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PENTOBARBITONE PHARMACOLOGY OF MAMMALIAN CENTRAL NEURONES GROWN IN TISSUE CULTURE

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SUMMARY

1. The effects of the barbiturate anaesthetic pentobarbitone on the membrane properties and amino acid pharmacology of mammalian C.N.S. neurones grown in tissue culture were studied using intracellular recording coupled with bath application, extracellular ionophoresis, or focal diffusion.

2. The addition of an anaesthetic concentration of pentobarbitone to the bathing medium abolished all spontaneous synaptic activity, but did not render individual cells electrically inexcitable nor prevent evoked synaptic activity.

3. Focal ionophoresis of pentobarbitone or diffusion from blunt micropipettes reversibly increased membrane conductance, effectively dampening excitability without directly affecting individual action potential characteristics.

4. Pentobarbitone-induced membrane conductance was reversibly blocked by picrotoxin. The inversion potential of the pentobarbitone voltage response depended on Cl^- ion gradients and was similar to that of GABA.

5. Pentobarbitone reversibly enhanced the conductance increase produced by GABA with a variable slowing of response kinetics, shifting GABA dose–response curves to the left. Responses to glycine and β-alanine were not affected.

6. Higher ionophoretic currents of pentobarbitone, which measurably increased membrane conductance, attenuated and markedly slowed GABA responses. Similar effects on GABA responses were observed by superimposing GABA pulses on low level GABA currents.

7. Pentobarbitone, in the absence of an increase in membrane conductance, reversibly depressed depolarizing responses to glutamate without changing response kinetics. Slower responses to acetylcholine which were associated with an apparent decrease in membrane conductance were not affected by the drug.

8. Analysis of double-reciprocal plot data suggested a non-competitive type of antagonism between pentobarbitone and glutamate. Pentobarbitone depression of glutamate was not affected by picrotoxin.

9. Both GABA and glutamate responses appeared to be equally sensitive to pentobarbitone. Specific interaction of the drug with amino acid receptor-coupled events is indicated by the requirement for pentobarbitone pipette placement close to the amino acid response site.

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10. The results suggest that pentobarbitone depresses neuronal excitability by (1) directly activating post-synaptic GABA-receptor coupled Cl^- conductance, (2) potentiating post-synaptic GABA-induced conductance events, probably at the level of the GABA receptor, and (3) depressing post-synaptic glutamate-induced excitation, probably at the level of the conductance mechanism.

INTRODUCTION

The barbiturate pentobarbitone is commonly used to induce or maintain anaesthesia in vertebrates. The cellular mechanisms underlying the generalized depression of excitability have been studied using a wide variety of vertebrate and invertebrate preparations with various electrophysiological and biochemical methods. Several frequently made observations include (1) depression of axonal conduction (Staiman & Seeman, 1974) and voltage-dependent Na^+ spike conductance (Thesleff, 1956; Blaustein, 1968; Narahashi, Frazier, Deguchi, Cleaves & Ernaw, 1971), (2) direct depression of post-synaptic excitability either through cytoplasmic (Krnjević, 1974) or membrane effects (Ransom & Barker, 1975; Nicoll, 1975*a*; Nicoll, Eccles, Rubia & Oshima, 1975), (3) depression of excitatory synaptic transmission at neuromuscular junctions (Galindo, 1971; Thomson & Turkanis, 1973; Seyama & Narahashi, 1975; Adams, 1976; Proctor & Weakly, 1976) with evidence of enhanced transmitter release, (4) depression of excitatory synaptic transmission at peripheral vertebrate ganglia (Larrabee & Posternak, 1952) and invertebrate ganglia (Barker & Gainer, 1973; Barker, 1975*a*, *b*, *c*), (5) depression of excitatory transmission in the spinal cord (Brooks & Eccles, 1947; Somjen, 1963; Somjen & Gill, 1963; Løyning, Oshima & Yokota, 1964; Weakly, 1969) and in supra-spinal preparations (Galindo, 1969; Richards, 1972), (6) preservation of inhibitory synaptic transmission in the C.N.S. (Larson & Major, 1970; Eccles, Faber & Taborikova, 1971), (7) prolongation of inhibitory post-synaptic (Nicoll, 1972; Nicoll, Eccles, Oshima & Rubia, 1975) and pre-synaptic transmission (Eccles & Malcolm, 1946; Eccles, Schmidt & Willis, 1963; Schmidt, 1963; Nicoll, 1975*b*) and (8) depression of K^+-depolarized Ca^{2+} uptake and/or transmitter release in peripheral nerve or C.N.S. nerve ending preparations reported by some laboratories (Quastel, Hackett & Okamoto, 1972; Blaustein & Ector, 1975; Blaustein, 1976; Haycock, Levy & Cotman, 1977; but not others (Olsen, Lamar & Bayless, 1977). Pentobarbitone thus appears to have multiple actions, most of which would be expected to contribute to a general depression of neuronal excitability.

The membrane events which account for pentobarbitone's C.N.S. effects have not been directly established using C.N.S. tissue, although considerable progress has been made with a number of the *in vitro* model systems. We have investigated some of the membrane mechanisms underlying the depression of neuronal excitability by pentobarbitone using a tissue-cultured C.N.S. preparation and report here three principal effects of the anaesthetic: (1) direct depression of membrane excitability through activation of GABA receptors, (2) specific modulation of GABA events to enhance and prolong the conductance change evoked by GABA, and (3) non-competitive depression of glutamate excitation. Some preliminary observations have appeared earlier (Ransom & Barker, 1975, 1976).

METHODS

The techniques for tissue culture, recording, solutions and ionophoresis were as described in the preceding paper. Post-synaptic pharmacology was carried out in medium containing elevated Mg^{2+} to depress synaptic activity and allow clearer study of post-synaptic interactions. Pentobarbitone (Abbott, Chicago) was ionophoresed from a freshly prepared 0·2 M solution (pH 9·2) or diffused from a blunt micropipette containing 0·1 mM drug in bathing medium (10 % heat-inactivated horse serum–90 % Eagle medium with 10 mM-$MgSO_4$) or added to the medium (giving a final concentration of 0·1 mM). In several experiments picrotoxin (Sigma, St Louis) was diffused near a penetrated cell from a blunt micropipette containing 1 mM drug in bathing medium.

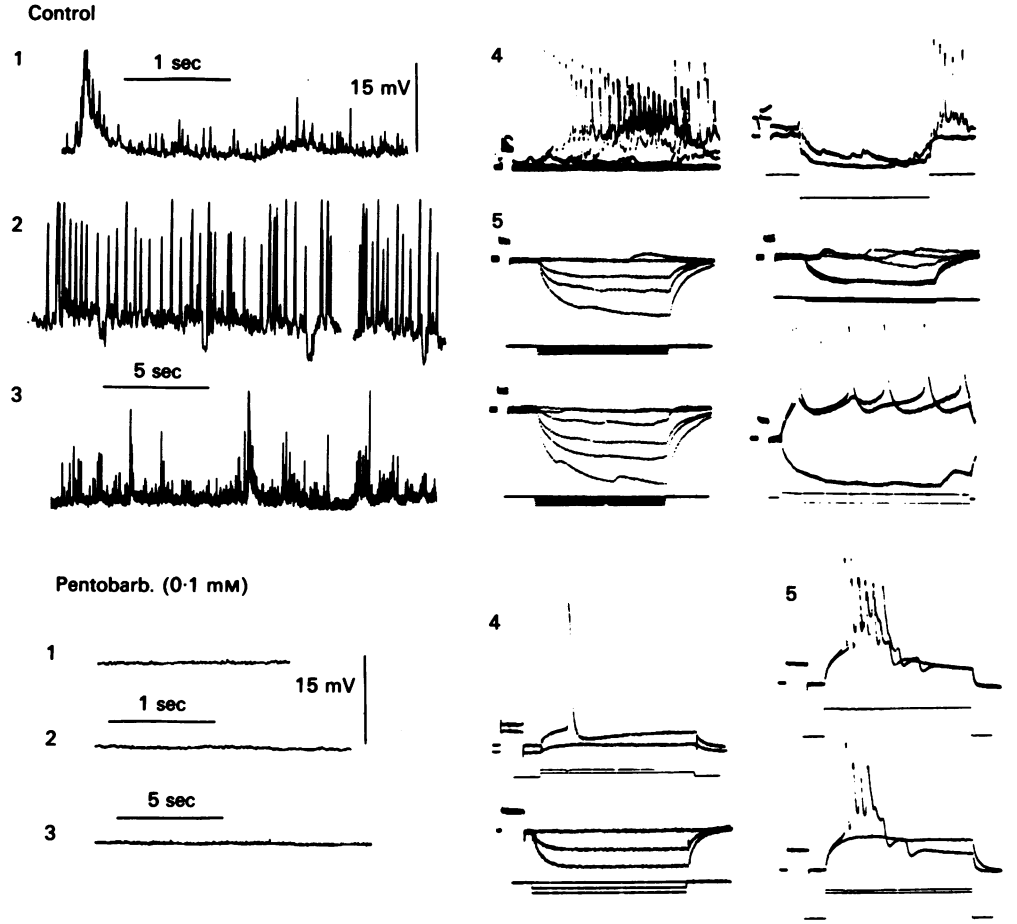

Fig. 1. Recordings from ten different neurones in the same culture. *Control*, five have been recorded in the maintenance medium (modified Eagle medium with 10 % horse serum) to show representative examples of spontaneous activity. 1–3 are rectilinear pen recordings (downward 'events' in 2 are voltage responses to constant current pulses). 4–5 are oscillographic recordings with potential traces above current traces (−0·5 nA in upper right panel of 4). Cal. pulse: 10 mV, 10 msec. Resting membrane potentials (mV): −57 (1), −51 (2), −54 (3), −49 (4), −62 (5). Pentobarbitone was added to same plate to give final concentration of 0·1 mM. All neurones recorded under these conditions were electrically silent. 1–3 are pen recordings. 4–5 are oscillographic records which show excitability and recurrent inhibitory activity (5). Cal. 10 mV, 10 msec. Resting membrane potentials (mV): −52 (1), −60 (2), −47 (3), −50 (4), −58 (5).

RESULTS

Direct effects of pentobarbitone

Intracellular recordings of spontaneous activity before and after addition of pentobarbitone in the bath were carried out on two different occasions. All 33 neurones recorded from in control medium showed spontaneous activity of varying degree (Fig. 1*A*), while all twenty-four neurones recorded from after addition of pentobarbitone were electrically silent (Fig. 1*B*). Although the latter were not spontaneously active, they were excitable, showing full-amplitude action potentials, repetitive activity and recurrent synaptic activity when depolarized to threshold by injected current (Fig. 1, B_{4-5}). Due to variation in input resistance from cell to cell, any change in this membrane property could not be properly evaluated.

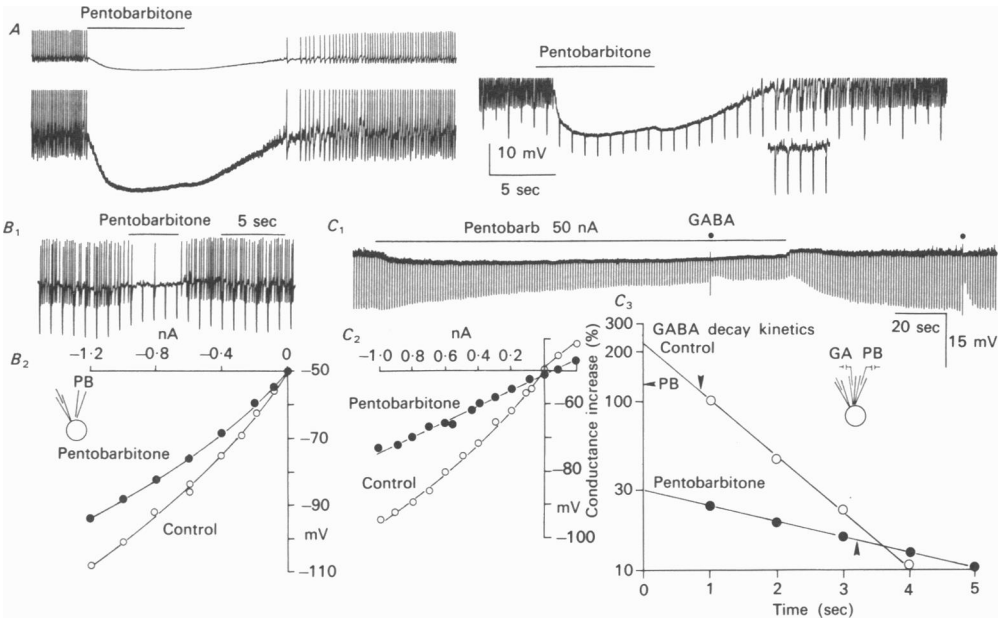

Fig. 2. Pentobarbitone directly alters resting membrane properties. *A* and *C* are recordings from different spinal cord cells, *B* is from a cerebellar-brainstem neurone. Pentobarbitone applied by local diffusion from a blunt micropipette containing 0·1 mM of the drug in *A* and *B*, and by ionophoresis in *C*. The drug depresses excitability (*A*, *B*) by increasing membrane conductance (*A–C*) with (*A*, *C*), or without (B_1) an increase in membrane potential. Uppermost trace in *A* is at one third the gain of other two traces in *A*. Pentobarbitone alters the steady-state *I–V* curve (B_2, C_2) and depresses and prolongs the conductance increase to ionophoresed GABA (C_3). Vertical arrowheads in C_3 indicate half-decay times. Horizontal arrowhead marked PB gives PB-induced membrane conductance. Constant current pulses (nA): −0·5 (*A*), −0·3 (*B*, *C*). GABA pulses: 25 nA, 100 msec (*C*). Resting membrane potentials (mV): −41 (*A*), −40 (*B*, *D*), −53 (*C*). Calibration in *A* applies to *B*. Inserts in this and following Figures are schematic diagrams of intracellular and extracellular pipettes used in each experiment.

Diffusion or ionophoresis of pentobarbitone from micropipettes placed at the cell body invariably depressed the excitability of spinal and cerebellar–brainstem neurones, altering membrane potential and increasing membrane conductance to a

variable degree (Fig. 2). These effects were associated with a decrease in the slope resistance the steady-state current–voltage curve (n = 22 cells) (Fig. 2B_2, C_2). The magnitude of the conductance change which could be induced by sustained ionophoresis of pentobarbitone was considerably less, and the kinetics of the conductance change obviously slower than that induced by GABA (Fig. 2C_3) (see also Figs. 7, 10).

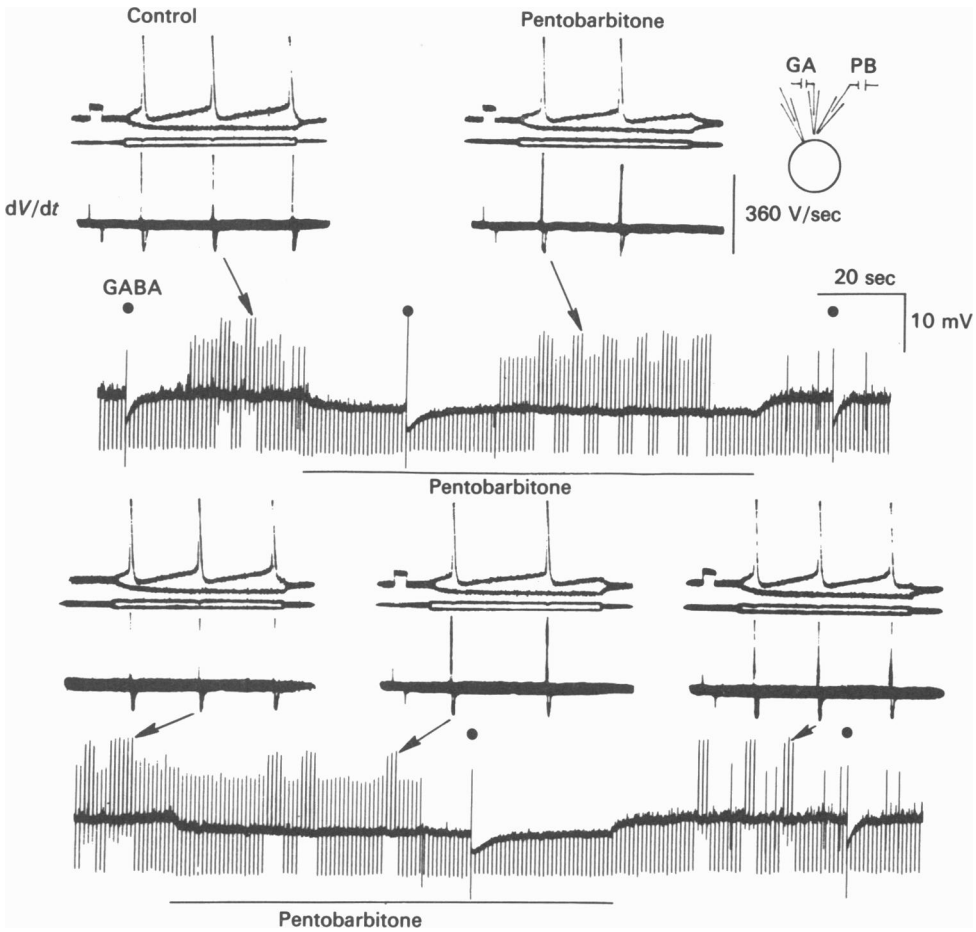

Fig. 3. Pentobarbitone increases the membrane conductance and alters the GABA response without altering rate of rise of action potential of a spinal cord cell. Top: oscillographic records of action potentials with potential and current traces aligned above dV/dt traces before (control) and during pentobarbitone ionophoresis (pentobarbitone). Arrows indicate where oscillographic data come from on continuous pen-writer record. GABA pulse (100 msec, 20 nA) used to monitor pentobarbitone efficacy (40 nA ionophoresis indicated by bars beneath trace) is enhanced and prolonged during concomitant ionophoresis with pentobarbitone. Sequence repeated and illustrated in lower oscillographic and pen-writer records. Calibration on potential traces: 10 mV, 10 msec.

Voltage responses to ionophoresed pentobarbitone were also slower than those of comparable size in response to pulsed GABA (Figs. 6A, 9D_2). A large increase in conductance induced by pentobarbitone greatly attenuated that produced by a pulse of GABA (Figs. 2C_1, C_3, 10A_1). Voltage responses were likewise attenuated and their

kinetics altered by diffused and ionophoresed pentobarbitone (Figs. $2C_3$, D, 7). While local application of pentobarbitone appreciably inhibited spontaneous activity and increased membrane conductance, it did not render the cell inexcitable nor did it depress the rate of rise of the action potential ($n = 5$ cells) (Fig. 3).

Picrotoxin diffused from a micropipette containing a 1 mM solution prevented (Fig. 4A) or reversed (Fig. 4B) the direct effects of ionophoresed pentobarbitone on the cell ($n = 6$ cells) (Fig. 4A, B). Under these conditions picrotoxin directly excited the cell and completely blocked the voltage response and conductance increase in response to GABA. A barbiturate reversal of convulsant antagonism of GABA

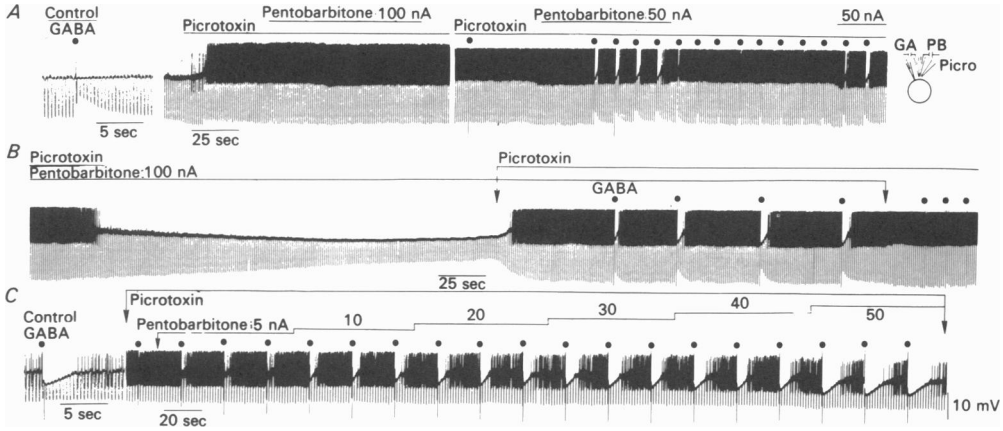

Fig. 4. Picrotoxin blocks inhibitory effects of GABA and pentobarbitone on a spinal cord cell. A, picrotoxin diffused from blunt pipette containing 1 mM drug directly excites cell, increases apparent input resistance, and decreases depressant effects of ionophoresed pentobarbitone and GABA (marked by filled circles). Coincident ionophoresis of GABA and pentobarbitone brings out GABA's inhibitory effect. Trace continued in B. Withdrawal of picrotoxin pipette during ionophoresis of pentobarbitone reveals drug-induced increase (45 %) in membrane conductance. Replacement of picrotoxin pipette reverses pentobarbitone effect but GABA responses remain inhibitory during, but not after, pentobarbitone ionophoresis. C, dose–response curve of pentobarbitone restoration of picrotoxin-blocked GABA response of a spinal cord cell. Quantitative data graphed in Fig. 5. Resting membrane potentials (mV): -47 (A), -44 (C). Constant current pulses: $-0{\cdot}5$ nA (A), $-0{\cdot}3$ nA (B, C).

responses elicited at the superior cervical ganglion has been reported by Bowery & Dray (1976). Concomitant ionophoresis of pentobarbitone at doses which did *not* directly affect membrane properties restored the blocked GABA response (Fig. 4A, B) in a dose-dependent manner (Fig. 4C). In fact, it was possible more than fully to recover the completely blocked GABA response with sufficient ionophoresed pentobarbitone. Thus, pentobarbitone could restore and potentiate GABA responses before it directly increased membrane conductance. The picrotoxin-sensitivity of the pentobarbitone-induced change in membrane conductance suggests that the drug effect is due, in some way, to activation of GABA receptors.

The ionic mechanism of the pentobarbitone-induced change in membrane properties was assessed by comparing the inversion potential of the pentobarbitone response to that of GABA, the latter being due primarily to a Cl^- conductance (see preceding paper). In Fig. $2B_1$ both pentobarbitone and GABA increased membrane

conductance without changing membrane potential since the resting potential of the cell was equivalent to the inversion potential of the voltage responses. A similar inversion potential for both GABA and pentobarbitone was observed on seven cells under control conditions using K-acetate or KCl recording pipettes. In low Cl^- solution the inversion potentials of both GABA and pentobarbitone responses were similar and were shifted in a hyperpolarizing direction upon placement of a diffusion micropipette filled with 159 mM-Cl^- ion concentration (n = 4 cells) (Fig. 5). Thus, the similarity of inversion potentials between pentobarbitone and GABA and their Cl^- dependency coupled with the picrotoxin sensitivity of both responses suggests that pentobarbitone directly depresses membrane excitability through its 'GABA-mimetic' properties by activation of GABA-receptor-coupled Cl^- conductance.

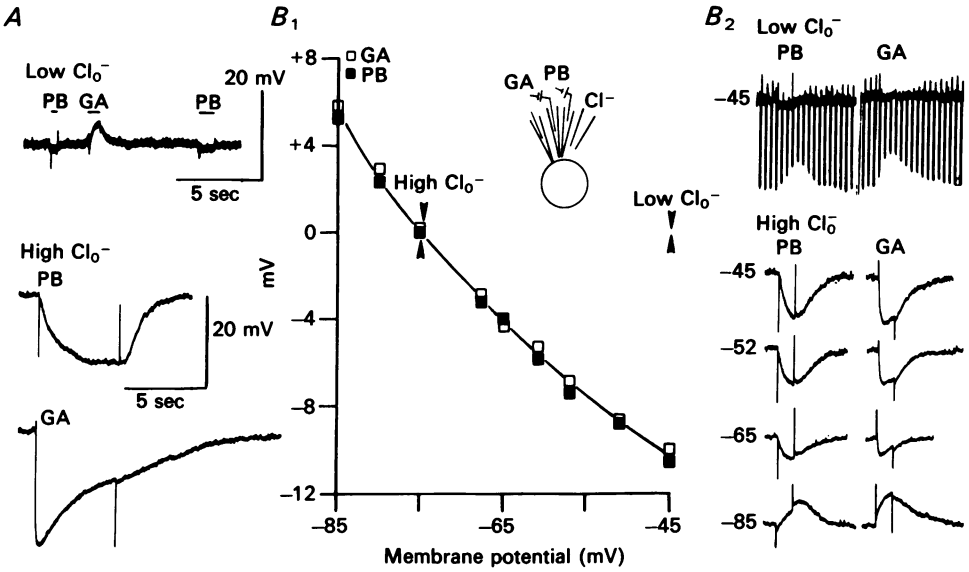

Fig. 5. Cl^- dependency of pentobarbitone response in spinal cord cells. Cells bathed in 9 mM-Cl^- ('low Cl_o^-') with blunt pipette near cell used to diffuse Cl^- ions (159 mM-Cl^-) ('high Cl_o^-'). *A*, pentobarbitone ionophoresis (40 nA) produces no observable response in 9 mM-Cl^- (coupling artifact evident), while response to 20 nA–2 sec GABA pulse is depolarizing. After placement of Cl^- diffusion pipette pentobarbitone and GABA hyperpolarize cell membrane, but at quite different rates. Sag in voltage during GABA response is likely due to Cl^- redistribution. *B*, inversion potentials of GABA and pentobarbitone responses (arrowheads) shift from −45 mV in 9 mM-Cl^- to −75 mV after placement of Cl^- diffusion pipette (B_1). Specimen records of responses to 20 nA, 2 sec GABA pulse and 50 nA, 2 sec pentobarbitone pulse shown in B_2. Small conductance increases to pentobarbitone and GABA (30–50 %) seen in 'low Cl_o^-'. Resting potentials (mV): −56 (*A*), −45 (*B*). Constant current pulse: −1 nA (*B*).

Modulation of GABA responses

Pentobarbitone could either attenuate certain aspects of GABA responses (Fig. 2) or restore and potentiate picrotoxin-blocked GABA responses (Fig. 4). Even in the absence of picrotoxin, pentobarbitone, in concentrations which either did not increase membrane conductance or increased it only slightly, potentiated GABA responses, enhancing the conductance increase to GABA and altering the kinetics of the

response (Ransom & Barker, 1975, 1976; Figs. 6, 8). The spectrum of potentiation, as well as its time course of effect (when diffused from a pipette) can be observed in Fig. 6 where a 54% enhancement in extrapolated GABA conductance increase is associated with a 45% increase in decay rate (*A*), while in another cell a 300% enhancement is associated with a 15% increase in decay (*B*). Thus, enhancement of the GABA-induced conductance increase was associated with a variable slowing of the kinetics. As can be seen in the traces illustrating Fig. 6*A*, potentiation of the GABA response by pentobarbitone was functionally important, leading to an increase in the duration of the inhibitory effect of GABA on spontaneous activity.

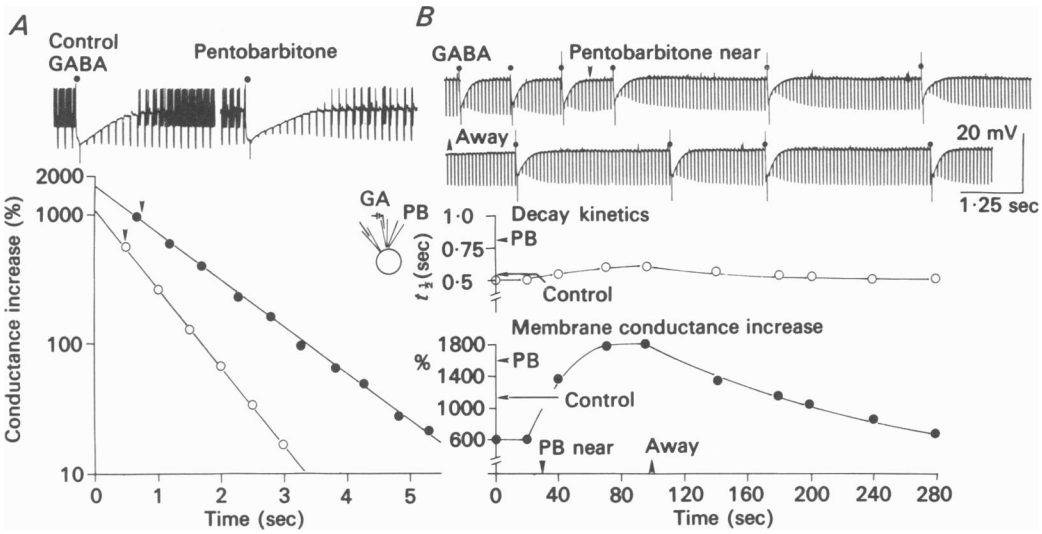

Fig. 6. Pentobarbitone diffused from a blunt pipette containing 0·1 mM drug potentiates GABA response on spinal cord neurone (*A*) and cerebellar–brain-stem neurone (*B*). Pentobarbitone enhancement of GABA-induced conductance increase associated with variable slowing of decay kinetics (filled circles; control: open circles). Horizontal arrowheads in plots in *B* give values for data in *A*. Duration of GABA inhibition of spike generation is also increased during pentobarbitone. *B*, time course of pentobarbitone potentiation with relatively greater enhancement of GABA-induced conductance change than decay kinetics. GABA pulses: 14 nA, 100 msec (*A*), 20 nA, 100 msec (*B*). Resting membrane potentials: −41 mV (*A*), −52 mV (*B*). Constant current pulses: −0·5 nA (*A*), −0·4 nA (*B*).

Initial pharmacological analysis of pentobarbitone's potentiating action was done by examining the effect of low concentrations of the drug on the GABA dose-response curve (n = 8 cells). Pentobarbitone shifted the curve of both GABA voltage responses (Fig. 7*A*, B_1) and conductance changes (not illustrated) to the left. It also delayed time-to-peak (Fig. 7*C*) and prolonged decay kinetics (Fig. 7*D*), the net result of which was to enhance the integral of the voltage response considerably more than the amplitude of the response (Fig. 7B_2). The change in the kinetics of the GABA response during ionophoresis of pentobarbitone would thus effectively enhance GABA inhibition by more than would be expected from consideration of the amplitude enhancement alone. Analysis of double-reciprocal plots of GABA dose–response

curves and of pentobarbitone's effects on the plots was complicated by the nature of the GABA response, which involved more than one GABA molecule (see preceding paper).

At higher ionophoretic currents pentobarbitone depressed GABA responses (as illustrated in Fig. 2*C*). The peak conductance increases in response to GABA declined, the kinetics of the GABA conductance change slowed and the input conductance of the membrane increased in response to increasing pentobarbitone current (n = 8 cells). Since the rate of change of these parameters was not identical, the conductance event (as reflected in its magnitude and duration) was potentiated at lower barbiturate currents relative to that observed in control or at higher barbiturate currents.

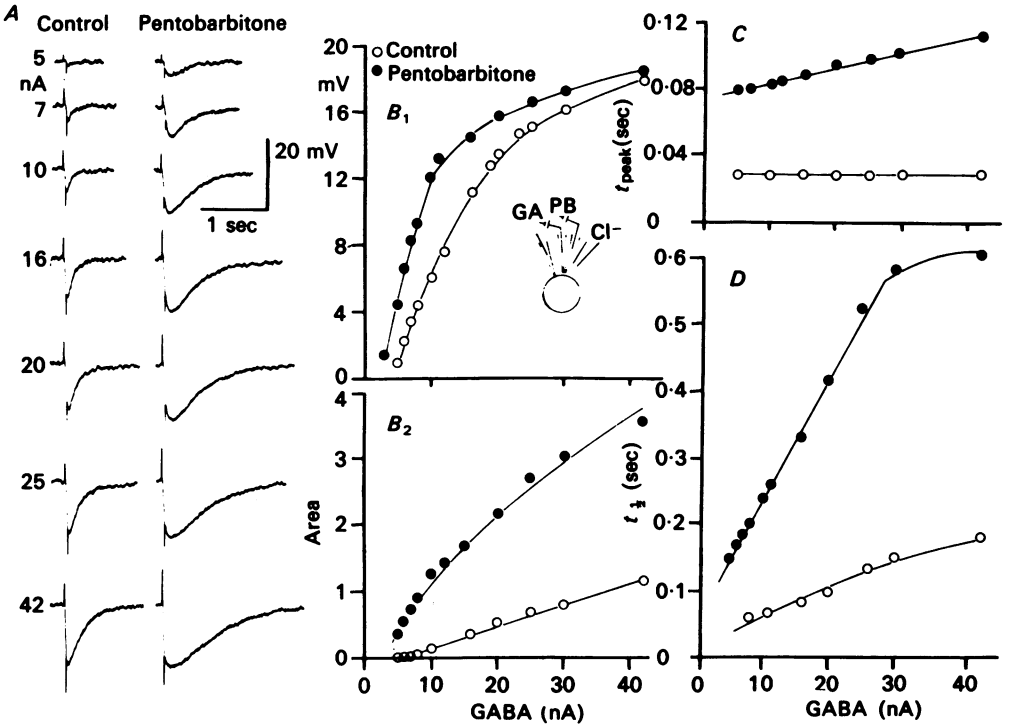

Fig. 7. Pentobarbitone alterations in parameters of GABA response as a function of GABA ionophoretic current. Recording from a spinal cord cell bathed in low Cl^- with Cl^- diffusion pipette in place. Pentobarbitone ionophoresis (15 nA) potentiates response amplitude (B_1) and slows kinetics (*C* and *D*) of voltage responses to 50 msec GABA pulses. Hyperpolarizing GABA response amplitude plotted as positive integer in B_1. The change in response kinetics effectively yields a greater overall increase in response, B_2, (estimated from integral of response), than is seen when simply comparing amplitude enhancement. Resting membrane potential −46 mV.

When present, the barbiturate-induced membrane conductance summed with the GABA peak conductance increase to produce a total membrane conductance which approached, but was typically less than the control GABA peak conductance increase, suggesting that much of the apparent decline in the GABA response was due to concomitant pentobarbitone activation of GABA-mediated Cl^- conductance with

some 'desensitization'. Significant enhancement of GABA peak conductance increase of pentobarbitone was usually obtained in the *absence* of measurable barbiturate-induced conductance (as in Figs. 6 and 8; Fig. 1 of Ransom & Barker, 1976).

Since the pentobarbitone-induced increase in membrane conductance appears to occur through activation of GABA receptors, we examined the effects of stepwise increments of sustained GABA ionophoretic application on the conductance increase to a pulse of GABA and compared this interaction with that of pentobarbitone on the same cell ($n = 5$ cells). Continuous, low current

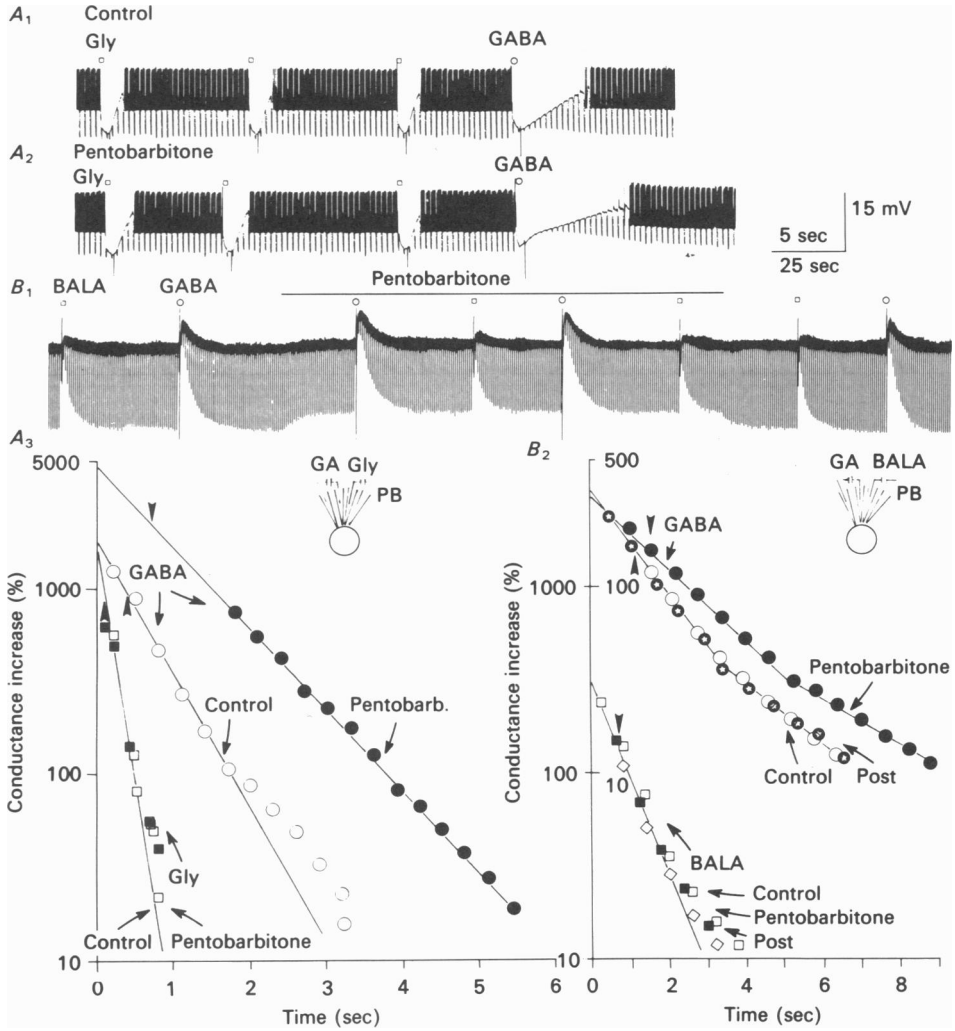

Fig. 8. Selectivity of pentobarbitone potentiation of GABA. *A*, recordings from a cerebellar–brainstem neurone (*A*) and a spinal neurone (*B*). Specimen records in A_{1-2}, B_1; plots of kinetics and conductance in A_3, B_2. Pentobarbitone diffused from 0·1 mM solution potentiates and prolongs GABA-induced conductance (A_3) and prolongs inhibitory effect of GABA on spontaneous excitation (A_{1-2}) (open circles), without affecting glycine response (open squares). *B*, pentobarbitone selectively prolongs GABA-induced conductance change (open circles) without affecting that of BALA (open squares). Resting membrane potentials (mV): −43 (*A*), −59 (*B*). GABA pulses: 24 nA, 300 msec (*A*), 14 nA, 200 msec (*B*). Glycine pulse: 30 nA, 300 msec; BALA pulse: 24 nA, 200 msec. Constant current pulses: −0·5 nA (*A*), −1 nA (*B*).

ionophoresis of GABA increased membrane conductance, decreased the subsequent conductance increase to a pulse of GABA and slowed the kinetics of the GABA-induced conductance change. All of these effects occurred in a dose-dependent manner. The depression of the conductance increase to GABA pulses was considerably more than could be accounted for by the increasing membrane conductance to continuous GABA.

Presumably, the relatively greater depression of the GABA response by GABA reflects a relatively more complete 'desensitization' of the GABA receptor-coupled conductance by GABA. Thus, the effects of low-current ionophoresis of GABA on GABA responses, while qualitatively similar to those of pentobarbitone, can be distinguished from the drug mainly by the lack of enhancement, and relatively greater depression of GABA responses by sustained GABA current.

The specificity of pentobarbitone's enhancement of GABA responses was tested by examining the effects of pentobarbitone ionophoresis or diffusion application on glycine (n = 26 cells) and β-alanine responses (n = 12 cells). GABA responses were used as a monitor of pentobarbitone's efficacy. Pentobarbitone specifically enhanced GABA voltage responses and conductance changes in all cells tested without measurably affecting those to glycine and β-alanine (Fig. 8). The drug prolonged the ability of GABA to inhibit either spontaneously present excitation (Figs. 6 and 8) or excitation of the cell in response to suprathreshold constant current depolarizing pulses (n = 8 cells). Pentobarbitone did depress, but did not prolong glycine and β-alanine responses, when applied in concentrations sufficient to increase membrane conductance. The specificity of the enhancing effect for GABA-mediated Cl^- conductance and not other neutral amino acid-mediated Cl^- conductance (see preceding paper) suggests that pentobarbitone interacts with the GABA receptor-coupled conductance mechanism at the receptor or coupling-step level rather than at the Cl^- conductance stage.

Modulation of glutamate responses

Pentobarbitone depressed depolarizing glutamate responses directly (n = 24 cells) (Ransom & Barker, 1975, 1976) and indirectly by increasing membrane conductance (n = 18 cells). The two types of depression of the glutamate response occurred at pentobarbitone concentrations which enhanced and prolonged GABA responses (n = 6 cells). Direct depression of glutamate excitation was thus about as sensitive to pentobarbitone as enhancement of GABA inhibition. In either case, the interaction required application of the drug at the same site as the ionophoretic response was obtained. Furthermore, the direct interaction between pentobarbitone and glutamate was not affected by application of picrotoxin in doses which depressed the GABA conductance change (n = 6 cells, not illustrated).

On occasion glutamate ionophoresis evoked rapid events superimposed on the primary post-synaptic response. These events were presumably derived from glutamate-induced release of transmitter from terminals pre-synaptic to the cell recorded from. Pentobarbitone blocked the appearance of these events in the absence of direct effects on post-synaptic membrane properties, suggesting that the drug can also block chemically induced release of transmitter.

Pharmacological analysis of the direct interaction between pentobarbitone and glutamate was carried out by examining the effect of the drug on the glutamate dose–response curve (n = 6 cells). Pentobarbitone depressed glutamate responses without changing their kinetics (Fig. 9). Double-reciprocal plot analysis of the antagonism, assuming one glutamate molecule participates in the conductance event and that this

is unchanged in the presence of the drug, reveals no change in the affinity parameter of the glutamate response (Fig. 9*C*). Rather, the results indicate that the direct antagonism of glutamate is likely occurring at a coupling step or at the conductance mechanism of the responses and is independent of pentobarbitone activation of GABA receptor-coupled conductance.

Pentobarbitone did not depress depolarizing responses to acetylcholine (ACh) ionophoresis on four cells tested where it did antagonize glutamate responses (Fig. 10*C*). Although not completely characterized, the ACh responses recorded had

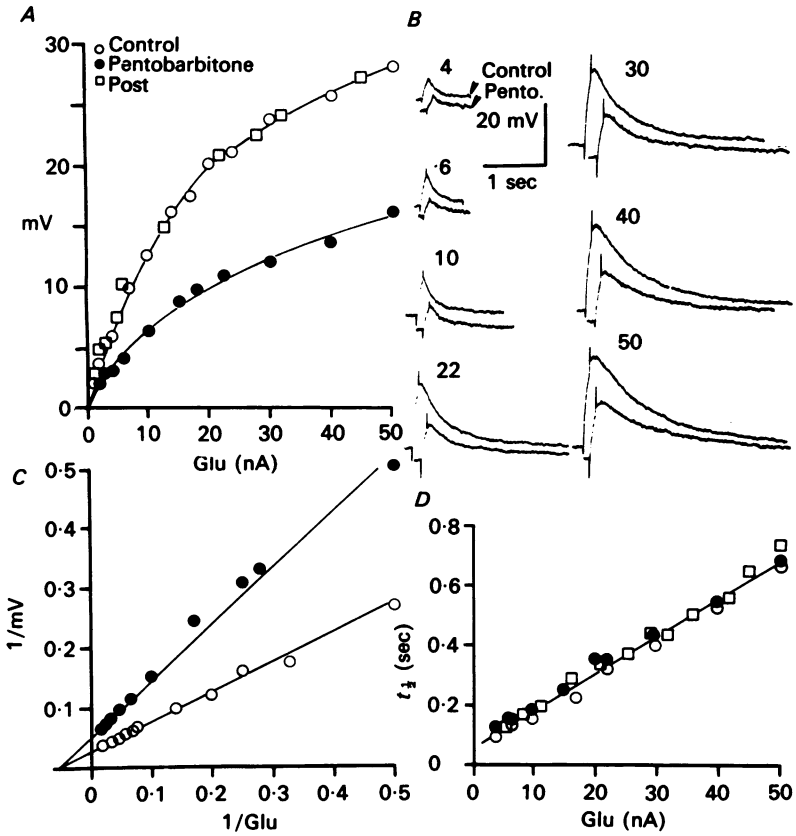

Fig. 9. Pentobarbitone depression of glutamate dose–response curve. Recording from a spinal cord cell. *A*, plot of depolarizing effect of increasing glutamate current before, during and after concomitant ionophoresis of 20 nA pentobarbitone. Specimen records in *B* and double-reciprocal plot of data in *C*. Numbers above each set of records indicate glutamate ionophoretic currents in nA. *D*, half-decay time of glutamate response as function of glutamate current before, during and after pentobarbitone. Glutamate pulse duration 50 msec. Resting membrane potential −67 mV.

considerably slower kinetics than amino acid responses and were associated with an apparent *increase* in membrane resistance independent of potential-dependent changes in membrane resistance (Fig. 10*A*). Superimposition of glutamate pulses on ACh responses revealed an enhancement of the glutamate voltage response, as would be

expected from the increase in input resistance (Fig. 10*B*). ACh ionophoresis also gave rise to rapid events superimposed on the slow post-synaptic depolarization (Fig. 10*B*, *C*). These presumably reflect ACh-induced release of transmitter from terminals presynaptic to the cell recorded from. They were not blocked by pentobarbitone (Fig. 10*C*).

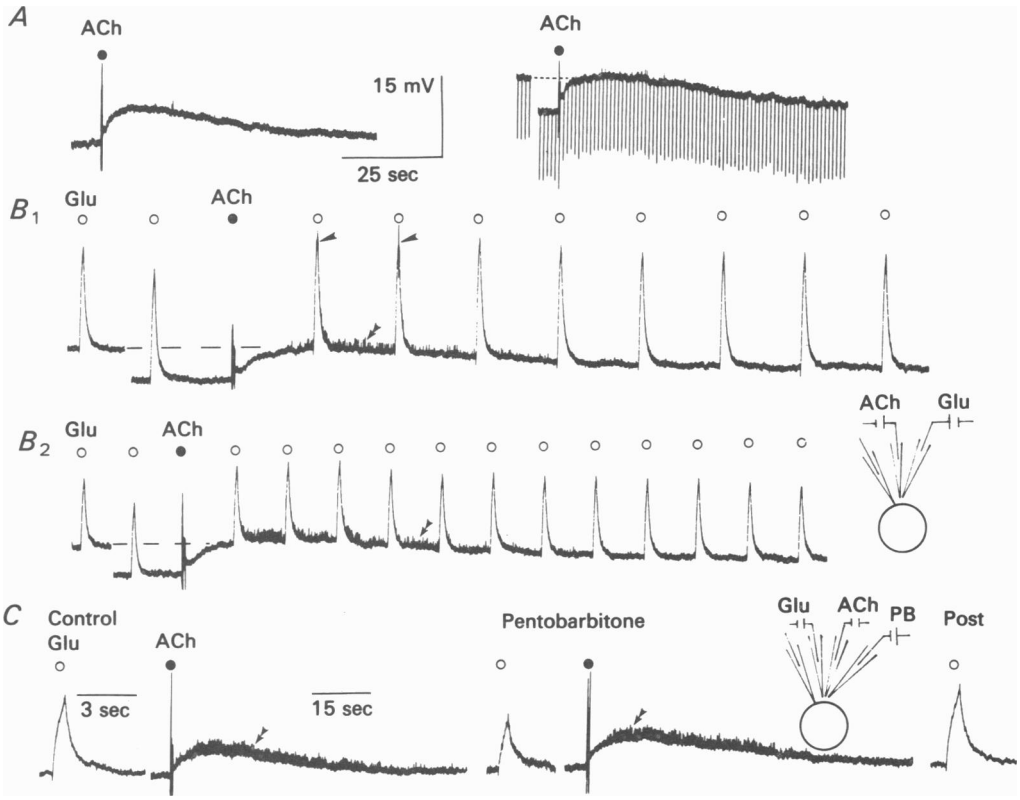

Fig. 10. Pentobarbitone does not block slow depolarization to acetylcholine (ACh). Recording from two spinal cord cells. ACh ionophoresed from a 1 M solution. *A*, depolarizing response and conductance decrease long outlast ionophoretic pulse (25 nA, 200 msec). B_{1-2}, same cell as in *A*. Glutamate pulses superimposed on ACh response are potentiated during the ACh response. Large arrowheads indicate spikes evoked during glutamate response. Small events (double arrow heads) are presumably post-synaptic events reflecting presynaptic effects of ACh. *C*, pentobarbitone depresses glutamate response (20 nA, 100 msec) but not ACh (40 nA, 400 msec). Resting membrane potentials (mV) −56 (*A*), −61 (*C*). Constant current pulses −0·6 nA.

DISCUSSION

Studies on pentobarbitone effects in vivo and in vitro

Pentobarbitone's depression of neuronal excitability has been studied in many different vertebrate and invertebrate preparations, using a variety of methods. The results derived from these varied investigations have forced the conclusion that pentobarbitone's anaesthetic properties appear tc be due to a wide array of pharmacological actions. Pharmacological effects in five distinct areas of neuronal function are commonly thought to contribute to the depressant effects of the drug.

(1) Depression of excitatory transmission has been correlated with depressant effects on post-synaptic receptor coupled-conductance changes in a variety of preparations (Galindo, 1969; Thomson & Turkanis, 1973; Barker & Gainer, 1973; Barker, 1975*a*,*b*; Seyama & Narahashi, 1975; Nicoll, 1975*a*; Ransom & Barker, 1975, 1976; Adams, 1976; Lee Son, Waud & Waud, 1974; Richards & Smaje, 1976). This depression appears to be non-competitive (Barker & Gainer, 1973; Barker, 1975*b*; Seyama & Narahashi, 1975) and is likely not due to some effect on the affinity of membrane receptor for agonist (Barker & Gainer, 1973; Barker, 1975*b*; Adams, 1976). Barbiturate depression of excitatory transmission at peripheral neuromuscular junctions does not appear to include depression of presynaptic transmitter release since an increase or no change in the presynaptic phases of transmission has been reported (Westmoreland, Ward & Johns, 1971; Thomson & Turkanis, 1973; Seyama & Narahashi, 1975; Proctor & Weakly, 1976).

(2–4) Barbiturate depression of excitatory transmission at the initial synapse into the C.N.S. (primary sensory afferent-motoneurone or interneurone) is, however, correlated with a decrease in transmitter release (Weakly, 1969) which could be due to direct inhibition of release and/or to an indirect enhancing effect of barbiturates on 'presynaptic' inhibitory pathways which regulate transmission at this synapse into the C.N.S. (Eccles & Malcolm, 1946; Eccles *et al.* 1963; Schmidt, 1963; Nicoll, 1975*b*). Not yet understood are the mechanisms underlying either barbiturate enhancement of presynaptic inhibitory transmission, the latter thought to be mediated partly by GABA (Davidoff, 1972; Barker & Nicoll, 1972, 1973; Barker, Nicoll & Padjen, 1975*a*,*b*), and of GABA responses on primary afferents (Nicoll, 1975*b*) or barbiturate enhancement of post-synaptic inhibitory transmission, presumed to be mediated by GABA (Nicoll *et al.* 1975), as well as post-synaptic inhibitory responses to GABA (Ransom & Barker, 1975, 1976; Nicoll, 1975*a*). The notion that barbiturate anaesthesia derives partly from a generalized depression of evoked transmitter release throughout the C.N.S. is largely based on studies of the release of labelled transmitter from synaptosomal fractions (Blaustein & Ector, 1975; Haycock *et al.* 1977). A later investigation, also using a C.N.S. synaptosomal fraction as a model of presynaptic terminal physiology, has, however, not confirmed the earlier observations (Olsen *et al.* 1977). If barbiturates can decrease excitability by acting at presynaptic terminals in the C.N.S. is this effect generalized to all presynaptic terminals?

(5) The direct inhibitory effect of barbiturates on post-synaptic membranes (Krnjević, 1975; Nicoll, 1975*b*; Ransom & Barker, 1975) is blocked by picrotoxin (Nicoll, 1975*a*), suggesting that the barbiturate action may involve activation of GABA receptors, rather than depression of cellular metabolism as proposed by Krnjević (1975).

Pentobarbitone activation of GABA receptors

The observations presented in this paper indicate that pentobarbitone depresses excitability by (1) activating GABA receptor-coupled Cl^- conductance, (2) enhancing and prolonging GABA-mediated synaptic events, and (3) depressing glutamate-mediated excitatory synaptic events.

Activation of GABA receptors by pentobarbitone does not appear to be due to pentobarbitone-induced release of GABA from terminals presynaptic to the recorded

cell, since pentobarbitone either depresses (Haycock *et al.* 1977) or does not alter GABA release from synaptosomes (Olsen *et al.* 1977). The time course of the membrane's response to pentobarbitone, which is much slower than that to ionophoresed GABA, is also quite unlike that observed during glutamate-induced release of inhibitory transmitter where discrete events are evident. Finally, Nicoll (1975*b*) has demonstrated a picrotoxin-sensitive, pentobarbitone-induced depolarization of dorsal root ganglion cells which are devoid of synapses, although invested with glia. Pentobarbitone would thus appear directly to increase membrane conductance by activating GABA receptor-coupled Cl^- conductance.

Pentobarbitone potentiation of GABA responses

Enhancement and prolongation of post-synaptic responses to GABA pulses by pentobarbitone may help to explain the enhancement and prolongation of synaptic events thought to be mediated by GABA. Depression of GABA uptake mechanisms would effectively enhance and prolong time-to-peak and decay kinetics of responses to GABA pulses, especially if these mechanisms regulate the kinetics and amplitude of the response. However, anaesthetic concentrations of pentobarbitone do *not* depress uptake by, and transport into synaptosomes (Peck, Miller & Lester, 1976; Olsen *et al.* 1977). That pentobarbitone itself can apparently activate GABA receptor-coupled conductance suggests that the drug might participate in the response to GABA when the two are applied coincidentally. Some interaction between pentobarbitone and GABA at the level of the GABA receptor is suggested by the 'cross-desensitization' between the two agents observed by Nicoll (1975*b*) on amphibian sensory neurones. A *receptor* level of interaction is also indicated in the present study from the selectivity of pentobarbitone for GABA receptors and responses and not other neutral amino acids utilizing Cl^- conductance mechanisms. The mechanism of pentobarbitone prolongation of GABA responses is unclear, but in the absence of any reported effects on GABA uptake (Peck *et al.* 1977; Olsen *et al.* 1977), it most likely reflects some interaction of the drug with GABA receptors. The drug might enhance the affinity of GABA receptors for GABA or act at later steps in the GABA response so as to prolong the conductance change. Alternatively, the modulation of GABA responses by pentobarbitone might reflect both binding to an operationally distinct 'activator site' in proximity with GABA receptors, as well as binding to GABA receptors. In fact, GABA responses can be markedly enhanced by phenobarbitone *without* change in response kinetics (Barker, Macdonald & Ransom, 1977; Macdonald & Barker, 1978).

Pentobarbitone depression of glutamate excitation

Depression of glutamate-induced depolarization occurred through a direct interaction between pentobarbitone and the receptor-coupled conductance as well as indirectly through activation of GABA receptor-coupled Cl^- conductance. Analysis of the direct depression indicated a non-competitive antagonism, excluding depression of receptor affinity for glutamate and suggesting an interaction at the level of the conductance mechanism, as has been proposed previously from invertebrate (Barker & Gainer, 1973; Barker, 1975*b*) and peripheral vertebrate studies (Seyama & Narahashi, 1975; Adams, 1976). Although various putative excitatory transmitters have

not been tested, the fact that depolarizing responses to ACh, which have distinctly slower kinetics than glutamate responses and are associated with an apparent decrease in membrane conductance, were not affected by pentobarbitone argues against a non-specific depression of all excitatory events. A similar lack of interaction between pentobarbitone and slow excitatory responses coupled to membrane conductance decreases in response to ACh and serotonin has been reported in invertebrate C.N.S. studies (Barker, 1975*a*, *b*), leading to the proposal that pentobarbitone interacts only with the conductance mechanism of excitatory receptor-coupled events where the underlying ionic conductance is Na^+ or Na^+–K^+ (Barker, 1975*a*, *b*, *c*). The ionic conductance associated with the glutamate response recorded from frog motoneurones is dependent on $[Na^+]_o$ (Nicoll, Padjen & Barker, 1976). Although the response observed in the present study is unknown, sustained application of glutamate leads to a post-response hyperpolarization which is abolished by ouabain (Ransom, Barker & Nelson, 1976). The latter has been taken as evidence for measurable Na^+ pump activity stimulated by an excessive influx of Na^+ during the glutamate response. The mechanism by which pentobarbitone affects receptor-coupled Na^+ and Na^+–K^+ conductance has not been elucidated. It does not appear to involve GABA-mimetic properties of the drug since depression of glutamate responses by pentobarbitone is *not* reversed by picrotoxin.

Conclusions

The results presented in this study provide evidence, based on intracellular recordings of mammalian central neurones, that pentobarbitone's depression of spontaneous activity derives from a variety of effects. These include (1) activation of GABA receptor-coupled Cl^- conductance, rendering all cells with GABA receptors less excitable, thus decreasing their output, (2) potentiation of GABA-mediated synaptic events at the GABA-receptor level, thereby enhancing this important form of synaptic inhibition, and (3) depression of glutamate-mediated synaptic events at the conductance level, thereby depressing this important form of synaptic excitation. Missing from the present report are details regarding direct actions of pentobarbitone on C.N.S. presynaptic release mechanisms, although blockade of glutamate-induced release has been observed. This aspect of pentobarbitone's anaesthetic actions requires further study. While all of these effects of pentobarbitone likely contribute to the anaesthetic properties of the drug, the relative contribution of each will depend on the distribution of GABA receptors and inhibitory pathways, as well as the distribution of excitatory pathways utilizing receptor-coupled Na^+ and Na^+–K^+ conductance mechanisms. Assessing these parameters may be difficult, if not impossible.

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REFERENCES

ADAMS, P. R. (1976). Drug blockade of open end-plate channels. *J. Physiol.* **260**, 531–552.

BARKER, J. L. (1975*a*). Selective depression of post-synaptic excitation by general anesthetics. *Prog. Anesthesiol.* **1**, 135–156.

BARKER, J. L. (1975*b*). CNS depressants: effects on post-synaptic pharmacology. *Brain Res.* **92**, 35–55.

BARKER, J. L. (1975*c*). Inhibitory and excitatory effects of CNS depressants on invertebrate synapses. *Brain Res.* **93**, 77–90.

BARKER, J. L. & GAINER, H. (1973). Pentobarbital: selective depression of excitatory post-synaptic potentials. *Science, N.Y.* **182**, 720–722.

BARKER, J. L., MACDONALD, R. L. & RANSOM, B. R. (1977). Post-synaptic pharmacology of GABA. In *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System*, ed. RYALL, R. W. & KELLY, J. S. Amsterdam: Elsevier. (In the Press.)

BARKER, J. L. & NICOLL, R. A. (1972). GABA: role in primary afferent depolarization. *Science, N.Y.* **176**, 1043–1045.

BARKER, J. L. & NICOLL, R. A. (1973). The pharmacology and ionic dependency of amino acid responses in the frog spinal cord. *J. Physiol.* **228**, 259–277.

BARKER, J. L., NICOLL, R. A. & PADJEN, A. (1975*a*). Studies on convulsants in the isolated frog spinal cord. I. Antagonism of amino acid responses. *J. Physiol.* **245**, 521–536.

BARKER, J. L., NICOLL, R. A. & PADJEN, A. (1975*b*). Studies on convulsants in the isolated frog spinal cord. II. Effects on dorsal root potentials. *J. Physiol.* **245**, 537–548.

BLAUSTEIN, M. P. (1968). Barbiturates block sodium and potassium conductance increase in voltage-clamped lobster axons. *J. gen. Physiol.* **51**, 293–307.

BLAUSTEIN, M. P. (1976). Barbiturates block calcium uptake by stimulated and potassium-depolarized rat sympathetic ganglia. *J. Pharmac. exp. Ther.* **196**, 80–86.

BLAUSTEIN, M. P. & ECTOR, A. C. (1975). Barbiturate inhibition of calcium uptake by depolarized nerve terminals *in vitro*. *Molec. Pharmacol.* **11**, 369–378.

BOWERY, N. G. & DRAY, A. (1976). Barbiturate reversal of amino acid antagonism produced by convulsant agents. *Nature, Lond.* **264**, 276–278.

BROOKS, C. M. & Eccles, J. C. (1947). A study of the effects of anesthesia on the monosynaptic pathway of the spinal cord. *J. Neurophysiol.* **10**, 349-360.

DAVIDOFF, R. A. (1972). Gamma-aminobutyric acid antagonism and pre-synaptic inhibition in the frog spinal cord. *Science, N.Y.* **175**, 331-333.

ECCLES, J. C., FABER, D. S. & TABORIKOVA, H. (1971). The action of a parallel fiber volley on the antidromic invasion of Purkyne cells of cat cerebellum. *Brain Res.* **25**, 335-356.

ECCLES, J. C. & MALCOLM, J. L. (1946). Dorsal root potentials of the spinal cord. *J. Neurophysiol.* **9**, 139–160.

ECCLES, J. C., SCHMIDT, R. F. & WILLIS, W. D. (1963). Pharmacological studies on presynaptic inhibition. *J. Physiol.* **168**, 500–530.

GALINDO, A. (1971). Procaive, pentobarbital and halothane: effects on the mammalian myoneural junction. *J. Pharmac. exp. Ther.* **177**, 360–368.

HAYCOCK, J. W., LEVY, W. B. & COTMAN, C. W. (1977). Pentobarbital depression of stimulus-secretion coupling in brain-selective inhibition of depolarization-induced calcium-dependent release. *Biochem. Pharmacol.* **26**, 159–161.

HOLMES, J. C. & SCHNEIDER, F. H. (1973). Pentobarbitone inhibition of catecholamine secretion. *Br. J. Pharm.* **49**, 205–213.

KRNJEVIČ, R. (1975). Is general anesthesia induced by neuronal asphyxia? In *Molecular Mechanisms of Anesthesia*, ed. FINK, B. R., pp. 93–98. New York: Raven.

LARRABEE, M. G. & POSTERNAK, J. M. (1952). Selective action of anesthetics on synapses and axons in mammalian sympathetic ganglia. *J. Neurophysiol.* **15**, 91–114.

LARSON, M. D. & MAJOR, M. A. (1970). The effect of hexobarbital on the duration of the recurrent IPSP in cat motoneurons. *Brain Res.* **21**, 309–311.

LEE SON, S., WAUD, B. E. & WAUD, D. R. (1974). The effects of barbiturates on depolarization at the neuromuscular junction. *Fedn Proc.* **33**, 510.

LØYNING, Y., OSHIMA, T. & YOKOTA, J. (1964). Site of action of thiamylal sodium on the monosynaptic spinal reflex pathway in cats. *J. Neurophysiol.* **27**, 408–428.

MACDONALD, R. L. & BARKER, J. L. (1978). Different actions of anticonvulsant and anesthetic barbiturates revealed using cultured mammalian neurones. *Science, N.Y.* (In the Press.)

NARAHASHI, T., FRAZIER, D. T., DEGUCHI, T., CLEAVES, C. A. & ERNAW, M. C. (1971). The active form of pentobarbital in squid giant axons. *J. Pharmac. exp. Ther.* **177**, 25–33.

NICOLL, R. A. (1972). The effects of anesthetics on synaptic excitation and inhibition in the olfactory bulb. *J. Physiol.* **223**, 803–814.

NICOLL, R. A. (1975*a*). Pentobarbital: action on frog motoneurons. *Brain Res.* **96**, 119–123.

NICOLL, R. A. (1975*b*). Pre-synaptic action of barbiturates in the frog spinal cord. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1460-1463.

NICOLL, R. A., ECCLES, J. C., OSHIMA, T. C. & RUBIA, F. (1975). Prolongation of hippocampal inhibitory post-synaptic potentials by barbiturates. *Nature, Lond.* **258**, 625–627.

NICOLL, R. A., PADJEN, A. & BARKER, J. L. (1976). Analysis of amino acid responses on frog motoneurones. *Neuropharmacology* **15**, 45–53.

OLSEN, R. W., LAMAR, E. E. & BAYLESS, J. D. (1957). Calcium-induced release of γ-aminobutyric acid from synaptosomes: effects of tranquilizer drugs. *J. Neurochem.* **28**, 299–305.

PECK, E. J., MILLER, A. L. & LESTER, B. R. (1976). Pentobarbital and synaptic high-affinity receptor sites for gamma-amino butyric acid. *Brain Res. Bull.* **1**, 595–597.

PEPER, K., DREYER, F. & MÜLLER, D-D. (1975). Analysis of cooperativity of drug receptor interaction by quantitative iontophoresis at frog motor end plates. In *Cold Spring Harb. Symp. quant. Biol.* **40**, 187–192.

PROCTOR, W. R. & WEAKLY, J. N. (1976). A comparison of the presynaptic and post-synaptic actions of pentobarbitone and phenobarbitone in the neuromuscular junction of the frog. *J. Physiol.* **258**, 257–268.

QUASTEL, D. M. J. & LINDER, T. M. (1975). Pre- and post-synaptic actions of central depressants at the mammalian neuromuscular junction. In *Molecular Mechanisms of Anesthesia*, ed. FINK, B. R., pp. 151–165. New York: Raven.

RANSOM, B. R. & BARKER, J. L. (1975). Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture. *Nature, Lond.* **254**, 703–705.

RANSOM, B. R. & BARKER, J. L. (1976). Pentobarbital selectively enhances GABA-mediated post-synaptic inhibition in tissue cultured mouse spinal neurons. *Brain Res.* **114**, 530–535.

RANSOM, B. R., BARKER, J. L. & NELSON, P. G. (1976). Two mechanisms for poststimulus hyperpolarizations in cultured mammalian neurones. *Nature, Lond.* **256**, 424–425.

RICHARDS, C. D. (1972). On the mechanism of barbiturate anesthesia. *J. Physiol.* **227**, 749–767.

RICHARDS, C. D. & SMAJE, J. C. (1976). Anaesthestics depress the sensitivity of cortical neurones to L-glutamate. *Br. J. Pharmac.* **58**, 347–357.

SCHMIDT, R. F. (1963). Pharmacological studies on primary afferent depolarization of the toad spinal cord. *Plügers Arch. ges. Physiol.* **277**, 325–346.

SEYAMA, I. & NARAHASHI, T. (1975). Mechanism of blockade of neuromuscular transmission by pentobarbital. *J. Pharmac. exp. Ther.* **192**, 95–104.

SOMJEN, G. G. (1963). Effects of ether and thiopental on spinal pre-synaptic terminals. *J. Pharmac. exp. Ther.* **140**, 393–402.

SOMJEN, G. G. & GILL, M. (1963). The mechanism of the blockade of synaptic transmission in the mammalian spinal cord by diethyl ether and by thiopental. *J. Pharmac. exp. Ther.* **140**, 19–30.

STAIMAN, A. & SEEMAN, P. (1974). The impulse-blocking concentrations of anesthetics, alcohols, anticonvulsants, barbiturates and narcotics on phrenic and sciatic nerves. *Can. J. Physiol. Pharmacol.* **52**, 535–550.

THESLEFF, S. (1956). The effect of anesthetic agents in skeletal muscle membrane. *Acta physiol. scand.* **37**, 335–349.

THOMSON, T. D. & TURKANIS, S. A. (1973). Barbiturate-induced transmitter release at a frog neuromuscular junction. *Br. J. Pharmac.* **48**, 48–58.

WEAKLY, J. N. (1969). Effect of barbiturates on 'quantal' synaptic transmission in spinal motoneurones. *J. Physiol.* **204**, 63–77.

WERMAN, R. (1976). Desensitization and stoichiometry of transmitter-receptor interactions. In *Electrobiology of Nerve Synapse and Muscle*, ed. REUBEN, J. P., PURPURA, D. P., BENNETT, M. V. L. & KANDEL, E. R., pp. 45–60. New York: Raven.

WESTMORELAND, B. F., WARD, D. & JOHNS, T. R. (1971). The effect of methohexital at the neuromuscular junction. *Brain Res.* **26**, 465–468.