potential to 0 to −20 mV. Following membrane hyperpolarization to about −60 mV, calcium-dependent action potentials with durations of 100 msec–2 sec, but without after-hyperpolarization, could be evoked, consistent with substantial blockade of potassium conductance (Fig. 1, C_1). Application of cadmium (100 μM) blocked the long duration action potential, leaving only the short sodium-dependent component (Fig. 1, C_2).

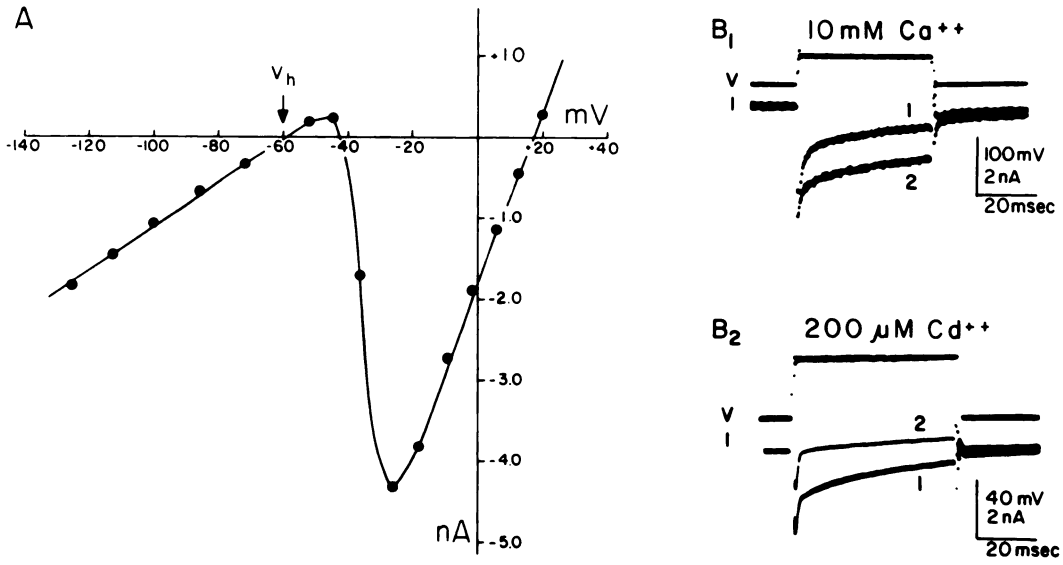

FIG. 6. *Depolarizing commands evoked calcium-dependent currents*

The current-voltage relation obtained when command steps were applied from a holding potential of −60 mV to a dorsal root ganglion neuron bathed in potassium-free medium that substituted cesium for potassium and contained 5 mM tetraethylammonium chloride is illustrated in *A*. The recording micropipette contained 3 M CsCl. B_1 and B_2 (trace 1 in each case) illustrate the inward currents obtained from neurons bathed in 1 mM Ca^{2+} and 5 mM Ca^{2+}, respectively. Local application of 10 mM calcium augmented inward current (B_1; trace *2*) while the calcium channel blocker, cadmium, at 200 μM reduced the inward current (B_2; trace *2*).

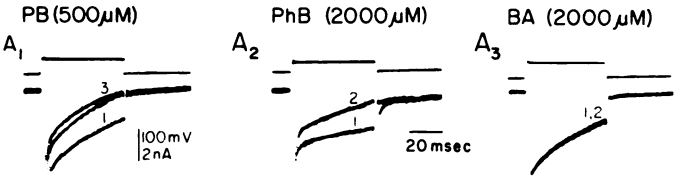

FIG. 7. *Barbiturates decreased inward currents evoked by depolarizing voltage commands*

Pentobarbital (*PB*), phenobarbital (*PhB*), but not barbituric acid (*BA*) decreased the amplitude of inward currents evoked by depolarizing voltage commands from a holding potential of −60 mV. Data represent superimposed traces evoked prior to (*1*) and during (*2*) barbiturate application. Trace *2* (pentobarbital) was obtained immediately after the onset of barbiturate application by local pressure ejection from blunt-tipped micropipettes. Trace *3* (pentobarbital) was evoked 5 sec later and was the maximal obtained response.

Barbiturates decreased DRG neuron somatic calcium-dependent action potential duration without affecting resting membrane potential or conductance. We determined barbiturate actions on somatic calcium-dependent action potentials obtained from DRG neurons bathed in 5.3 mM potassium and impaled with KAc-filled micropipettes (Fig. 2). Application of pentobarbital (250 μM) for 2 sec by pressure ejection delivered 4 sec prior to evoking an action potential decreased DRG neuron somatic calcium-dependent action potential duration of 10 neurons tested and slightly augmented action potential duration of 4 neurons. Phenobarbital at 1 mM decreased DRG neuron somatic calcium-dependent action potential duration in 5 neurons tested and slightly augmented action potential duration in 4 neurons. The inactive barbiturate, barbituric acid (2 mM), did not affect action potential duration or configuration.

Effects on DRG neuron somatic calcium-dependent action potential duration by pentobarbital, phenobarbital, and cadmium were not associated with a change of either resting membrane potential (20 neurons) or resting membrane conductance (4 neurons).

Barbiturates reduced calcium-dependent action potential after-hyperpolarization. DRG neuron action potentials have large after-hyperpolarizations of 10–25 mV with a mean after-hyperpolarization amplitude of 19 mV ± 0.7 (n = 19). Pentobarbital and phenobarbital effects on calcium-dependent action potential duration were associated with a decrease in the amplitude of action potential after-hyperpolarizations (Fig. 3). The peak amplitude of action potential after-hyperpolarizations was decreased 0.9 to 3.7 mV (mean 2.2 mV ± 0.2) (13 of 13 neurons) by 500 μM pentobarbital and 2.5 to 4.9 mV (mean 3.5 mV ± 0.4) by 1 mM phenobarbital (5 of 5 neurons). The calcium channel blocker cadmium (100 μM) had a similar action on after-hyperpolarizations producing decreases of 1–10 mV (mean 3.3 mV ± 1.0) (9 of 9 neurons).

Barbiturates decreased calcium-dependent action potential duration following intracellular cesium injection. Pentobarbital and phenobarbital, but not barbituric acid, decreased somatic calcium-dependent action potential duration of DRG neurons bathed in medium substituting cesium for potassium and impaled with CsAc-filled micropipettes (18 neurons). We compared barbiturate actions on single DRG neurons bathed in potassium-containing medium during recording with KAc-filled and CsAc-filled micropipettes (4 neurons). Barbiturate decreases of calcium-dependent action potential duration were consistently larger during CsAc recording. In contrast to the results obtained with KAc-filled micropipettes, barbiturates decreased calcium-dependent action potentials of all DRG neurons during CsAc recording. Indeed, DRG neuron action potentials that had durations

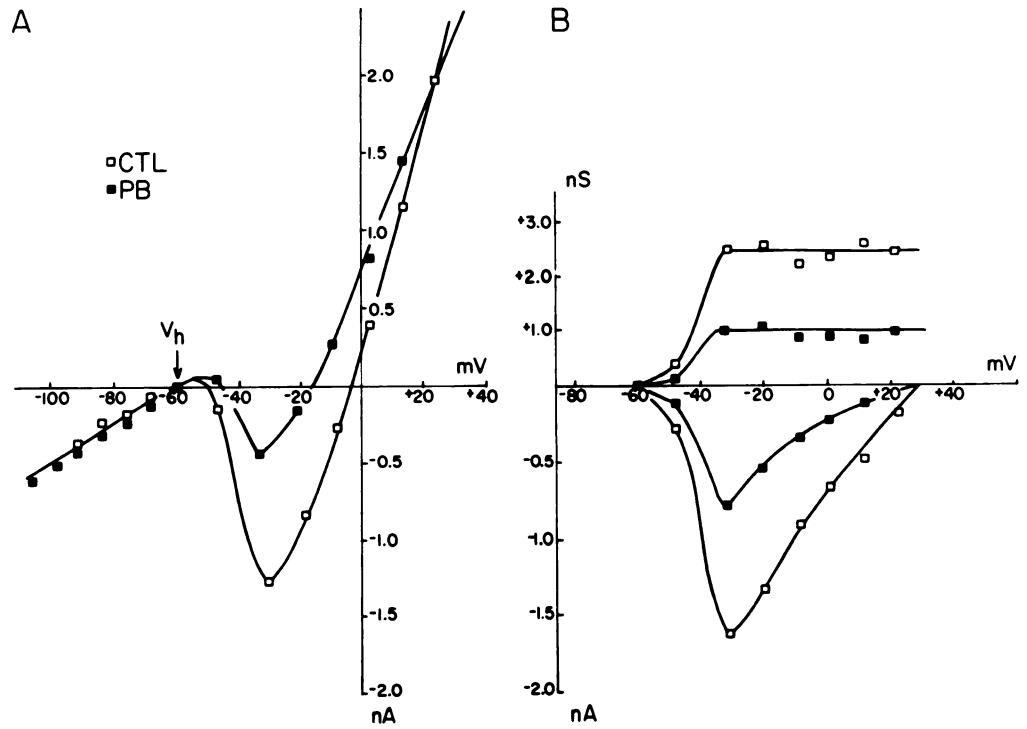

FIG. 8. *Pentobarbital reduced the amplitude of inward currents but did not affect their voltage dependence or extrapolated reversal potential*
The current-voltage relation obtained from a DRG neuron prior to (□) and following (■) pentobarbital (500 μM) application is plotted. *B* (*lower*) illustrates the current-voltage relation after leak current has been subtracted. The leakage curve was estimated by averaging the current-voltage curve obtained in 7 neurons with an input resistance comparable (±10%) to the neuron illustrated. *B* (*upper*) is the conductance-voltage relation following leak subtraction and is derived from *B* (*lower*).

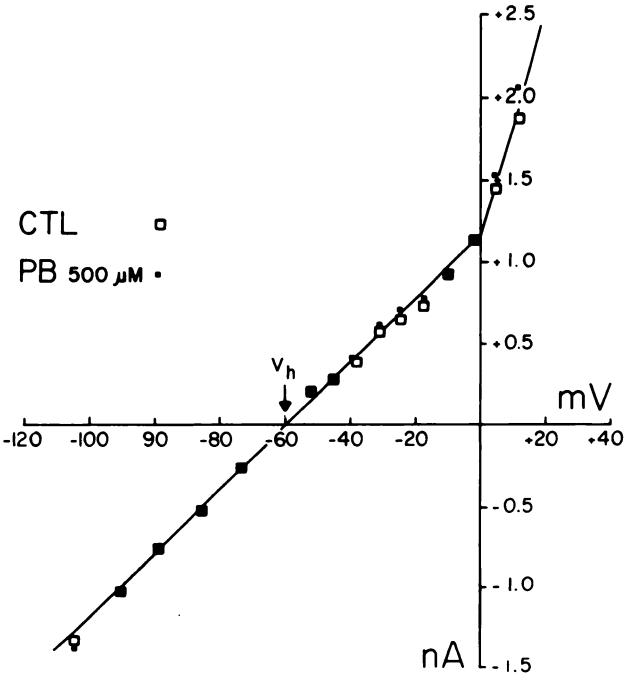

FIG. 9. *Barbiturates did not affect leak conductance*
Pentobarbital decreased inward current when potassium conductance was largely blocked by cesium but did not affect leak currents when the inward calcium current was blocked by 500 μM cadmium. The plot is the current-voltage relationship obtained in the presence of cadmium (□) and cadmium plus pentobarbital (■).

unaffected or slightly prolonged by barbiturates during recording with KAc-filled micropipettes had large decreases of action potential duration during recording with CsAc-filled micropipettes (Fig. 4). During recording with CsAc-filled micropipettes, pentobarbital and phenobarbital produced dose-dependent decreases of calcium-dependent action potential duration over 50–500 μM (IC_{50} = 220 μM 6 of 6 neurons) and 500–2000 μM (IC_{50} = 1200 μM) (8 of 8 neurons) respectively (Fig. 5).

Membrane currents of DRG neuron somata. For voltage clamp, neurons were bathed in potassium-free medium containing cesium and TTX and were impaled with CsCl-filled micropipettes. Neurons were held at −60 mV and step depolarizations to potentials between −40 to +25 mV produced net inward currents which had several components (Fig. 6). Despite the present of the sodium channel blocker, TTX, an early inward current was occasionally recorded which was presumably at least partially sodium dependent. Following this early current, a later slowly inactivating current was recorded. The magnitude of the late inward current was dependent upon extracellular calcium concentration (Fig. 6, B_1), and the currents were reduced by the calcium channel blocker, cadmium (Fig. 6, B_2). Since the single electrode clamp technique is relatively slow and allowed voltage control only after 1–3 msec and since there was occasionally some TTX-resistant early inward sodium current, all current measurements were made after the early inward current had diminished (5–10 msec after the onset of the clamp step). The current-voltage relation-

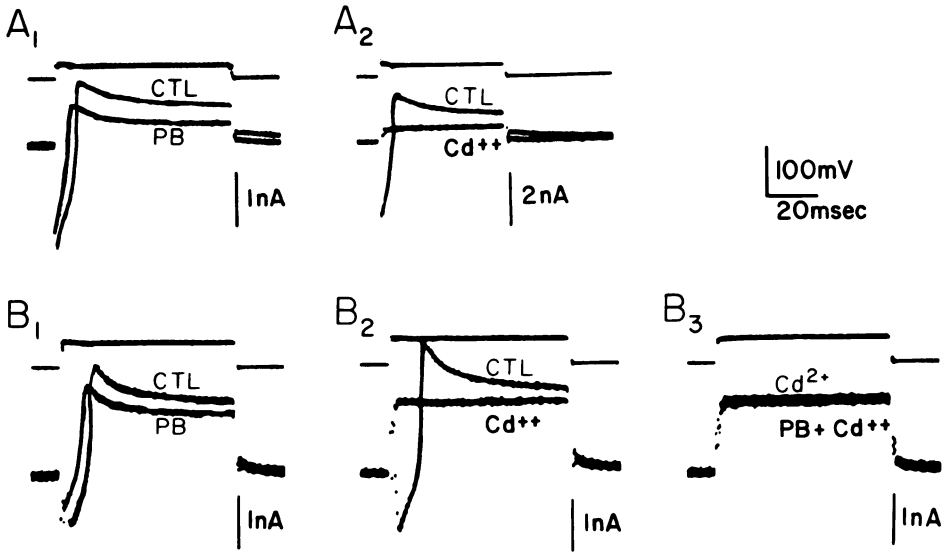

FIG. 10. *Barbiturates decreased the magnitude of late outward current when they were not blocked by cesium*

Voltage clamp recordings obtained from neurons bathed in medium containing normal potassium. Depolarizing clamp commands from a holding potential of −60 mV produced a transient inward current which rapidly declined and developed into an outward current. Pentobarbital decreased (A_1) and cadmium totally suppressed (A_2) the inward current. Both substances also decreased the outward potassium current. *B.* Recording from a single neuron illustrating that pentobarbital decreased (B_1) and cadmium totally abolished (B_2) the initial inward current and that both substances reduced the late outward current. On the same neuron, pentobarbital did not affect that magnitude of the outward current measured in the presence of cadmium (B_3). Calibrations for A_1 and *B* are: voltage, 100 mV; current, 1 nA; and time, 20 msec. Calibrations for A_2 are the same except that for current the calibration bar represents 2 nA.

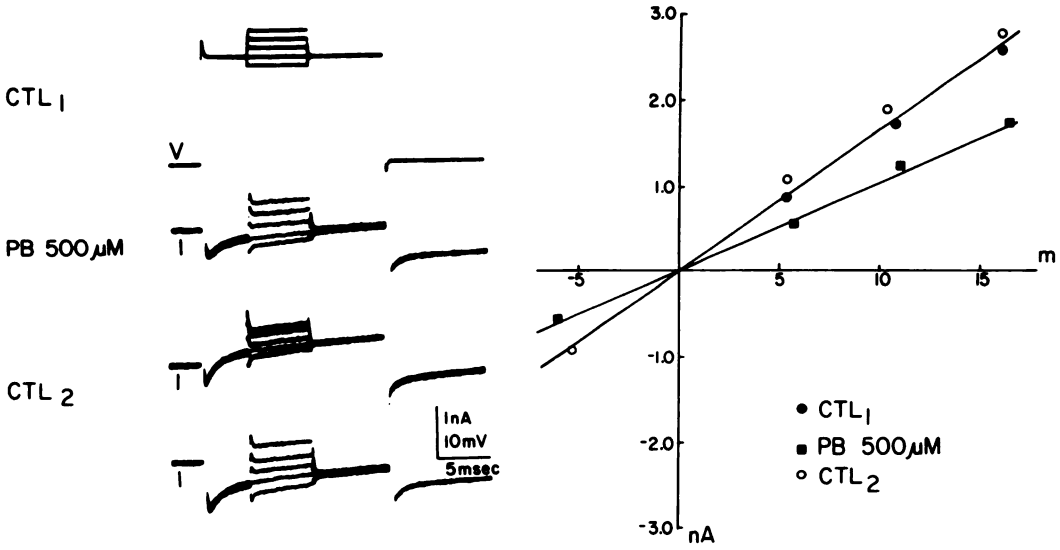

FIG. 11. *Pentobarbital reduced chord conductance by about 50% at step commands that fully activated calcium conductance*

From a holding potential of −60 mV, depolarizing step commands were applied to fully activate voltage-dependent calcium conductance. During the large depolarizing command, small hyperpolarizing and depolarizing voltage steps (±10 mV) were applied. Barbiturate application resulted in less current being required to attain a given voltage during step commands that fully activated calcium conductance.

ship was linear with hyperpolarizing and small depolarizing command voltages (Fig. 6, A). With depolarizing commands greater than 20 mV, net inward currents of 1–12 nA were produced with the maximal inward current being recorded at about −20 mV. Currents became net outward above +20 mV. In the presence of cadmium (500 μM) the current-voltage relationship was linear over the voltage range of −140 to 0 mV consistent with substantial blockade of potassium conductance by cesium and calcium conductance by cadmium (7 neurons).

Barbiturates decreased the magnitude of inward currents. Pentobarbital and phenobarbital, but not barbituric acid, reversibly decreased the magnitude of depolarization-induced inward currents (19 neurons) (Fig. 7). Barbiturates did not affect the potential at which net inward current was recorded, the potential at which the inward current was maximal, or the extrapolated reversal potential for calcium current (4 neurons) (Fig. 8). When the inward calcium current was blocked by cadmium, the

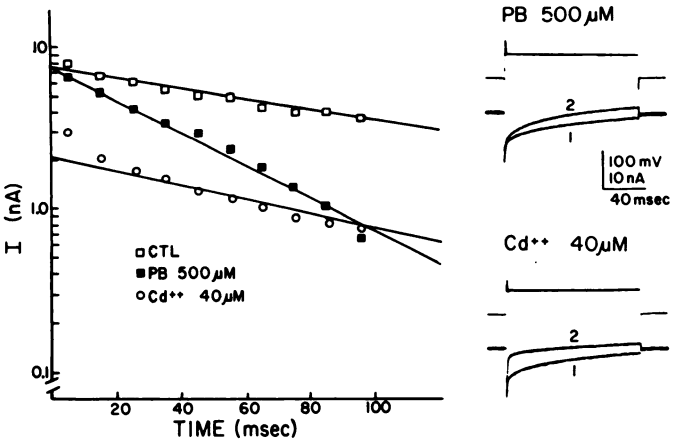

FIG. 12. *Pentobarbital increased the rate at which calcium currents decayed*

The decay of calcium current as a function of time is plotted semilogarithmically. Calcium current activated by a step depolarization from a holding potential of −60 mV inactivated with a time constance of about 180 msec. The decay time constant in the presence of pentobarbital, however, was about 70 msec. In contrast, calcium current inactivation in the presence of 40 μM cadmium had a time constant of 160 msec and thus did not differ from control.

remaining leak current was unaffected by pentobarbital (5 neurons) (Fig. 9).

The absence of a barbiturate effect on cadmium-insensitive outward currents does not definitively indicate a barbiturate action on voltage-dependent calcium conductance since an augmentation of calcium-dependent potassium current is not excluded. We have obtained an indirect measure of barbiturate effects on calcium-dependent potassium current. In the presence of normal potassium, depolarizing commands produced a transient inward current that declined rapidly and developed into an outward current (Fig. 10). Cadmium chloride (200–500 μM) abolished the inward current and also reduced the magnitude of the later outward current (Fig. 10, A_2, B_2). Similarly, barbiturates decreased the duration of the early inward current and reduced the magnitude of the late outward current ($n = 28$) (Fig. 10, A_1, B_1). However, barbiturates did not affect the magnitude of the outward current in the presence of cadmium (Fig. 10, B_3). Thus, cadmium and barbiturates block the calcium-dependent inward current and also calcium-dependent outward currents.

Barbiturates decreased calcium conductance. Finally, we have used a more direct approach to determine that barbiturates decrease voltage-dependent calcium conductance rather than augment calcium-dependent potassium conductance. From a holding potential of −60 mV, depolarizing step commands were applied to fully activate voltage-dependent calcium conductance. During the large depolarizing commands, small hyperpolarizing and depolarizing voltage steps (±10 mV) were applied. Barbiturate application resulted in less current being required to attain a given voltage during step commands that fully activated calcium conductance (Fig. 11). Thus, the reduction in inward currents by barbiturates was associated with a decrease in membrane chord conductance consistent with an action to decrease calcium conductance rather than to augment an outward potassium conductance. At step commands that fully activated calcium conductance, 500 μM pentobarbital reduced chord conductance by 48% ± 5 (mean of 5 neurons).

Barbiturates increased the rate at which calcium currents decayed. Calcium currents activated by depolarizing step commands decayed with a time constant between 120 and 180 msec ($n = 3$). During step commands 150 to 750 msec, pentobarbital (500 μM) increased the rate at which calcium currents decayed by about a factor of three. In three neurons, we obtained time constants of 120, 160, and 180 in control, but 45, 45, and 70 in 500 μM pentobarbital. While 40 μM cadmium also reduced the magnitude of calcium currents, it did not affect the rate at which calcium currents decayed (Fig. 12).

DISCUSSION

The barbiturates, pentobarbital and phenobarbital, directly reduced voltage-dependent calcium conductance of mouse DRG neurons in cell culture since they: 1) reduced the duration of calcium-dependent action potentials and inward currents following extracellular replacement of potassium by the potassium channel blocker cesium and intracellular cesium injection; 2) reduced peak calcium conductance without altering the extrapolated calcium equilibrium potential; 3) reduced the instantaneous current-voltage slope (calcium chord conductance); and 4) did not alter membrane conductance following blockade of calcium channels by cadmium.

It is possible that the reduction in inward current produced by the barbiturates was due to an enhancement of an outward cesium current. However, the absence of effect of barbiturates in the presence of the calcium channel blocker cadmium argues strongly against an effect on a "leak" outward cesium current or a voltage-activated outward cesium current. It does not rule out the possibility that barbiturates enhanced calcium-activated outward cation current. The finding that barbiturates reduced the peak conductance achieved at large depolarizing commands, however, effectively rules out a major action on a calcium-activated cesium outward current. Thus, it is likely that the barbiturates directly reduce voltage-dependent calcium conductance.

The decay of the inward current during the maintained depolarizing step was accelerated in the presence of barbiturates. A similar enhancement of the rate of calcium current decline by barbiturates has been observed in *Helix* neurons (39). The decay of the inward current is likely to be due to either calcium- and/or voltage-dependent inactivation of calcium conductance. Thus, barbiturates may increase the rate of decline of the inward current by accelerating calcium-dependent or voltage-dependent inactivation of calcium channels. Alternatively, an enhanced rate of current decay would be expected if barbiturates produce a blockade of open calcium channels similar in mechanism to the barbiturate blockade of open end-plate channels that has been reported (40).

Barbiturate reduction of calcium-dependent action potentials of mouse dorsal root ganglion neurons in cell culture was similar to that of mouse spinal cord neurons

in cell culture (32). However, the action potentials had to be recorded using different methodologies. Spinal cord neurons have minimal somatic calcium conductance and calcium-activated potassium conductance while dorsal root ganglion neurons have a large somatic calcium conductance and calcium-activated potassium conductance (33). In spinal cord neurons, 25 mM extracellular TEA produced substantial block of voltage-dependent potassium conductance and allowed long duration calcium-dependent action potentials to be evoked. It was difficult to consistently evoke calcium-dependent action potentials at lower TEA concentrations in spinal cord neurons. In dorsal root ganglion neurons, considerable calcium-activated potassium conductance remained following 5 mM extracellular TEA application, and thus the calcium-dependent action potentials were of shorter duration and were followed by large after-hyperpolarizations. Under these conditions, barbiturates shortened calcium-dependent action potentials of spinal cord neurons but had variable actions on the calcium-dependent action potentials of dorsal root ganglion neurons. It is likely that the variable result on dorsal root ganglion neurons was due to insufficient blockade of calcium-activated potassium conductance. Thus, reduction of calcium entry also reduced the calcium-activated outward potassium current. The net effect on calcium-dependent action potential duration, then, depended on the relative reduction in the two currents.

These results have several implications for the action of barbiturates. First, as with spinal cord neurons, barbiturates reduce calcium conductance of DRG neurons at concentrations that are in the sedative/anesthetic range and are correlated with reduction in neurotransmitter release (32). This suggests that calcium channel block and reduction of neurotransmitter release may be causally related and that the effect may contribute to the development of sedation and anesthesia. In addition, if barbiturates hasten calcium channel inactivation, it is possible that barbiturates would reduce release of neurotransmitter due to rapid repetitive stimulation more effectively than to slow repetitive stimulation.

ACKNOWLEDGMENTS

We thank Nancy Fox for illustrative work and Michaela Weeks, Debbra Neuvirth, and Marjorie Mills for secretarial assistance. We thank Dr. Gordon Guroff for kindly supplying us with nerve growth factor.

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