Br. J. Pharmac. (1978), **63**, 197–215.

REVERSAL OF THE ACTION OF AMINO ACID ANTAGONISTS BY BARBITURATES AND OTHER HYPNOTIC DRUGS

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1 The effects of pentobarbitone (PB) and other sedative/hypnotic drugs have been examined in relation to γ-aminobutyric acid (GABA) *in vitro* on the superfused isolated superior cervical ganglion of the rat and *in vivo* on single units in the brain stem of the anaesthetized rat.

2 PB, and other barbiturates, depolarized the ganglion in a dose-dependent manner (threshold concentration 100–300 μM, cf. GABA depolarization threshold 1 μM). The depolarization was reduced in the presence of the selective GABA antagonist (+)-bicuculline methochloride (Bic). Other non-barbiturate sedatives e.g. chlordiazepoxide, amitriptyline, promethazine at concentrations up to 2mM produced no depolarization.

3 PB, tested at concentrations up to 80 μM, produced variable effects on the dose-response curve to GABA. On most occasions a slight potentiation occurred in responses to low concentrations of GABA (below 10 μM) coupled with a depression in the responses to concentrations of GABA greater than 10 μM.

4 Superfusion with PB in the presence of Bic reversed the depression in the response to GABA produced by Bic. This reversal phenomenon occurred at concentrations of PB too low to depolarize the ganglion and was dependent not only on the concentration of PB but also on that of Bic.

5 The reversal potency within an homologous series of barbiturates increased with the size of the alkyl substituent (R2) at C5 on the barbiturate ring. The most potent occurred when the substituent contained 5 carbon atoms (pentobarbitone and amylobarbitone); above this, activity decreased.

6 PB reversed the effects of the other GABA antagonists, tetramethylenedisulphotetramine and isopropyl bicyclophosphate and also the non-selective antagonism produced by strychnine. A concomitant reduction by strychnine of responses to the cholinomimetic, carbachol, was not reversed by PB.

7 Non-barbiturate sedative/hypnotics also reversed the GABA antagonism produced by Bic. The benzodiazepines were effective at lower concentrations than PB (chlordiazepoxide threshold concentration 0.5 μM, cf. PB 5 μM), however, they only produced a partial reversal even at concentrations much higher than the maximally effective concentration of PB.

8 The Bic reversal effect of chlordiazepoxide (and other benzodiazepines) lasted many hours after removal from the superfusion solution. By contrast the effect of PB lasted only 15–30 min after its removal.

9 Chlordiazepoxide (30 μM) applied in the absence of Bic did not affect the response to GABA but did reduce the depression produced by the subsequent application of Bic even though the chlordiazepoxide had been removed 40 min earlier.

10 In the rat brain stem *in vivo* PB, applied iontophoretically in amounts which neither decreased the spontaneous neuronal firing rate nor affected the response to GABA or glycine, reversed the GABA antagonism induced by iontophoretic application of Bic (in all 23 neurones tested). PB also reversed the antagonism produced by strychnine of responses to glycine although this was less readily observed (5 out of 14 neurones tested).

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11 Iontophoretic application of other barbiturates and chlordiazepoxide also reversed the effect of Bic. Chlordiazepoxide only produced a partial reversal, as in the isolated ganglion, and no reversal could be demonstrated with flurazepam.

12 Intravenous administration of thiopentone (1.3 mg/kg) pentobarbitone (0.4–5.5 mg/kg) hexobarbitone (0.4–0.8 mg/kg) and clonazepam (0.1–0.2 mg/kg) also reversed the effect of iontophoretically applied Bic. The reversal by clonazepam was of much longer duration than that produced by the barbiturates.

13 It is suggested that the reversal exhibited by PB and the other hypnotics may be explained by assuming that the amino acids and their antagonists bind to the membrane at separate sites. If the reversal agent has particular affinity only for the antagonist binding site then it may displace the antagonist without affecting the receptor.

Introduction

Fischer & von Mering introduced the first therapeutically useful barbiturate, diethylbarbituric acid, to medicine in 1903. Since then many more have emerged but even 70 years later their mechanism of action as hypnotics and anti-convulsants remains obscure although biochemical, local anaesthetic and synaptic blocking actions have been implicated in their action. Synaptic function within the central nervous system appears to be particularly sensitive to the barbiturates and may be influenced in a variety of ways. For example, barbiturates can alter tissue transmitter levels (Richter & Crosland, 1949; Anderson & Bonnycastle, 1960; Corrodi, Fuxe & Hökfelt, 1966), they can decrease or enhance transmitter release (Mitchell, 1963; Phillis & Chong, 1965; Phillis, 1968; Cutler, Markowitz & Dudzinski, 1974; Cutler & Dudzinski, 1974) they can interfere with the removal of transmitter from the synaptic cleft (Cutler *et al.*, 1974) and they can depress the sensitivity of the post-synaptic cell to excitatory substances such as glutamic acid (Crawford & Curtis, 1966; Crawford, 1970; Richards & Smaje, 1974). Perhaps of greater significance is the recent proposal that the hypnotic action of the barbiturates may relate, at least in part, to the prolongation of central inhibitory transmission processes which are thought to be mediated by γ-amino-*n*-butyric acid (GABA) (Nicoll, Eccles, Oshima & Rubia, 1975). This possibility stems from the observed increase in the duration of pre- and post-synaptic inhibition produced by the barbiturates, in particular pentobarbitone at spinal and supraspinal sites (Eccles, Schmidt & Willis, 1963; Eccles, Faber & Táboříková, 1971; Nicoll *et al.*, 1975). The results of Nicoll (1975a, b) indicate that a direct action on GABA receptors may account for this effect of barbiturates and furthermore, may not be confined to this group of hypnotics but may also be a property of others (Nicoll, 1972). We have attempted to examine this association with GABA receptors further in the rat superior cervical ganglion *in vitro* and rat brainstem *in vivo*. During the course of this study we observed that the barbiturates and other hypnotics prevented or reversed the actions of GABA antagonists and to a lesser extent the glycine antagonist, strychnine. Some of these results have already appeared in preliminary form (Bowery, 1976; Bowery & Dray, 1976).

Methods

Isolated superior cervical ganglion preparation

The method was essentially that described previously (Bowery & Brown, 1974) for measurement of changes in surface potential produced by GABA in the rat superior cervical ganglion with the modification for superfusion described by Brown & Marsh (1975). Briefly, superior cervical ganglia with attached trunks were excised from male Wistar rats (220–280 g) anaesthetized with urethane (1.4 g/kg). The ganglia were desheathed and suspended vertically with a non-polarizable $Ag^+/AgCl$ electrode in contact with the post-ganglionic trunk and another in contact with the ganglion body. The potential difference between these electrodes was monitored continuously on a Servoscribe Is potentiometric recorder. The tissue was superfused at 1 ml/min with Krebs solution at ambient temperature bubbled with 95% O_2 and 5% CO_2. The Krebs solution composition (mM) was as follows: NaCl 118, KCl 4.8, $CaCl_2$ 2.52, KH_2PO_4 1.18, $MgSO_4 7H_2O$ 1.19, D-glucose 11 and $NaHCO_3$ 25 (pH 7.4). Drugs were diluted in Krebs solution in all cases. Solutions of quinalbarbitone, glutethimide and tetramethylenedisulphotetramine contained acetone to facilitate solubilization but the concentration of acetone in contact with the tissue never exceeded 0.3%. This did not affect the ganglion directly or interfere with responses to GABA or the other agonists (cf. Bowery, Brown & Collins, 1975).

Single unit recording in the rat brain stem

Experiments were performed on 15 male albino rats (200–250 g) lightly anaesthetized with urethane (1.4 g/kg) and prepared for recording from the brain stem as previously described (Bradley & Dray, 1973). Seven barrelled glass micropipettes were used to record extracellular action potentials from single spontaneously firing neurones in the medulla, and to administer substances close to them by standard micro-electrophoretic techniques. The centre recording barrel contained 3 M sodium chloride. Another barrel containing 1 M sodium chloride was used to balance the net current at the micropipette tip to zero or to expel Na^+ or Cl^- to test for electrophoretic current effects. The other barrels contained freshly prepared solutions of GABA 0.2 M, pH 3.5; glycine 0.2 M, pH 3.5; (+)-bicuculline methochloride 5 mM, pH 3.5; strychnine 5 mM, pH 3.5; pentobarbitone sodium 20–40 mM, pH 9.5; quinalbarbitone sodium 20–40 mM, pH 9.5; chlordiazepoxide hydrochloride 6–20 mM, pH 4.0; flurazepam hydrochloride 20 mM, pH 4.0.

Micropipettes were filled immediately before use by centrifugation and a glass fibre method. Action potentials were recorded and displayed by conventional methods. Spike shape and amplitude were routinely monitored from filmed records obtained on line from a Medilec oscilloscope u.v. recorder.

Reproducible responses to GABA and glycine were obtained during consecutive electrophoretic administrations of these compounds during a fixed time sequence. The effects on these responses of electrophoretically administered bicuculline or strychnine were determined and subsequent interactions with electrophoretically or intravenously administered barbiturates and benzodiazepines were studied.

Drugs

The following drugs were used: γ-amino-*n*-butyric acid (BDH); amitriptyline hydrochloride (Merck, Sharp & Dohme); amylobarbitone sodium (E. Lilly); aprobarbitone sodium (Roche); atropine sulphate (BDH); barbitone sodium (BDH); barbituric acid, sodium salt (kindly given by Dr D.A. Brown); bemegride (Nicholas Products Lab); (+)-bicuculline methochloride (kindly prepared by Dr J.F. Collins, Sir John Cass School of Science, London); butobarbitone (Amber Pharmaceuticals); caffeine (BDH); clonazepam (Rivotril–Roche); carbachol chloride (BDH); chloral hydrate (BDH); chlordiazepoxide hydrochloride (Roche); chlorpromazine hydrochloride (Largactil, May & Baker); cyclobarbitone calcium (Amber Pharmaceuticals); diazepam (Valium, Roche); flurazepam (Roche); glutethimide (CIBA); haloperidol (G.D. Searle & Co); *n*-heptyl ethyl barbituric acid (kindly prepared by Dr J.F. Collins); hexobarbitone sodium (May & Baker); hyoscine hydrobromide (Burroughs Wellcome); iproniazid (Roche); leptazol (Martindale Samoore); lignocaine hydrochloride (Antigen); mephobarbitone sodium (Bayer); mepyramine maleate (May & Baker); morphine hydrochloride (Martindale Samoore); nikethamide (Antigen); (±)-nipecotic acid (kindly prepared by Mr C. Cooksey, University College, London, from the method of Freifelder, 1963); pentobarbitone sodium (McCarthys); phenobarbitone sodium (BDH); phenytoin sodium (Parke Davis); picrotoxin (Sigma); probarbitone sodium (kindly given by Dr D.A. Brown); procaine hydrochloride (BDH); promethazine hydrochloride (May & Baker); quinalbarbitone (May & Baker); strychnine hydrochloride (BDH); tetramethylenedisulphotetramine (TETS, kindly prepared by Dr J.F. Collins); thiopentone sodium (May & Baker) tranylcypromine sulphate (SKF); trifluoperazine dihydrochloride (SKF); urethane (BDH).

Results

Superior cervical ganglia

Effect of hypnotics on the surface potential Pentobarbitone (PB) depolarized the isolated ganglion in a dose-dependent manner. This effect was examined in 9 preparations and the data from one of them are shown in Figure 1. The threshold concentration for depolarization was always between 100–300 μM whereas the threshold for GABA-induced depolarization in the same preparations was 0.5 – 1 μM (cf. Bowery & Brown, 1974). The response to pentobarbitone was much more prolonged than responses to GABA and the duration appeared to increase with the concentration of the barbiturate. The potency of pentobarbitone measured in terms of peak depolarization relative to GABA was 0.0047 ± 0.0008 (mean ± s.e. mean of 6 experiments) determined at the GABA ED_{50} level.

On superfusing the ganglion (Figure 1) with the selective GABA antagonist, bicuculline methochloride (Bic 20 μM), there was a displacement to the right of both the GABA and PB log dose-response curves but as shown in the example of Figure 1 there was a greater shift of the GABA curve than of the PB curve (0.88 ± 0.083 and 0.33 ± 0.037 log units displacement respectively ± s.e. mean of 3 experiments). Thirty minutes after removal of Bic from the superfusing fluid, responses to GABA and PB returned to the control level.

Other hypnotic/sedative drugs were tested for any direct effects on the ganglia. Quinalbarbitone and phenobarbitone also depolarized the ganglion but whereas quinalbarbitone was as active as PB, phenobarbitone was only 0.025 – 0.05 as active. Chlordi-

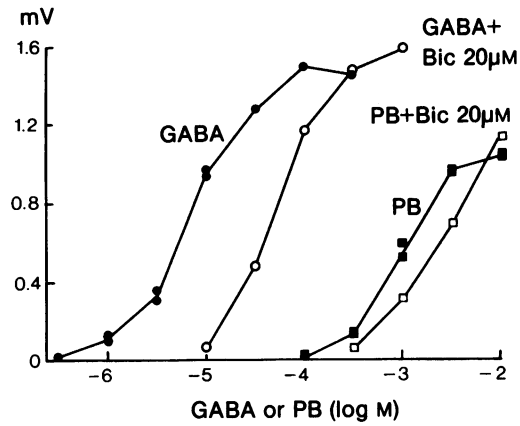


Figure 1 Dose-dependent depolarization of an isolated superior cervical ganglion produced by γ-aminobutyric acid (GABA, ●, ○) and pentobarbitone sodium (PB, ■, □) in the absence (filled symbols) and presence (open symbols) of (+)-bicuculline methochloride (Bic, 20 μM). Responses to each concentration of the agonists were determined by measurement of the peak depolarization (ordinate scale: millivolts) during 1 min periods of contact with the tissue. Intervals of 15 min were allowed between applications. Abscissa scale: log molar concentrations of either GABA or PB.

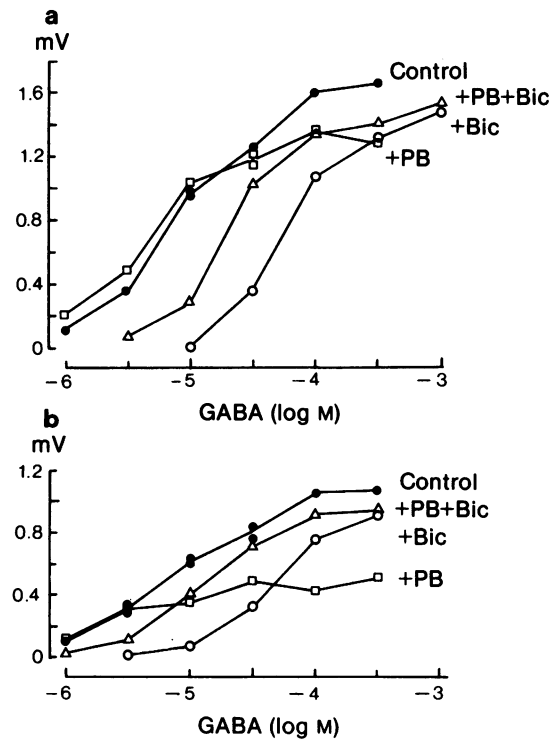


Figure 2 Effect of pentobarbitone sodium on the dose-response curve to γ-aminobutyric acid (GABA) obtained in the presence and absence of bicuculline methochloride. Data obtained from two superior cervical ganglia (a & b). Control: (●), plus pentobarbitone: (PB, 80 μM, □); plus bicuculline methochloride: (Bic, 20 μM, ○); plus PB (80 μM) and Bic (20 μM): (△). Ordinates response to GABA (peak depolarization in millivolts). Abscissae: log molar concentration of GABA. Each concentration of GABA was in contact with the tissue for 1 minute; 15 min intervals were allowed between doses. After removal of PB (and before the addition of Bic) a recovery curve was obtained in both tissues but these have been omitted as they were superimposed on the control curves. In (a) potentiation of the responses to GABA (<30 μM) occurred in the presence of PB whereas in (b) no potentiation occurred but there was a marked depression in the responses to GABA at concentrations above 3 μM.

azepoxide, promethazine and amitriptyline at concentrations up to 2 mM did not produce any depolarization.

Effects of hypnotics on the response to γ-aminobutyric acid PB at concentrations (4–80 μM) too low to depolarize the ganglion was added to the superfusion fluid and its effect on the responses to GABA observed. The change produced by PB varied between preparations but generally there was a small potentiation in the responses to GABA below the ED_{50} level (Figure 2a) with either a reduction or no change in the responses to higher GABA concentrations. In 3 experiments, an example of which is shown in Figure 2b, there was no alteration in the responses to low concentrations of GABA but a marked reduction occurred in the responses to larger concentrations. The effect of PB on responses to GABA was in all cases reversible within 30 min of its removal. Subsequent superfusion with Bic (20 μM) in the same preparations displaced the GABA curve as expected (Figure 2); PB (80 μM) was then added during superfusion with Bic. The GABA curve was now displaced back towards the control. An apparent reversal of the bicuculline antagonism had occurred. If the opposite procedure was employed, that is, superfusion with PB followed by concomitant superfusion with Bic then the PB reduced or prevented the expected decrease in the response to GABA produced by Bic.

The reversal of the antagonism by Bic did not appear to result from an increase in the response to GABA in a manner unrelated to the presence of the antagonist. The evidence for this was three-fold; firstly, although a slight potentiation of responses to low concentrations of GABA occurred only in some

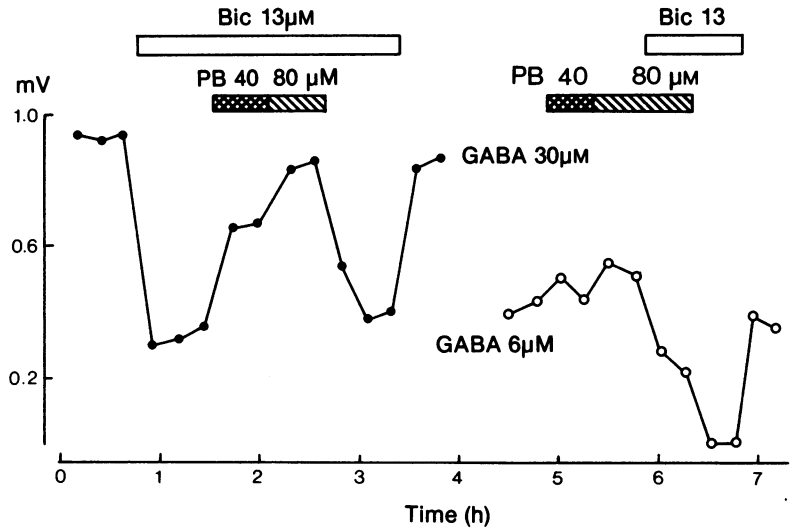


Figure 3 Comparison of the effect of pentobarbitone on responses to γ-aminobutyric acid (GABA) in the presence and absence of bicuculline methochloride. (●) Indicate the magnitude of responses of a single superior cervical ganglion to 1 min applications of GABA (30 μM). Bicuculline methochloride (Bic 13 μM) superfused over the tissue during the period indicated by the first open horizontal bar depressed the response to GABA. This depression was overcome by the addition of pentobarbitone (PB 40 and 80 μM) indicated by the hatched bars. After pentobarbitone and bicuculline methochloride had been removed and the control response to GABA re-established, the GABA concentration was reduced (to 6 μM) so that the responses (○) now matched the previous responses to GABA (30 μM) obtained in the presence of Bic. PB (40 and 80 μM) alone did not potentiate the response to 6 μM GABA, but in the presence of Bic reduced the expected GABA antagonism which can be seen to occur following the removal of PB. Ordinate scale: peak depolarization in millivolts. Abscissa scale: time in hours.

experiments as illustrated in Figure 2a, PB reversed the antagonism by Bic in every experiment. Secondly, PB did not enhance responses to GABA which were of equal magnitude to those obtained at a higher concentration in the presence of Bic. The experiment shown in Figure 3 illustrates this point. Repeated responses to GABA (30 μM) were obtained in the absence and presence of Bic (13 μM). PB (40 μM) applied concomitantly with Bic reversed the antagonism as expected. Removal of the PB re-established the antagonism. Following the recovery of the response to GABA in the absence of Bic the concentration of applied GABA was reduced to 6 μM, a level which matched the previous responses to 30 μM in the presence of Bic. Superfusion with PB (40 μM) alone produced no clear enhancement in the responses to 6 μM GABA although it did prevent Bic exerting its full antagonistic effect. Thirdly, nipecotic acid which potentiates the response to GABA in the superior cervical ganglion (Brown & Galvan, 1977) neither reversed nor prevented the antagonism produced by Bic (3 experiments). This is illustrated in the experiment of Figure 4. Responses to 3 μM and 30 μM GABA were obtained alternately and the effect of Bic (13 μM) on these responses was determined. Following recovery from Bic, the tissue was superfused continuously with 1 mM nipecotic acid which inhibits 3 μM [^{3}H]-GABA uptake in ganglia by 70% (Brown & Galvan, 1977). This potentiated the response to 3 μM GABA but had little or no effect on the response to 30 μM GABA. When Bic (13 μM) was reapplied in the presence of nipecotic acid the resulting antagonism was the same as obtained in the absence of nipecotic acid and only when PB (40 μM) was added was there any enhancement in the response to GABA at either dose level. Thus although there was some similarity between the effects of PB and nipecotic acid in the absence of Bic, in its presence, they appeared to exert different actions.

Reversal of the action of other γ-aminobutyric acid antagonists by pentobarbitone Bic was not the only GABA antagonist affected by PB; the actions of picrotoxin, tetramethylenedisulphotetramine and isopropyl bicyclophosphate (IPTBO) were also prevented or reversed by PB. The experiment shown in Figure 5 demonstrates the effect of PB (40 and 80 μM) on the antagonism of responses to GABA (30 μM) pro-

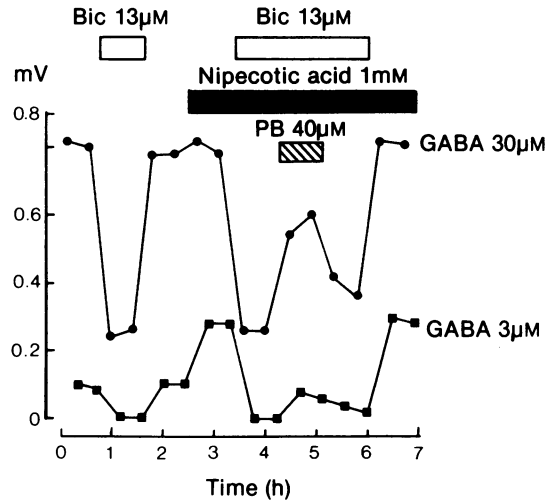


Figure 4 The inability of nipecotic acid to prevent or reduce the γ-aminobutyric acid (GABA) antagonism produced by bicuculline methochloride. Two concentrations of GABA (3 and 30 μM) were superfused over the ganglia alternately for 1 min periods at 12–15 min intervals. Ordinate scale: response in millivolts. Abscissa scale: time in hours. Continuous superfusion with nipecotic acid (1 mM) indicated by the solid horizontal bar, potentiated the responses to 3 μM GABA but had little or no effect on responses to 30 μM GABA (cf. Brown & Galvan, 1977). However, the addition of bicuculline methochloride (Bic 13 μM, open bars) still antagonized the response to GABA to the same extent as in the absence of nipecotic acid. Pentobarbitone (40 μM, hatched bar) decreased the antagonism.

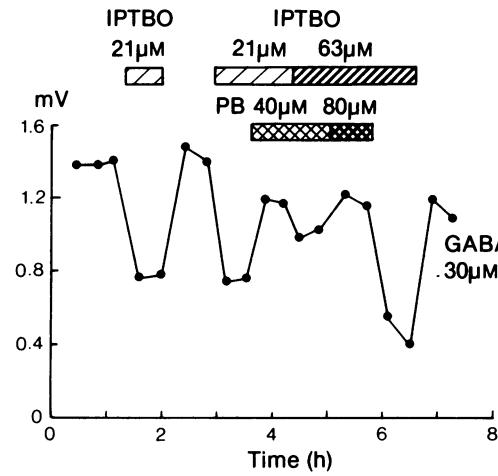


Figure 5 Reversal by pentobarbitone of γ-aminobutyric acid (GABA) antagonism produced by isopropyl bicyclophosphate (IPTBO). A single concentration of GABA, 30 μM, was applied repeatedly to a superior cervical ganglion and the magnitude of the individual responses obtained during 1 min contact periods is represented by the symbols (●). During the periods indicated by bars at the top of the figure IPTBO (21 or 63 μM) alone or in combination with pentobarbitone (PB 40 or 80 μM) was continuously superfused over the tissue. Ordinate scale: magnitude of response to GABA in millivolts. Abscissa scale: time in hours.

duced by IPTBO (21 and 63 μM). IPTBO (21 μM) reproducibly antagonized the response to GABA (30 μM) by about 50%. The addition of PB (40 μM) in the presence of IPTBO produced a partial reversal in the response to GABA. On increasing the concentration of IPTBO to 63 μM whilst maintaining the same concentration of PB (40 μM) the response to GABA diminished. This diminution was overcome by increasing the PB concentration to 80 μM. A similar interdependence between othe concentrations of Bic and PB was also observed.

Strychnine will also antagonize responses to GABA in this preparation but it appears to be non-selective since it depressed matched responses to carbachol to the same extent (cf. Bowery & Brown, 1974). Moreover, even partial recovery from strychnine was extremely slow to occur. PB readily reversed the action of strychnine on responses to GABA but did not affect the depression in the responses to carbachol. This is illustrated in Figure 6. In this experiment, repeated responses to GABA (30 μM) and carbachol (33 μM) were obtained alternately. Strychnine (74 μM) depressed the response to both agonists. However, when PB (40 μM) was introduced only the response to GABA was enhanced. The response to carbachol was not increased and was further depressed when the PB concentration was increased to 120 μM. This lack of effect on responses to carbachol accords with the inability of PB to reverse the antagonism of responses to carbachol produced by hexamethonium (Bowery, 1976).

Bicuculline reversal by other barbiturates A comparison of the relative molar potencies of some other barbiturates in their ability to reduce or reverse the action of Bic on responses to GABA is given in Table 1. The values were obtained by comparing the concentration of PB with that of other barbiturates required to produce a 50% reversal of the action of Bic within the same experiments. Thiopentone, the sulphur substituted analogue of PB, was similar in activity to PB as was its isomer, amylobarbitone. Allyl substitution at R_1 in PB (quinalbarbitone) increased activity two to three fold. Reducing or increasing the number of carbon atoms below and

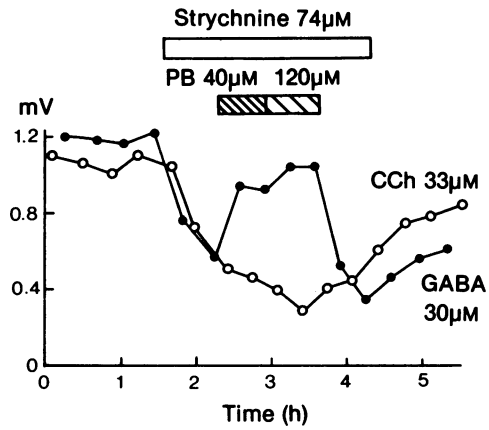


Figure 6 Selective reversal by pentobarbitone of the antagonism of responses to γ-aminobutyric acid (GABA) produced by strychnine. Repeated responses to GABA (30 μM, ●) and carbachol (CCh 33 μM, ○) were obtained alternatively in a single superior cervical ganglion at 10–12 min intervals. Strychnine (74 μM) was superfused over the tissue during the period indicated by the bar at the top of the figure. Pentobarbitone (PB, 40 and 120 μM) was additionally superfused during the period indicated by the hatched bar. Ordinate scale: magnitude of depolarization in millivolts produced by 1 min applications of GABA or carbachol. Abscissa scale: time in hours. Strychnine antagonized responses to GABA and carbachol but pentobarbitone only restored the responses to GABA and not those to carbachol. Hyoscine hydrobromide (2.5 μM) was present throughout this experiment to limit the action of carbachol to nicotinic receptors (see Bowery & Brown, 1974).

above 5 at R_2 decreased activity. The anticonvulsant barbiturates, phenobarbitone and mephobarbitone, were less active than PB. The column on the right of Table 1 gives the relative anaesthetic potencies obtained from the data of Butler (1942). Interestingly these figures correspond quite well with those for reversal.

Bicuculline reversal by other hypnotics Table 2 summarizes results obtained with other non-barbiturate drugs. The list of drugs divides into those which were effective and those which were ineffective in reversing Bic. The threshold concentration, for each effective drug has been tabulated instead of relative potency because as is shown in Figure 7 not all were capable of producing as extensive a reversal as PB. Moreover, the slopes of the apparent dose-response curves were not the same.

All of the hypnotics tested were able to reduce Bic-induced antagonism. In addition, other classes of drugs shared this property notably the neuroleptics, chlorpromazine and haloperidol, the anti-histamines, promethazine and mepyramine and the tricyclic antidepressant, amitriptyline. By comparison, the monoamine oxidase inhibitor antidepressants, tranylcypromine and iproniazid were ineffective as was the neuroleptic, trifluoperazine.

Benzodiazepines In view of the low threshold concentration of the benzodiazepines, their effectiveness in reversing the action of Bic was studied further. Figure 7 illustrates the results from an experiment in which the effectiveness of increasing concentrations of PB was compared with a similar range of concentrations of chlordiazepoxide. The threshold for PB in this experiment was between 0.4–4 μM whereas 0.3 μM chlordiazepoxide produced some enhancement in the GABA response. However, whereas 40 μM PB produced an 80% reversal even 90 μM chlordiazepoxide produced less than 60% reversal. The benzodiazepines always produced such a partial reversal despite any further increase in concentration, and was always much less than the maximal effect of PB.

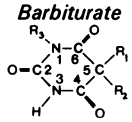
A feature of the effect of chlordiazepoxide was the duration of its effect even after removal from the superfusion fluid. An example of this prolonged action is illustrated in Figure 8. Chlordiazepoxide 90 μM partially reversed the antagonism produced by Bic (13 μM) but even 200 min after its removal the original level of antagonism was not re-established. In contrast the effect of PB was much more rapid in offset. In an attempt to accelerate the rate of recovery from the effect of chlordiazepoxide the concentration of Bic was temporarily increased to 39 μM. However, on returning to 13 μM Bic there was no change in the level of antagonism.

Superfusion with chlordiazepoxide (90 μM) in the absence of Bic did not affect the dose–response curve to GABA. However, chlordiazepoxide (90 μM) did reduce the GABA antagonistic effect of a subsequent period of perfusion with Bic even though the benzodiazepine had been removed 40 min previously. This accords with its prolonged effect illustrated in Figure 8.

Rat brain stem

These results are based on studies of 51 brain stem neurones where the continuous administration of Bic (7–30 nA: mean 17.4 nA) during the sequential administration of GABA or glycine (2–40 nA) produced reversible antagonism of one or other of these substances. On most cells Bic produced a selective reduction of GABA responses. However, higher currents of Bic (20–30 nA) were used intentionally in some studies to produce additional reduction of glycine responses. Care was taken to ensure that barrels con-

Table 1 Comparative activities of barbiturates in reversing antagonism produced by bicuculline methochloride of responses to γ-aminobutyric acid (GABA) in the rat superior cervical ganglion

Barbiturate 	*Structure* R_3	R_1	R_2	*Relative Molar* potency (PB = 1)*	*Relative anaesthetic** potency (in mice) (Butler, 1942)*
Pentobarbitone	H	ethyl	1-methylbutyl	1	1
Thiopentone (at C2 S instead of 0)	H	ethyl	1-methylbutyl	0.8	2.74
Amylobarbitone	H	ethyl	iso-pentyl	0.9, 0.5	0.61
Quinalbarbitone	H	allyl	1-methylbutyl	3, 2	1.18
Aprobarbitone	H	allyl	isopropyl	0.125	0.57
Barbituric acid	H	H	H	0.018	
Barbitone	H	ethyl	ethyl	0.025, 0.025	0.14
Probarbitone	H	ethyl	isopropyl	0.1	0.30
Butobarbitone	H	ethyl	*n*-butyl	0.35, 0.3	0.43
n-Hexyl ethyl	H	ethyl	*n*-hexyl		0.42
n-Heptyl ethyl	H	ethyl	*n*-heptyl	0.03, 0.075	
Cyclobarbitone	H	ethyl	cyclohexen-1-yl	0.3 0.15	0.30
Hexobarbitone	methyl	methyl	cyclohexen-1-yl	0.7	1.13
Phenobarbitone	H	ethyl	phenyl	0.03, 0.06, 0.05	0.24
Mephobarbitone	methyl	ethyl	phenyl	0.16, 0.25	

* Values determined by comparison with pentobarbitone (PB) about the 50% reversal concentration (ED_{50}) and expressed as a fraction of the PB ED_{50} in the same experiment. In every experiment the concentrations of GABA and bicuculline methochloride were 30 and 13 μM respectively.

** Median anaesthetic doses (AD_{50}) for each barbiturate as determined by Butler (1942) have been expressed as a fraction of the PB AD_{50} (=1).

taining Bic passed steady electrophoretic current without fluctuations of periodic 'blocking'. Hence continuous monitoring of the Bic expelling current was routinely employed. Most electrodes where the Bic barrel had an impedance of 50 MΩ or less were usually satisfactory throughout the experiment. Any electrode where changes in electrophoretic expelling current were noted during an experiment was rejected.

Electrophoretic barbiturates and benzodiazepines As with Bic, great care was taken to ensure that barrels containing barbiturate or benzodiazepines passed steady electrophoretic currents during an experiment. Only micropipettes with impedance of 30–50 MΩ or less were selected for use. Little difficulty was encountered with electrophoresis of PB, chlordiazepoxide and flurazepam at the concentrations used in this study. Quinalbarbitone was more difficult to eject with steady current and this sometimes necessitated aborting partially completed studies and continuing the experiment with a more satisfactory micropipette.

Pentobarbitone The effects of electrophoretic PB were studied on 23 neurones. PB ejected (30–80 nA, mean 56.7 nA, for 2–8.2 min, mean 4.2 min) from an adjacent barrel of the same micropipette during the continuous administration of Bic, partially (50%) or completely reversed the GABA antagonistic effect of Bic (Figure 9). This reversal occurred in all neurones tested, was never accompanied by changes in spike shape or amplitude and the antagonism of GABA by Bic was rapidly restored after the end of the PB administration (Figures 9, 10, 11). PB reversal was not accompanied by changes in background firing except in one neurone where slight depression (10%) was observed. In 7 of these neurones the PB administration was repeated and reversal was observed on each occasion. On 4 neurones tested, the antagonism of GABA by Bic could be restored during a continuous PB administration by increasing the ejecting current of Bic (see Figure 10). Further tests on 8 cells where a reversal of Bic antagonism had already been demonstrated, showed that PB administration with the same current for similar or more prolonged periods in the absence of Bic, produced no changes in the submaximal responses to GABA or glycine (5 cells). Slight potentiation of both GABA and glycine responses were observed in 1 cell and reduction of these responses was observed in the 2 other cells

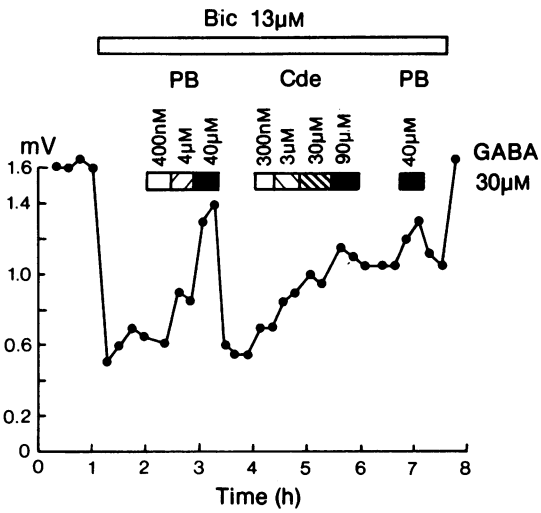


Figure 7 Reversal by pentobarbitone and chlordiazepoxide of γ-aminobutyric acid (GABA) antagonism produced by bicuculline methochloride (Bic). Each dot represents the magnitude of a single response to GABA (30 μM) (ordinate scale: millivolts) applied for 1 min and plotted against time (abscissa scale: hours). Data are from a single ganglion. Bic (13 μM) was continuously superfused over the tissue during the period indicated by the open horizontal bar. Pentobarbitone (PB) and chlordiazepoxide (Cde) were applied in increasing concentrations (0.4–40 μM and 0.3–90 μM respectively) during the periods shown by the hatched/closed horizontal bars. Note (i) the dose-dependent increase in the response to GABA in the presence of either hypnotic (ii) the threshold concentration for chlordiazepoxide was lower than for pentobarbitone although the latter produced a more effective enhancement at higher concentrations (iii) the more rapid decline of the action of pentobarbitone.

(Figure 11). PB also produced a gradual depression in background firing in 2 of these cells otherwise the firing rate was unchanged. On the other hand consistent depression of background firing was observed following expulsion of PB with higher currents (100–150 nA).

When additional reduction of glycine responses was obtained with Bic (7 cells) PB always reversed this antagonism sooner than that of GABA. However, it was also noted that occasionally PB produced a slight potentiation of GABA (Figure 11) or glycine (Figure 10) responses indicated by a faster onset of the inhibitory response (e.g. see Figure 10). From a technical point of view, reversal by PB was more easily demonstrated if the PB administration was started immediately after the first satisfactory demonstration of GABA blockade (60–80%) by Bic. Otherwise more prolonged administration was necessary (up to 12 min in 2 cells) to show adequate reversal.

Strychnine (2–5 nA) reversibly reduced the effects of glycine on 14 neurones. Larger ejecting currents (10–15 nA) produced additional reduction of the responses to GABA (3 cells) (Figure 9). All the tests with strychnine were performed on neurones which had also been tested previously with Bic and reversal shown with PB. The concurrent administration of PB (30–80 nA, mean 66.5 nA, for 1–13 min, mean 5.8 min), during a partial or complete suppression of glycine by strychnine, reversed this antagonism in 5 of the 14 neurones (Figure 9). The antagonist effect of strychnine was rapidly restored following the end of the PB administration. While on most cells strychnine antagonism of glycine was unaffected by amounts of PB in excess of those previously shown to reverse Bic antagonism of GABA, additional non-selective blockade of GABA by strychnine was readily and reversibly restored by PB (Figure 9).

Quinalbarbitone Quinalbarbitone (20–80 nA, mean 51.0 nA, for 2–7 min, mean 4.7 min) was tested during Bic antagonism of GABA in 9 cells. In each case quinalbarbitone partially or completely restored the GABA response. As with PB this could be shown on repeated tests on the same cells (3 cells), was unaccompanied by changes in background firing or spike amplitude and was readily and rapidly reversible (Figure 12). On 3 neurones where the responses to glycine were also depressed by Bic, quinalbarbitone restored the glycine response before that of GABA. When tested against submaximal GABA and glycine responses (3 cells) quinalbarbitone by itself had no effect on these responses in amounts which had previously reversed Bic antagonism of GABA on the same neurones. These amounts of quinalbarbitone had no significant effect on background firing in any of these neurones.

Flurazepam This compound, (5–40 nA, mean 12.7 nA, for 3.5–8.0 min, mean 5.3 min) was tested on 11 neurones during the selective antagonism of GABA responses by Bic. No significant reversal of the Bic antagonistic effect was observed in any of these cells. However, in 2 of these neurones, tests with quinalbarbitone had produced a reversal. This lack of significant reversal by flurazepam was not considered to be due to its inadequate release from micropipettes since its continuous administration with low currents often produced a gradual depression of spontaneous firing (Figure 13). Thus of the 11 cells tested flurazepam depressed firing in 9, though this was less marked in the other 2. Higher currents of flurazepam (70–100 nA) produced a marked but reversible depression of spike amplitude. It was notable that the prolonged administration of flurazepam appeared to

depress responses to GABA and glycine even in the presence of Bic (Figure 13). When tested alone (3 cells), the prolonged administration of flurazepam also reduced GABA and glycine responses in 2 cells but had no effect on either compound on the other. On one neurone reproducible depressant responses to pulse administrations of flurazepam were reduced by Bic together with those of GABA but not glycine. A concomitant administration of PB restored both the GABA and flurazepam effects in this cell.

Chlordiazepoxide Chlordiazepoxide (20–80 nA, mean 43.2 nA, for 1.5–9.0 min, mean 4.4 min) was tested on 13 neurones during the administration of Bic. Reversal of the GABA antagonistic effect of Bic was observed in 7 cells. However, unlike the reversal seen with barbiturates, chlordiazepoxide reversal was always only partial (max. 20–40% of control) (Figure 14). It was unaccompanied by spike changes and recovery from its effects was rapid. On 4 cells the continuous administration of chlordiazepoxide, like flurazepam, depressed responses to GABA and glycine even in the presence of Bic (Figure 13). This, however, usually occurred after a partial reversal of the GABA response was seen. Similar depression of GABA and glycine responses was observed when chlordiazepoxide was tested alone.

Chlordiazepoxide sometimes reversibly increased background firing (4 cells) an effect opposite to that seen with flurazepam (Figure 13). In one cell firing was gradually depressed and in the others (8 cells) no significant changes were noted.

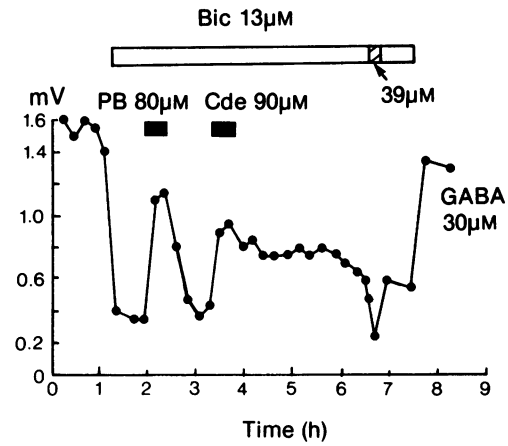


Figure 8 Enhancement of the response to γ-aminobutyric acid (GABA) produced by pentobarbitone (PB) and chlordiazepoxide in the presence of bicuculline methochloride (Bic). Details are the same as in the legend of Figure 7, except that only single doses of pentobarbitone (80 μM) and chlordiazepoxide (90 μM) were applied. Following the removal of pentobarbitone the response to GABA declined rapidly towards the antagonized level. By contrast the recovery from chlordiazepoxide was much slower. During the brief period indicated towards the end of the experiment the concentration of Bic was increased to 39 μM. This did not appear to alter the rate of recovery at the lower concentration (13 μM) of the antagonist.

Table 2 Reversal of action of bicuculline methochloride

Effective	*Threshold concentration*†	*Ineffective*	*Maximum concentration tested*
Pentobarbitone	5 μM	Bemegride	645 μM
Chloral hydrate	500 μM	Nikethamide	720 μM
Urethane	1 mM	Leptazol	724 μM
Glutethimide	35 μM	Caffeine	515 μM
Chlordiazepoxide	0.5 μM	Morphine	266 μM
Diazepam	12 μM		
Flurazepam	4 μM	Lignocaine	1 mM
Clonazepam	0.5 μM	Procaine	10 mM
Phenytoin	80 μM	Iproniazid	720 μM
Chlorpromazine	90 μM	Tranylcypromine	1.5 mM
Haloperidol	70 μM	Atropine	273 μM
Promethazine	70 μM	Hyoscine	260 μM
Mepyramine	100 μM	Trifluoperazine	208 μM
Amitriptyline	80 μM		

† The concentration which produced a just-measurable increase in the response to γ-aminobutyric acid (30 μM) in the presence of bicuculline methochloride (13 μM) in at least 3 experiments for each substance.

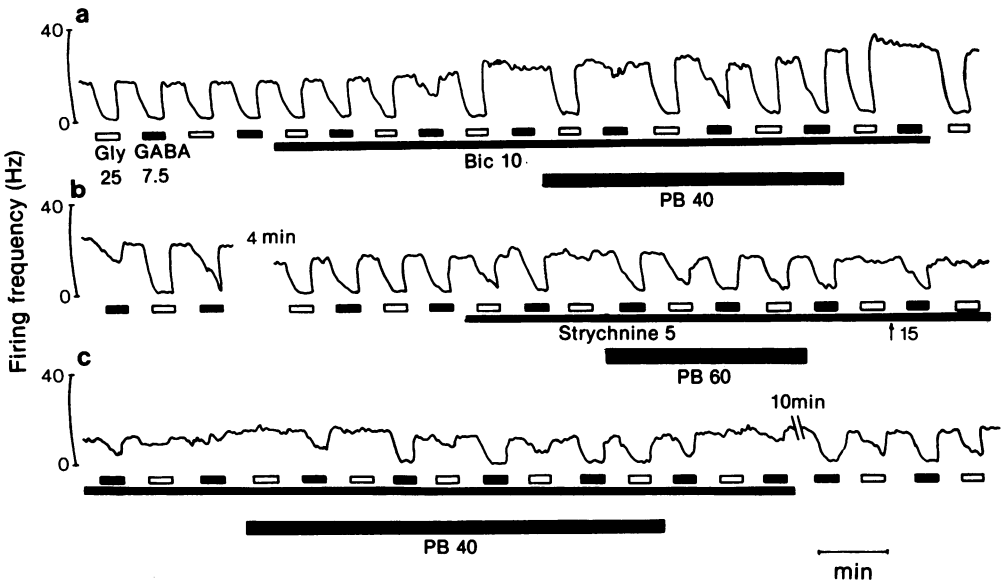


Figure 9 The effects of bicuculline and strychnine on responses to γ-aminobutyric acid (GABA) and glycine (Gly) and the interactions of pentobarbitone with these effects. The continuous rate meter records (firing frequency in Hz against time in min) of a spontaneous brain stem neurone showing reproducible depression by glycine and GABA. (a) Shows that the continuous administration of bicuculline methochloride (Bic) selectively reduced the responses to GABA. The additional continuous administration of pentobarbitone (PB) restores the GABA response without producing any significant effect on background firing rate. Shortly after the cessation of PB administration the GABA response is again completely blocked by Bic. After complete recovery of the responses to GABA a continuous administration of strychnine selectively reduces the glycine response (b). An additional administration of PB restores the glycine response which is again blocked after the PB current is switched off. Increasing the ejecting current of strychnine to 15 nA produced additional antagonism of the GABA response. A repeated administration of PB rapidly restored the GABA response and also that of glycine. Responses to both GABA and glycine were blocked after PB was switched off, and recovered some 10 min after the strychnine administration was stopped. In all records the horizontal bars indicate the duration of drug ejection and the ejecting currents are indicated in nA.

Intravenous studies

Barbiturates The effects of intravenous injections of PB (0.4–5.5 mg/kg) thiopentone (1–3 mg/kg) or hexobarbitone (0.4–0.8 mg/kg) were tested on neuronal responses to GABA and glycine during the continuous administration of Bic by electrophoresis. Because of possible residual effects after intravenous administration only one cell in any experiment was tested by this route of administration. Injections were usually made over a 30 s period in a volume of 0.1–0.2 ml or by a slow continuous infusion over several minutes.

PB was tested on 3 neurones which had previously been tested with electrophoretic PB and reversal of Bic shown. In one cell a slow infusion of PB over 3 min (total dose 5.5 mg/kg) produced almost complete restoration of the GABA response, while an infusion of up to 3 mg/kg over a 5 min period in another cell was ineffective. In the third cell 0.8 mg/kg PB administered in divided doses (2 × 0.4 mg/kg) produced only a partial reversal. The reversal effects lasted from 4–7 min and were not accompanied by depression of background firing.

Thiopentone (4 cells) partially or completely restored the GABA response in all cases (Figure 15a). On two cells the additional reduction of glycine by Bic was also reversed (Figure 15a), and in another the response to GABA was reversed but more prolonged. Background firing was only slightly depressed in one cell. The effects of thiopentone lasted some 2–6 minutes. Hexobarbitone reversed the Bic antagonism of GABA in 2 cells tested (Figure 15b). In one cell the background firing was slightly depressed. The effects of hexobarbitone lasted some 3–7 minutes.

Intravenous clonazepam (100–200 μg/kg) was tested on 2 cells. On both cells only weak, partial recovery of GABA responses was observed and these could

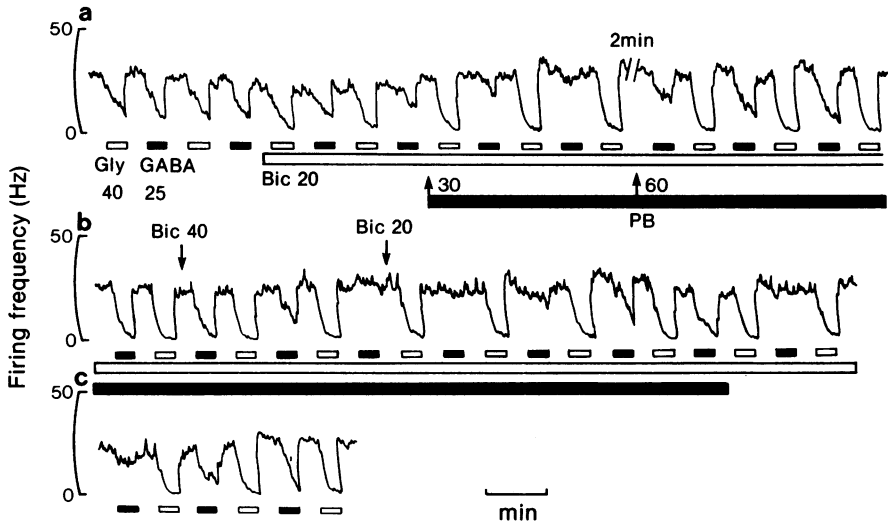


Figure 10 The dose-dependent nature of the interactions of pentobarbitone (PB) with the effects of bicuculline (Bic). (a) Shows that the ejection of PB at 30 nA had no significant effect on γ-aminobutyric acid (GABA) responses during the selective block by Bic. On increasing the pentobarbitone ejecting current to 60 nA the effect of GABA was gradually restored and in fact the responses to both GABA and glycine (Gly) were slightly potentiated. When the Bic ejecting current was then increased from 20–40 nA (b) the response to GABA was again blocked. Reduction of the Bic ejecting current to 20 nA again allowed the GABA response to be gradually revealed. Note that no significant changes in background firing occurred throughout this test and that the blockade of GABA rapidly follows the cessation of pentobarbitone administration. (c) Shows recovery of GABA responses soon after Bic was switched off. All three traces are a continuous record.

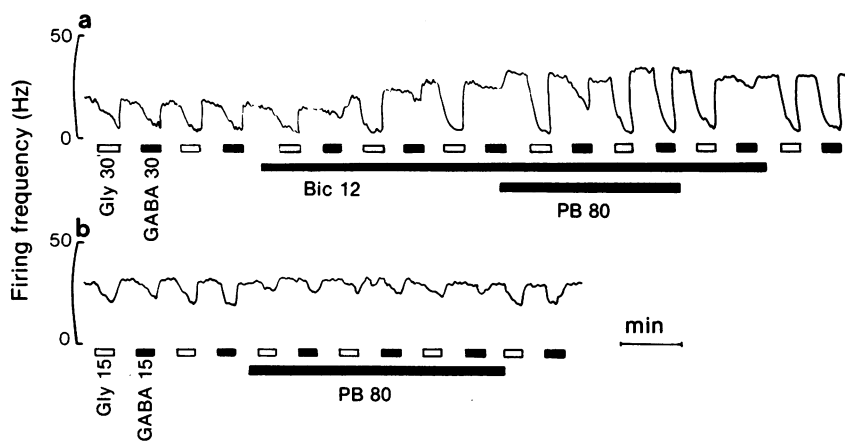


Figure 11 The effects of pentobarbitone (PB) on γ-aminobutyric acid (GABA) and glycine (Gly) responses in the presence and absence of bicuculline methochloride (Bic). (a) Shows that the selective antagonism of GABA by Bic was reversed by pentobarbitone 80 nA. Following recovery of the responses to GABA after the cessation of Bic ejection the expelling currents for GABA and glycine were reduced from 30 to 15 nA to produce clearly submaximal responses. (b) Pentobarbitone administered with the same current as before but for a longer period had no significant effect on background firing and slightly reduced the effects of GABA and glycine.

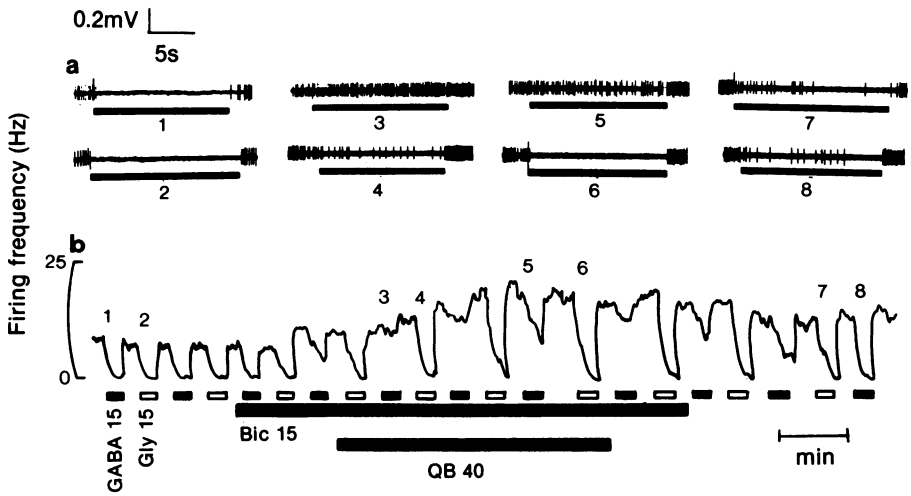


Figure 12 Effects of quinalbarbitone (QB) and the antagonism of γ-aminobutyric acid (GABA) by bicuculline methochloride (Bic). The ratemeter trace (b) shows that QB partially restored the response to GABA during the concurrent administration of Bic. The numbered extracellular spike records (a) show the responses to GABA and glycine (Gly) which correspond to those numbered on the ratemeter record. Note that the partial reversal produced by QB was not accompanied by changes in spike amplitude. The increase in background firing rate is probably due to the continuous administration of Bic.

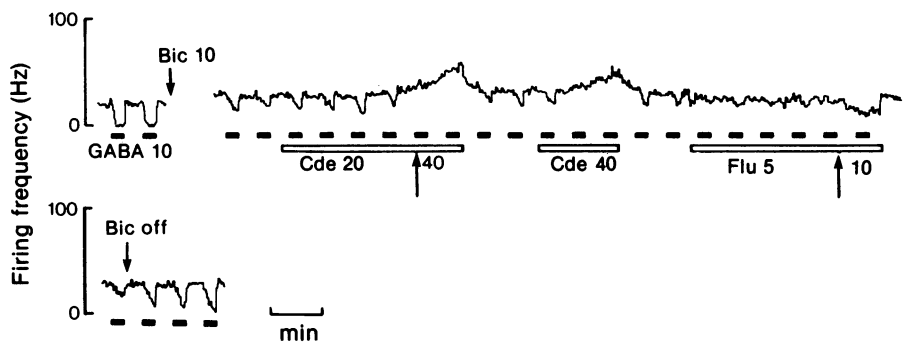


Figure 13 The effects of chlordiazepoxide (Cde) and flurazepam (Flu) on the response to γ-aminobutyric acid (GABA) during the administration of bicuculline methochloride (Bic). Cde (20 nA) partially restored the GABA response, but then reduced it. Increasing the expelling current of Cde also increased the background firing and this effect was reproducible and rapidly reversible. Flu (5 nA) produced a gradual depression of background firing, and reduced GABA responses. When the ejecting current of Flu was increased to 10 nA a marked depression of firing was produced. Bic (10 nA) was administered continuously during the period between the vertical arrows above the record.

be completely suppressed by increasing the Bic expelling current (30–40 nA). On one neurone the partial restoration of the GABA response was accompanied by the depression of background firing which lasted some 8 minutes. However, the response to GABA was still observed for up to 25 minutes. In the other cell 100 μg/kg of clonazepam partially restored the GABA response (Figure 16). However, a repeat injection at the same dose had no further effect on the magnitude of the GABA response. The effect of clonazepam in

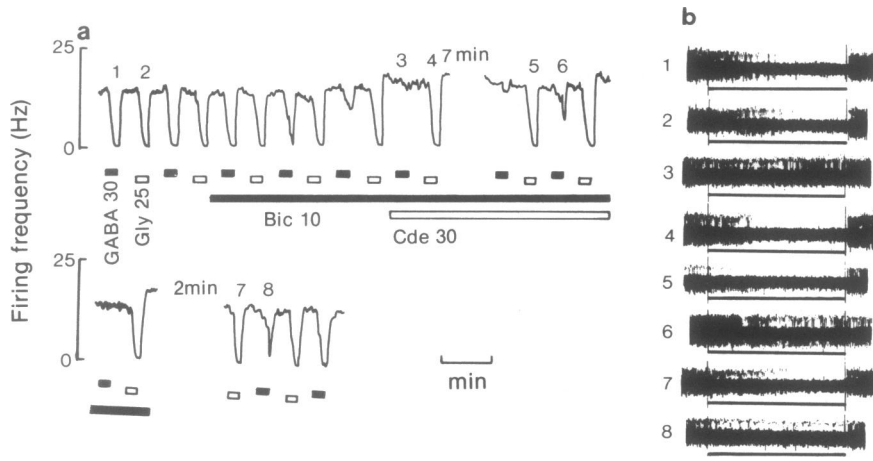


Figure 14 Effects of chlordiazepoxide (Cde) on responses to γ-aminobutyric acid (GABA) and glycine (Gly) during the administration of bicuculline methochloride (Bic). A prolonged administration of Cde partially restored the GABA response and had little effect on the response to glycine. Spike records from this neurone (b) show responses to GABA and glycine which correspond to the numbered responses on the ratemeter record. Little change in the spike amplitude occurred in this study.

this case lasted some 112 min and the GABA response recovered rapidly following the termination of the Bic ejection.

Discussion

The hypnotic and anaesthetic properties of barbiturates have been considered to result from an interaction, at least in part, with central inhibitory processes mediated by GABA (Nicoll *et al.*, 1975). The present experiments suggest that barbiturates also interact at post-synaptic membranes at sites indirectly associated with inhibitory amino acid receptors. Thus both *in vitro* and *in vivo* PB reversed the antagonistic effects of Bic and strychnine at concentrations which had no obvious indirect effect and at which no significant modification of GABA, glycine or other agonist responses were observed.

The *in vitro* studies revealed that PB may exhibit GABA-like bicuculline-sensitive activity but only at concentrations in excess of those required to reverse the effects of antagonists. It is conceivable, however, that PB may potentiate the action of GABA (Ransom & Barker, 1976) and thereby produce an apparent reversal of Bic antagonism. However, even though we could observe such a potentiation of responses to low concentrations of GABA this was inconsistent, and the effects of higher concentrations of GABA were invariably depressed by PB.

In similar studies by Evans (1977), it was suggested that the GABA-mimetic properties of PB are sufficient to account for the reversal of the action of Bic. Also in intracellular ganglionic studies performed by Brown & Constanti (1978) it was observed that PB prolongs and potentiates the depolarization produced by GABA perhaps sufficiently to account for the reversal of Bic. However, in these experiments PB, unlike GABA, hyperpolarized ganglionic neurones. These observations of Evans and of Brown & Constanti are difficult to reconcile completely with our own. In our experiments, PB at low concentrations (threshold 5 μM) clearly restored the effects of GABA in the presence of Bic by an action which was not related to a direct GABA-mimetic action, potentiation of GABA (cf. nipecotic acid experiments) or by interfering with GABA inactivation processes. PB at concentrations up to 1 mM does not reduce the glial uptake of [^{3}H]-GABA in ganglia (personal observation).

PB reversed the effects of other GABA antagonists such as picrotoxin, IPTBO and tetramethylenedisulphotetramine. This action appeared to be related to GABA receptors since neither the effects of carbachol nor its antagonist hexamethonium were modified by similar concentrations of PB (Bowery, 1976). Interestingly, strychnine, produced prolonged non-selective antagonism of GABA and carbachol. PB, however, only reversed the antagonism of GABA by this agent.

Other barbiturates also reversed the action of Bic

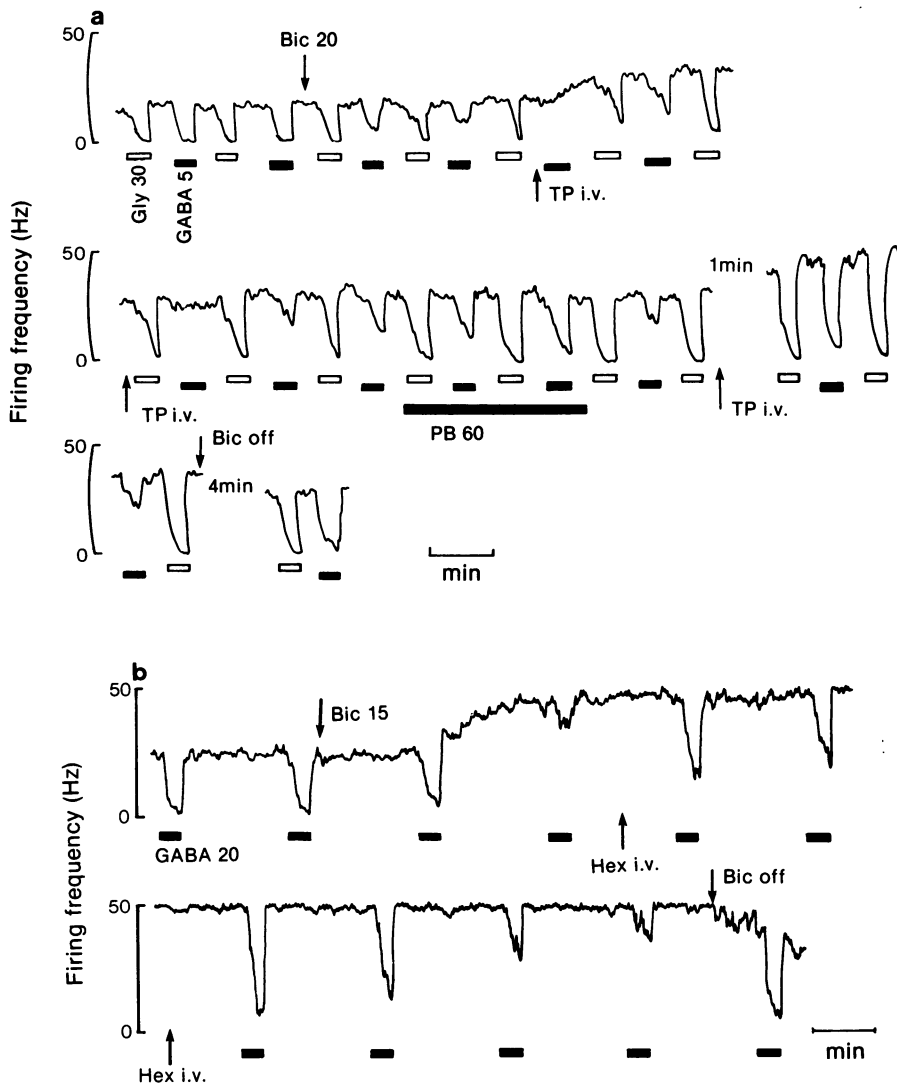


Figure 15 The effects of intravenously administered barbiturates on the responses to γ-aminobutyric acid (GABA) during an administration of bicuculline methochloride: (a) Bicuculline methochloride (Bic 20 nA, arrows) reduced the response to GABA and also delayed that to glycine (Gly). The administration of thiopentone (TP 1 mg/kg) produced a short lasting restoration of the GABA response and also improved that to glycine. A repeated injection of TP at the same dose again partially restored the GABA response. This was improved even further by the additional electrophoretic administration of PB. A further administration of TP (1 mg/kg) restored the GABA response. (b) Reversal of Bic antagonism of GABA by repeated doses of hexobarbitone (Hex 0.4 mg/kg each dose).

and there appeared to be a structural requirement for this action, being particularly dependent on substitution at R2 in the barbituric acid moiety (see Table 1). Reversal potency increased with the number of carbon atoms in the alkyl chain at R2 to a maximum at 5; above this activity decreased. A similar relationship has been described for anaesthetic potency by Butler (1942). However, the relationship can also be observed for the lipid: water partition coefficients (Tabern & Shelberg, 1933). A number of other structurally dissimilar hypnotics/sedatives were also capable of reversing the action of Bic (see

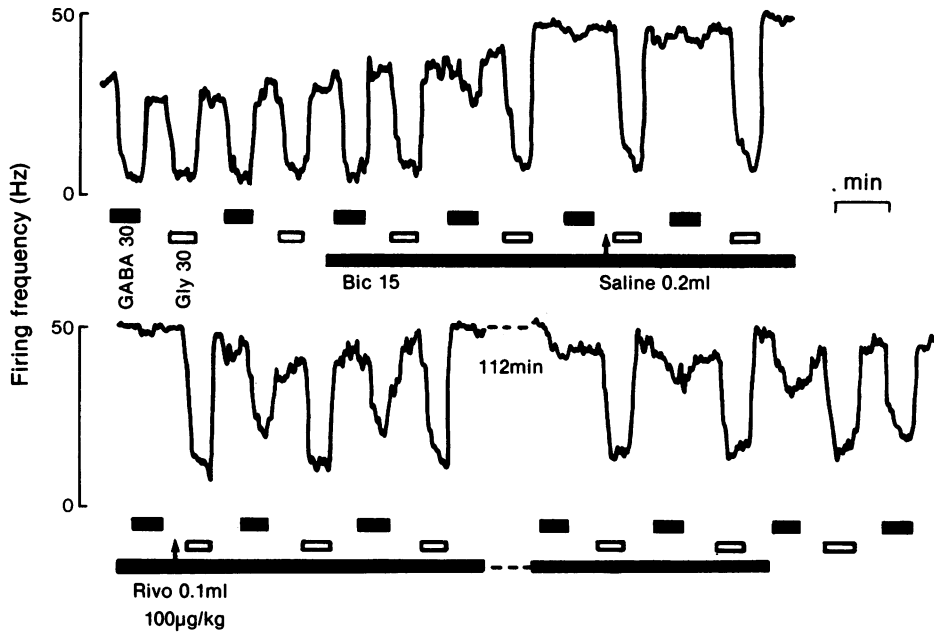


Figure 16 Effects of intravenous clonazepam (Rivo) on the antagonism of γ-aminobutyric acid (GABA) by the continuous electrophoretic administration of bicuculline (Bic). A control injection of saline (0.2 ml) had no effect on the response to GABA. However, the administration of clonazepam (100 µg/kg) partially restored the response to GABA and slightly depressed background firing. A repeat injection of clonazepam at the same dose (not shown) produced no additional improvement of the GABA response. The responses to GABA were again blocked by the continued Bic ejection some 112 min after the first injection of clonazepam. The effect of GABA was rapidly restored after the termination of the Bic ejection.

Table 2). None of these latter compounds appeared to have any significant GABA-mimetic action. The benzodiazepines were effective at lower concentrations than PB, but produced only a partial, although prolonged, reversal of Bic.

Similar observations were made *in vivo* with electrophoretic administration of the substances. As in the ganglion, PB and quinalbarbitone produced a clear consistent reversal of the GABA, or non-specific glycine, antagonistic action of Bic. These interactions were dose-related and were not accompanied by depression of background firing. Lodge & Curtis (1977) have reported that the reversal of Bic by PB in feline dorsal horn neurones may be accounted for by potentiation of GABA depression by PB. These authors also show that the effects of PB were accompanied by depression of background firing. Possibly the concentration range for reversal of Bic, potentiation of GABA, or direct depression of neuronal activity may be narrow and not always easily separated when electrophoretic methods of drug are used.

In the present experiments the effects of PB were not restricted to GABA antagonists since the antagonistic action of strychnine towards glycine depression was also reversed. Reversal of Bic was, however, more easily demonstrable than that of strychnine possibly because of the difference in the binding affinity of these substances to their site of action (Zukin, Young & Snyder, 1974). The reversal of Bic was also demonstrable following the intravenous administration of barbiturates. This reversal was usually brief but was unaccompanied by changes in background firing.

Reversal of Bic with electrophoretically administered benzodiazepines was more difficult to demonstrate. No reversal was observed with flurazepam, though prolonged administration of this compound depressed neuronal firing and reduced the effects of GABA and glycine. Neuronal depression by brief administrations of flurazepam was antagonized by Bic as in previous experiments (Dray & Straughan, 1976) and this was reversed by the concurrent administration of PB. Chlordiazepoxide produced a partial, but more consistent reversal of Bic but as with flurazepam

pam, prolonged administration also reduced the effect of GABA and glycine. Similar complex interactions of benzodiazepines have been reported in other studies. Thus benzodiazepines have been proposed on the one hand to mimic (Young, Zukin & Snyder, 1974; Dray & Straughan, 1976) or facilitate (Costa, Guidotti, Mao & Suria, 1975; Costa, Guidotti & Mao, 1975; Haefely, Kulczar, Möhler, Pieri, Polc & Schaffner, 1975; Choi, Farb & Fischbach, 1977), and on the other to depress (Gähwiler, 1976; Steiner & Felix, 1976) the action of GABA or glycine, though the mimetic and antagonistic activities have been disputed (Haefely, Pieri, Polc & Schaffner, 1976; Curtis, Game & Lodge, 1976; Curtis, Lodge, Johnston & Brand, 1976; Hunt & Raynaud, 1977; Kozhechkin & Ostrovskaya, 1977). From the present data the benzodiazepines seem also to have the additional property of reversing the action of antagonists. This action was more clearly demonstrated with clonazepam after its intravenous administration when prolonged partial reversal of Bic was observed.

The observed interactions of barbiturates with convulsant agents can be tentatively explained by assuming that amino acids and their antagonists bind to the cell surface at different sites. Thus when an antagonist is present it prevents access of the amino acid to its receptor, not because of preferential binding but by occluding or distorting it. Barbiturates may have particular affinity for the antagonistic binding site and could therefore displace the antagonist without affecting the receptor. The possibility of differences in agonist and antagonistic sites has been suggested (Young & Snyder, 1974) in the case of glycine and strychnine. Additionally the binding sites for GABA and [^{3}H]α-dihydropicrotoxinin appear to be distinct (Olsen, 1978). As yet there are few data concerning separate binding sites for GABA and Bic although the results of Collins & Cryer (1978) support this possibility. These authors have shown that although PB does not interfere with [^{3}H]-GABA binding as reported previously by Zukin *et al.* (1974) and Peck, Miller & Lester (1976), it will prevent Bic from displacing GABA. On the other hand, Möhler & Okada (1977) have described specific binding of bicuculline methiodide (BMI) to synaptosomal membranes from cerebellar cortex. PB in common with many other drugs did not interfere with BMI binding. An interesting feature of these studies was the apparent lack of GABA-mimetic activity attributed to PB in intact preparations (Nicoll, 1975a & b). Other more recognized GABA-mimetics (3-aminopropane sulphonic acid and imidazole acetic acid) displace [^{3}H]-GABA and [^{3}H]-BMI from specific binding sites but PB does not.

While our results do not necessarily indicate that barbiturates produce their hypnotic effects by interacting with endogenous substances, they may provide a rational basis to explain the efficacy of these drugs in the treatment of acute convulsant poisoning (Esplin & Zablocka-Esplin, 1970; Franz, 1975). It could be reasoned that the effects of barbiturates on various conductance mechanisms e.g. Na^+, Ca^{++}, K^+ (Sato, Austin & Yai, 1967; Blaustein, 1968; 1976) and possibly Cl^-, does not require an interaction with neurotransmitter receptors but may be due to effects on closely associated parts of the membrane which are linked to the receptor and/or the ionophore.

The authors wish to acknowledge the assistance of Alan Hudson.

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(*Received November* 17, 1977.)