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Two distinct interactions of barbiturates and chlormethiazole with the $GABA_A$ receptor complex in rat cuneate nucleus *in vitro*

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1 Some pharmacological properties of the $GABA_A$ receptor complex in the rat cuneate nucleus slice have been assessed from depolarization responses to the γ-aminobutyric acid (GABA) analogue muscimol and antagonism of the responses by bicuculline and picrotoxin.

2 Responses to muscimol were potentiated by the following drugs, in descending order of potency with regard to the concentrations required in the Krebs medium: (±)-5-(1,3-dimethylbutyl)-5-ethylbarbituric acid ((±)-DMBB) = (±)-quinalbarbitone = (±)-pentobarbitone > (±)-methyl-phenobarbitone = (−)-methylphenobarbitone > butobarbitone = chlormethiazole > phenobarbitone > barbitone = (+)-methylphenobarbitone. Primidone and phenylethylmalonamide were inactive. Calculation of the concentrations likely to be present in membrane lipids for equal potentiations of muscimol revealed little difference between quinalbarbitone, pentobarbitone, phenobarbitone and barbitone.

3 The effect of picrotoxin as a muscimol antagonist was selectively reduced only by DMBB, chlormethiazole, phenobarbitone and (−)-methylphenobarbitone in concentrations that caused only a modest potentiation of muscimol.

4 It is suggested that a specific site of action in the $GABA_A$ receptor complex is involved in the reduction of picrotoxin effect and that this may be relevant to the anticonvulsant properties of chlormethiazole, phenobarbitone and (−)-methylphenobarbitone. The potentiation of muscimol by chlormethiazole and the barbiturates in general involves a distinctly different site that is less selective and this may underlie the hypnotic properties of these drugs.

Introduction

The $GABA_A$ receptor complex in vertebrate neurones contains a bicuculline-sensitive receptor for γ-aminobutyric acid (GABA) in association with sites for picrotoxin and benzodiazepines that regulate responses to GABA (Olsen, 1982; Simmonds, 1983). Picrotoxin antagonizes GABA and its analogue muscimol in a manner that is not competitive (Simmonds, 1980), while benzodiazepines potentiate responses to GABA and muscimol (Choi, Farb & Fischbach, 1977; MacDonald & Barker, 1978; Simmonds, 1981). Barbiturates also have a modulatory influence on this complex that results in potentiations of GABA and muscimol (Nicoll, 1975; Ransom & Barker, 1976; Barker & Ransom, 1978; Brown & Constanti, 1978; Schlosser & Franco, 1979; Simmonds, 1981). In addition, evidence has been obtained which suggests that both barbiturates and benzodiazepines can reduce the potency of picrotoxin as an antagonist of muscimol (Simmonds, 1981). This latter effect can be seen with phenobarbitone in concentrations that evoke only modest potentiations of muscimol, whereas a similar effect of pentobarbitone is seen only with concentrations that more markedly potentiate muscimol.

In the present experiments, several more barbiturates, chlormethiazole, primidone and phenylethylmalonamide have been tested. The results confirm the suggestion from the phenobarbitone and pentobarbitone data (Simmonds, 1981) that distinctly different interactions are involved in the potentiation of muscimol compared with the reduction in picrotoxin potency. They also point to a possible explanation of the differential distribution of hypnotic and anticonvulsant potencies within this series of drugs.

Methods

Experiments were performed on slices of cuneate nucleus prepared from male Wistar rats (Tuck) as previously described (Simmonds, 1978; 1980). Each slice was placed in a two-compartment bath and superfused with Krebs medium at room temperature so that depolarizations of the dorsal funiculus fibres and terminals mediated by the GABA receptor could be recorded as population responses.

Muscimol was used routinely as the GABA receptor agonist. The concentrations of muscimol used under control conditions, usually 2.5 and 5 μM, were selected to give responses in the lower part of the dose-response curve to minimize problems of desensitization. The barbiturates and related drugs to be tested were superfused alone and in combination with picrotoxin, 3 μM and 30 μM, for 30 min before and during the redetermination of responses to muscimol. In some experiments, picrotoxin was superfused in the absence of a test drug. The concentrations of muscimol were adjusted so that two concentrations, a factor of two apart, evoked responses in the same range as the control responses (Figure 1). The muscimol dose-response lines were always displaced in an approximately parallel fashion so the effects of the drugs were measured as equipotent muscimol dose-ratios (Simmonds, 1980).

Two actions of the barbiturates and related drugs were determined. Firstly, the potentiation of muscimol was expressed as the leftward shift of the muscimol log dose-response line (log muscimol dose-ratio). Secondly, the effect on picrotoxin potency was determined by comparing the Schild plots for picrotoxin (Figure 1) in the absence and presence of the barbiturate. Reduction in picrotoxin potency was expressed as the rightward shift of the Schild plot at the arbitrarily chosen level of (muscimol dose-ratio − 1) = 4. A few experiments were performed with bicuculline instead of picrotoxin.

The Krebs medium contained (mM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 2.2, $NaHCO_3$ 25 and glucose 11 and was continuously bubbled with 95% O_2/5% CO_2 to give pH 7.4. Muscimol (Fluka) and picrotoxin (Sigma) were dissolved directly in the Krebs medium. (+)-Bicuculline (Sigma) was prepared as a 10 mM solution in 0.02 M HCl and diluted in the Krebs medium just before use. Barbiturates obtained as sodium salts were dissolved directly in the Krebs medium and the others were dissolved in a little 0.05 M NaOH and then diluted into the Krebs medium. The following barbiturates were used: (±)-pentobarbitone sodium (Sigma); phenobarbitone sodium (BDH); (±)-5-(1,3-dimethylbutyl)-5-ethylbarbituric acid (DMBB) (Eli Lilly); (±)-methylphenobarbitone (Ward Blenkinsop); (−)-methylphenobarbitone and (+)-methylphenobarbitone (gifts from Dr R.W. Olsen); (±)-quinalbarbitone, butobarbitone and barbitone (May & Baker). The non-barbiturates used were: primidone and phenylethylmalonamide (ICI); chlormethiazole ethanedisulphonate (Astra).

Statistical analyses of the drug effects were made with Student's *t* test.

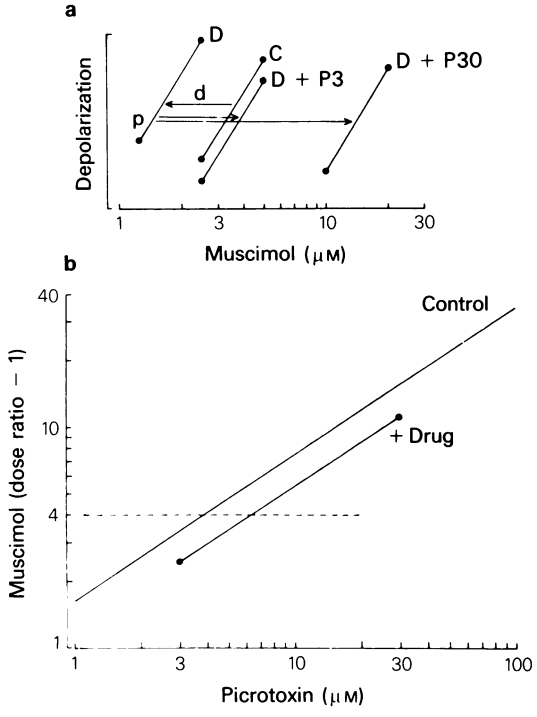

Figure 1 The experimental design. (a) Two-point muscimol log dose-response lines were obtained under control (C) conditions and then in the presence of the barbiturate or other test drug (D) and subsequently test drug + picrotoxin 3 μM (D + P3) and 30 μM (D + P30). The potentiation of muscimol (d) and the antagonism of muscimol (p) by picrotoxin in the presence of test drug were determined. (b) A Schild plot of (log muscimol dose-ratio − 1) versus log concentration of picrotoxin was constructed from the results of at least 4 (usually 6) experiments. This was compared with the control Schild plot for picrotoxin in the absence of test drug determined over a wider range of picrotoxin concentrations from 28 data points. The comparison of picrotoxin potencies was made at the level of (muscimol dose-ratio − 1) = 4. An original example is given in Simmonds (1981).

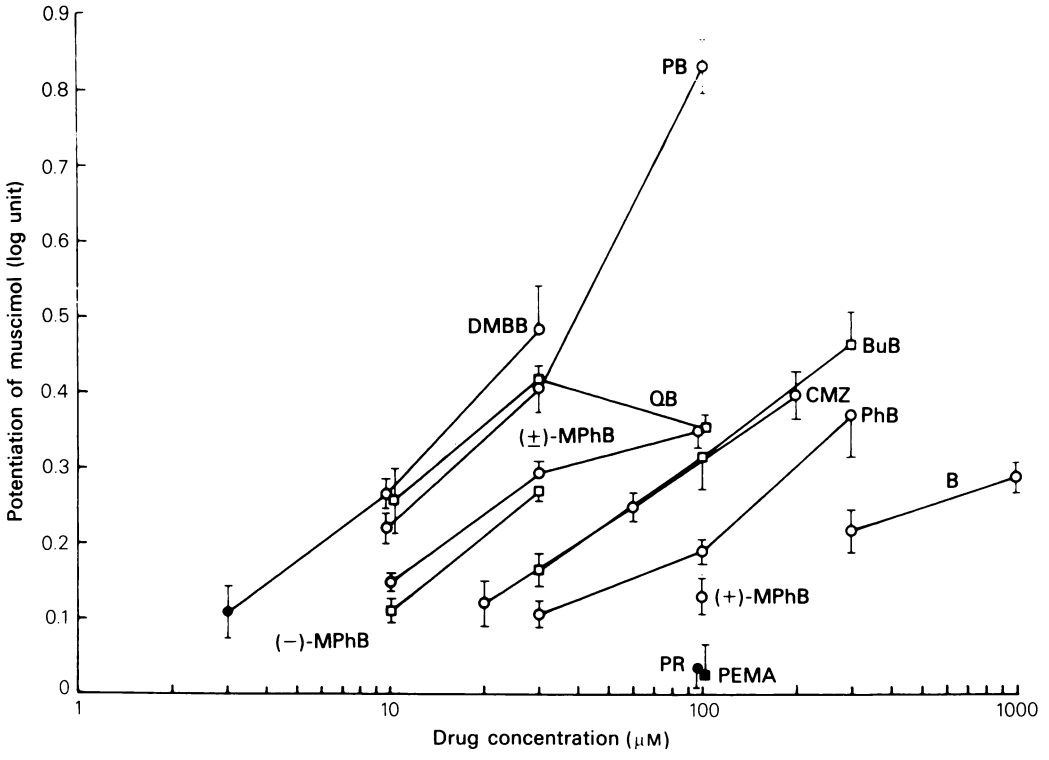

Figure 2 Potentiation of muscimol expressed as the leftward shift of the muscimol log dose-response line by each of the following drugs: DMBB, (±)-5-(1,3-dimethylbutyl)-5-ethylbarbituric acid; PB, (±)-pentobarbitone; QB, (±)-quinalbarbitone; (+)-, (−)- and (±)-MPhB, methylphenobarbitone; BuB, butobarbitone; PhB, phenobarbitone; B, barbitone; CMZ, chlormethiazole; PR, primidone; PEMA, phenylethylmalonamide. Each point is the mean of at least 4 values; vertical lines show s.e.mean. Open symbols indicate statistically significant potentiations of muscimol ($P<0.05$). The data for pentobarbitone and phenobarbitone are taken from Simmonds (1981) for comparison.

Results

Direct effects of barbiturates

With the exception of 100 μM pentobarbitone which causes a small but consistent depolarization (Simmonds, 1981), none of the remaining barbiturates, chlormethiazole, primidone or phenylethylmalonamide caused anything more than the occasional very small depolarization in the concentrations tested.

Potentiation of muscimol

Most of the drugs tested caused a concentration-dependent leftwards shift of the muscimol log dose-response line (Figure 2). It is apparent that pentobarbitone, DMBB and quinalbarbitone were approximately equipotent up to 30 μM but, at 100 μM, the effect of pentobarbitone further increased while that of quinalbarbitone decreased slightly. (±)-Methylphenobarbitone was about half as potent as these three barbiturates. (−)-Methylphenobarbitone was approximately equipotent with the racemate even though (+)-methylphenobarbitone was only one tenth as potent as the (−)-isomer. Phenobarbitone was about one sixth as potent as (±)-methylphenobarbitone. Of the remaining drugs tested, butobarbitone and chlormethiazole were intermediate in potency between (±)-methylphenobarbitone and phenobarbitone while barbitone was very weak and primidone and its metabolite phenylethylmalonamide (PEMA) were ineffective at 100 μM. The concentrations of each of these drugs that were equieffective in shifting the muscimol log dose-response line to the left by 0.25 log unit are shown in Table 1.

Table 1 Drug potencies in potentiating muscimol compared with their potencies in other systems

	Concentration that potentiates muscimol by 0.25 log unit (μM)	*Potentiating potency relative to pentobarbitone*	A	B	C
(±)-DMBB	8.8	1.35		1.91	*
(±)-Quinalbarbitone	9.4	1.27	2.5	1.03	1.25
(±)-Pentobarbitone	11.9	1.00	1.00	1.00	1.00
(±)-Methylphenobarbitone	21.5	0.553	0.2		
(−)-Methylphenobarbitone	26.3	0.452		0.57	
Butobarbitone	59.5	0.200	0.3		0.42
Chlormethiazole	60.0	0.198			
Phenobarbitone	143	0.083	0.05	0	0.26
Barbitone	505	0.024	0.025	0	0.12

A Reversal of bicuculline antagonism of GABA on rat superior cervical ganglion: potency relative to pentobarbitone (Bowery & Dray, 1978).
B Enhancement of [^{3}H]-GABA binding to rat brain membranes, relative to the effect of pentobarbitone, at 500 μM concentrations (Olsen & Snowman, 1982).
C Anaesthesia in mice: potency relative to pentobarbitone (Butler, 1942).
* Convulsant.

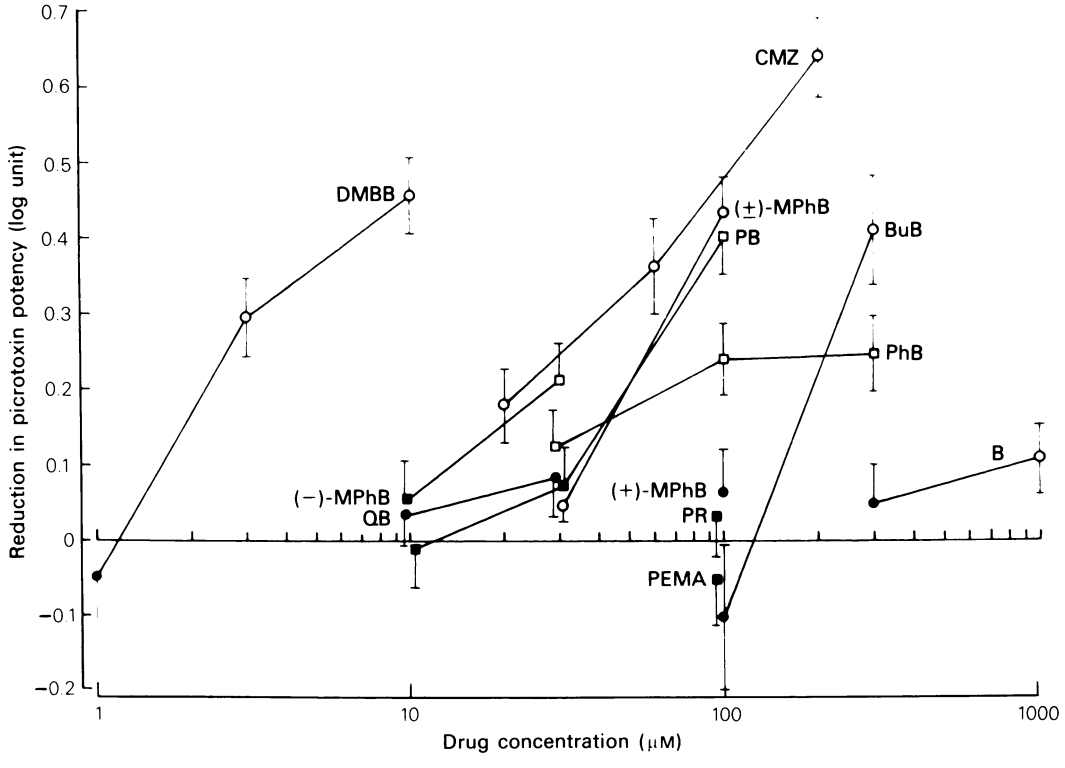

Figure 3 Reduction in the potency of picrotoxin as an antagonist of muscimol expressed as the rightward shift (mean ± s.e.mean) of the Schild plot for picrotoxin in the presence of a test drug. The abbreviations are the same as those in Figure 2. The open symbols indicate a significant reduction in picrotoxin potency ($P < 0.05$). The data for pentobarbitone and phenobarbitone are taken from Simmonds (1981) for comparison. Note: the values for 100 μM (±)-methylphenobarbitone and 30 μM quinalbarbitone are derived from non-parallel shifts of the Schild plots.

Reduction in picrotoxin potency

The Schild plots for picrotoxin may be regarded as log dose-effect relationships for picrotoxin. To assign a single numerical value to the effect of a drug on picrotoxin potency, a parallel shift of the Schild plot is required. This condition was met within 95% confidence limits for all but the highest concentration of (±)-methylphenobarbitone (100 μM), which significantly increased the slope of the Schild plot for picrotoxin, and quinalbarbitone (30 and 100 μM) which significantly decreased the slopes.

The rightward shifts of the Schild plots for picrotoxin are shown in Figure 3. It is apparent that the relative potencies between the barbiturates and chlormethiazole (Table 2) differ from the pattern for potentiation of muscimol. These comparisons are complicated, however, by the possibility that picrotoxin not only antagonizes muscimol but reduces the potentiating effect of the barbiturates and chlormethiazole on muscimol responses. Such an effect would result in an underestimate of the reduction in picrotoxin potency by these drugs, the extent of the underestimate depending on the degree of potentiation of the responses to muscimol by the drugs. To circumvent this problem, the equieffective concentrations of barbiturates and chlormethiazole for potentiation of muscimol by 0.25 log unit (Table 1) were taken and the reductions in picrotoxin potency by those concentrations of drug were interpolated from Figure 3. The values presented in Table 2 show that only DMBB, chlormethiazole, phenobarbitone and (−)-methylphenobarbitone caused reductions in picrotoxin potency at these concentrations.

Of all the drugs tested, quinalbarbitone was noted for its unusual properties. With increasing concentration it progressively flattened the Schild plot for picrotoxin (Figure 4). This effect of 100 μM quinalbarbitone could be substantially reversed during a 2 h

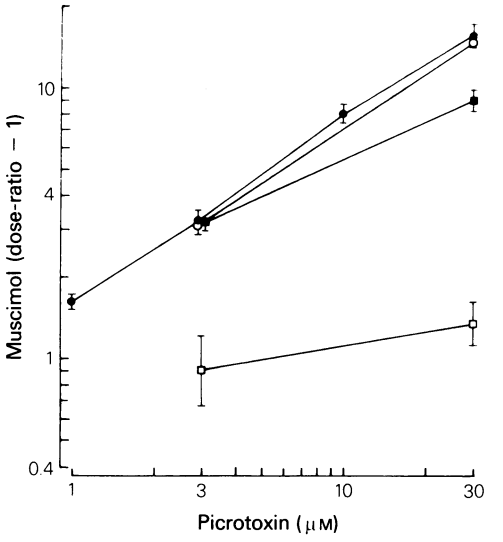

Figure 4 Schild plots for picrotoxin antagonism of muscimol in the absence (control, ●) and presence of quinalbarbitone 10 (○), 30 (■) and 100 μM (□). Each point is the mean ± s.e.mean of at least 4 values.

Table 2 Drug potencies in reducing the effect of picrotoxin compared with their potencies in inhibiting [^{3}H]-dihydropicrotoxinin binding

	Concentration that reduces picrotoxin effect by 0.2 log unit (μM)	*Potency relative to pentobarbitone*	A	B
(±)-DMBB	2.2	21.6	0.44	1000
Chlormethiazole	22.5	2.11	0.36	<0.1
(−)-Methylphenobarbitone	27.5	1.73	0.19	12.5
(±)-Quinalbarbitone	approx. 30 *	approx. 1.6	<0.03	10
(±)-Pentobarbitone	47.5	1.00	0	1.00
(±)-Methylphenobarbitone	48.0	0.990	<0.05	10
Phenobarbitone	66.0	0.720	0.24	0.13
Butobarbitone	190	0.250	0	
Barbitone	>1000	<0.048	0.07	1.00

A Reduction in picrotoxin effect (log unit) at drug concentrations that potentiate muscimol by 0.25 log unit (first column in Table 1).

B Inhibition of [^{3}H]-dihydropicrotoxinin binding: potency relative to pentobarbitone (Olsen, Ticku, Greenlee & Van Ness, 1979; Leeb-Lundberg, Snowman & Olsen, 1981; Ticku, Burch, Thyagarajan & Ramanjaneyulu, 1983).

* Depends on concentration of picrotoxin (see Figure 4).

washout period. The potentiation of muscimol by quinalbarbitone was also unusual in that it did not further increase upon raising the quinalbarbitone concentration from 30 to 100 μM (Figure 2).

Reduction in bicuculline potency

In the original experiments with pentobarbitone and phenobarbitone it was found that the potency of bicuculline as a muscimol antagonist was reduced by concentrations of barbiturate that caused potentiations of muscimol of more than 0.3 log unit (Simmonds, 1981). A similar experiment with chlormethiazole resulted in no significant reduction in bicuculline potency with 60 μM chlormethiazole (rightwards shift of bicuculline Schild plot by 0.042 ± 0.048 log unit) but a significant reduction ($P < 0.05$) with 200 μM chlormethiazole (rightwards shift of bicuculline Schild plot by 0.162 ± 0.049 log unit). Thus, again, a significant effect was seen at a concentration of chlormethiazole that potentiated muscimol by more than 0.3 log unit.

Discussion

The most obvious feature of the interaction of barbiturates with the $GABA_A$ receptor complex was the enhancement of responses to activation of the GABA receptor by muscimol. A similar enhancement was seen with chlormethiazole which, like the barbiturates, has hypnotic and anticonvulsant properties (Harvey, Higenbottam & Loh, 1975; Briggs, Castleden & Kraft, 1980). However, primidone, which is partially metabolized to phenobarbitone *in vivo*, was itself inactive at 100 μM as was its other *in vivo* metabolite PEMA 100 μM.

Several of the barbiturates used in the present experiments have been compared on other systems. On rat superior cervical ganglion (Bowery & Dray, 1978), for example, the reversal by barbiturates of bicuculline antagonism of GABA gave similar relative potencies to those found here for potentiation of muscimol (Table 1). Although Bowery & Dray could find little potentiation of GABA by the barbiturates on their preparation, substantial potentiations can be seen when a compartmented bath is used (S. Marsh, personal communication). In binding studies, it is found that barbiturates enhance [^{3}H]-GABA binding to rat brain membranes in the presence of chloride (Asano & Ogasawara, 1982; Olsen & Snowman, 1982; Whittle & Turner, 1982) and the relative potencies of the barbiturates are similar to those found here for potentiation of muscimol (Table 1).

On a behavioural measure of anaesthesia in mice (Butler, 1942), the relative potencies of the barbiturates are also in the same rank order as for potentiation of muscimol (Table 1). An apparent discrepancy is the potent potentiation of muscimol by the convulsant (±)-DMBB (Downes, Perry, Ostlund & Karler, 1970). Since it is (+)-DMBB that is convulsant, while (−)-DMBB is slightly more potent than (±)-pentobarbitone as a central nervous system depressant (Downes *et al.*, 1970), it seems likely that the convulsant properties of (+)-DMBB have nothing directly to do with the $GABA_A$ receptor complex.

Although in the present experiments there was a wide range of barbiturate potencies with regard to the concentrations required in the aqueous medium to potentiate muscimol, calculations based on the octanol/water partition coefficients indicate that both high and low potency barbiturates were likely to achieve similar concentrations in lipids for the same potentiation of muscimol (Table 3). An analogous situation occurs with the local anaesthetic effect of barbiturates on frog sciatic nerve (Seeman, 1972). However, we do not know whether it was the barbiturate in the aqueous or lipid phases that potentiated muscimol. An attractive explanation of the potentiation is the prolonged lifetimes of chloride channels

Table 3 Estimated concentrations of drugs in lipids

	pKa	*Octanol/water* (a)	*Potentiation of muscimol*		*Reduction of picrotoxin effect*	
			Krebs (b) (μM)	*Lipid* (c) (mM)	*Krebs* (d) (μM)	*Lipid* (e) (mM)
(±)-Quinalbarbitone	7.9	219	9.4	1.6	30	5.0
(±)-Pentobarbitone	8.1	107	11.9	1.1	47.5	4.2
Phenobarbitone	7.4	26.3	143	1.9	66.0	0.87
Barbitone	8.0	4.5	505	1.8	>1000	>3.6

(a) Octanol/water partition coefficient of unionized drug (Leo, Hansch & Elkins, 1971).
(b) Aqueous concentration at pH 7.4 that potentiates muscimol by 0.25 log unit (from Table 1).
(c) Concentration in lipids in equilibrium with (b), calculated from pKa and (a).
(d) Aqueous concentration at pH 7.4 that reduces picrotoxin effect by 0.2 log unit (from Table 2).
(e) Concentration in lipids in equilibrium with (d), calculated from pKa and (a).

opened by GABA and muscimol (Barker & McBurney, 1979; Study & Barker, 1981; Barker, MacDonald, Mathers, McBurney & Oertel, 1981). This effect of pentobarbitone is stereospecific, the (−)-isomer being more potent (Huang & Barker, 1980), and along with the stereospecificity of methylphenobarbitone in the present experiments it suggests that something more than a non-specific effect on membrane lipids is involved.

The other action of barbiturates and chlormethiazole studied was the reduction in effect of picrotoxin. This gave a distinctly different rank order of potencies. The potencies of quinalbarbitone, (±)-methylphenobarbitone, butobarbitone and barbitone relative to pentobarbitone were all similar to those found for potentiation of muscimol. The relative potencies of DMBB, chlormethiazole, (−)-methylphenobarbitone and phenobarbitone for reducing the effect of picrotoxin were higher than their relative potencies for potentiation of muscimol by factors of 16, 11, 4 and 9, respectively. The calculated concentrations of barbiturate likely to be present in the lipid phase of the neuronal membranes indicated that phenobarbitone was required in about one fifth the concentration of pentobarbitone or quinalbarbitone for similar reductions in picrotoxin effect (Table 3). These data would suggest that, compared with potentiation of muscimol, a different and more selective site of action is involved in the reduction of picrotoxin effect. A non-specific reduction in picrotoxin effect may occur when the barbiturates accumulate to high concentrations (4 to 5 mM) in the lipids of the membranes, at which concentrations the effect of bicuculline is also reduced (Simmonds, 1981). Unfortunately, comparable data from other systems on barbiturate interactions with picrotoxin is lacking. Binding studies on the picrotoxin site are hampered by the low affinity of the [^{3}H]-dihydropicrotoxinin ligand (Ticku, Ban & Olsen, 1978). This may be one reason for the poor correlation between barbiturate potencies in displacing [^{3}H]-dihydropicrotoxinin binding (Olsen, Ticku, Greenlee & Van Ness, 1979) and their potencies in reducing the effect of picrotoxin as an antagonist of muscimol in the present experiments (Table 2).

The functional relevance of the interactions of barbiturates with the $GABA_A$ receptor complex is of obvious interest. Schulz & MacDonald (1981) have proposed that the potentiation of GABA is relevant to the anticonvulsant actions of barbiturates while direct GABA-mimetic effects of the barbiturates underlie the anaesthetic/sedative actions. Their interpretation is based on apparently similar effects of pentobarbitone and phenobarbitone in potentiating responses to a single dose level of GABA. Such an experimental design can, however, give a misleading impression of relative potencies. As with antagonists, lateral displacements of log dose-response curves are to be preferred. With the latter design, it is clear that pentobarbitone is 12–13 times more potent than phenobarbitone as a potentiator of muscimol (Simmonds, 1981). This casts doubts on the interpretation of Schulz & MacDonald (1981).

The present results support an alternative hypothesis. Four drugs were found to reduce selectively the effect of picrotoxin whilst causing only modest potentiation of muscimol. Of these, phenobarbitone is well known to be anticonvulsant in doses that do not cause unacceptable degrees of sedation (Raines, Blake, Richardson & Gilbert, 1979). Methylphenobarbitone is also used as an anticonvulsant but is metabolized to phenobarbitone *in vivo* (Butler, 1952) so the interactions of the parent compound with picrotoxin *in vitro* may be of minor relevance. Chlormethiazole is effective in the treatment of status epilepticus in doses that do not seriously impair consciousness (Harvey *et al.*, 1975). (±)-DMBB, however, is not used clinically because of the convulsant properties of the (+)-isomer. Nevertheless, the present results suggest that the (−)-isomer might be an effective anticonvulsant at doses that cause minimal sedation. None of the remaining barbiturates tested in the present experiments is used as an anticonvulsant.

We would suggest, therefore, that the ability of phenobarbitone, (−)-methylphenobarbitone and chlormethiazole to reduce selectively the effects of picrotoxin by an action at a specific site in the $GABA_A$ receptor complex may be relevant to their anticonvulsant properties. The potentiation of muscimol and, therefore, GABA by chlormethiazole and barbiturates in general involves a distinctly different site that is less selective and this may underlie the hypnotic properties of these drugs.

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