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EFFECT OF BARBITURATES ON THE GABA RECEPTOR OF CAT PRIMARY AFFERENT NEURONES

BY H. HIGASHI AND S. NISHI

From the Department of Physiology, Kurume University School of Medicine, Kurume, 830 Japan

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SUMMARY

1. The effects of the barbiturate anaesthetics, pentobarbitone and thiopentone, on the membrane properties and the γ-aminobutyric acid (GABA)-induced responses of cat primary afferent neurones were studied with intracellular recording and voltage-clamp techniques.

2. At low concentrations (10^{-7}–10^{-5} M) both barbiturates slightly enhanced and prolonged GABA-induced depolarizations or currents without affecting the membrane properties. At these concentrations, barbiturates have no effect on the apparent dissociation constant of the GABA–GABA receptor interaction or the reversal potential for GABA-induced depolarizations or currents.

3. At high concentrations (10^{-4}–10^{-3} M) barbiturates produced a few millivolts reduction in the resting membrane potential. Voltage-clamp analysis revealed that the depolarization was associated with one of the three types of conductance change, i.e., an initial increase followed by a decrease (40% of neurones examined), only an increase (40%) and only a decrease (20%).

4. Analysis in different ionic media indicated that the depolarization with a reduced membrane resistance is associated with an increased chloride conductance and that the one with an increased membrane resistance is accompanied by a reduction in potassium conductance. Bath-application of GABA (10^{-3} M) or picrotoxin (10^{-5} M) inhibited the increase in chloride conductance but not the reduction in potassium conductance.

5. Barbiturates at these high concentrations initially caused a marked augmentation and prolongation of GABA responses; this was followed by a depression. The depressant action did not appear to be voltage-dependent. These actions of barbiturates were not accompanied by changes in the apparent dissociation constant of the GABA-current dose–response curve or the reversal potential for GABA currents. In addition, the single exponential decay of GABA current was not changed despite a marked prolongation of its decay time.

6. Picrotoxin (10^{-5} M) antagonized the depressant effect of barbiturates at high concentrations on GABA currents, and barbiturates (5×10^{-6} M) reduced the inhibitory action of picrotoxin (5×10^{-6} M) on the GABA-currents.

7. From all these results, it is suggested that the site of barbiturate actions on GABA-responses is mainly the allosteric site (the ionic conductance regulatory

subunit) but not the agonist recognition site or the chloride channels linked with GABA receptors.

INTRODUCTION

The barbiturates, pentobarbitone and thiopentone, are known to enhance presynaptic inhibition in the spinal cord of frogs (Schmidt; 1963) and cats (Eccles, Schmidt & Willis, 1963). This action may be attributable to facilitation of the GABA–GABA receptor interaction since barbiturates markedly augment and prolong depolarization induced by exogenous GABA in primary afferent neurones of the frog (Nicoll, 1975), cat (Higashi, Gallagher, Dun & Nishi, 1975; Higashi & Nishi, 1979) and rat (Evans, 1979; Connors, 1981). On the other hand, it has been proposed that pentobarbitone depresses the release of transmitter from primary afferent nerve terminals (Weakly, 1969) either by acting as a local anaesthetic and blocking impulse invasion into the nerve terminals (Løyning, Oshima & Yokota, 1964; Somjen, 1967), or by blocking a step in excitation–secretion coupling, such as Ca^{2+} entry into the presynaptic terminal (Blaustein, 1976; Haycock, Levy & Cotman, 1977). The enhancing mechanism of barbiturates on presynaptic inhibition, thus, appears to be unclear.

Furthermore, the precise mechanism(s) by which barbiturates enhance and prolong GABA-mediated responses is also unknown. Barbiturates at relatively high concentrations may act as agonists at the GABA receptor (Nicoll, 1975; Barker & Ransom, 1978; Nicoll & Wojtowicz, 1980; Connors, 1981). On the other hand, barbiturates have also been suggested to act at an allosteric site (ionic conductance modulator site or regulatory subunit) coupled to the GABA receptor (Higashi & Nishi, 1979; Barker & McBurney, 1979) or at the GABA *antagonist* binding site without affecting the *agonist* recognition site (Bowery & Dray, 1976; 1978). Moreover, Barker & Mathers (1981) have recently reviewed the interaction between the GABA receptor of cultured neurones and pentobarbitone, and suggested that GABA mimetic effects of pentobarbitone involve a direct action at GABA agonist binding sites.

Recent investigations have revealed that cell bodies of primary afferent neurones (dorsal root ganglion) are endowed with GABA receptors which may be similar to those on their nerve terminals (Feltz & Rasminsky, 1974; Nishi, Minota & Karczmar, 1974; Deschenes, Feltz & Lamour, 1976; Gallagher, Higashi & Nishi, 1978). Exogenous GABA appears to act on the cell bodies of primary afferent neurones by a mechanism similar in nature to that generating depolarization of their intraspinal axon terminals (see Levy, 1977).

The present study employed intracellular recording and voltage-clamp techniques on isolated cat dorsal root ganglia to determine whether barbiturates act directly on the GABA receptor complex. Some preliminary observations have appeared earlier (Higashi & Nishi, 1979; Higashi, Inokuchi & Nishi, 1980).

METHODS

Isolation and intracellular recording techniques have been previously described (Gallagher *et al.* 1978). Briefly, spinal ganglia (L_5–S_1) were excised with attached dorsal rootlets from cats anaesthetized with α-chloralose (60 mg/kg, I.P.). After dissecting the thin capsule enveloping a ganglion mass, individual somata (50–110 μm, diameter) were visualized under binocular magnifi-

cation (25–100 ×). Double barrelled micro-electrodes filled with 2 M-potassium citrate (20–40 MΩ) were employed to allow application of a known current or voltage across the membrane. The coupling resistance between the two barrels was measured before and after each experiment; this resistance was on the order of 100 kΩ, i.e. less than 1 % of the input resistance of individual cells (Ginsborg & Kado, 1975). A WPI electrometer (M701) and Nihon Kodèn voltage-clamp circuits (MEZ-7107 and CEZ-1100) were employed for recording membrane voltage and current responses, respectively.

The ganglion was superfused with Krebs solution at 36 ± 1 °C at a constant rate of 2 ml/min, using a perfusion pump. The composition of the Krebs solution was (mM): NaCl, 117·0; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$ 25·0; NaH_2PO_4, 1·2, glucose 11·0. The solution was bubbled continuously with 95 % O_2–5 % CO_2. The bath volume was about 0·5 ml. Extracellular substitution of anions or cations was done iso-osmotically.

GABA was applied by ionophoresis from electrodes of tip resistance 60–150 MΩ when filled with 0·5 M-GABA (pH 4·5). During ionophoresis experiments a sufficient bias voltage was applied to the GABA-containing electrode to prevent continuous leakage onto the cell and possible desensitization. Sodium pentobarbitone (Abbott), sodium thiopentone (Tanabe) and picrotoxin (Sigma) were added to the superfusion fluid to yield a final bath concentration. All quantitative results are expressed as mean ± S.E. of mean.

RESULTS

Actions of barbiturates on membrane properties

Membrane potential. Neither thiopentone nor pentobarbitone altered resting membrane potential or input resistance at concentrations of 10^{-7}–10^{-5} M. High concentrations (10^{-4}–10^{-3} M) resulted in membrane depolarization. The amplitude of this depolarization was concentration-dependent, reaching 3–11 mV when barbiturate (10^{-3} M) was applied by perfusion for 30 s or 1 min (Fig. 1*A*). Depolarization occurred slowly, and during longer perfusions (> 2 min) reached its maximum 90 s after starting barbiturate application. During a long application (10 min), the amplitude of the depolarization equilibrated at 20–30 % of its peak value.

During the initial part of depolarization, the membrane resistance decreased, but during the later part resistance increased in five of twelve neurones. Depolarizations were also observed with only a resistance decrease (five of twelve), or a resistance increase (two of twelve).

Membrane current. Voltage-clamp analysis (holding potential: −60 mV) of the barbiturate action indicated an inward current associated with three types of conductance change (Fig. 1*B*). About one-third of neurones (sixteen of forty-four) showed a conductance increase followed by a conductance decrease during the barbiturate application. A second group of neurones (eighteen of forty-four) showed only a conductance increase and a third group (ten of forty-four) showed only a conductance decrease. The type of conductance change was not related to the peak amplitude of the barbiturate current.

The time-to-peak of the inward barbiturate current was, however, different for the three types of conductance change (Fig. 1*B*). In neurones showing an increase followed by a decrease, inward current peaked at 62·7 ± 6·5 s; this time-to-peak was 48·7 ± 5·2 s and 73·8 ± 8·2 s in cells showing only increases and only decreases in conductance, respectively. These times all differ from one another (*t* tests, $P < 0·05$).

Ionic mechanism. Changes in the extracellular ion concentrations were made during the recording of barbiturate depolarizations and barbiturate currents. Responses of all three conductance types were unaffected by substitution of sodium chloride by

choline chloride or by replacement of calcium chloride (2·5 mM) by magnesium chloride (7·7 mM).

Regardless of the associated pattern of conductance change, the barbiturate depolarization or current was increased by low chloride solutions or by 0 potassium solution. Fig. 2 illustrates a typical barbiturate depolarization in low chloride or potassium-free medium. These results suggest that the initial effect of barbiturate

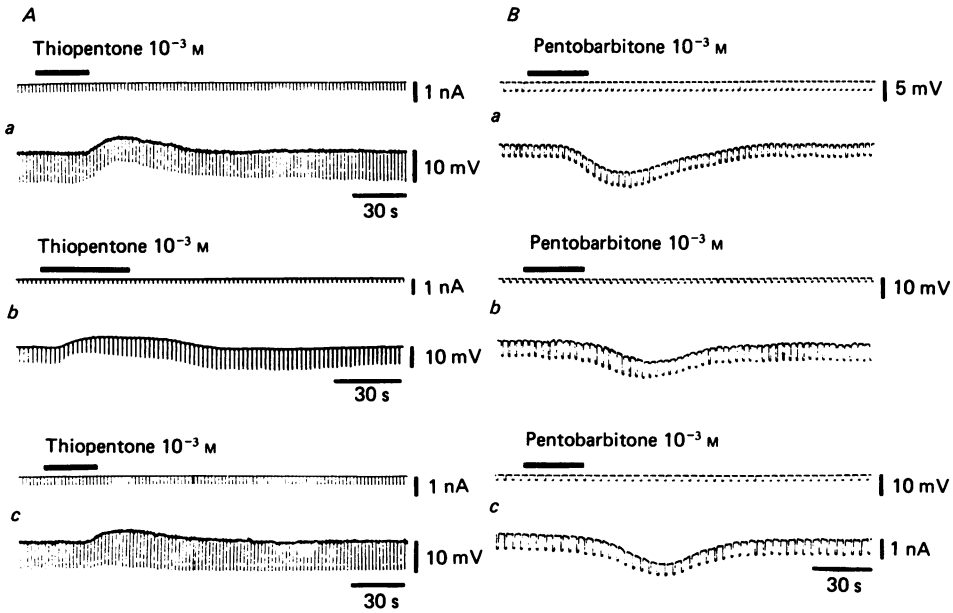

Fig. 1. Effect of barbiturates on resting membrane properties. Thiopentone or pentobarbitone was bath-applied during the period indicated by the bar. Each response was obtained from a different neurone. *A*, in each pair of recordings, the upper shows the current (200 ms, 0·5 Hz) passed across the membrane, and the lower depicts resting membrane potential and anelectrotonic potentials. The membrane resistance shown in *a* is only decreased throughout thiopentone-induced depolarization while the resistance in *b* is initially decreased and subsequently increased. The neurone in *c* shows only an increase in the membrane resistance. The resting membrane potential of each cell was −62 mV (*a*), −60 mV (*b*) and −58 mV (*c*), respectively. *B*, in each pair of recordings, the upper shows the clamped voltage and commanding pulses (1 s, 0·33–0·25 Hz) and the lower, the current. The holding potential in each neurone was −60 mV. The membrane conductance shown in *a* is only increased during pentobarbitone application, while in the neurone represented in *b* the conductance is initially increased and subsequently decreased. The neurone in *c* shows only a conductance decrease.

is an increase in chloride conductance and that this is associated with a more slowly developing reduction in potassium conductance.

Measurements of membrane conductance at various holding potentials were compatible with this mechanism. In neurones in which a conductance increase predominated, membrane depolarization (in the range −60 to −30 mV) progressively reduced the inward current but further depolarization had little effect. Membrane hyperpolarization increased the inward current, but no further increase was obtained

at potentials more negative than −80 mV (Fig. 3). However, in some cells, particularly those in which a conductance decrease predominated, membrane hyperpolarization progressively reduced the inward current.

The difficulties in analysing the underlying conductance changes are also illustrated in the tracings shown in Fig. 3*A*, obtained from a neurone which showed only a conductance increase at the resting potential (−57 mV). Application of barbiturate at more depolarized holding potentials caused a diminishing inward current which

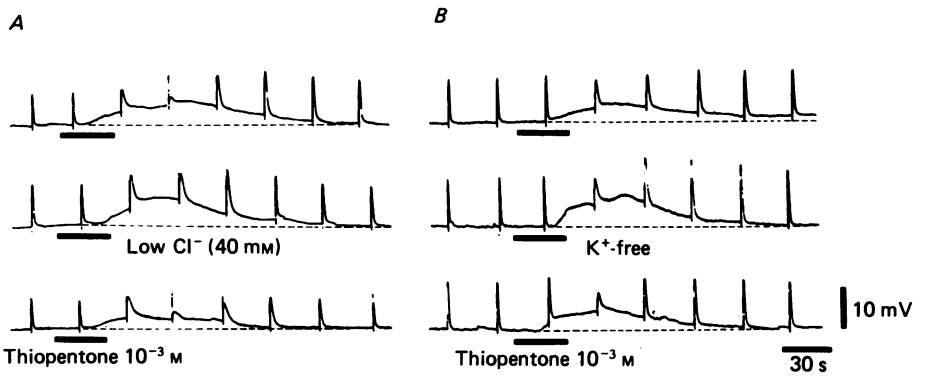

Fig. 2. Ionic mechanisms of thiopentone-induced depolarizations. Thiopentone was bath-applied (bar). GABA-depolarizations evoked ionophoretically have also been monitored every 30 s. In *A* and *B*, the upper, middle and lower recordings represent a control response, response in low Cl^- (The NaCl of Krebs solution was partially (89 mM) replaced by Na-isethionate, leaving 40 mM of chloride in the medium) (*A*) or K^+-free medium (*B*), and then after washing in normal Krebs solution, respectively. *A* and *B* were obtained from two different neurones with resting potentials (dashed line) of −60 and −63 mV, respectively. GABA ejection pulses were 50 nA and 50 ms in *A*, and 40 nA and 50 ms in *B*. In experiment *A*, there was a few millivolts reduction in membrane potential 5–10 min after starting superfusion of low Cl^- solution. Despite such a depolarization, both thiopentone-induced and GABA-induced depolarizations are markedly augmented. In addition, the rapid depression of the GABA responses during thiopentone depolarization are antagonized in low Cl^- solution. In the case of *B*, K^+-free solution caused a slight (3·5 mV) hyperpolarization and a small increase in input resistance only by 7% of the control value. This effect could be responsible for the enhancement of GABA-induced depolarizations, but not for augmentation of thiopentone-depolarization, since the former's enhancement (10% increase) was comparable in degree to the change in membrane potential and resistance while the latter's augmentation (70% increase) far exceeded the alteration in membrane properties.

was progressively associated with a conductance decrease. However, even at membrane potentials much less negative than the chloride equilibrium potential (see below), barbiturates still induced a net inward current associated with a conductance decrease. Membrane hyperpolarization only slightly increased the inward current. This result suggests that inactivation of potassium conductance is voltage-dependent. Because of this voltage-dependence no reversal potential for the inward current was obtained over holding potential levels ranging from −87 mV to +7 mV (Fig. 3*B*).

Separation of currents. On the assumption that the chloride conductance increasing effect of barbiturates was mediated by an action similar to that of GABA, we

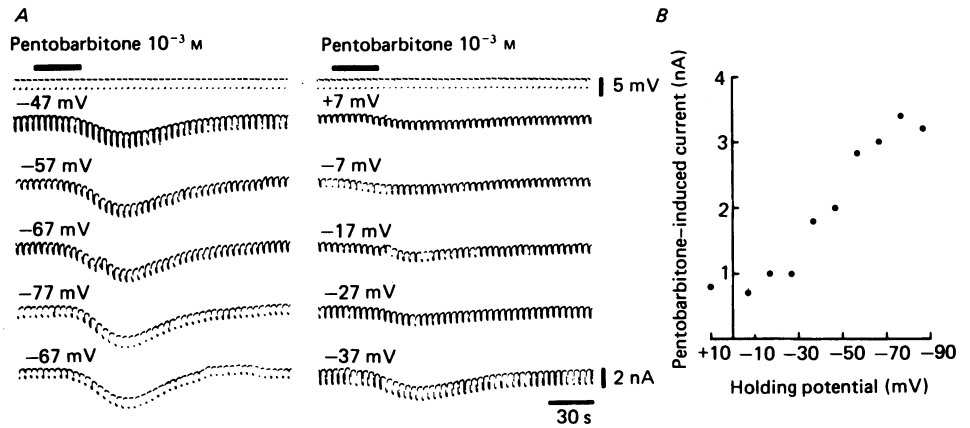

Fig. 3. Inward currents and conductance changes induced by pentobarbitone at different holding potentials. *A*, the top tracings in the left and right columns show hyperpolarizing steps from the holding potential level. Pentobarbitone was bath-applied (bar). The lower tracings show pentobarbitone-produced inward currents and conductance changes at each holding level which is indicated on the left side of the response. *B*, plot depicts graphically peak amplitudes of inward currents *vs.* holding potential shown in *A* (see text).

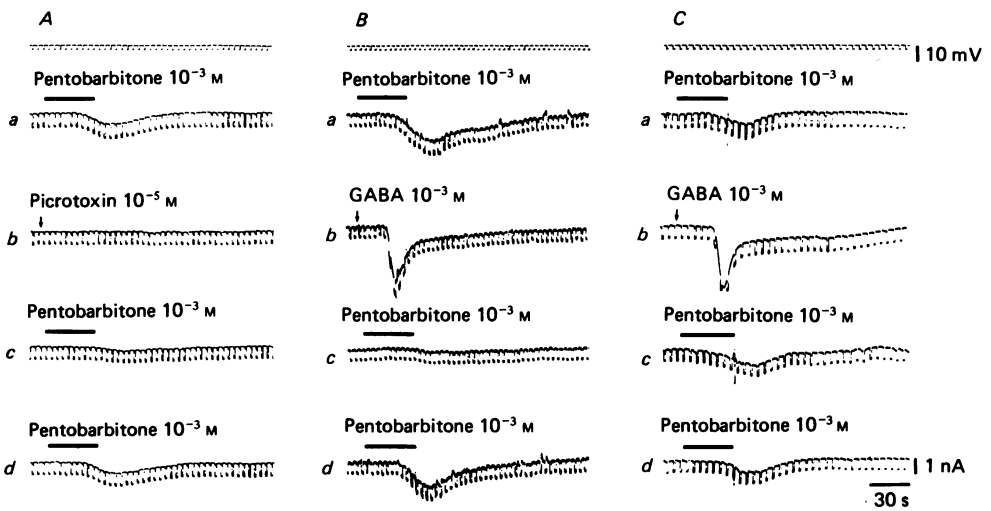

Fig. 4. Effects of continuous bath-application of picrotoxin or GABA on the pentobarbitone-induced current. The top tracing in each column represents the clamped voltage. The series of the lower tracings (*a–d*) in *A*, *B* and *C* represent a pentobarbitone-induced current in normal Krebs solution (*a*), a current change produced by continuous superfusion with picrotoxin (10^{-5} M) or GABA (10^{-3} M) (*b*), a pentobarbitone-induced current during continuous application of picrotoxin (*Ac*) or GABA (*Bc*, *Cc*), and the current evoked by pentobarbitone after washing out picrotoxin (*Ad*) or GABA (*Bd*, *Cd*) (see text). The beginning of picrotoxin or GABA application is indicated by a downward arrow. Pentobarbitone was also bath-applied (bar). The holding potentials in *A*, *B* and *C* were −60 mV.

attempted to block it selectively by picrotoxin and by application of desensitizing concentrations of GABA. Picrotoxin (10^{-5} M) prevented the initial part of the inward current. In the presence of picrotoxin, the barbiturate current was always associated with a conductance decrease (Fig. 4*A*).

The response to barbiturate was compared before and during application of GABA (10^{-3} M). The test dose of barbiturate was applied several minutes after the effect of GABA had completely desensitized. In some cells in which barbiturate caused a conductance increase, the barbiturate current was greatly reduced during GABA application (Fig. 4*B*). In other cells, prolonged application of GABA did not greatly change the inward barbiturate current, but it reversed the conductance increase to a conductance decrease (Fig. 4*C*). These findings indicate that one action of high barbiturate concentrations is similar to the GABA receptor agonist, as proposed by Nicoll (1975), with the additional accompaniment of potassium inactivation (see Discussion).

Other effects of barbiturate

Prolonged application (> 2 min) of very high concentrations (5×10^{-4}–10^{-3} M) appeared to block both resting chloride permeability and voltage-dependent sodium and potassium conductances. The former was measured by observing the slow depolarization which occurred after suddenly reducing the extracellular chloride concentration (substituted by isethionate or acetate). This depolarization presumably results from outward movement of chloride ions; it was insensitive to picrotoxin (10^{-5} M) but was depressed by barbiturate (10^{-3} M). The latter was observed by both the rate of rise and the amplitude of the after-hyperpolarization of the action potential which were reduced by barbiturate ($\geqslant 5 \times 10^{-4}$ M).

Actions of barbiturates on GABA responses

Ionophoretic application of GABA caused a rapidly rising membrane depolarization similar to that described by Gallagher *et al.* (1978). The effects of barbiturates were studied both on GABA-induced depolarization and, in the majority of the cells, on GABA-induced inward current under voltage clamp at -60 mV.

Amplitude of GABA response. Low concentrations (10^{-7}–10^{-5} M) of both barbiturates increased the amplitude of the GABA depolarization (Fig. 5*A*) or inward current (Fig. 6*A*). This effect of barbiturate began within 30 s after starting application of barbiturate and reached a maximum within 5–7 min. The maximum effect was related to the concentration applied and the effects were completely reversible (Fig. 5*B*). Thiopentone was approximately five to ten times more potent than pentobarbitone and the threshold concentration of thiopentone (10^{-7} M) was ten times lower than that of pentobarbitone. Higher concentrations of barbiturates (10^{-4}–10^{-3} M) initially caused a greater increase, and a subsequent decrease in the GABA response but these effects were associated with the membrane depolarization described above (Fig. 5*A*). Table 1 shows the relative alterations of the amplitude, the rise time, and the half decay time of the GABA currents after 10 min superfusion of pentobarbitone at different concentrations.

Since pentobarbitone at the concentrations ranging from 10^{-5} M to 10^{-4} M produced marked facilitatory effects on the GABA current amplitude in a majority of neurones

TABLE 1. Effect of pentobarbitone applied by superfusion on the amplitude, rise time and half-decay time of GABA-induced currents

Pentobarbitone (M)	No. of cells	GABA-induced current		
		Amplitude	Rise time	Half-decay time
10^{-8}	11	98·9±0·8	99·5±0·4	99·2±0·6
10^{-7}	22	102·6±2·6	101·1±0·6	103·8±1·4
10^{-6}	22	103·7±1·8	102·1±0·8	104·0±1·1
10^{-5}	23	105·7±2·8	106·1±1·6	110·4±2·0
10^{-4}	17	141·7±14·6	173·4±8·0	169·7±6·9
10^{-3}	6	27·0±13·2	425·2±69·8	427·2±28·7

Values are percentage of control ±S.E. of mean.

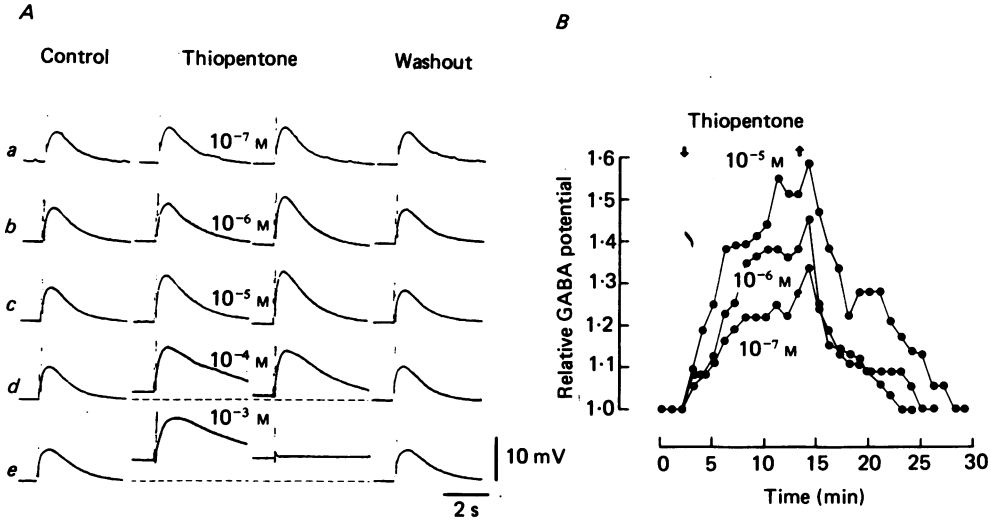

Fig. 5. Action of thiopentone at various concentrations on GABA-depolarizations. *A*, each series of four responses represents from left to right a control response, response after 5 min and 10 min of thiopentone superfusion, and then 15 min after washing off, respectively, except *e*. In *e*, the second and third GABA-depolarization shows a response after 1 and 5 min of superfusion, respectively. The cell membrane is depolarized from the resting level of −60 mV (dashed line) during superfusion of 10^{-4} M- or 10^{-3} M-thiopentone in *d* and *e*. GABA depolarizations were ionophoretically elicited in the same neurone by ejection pulses of 30 nA and 50 ms. *B*, graphic depiction of the effect of addition and removal of thiopentone (10^{-7}–10^{-5} M) on GABA depolarizations. Thiopentone at these concentrations did not affect membrane properties, i.e., membrane potentials or input resistances. All data have been normalized so that the control GABA response is equal to 1·0.

(about 80%) and depressant effect in a small number of neurones (approximately 20%), we chose these concentrations to examine the mechanism by which pentobarbitone facilitated or depressed the GABA currents.

Fig. 6 illustrates the facilitatory effect of pentobarbitone ($1–5 \times 10^{-5}$ M) on the dose–response curve of the GABA current. In this particular neurone, pentobarbitone only increased the GABA current independent of its initial value. Even the maximal GABA current showed a concentration-dependent increase and the ionophoretic

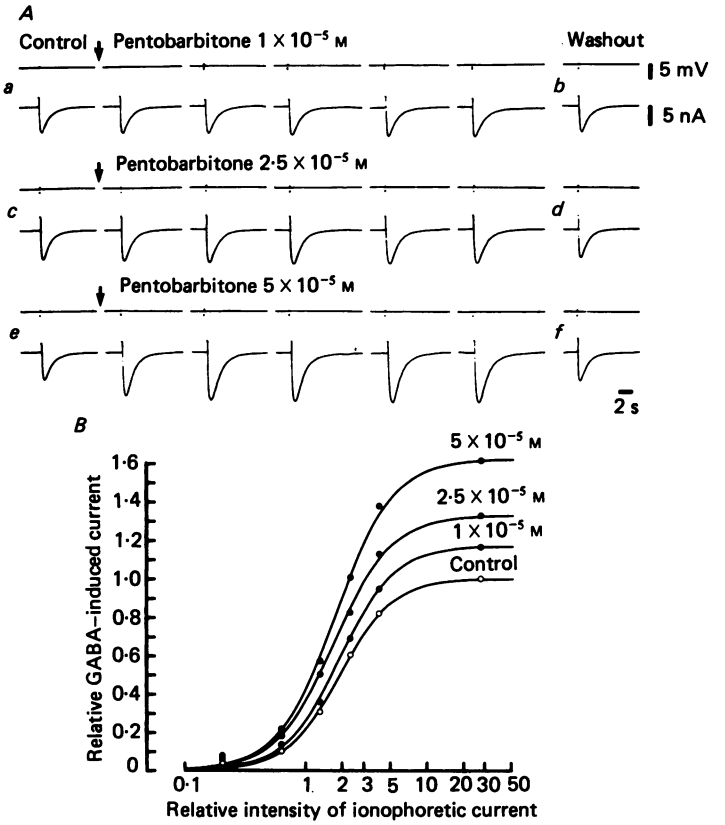

Fig. 6. Facilitatory effect of various concentrations of pentobarbitone upon GABA-currents. *A*, in each pair of records, the upper shows the clamped voltage (holding level: −60 mV) and the lower, the GABA-induced current by ionophoresis. Responses are evoked by GABA ejection pulses of 40 nA and 50 ms applied at 1 min intervals. *b*, *d* and *f* show the responses 20 min after washing off each concentration of the drug. All the traces were recorded from a single neurone. *B*, the dose–response curves measured from 8 to 14 min after start of the bath-application of pentobarbitone at various concentrations. The open and filled circles represent the relative GABA currents which were experimentally obtained in the absence and presence of pentobarbitone, respectively. The continuous lines are drawn according to the equation, $Y = I/I_{max} = a^n/a^n + K_m{}^n$, assuming $n = 2$, $K_m = 2$ and $I_{max} = 1{\cdot}0$, 1·1, 1·3 and 1·6, respectively. In this equation Y is the ratio of the GABA-induced current (I) to its maximal value (I_{max}), a is the relative ionophoretic current, K_m is the apparent dissociation constant of the GABA-receptor complex, and n is the Hill slope. Both K_m and I_{max} values have been estimated from the regression line of a double reciprocal plot of the GABA current *vs.* the ionophoretic current. The peaks of each GABA current are normalized to the maximum (1·0) response in normal Krebs solution.

current required to produce a half maximal response (the apparent dissociation constant of the dose–response curve) was unchanged by barbiturates. This implies that barbiturates do not affect the GABA receptor interaction directly, but amplify the effect of a given GABA receptor occupancy.

Fig. 7 illustrates the inhibitory effect of pentobarbitone (0·25–1 × 10^{-4} M) on the

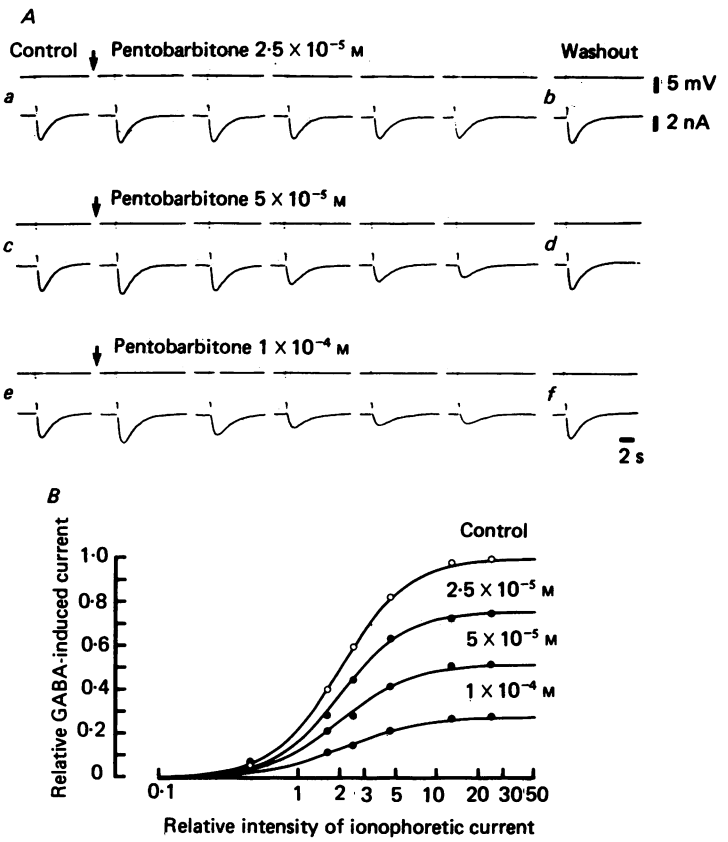

Fig. 7. Depressant effect of various concentrations of pentobarbitone upon GABA-currents. *A*, in each pair of records, the upper shows the clamped voltage (holding level: −60 mV) and the lower, the GABA-induced current by ionophoresis. The responses are evoked by GABA ejection pulses of 40 nA and 50 ms applied every 1 min. *b*, *d* and *f* show the responses 20 min after washing off each concentration of the drug. All the traces were recorded from a single neurone. *B*, plot depicts graphically the relative GABA-current *vs.* ionophoretic current intensity measured after 8–14 min exposure to each concentration of pentobarbitone. The open and filled circles represent the relative GABA-currents which were experimentally obtained in the absence and presence of pentobarbitone, respectively. The theoretical curves (continuous lines) are drawn by the equation, $Y = I/I_{max} = a^n/a^n + k_m{}^n$, used in Fig. 6, assuming $n = 2$, $K_m = 2$ and $I_{max} = 1{\cdot}0$, 0·75, 0·51 and 0·27, respectively.

GABA current dose–response curve obtained from another neurone. The initial enhancement of the GABA response gave way to an increasing depression. This action was concentration-dependent and was accompanied by a small inward current induced by the barbiturate itself. The dose–response curve to GABA was depressed in a non-competitive manner. The reversal potentials of the GABA currents (−25 mV and −27 mV in the neurones shown in Fig. 6 and Fig. 7, respectively) were unaltered after 15 min superfusion of pentobarbitone. Furthermore, there was no significant voltage-dependency in either the augmentative or inhibitory effects of barbiturates

on the GABA currents at different holding potentials ranging from +10 mV to −90 mV.

Time course of GABA response. Concentrations of pentobarbitone ($> 10^{-6}$ M) or thiopentone ($> 10^{-7}$ M) which increased the amplitude of the GABA response, also slowed the rate of rise and prolonged the decay (Table 1). Slowing of the rate of rise correlated well with the increase in half-decay time as the barbiturate concentration was increased. At the highest concentration of pentobarbitone (10^{-3} M) slowing of the

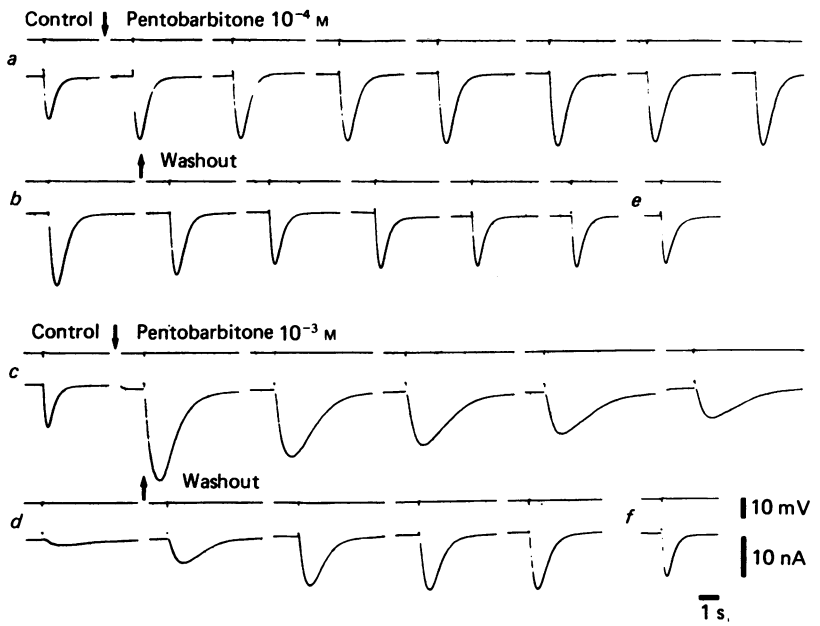

Fig. 8. Effect of pentobarbitone at high concentrations on GABA-induced currents. In each pair of records, the upper shows the clamped voltage (holding level: −60 mV) and the lower, the current. Responses are evoked by GABA ejection pulses of 30 nA and 50 ms applied at 1 min intervals. The first GABA current shown in *b* and *d* shows the response after 10 min of superfusion with pentobarbitone at the indicated concentration. The response in *e* and *f* are monitored 15 min after washing off the drug.

time course of the GABA response was more than four-fold, even though the amplitude was actually depressed (Table 1). In addition, decay of the GABA current remained a single exponential even during the marked prolongation of its decay time at high concentrations of barbiturates (Fig. 8).

Effect of picrotoxin. Picrotoxin (10^{-7}–10^{-5} M) reduced the amplitude of the GABA current, in confirmation of previous findings (Gallagher *et al.* 1978). This action was greatly reduced by low concentrations of barbiturate (5×10^{-6} M) (Fig. 9*A*). The reverse experiment also showed an interaction between barbiturate and picrotoxin (Fig. 9*B*). A high concentration of barbiturate was applied, and the GABA response was initially augmented and then depressed. The late depression of the GABA response was greatly reduced in the presence of picrotoxin (10^{-5} M).

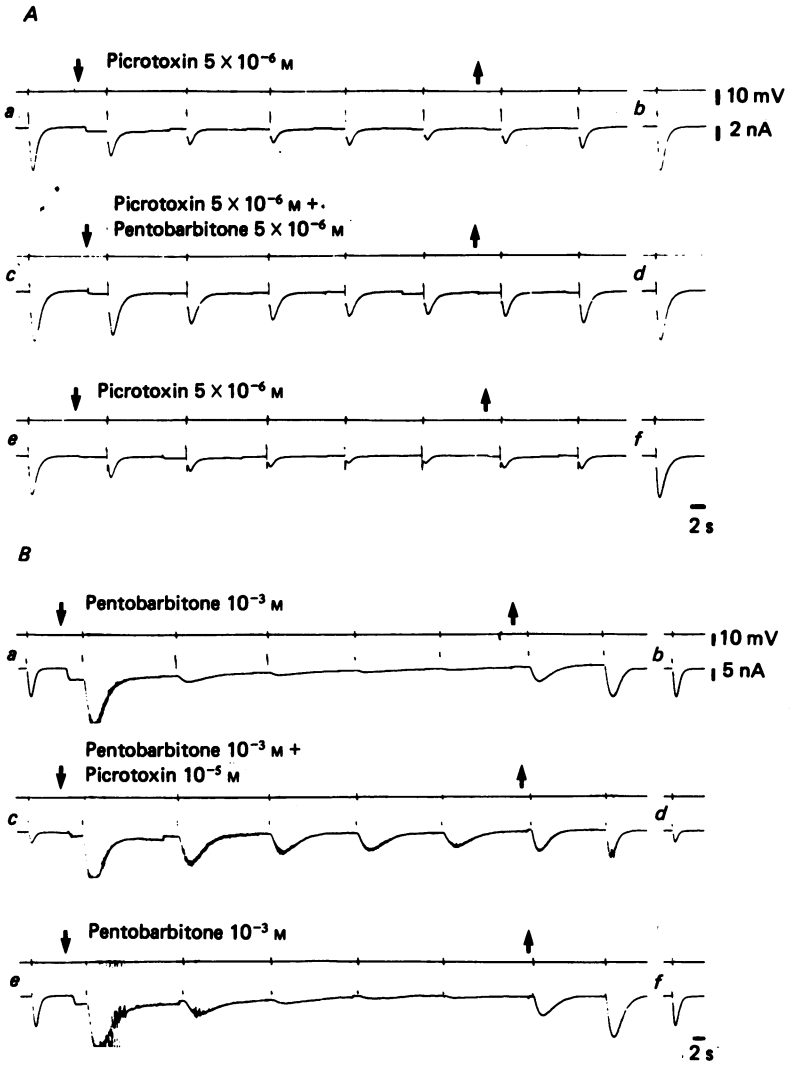

Fig. 9. Interaction between pentobarbitone and picrotoxin of GABA-induced currents. *A* and *B* show, respectively, the effects of pentobarbitone pre-treatment on picrotoxin action (*A*) and picrotoxin pretreatment on pentobarbitone action (*B*). In each pair of records, the upper shows the clamped voltage (holding level: −60 mV) and the lower, the current. The responses in *A* and *B* were evoked in two different neurones by GABA ejection pulses of 40 nA and 50 ms every 1 min. *A*, picrotoxin was first superfused for 5 min (between the arrows) in normal Krebs solution (*a*). The GABA current recovered fully 10 min after washing off the drug (*b*), and was then treated with pentobarbitone (5×10^{-6} M) for 10 min. As a result, the GABA-current was slightly enhanced and prolonged (first tracing in *c*). Subsequently, the solution was changed to a picrotoxin plus pentobarbitone containing solution. Ten min after washing out picrotoxin, the GABA current returned to the control value (*d*), then the pentobarbitone-containing solution was stopped and the preparation returned to normal Krebs solution (*e*). Picrotoxin (5×10^{-6} M) was re-applied for 5 min (between the arrows) and then washed off with Krebs solution. The GABA current fully recovered 10 min after washing off the drug (*f*). *B*, pentobarbitone was added for 5 min (between the arrows) in normal Krebs solution (*a*). The GABA current fully recovered

DISCUSSION

Augmentation of the GABA response. GABA selectively increases chloride conductance of cat spinal ganglion cells (Gallagher *et al.* 1978). The present experiment showed that thiopentone and pentobarbitone increased the amplitude and duration of the GABA-induced chloride current, at concentrations which had no effect on any resting membrane properties. The increase in the maximum amplitude of the GABA current and the unchanged apparent dissociation constant of the GABA–GABA receptor interaction, suggest that barbiturates do not act at the GABA recognition site, but on the coupling mechanism between the recognition site and the chloride channel (i.e. allosteric site). This effect of barbiturates was blocked by picrotoxin, and low concentrations of barbiturates themselves prevented the usual antagonism by picrotoxin of the GABA current. These results indicate that barbiturate and picrotoxin may act at the same site, allosteric to that of GABA recognition. Such an interpretation is supported by Ticku & Olsen (1978) who have shown that barbiturates competitively displace dihydropicrotoxin from binding sites on rat brain membranes.

Several other sites of action of barbiturates have been proposed. The first of these is an inhibition of GABA uptake (c.f. Aickin & Deisz, 1981), the second is an alteration of the chloride pump activity and the third is a prolongation of the mean channel life time by which barbiturate directly activate the chloride channel (Mathers & Barker, 1980, also see Barker & Mathers, 1981). The first mechanism would effectively enhance the amplitude and prolongs the rising and falling phases of the GABA current. However, we have found in unpublished experiments that the time course of the GABA current induced by a short (50–100 ms) ionophoretic pulse, was unaffected in nipecotic acid (10^{-3} M) containing or low Na^+ (2×10^{-3} M) Krebs solution. Under these conditions, GABA uptake was reported to be depressed in mammalian ganglia (Brown & Galvan, 1977). Furthermore, barbiturates (10^{-4}–10^{-3} M) still prolonged the GABA current in these media (H. Higashi & S. Nishi, unpublished observation). The latter two mechanisms are also unlikely since the present results showed that the reversal potential for the GABA current was unchanged by barbiturate and that the barbiturate enhancement of the GABA response was insensitive to different holding potentials.

The mechanism underlying the slowing of both the rising and falling phases of the GABA currents is difficult to explain because it still occurs after the inhibition of GABA uptake. A possibility would be that when the allosteric site had already been occupied by barbiturate, the GABA action on the recognition site might be prolonged

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10 min after washing off the drug (*b*), and was then pretreated with picrotoxin (10^{-5} M) for 10 min. As a result, the GABA-current was markedly depressed (first tracing in *c*). The solution was then changed to include pentobarbitone plus picrotoxin (between the arrows). 10 min after washing off pentobarbitone, the GABA current returned to the control value (*d*), then the picrotoxin-containing solution was replaced by normal Krebs solution. 20 min later (*e*), pentobarbitone was re-applied in normal Krebs solution (between the arrows). The GABA current recovered to the control value 10 min after washing off the drug (*f*) (see text).

and the rate of conformational changes associated with opening and closing of the channel might be retarded. However, further analysis is required to probe this possibility.

Direct action of barbiturate. At higher concentrations (10^{-4}–10^{-3} M) barbiturate applied for a short period of time (0·5–1 min) induced an increased chloride conductance and/or a decreased voltage-dependent potassium conductance. Which of the conductance changes appeared predominantly depended on individual neurones, but the latter usually occurred over a slower time course than the chloride activation. It should be noted that the barbiturate-induced chloride activation was abolished by picrotoxin (10^{-5} M) or by a continued application of GABA (10^{-3} M). However, we believe that barbiturate does not activate chloride conductance by occupying the GABA recognition site for three reasons. First, the time course of the barbiturate-induced chloride current was much slower than that of a similar amplitude evoked by GABA (Fig. 4*B*). Secondly, the response to GABA ionophoresis was not reduced when GABA was applied at the time of peak barbiturate-inward current (Fig. 8, 9*B*). Thirdly, prolonged application of barbiturate led to a reduction of its effect; at the time of this desensitization to barbiturate, the maximum GABA response was also reduced while the apparent dissociation constant of GABA–GABA receptor interaction was unaltered (Fig. 7*B*). Depression of the GABA response by prolonged barbiturate perfusion was not dependent on membrane potential, and the GABA current decay remained a single exponential. This is also compatible with an action of barbiturate at a site intermediate between the GABA recognition site and chloride channel, rather than a direct action on the channel. Several studies with the use of binding methods have already suggested that pentobarbitone does not inhibit the binding of GABA to its receptor site, but may combine with an allosteric regulation site of the GABA ionophore (c.f. Olsen & Leeb-Lundberg, 1981; also see Johnston & Willow, 1981).

We observed also that prolonged application (> 2 min) of very high concentrations ($> 5 \times 10^{-3}$ M) of barbiturate depressed both resting chloride conductance and voltage-dependent sodium and potassium conductances. Moreover, the GABA responses which were initially augmented showed a sequential depression in amplitude. It is possible that channel block by the barbiturates could explain depression of the GABA responses. However, since the depressant action of the barbiturates on GABA currents was not voltage-dependent, and since the decay of GABA currents remained a single exponential in the presence of the barbiturates, it seems unlikely that attenuation of the GABA responses by higher concentrations of barbiturates is due to a channel blocking action. Alternatively, it is conceivable that high concentrations of barbiturates could prevent agonist triggered channel opening by evoking a conformational change in the regulatory subunit or the ionophore, similar to that occurring during desensitization. This would be similar to a cyclic model of desensitization except that the conformational change to the desensitized state is triggered in the absence of ligand binding to the agonist recognition site (c.f. Adams, 1981).

Anaesthetic doses of pentobarbitone range from 5×10^{-5} to 6×10^{-4} M (Richards, 1972). The present result demonstrated that although GABA responses are depressed in the minority (20%) of neurones at these concentrations, they are strikingly

enhanced and prolonged in the majority (80%) of neurones, with no effect on the action potential. It is most likely, therefore that the enhancement of GABA action by barbiturates rather than the drug blocking action potential generation is the major factor leading to depression of sensory input.

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