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The effects of chlormethiazole on single unit activity in rat brain; interactions with inhibitory and excitatory neurotransmitters

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1 Extracellular recordings were made of single unit activity in the brainstem of urethane anaesthetized rats. Drugs were applied by microiontophoresis from multibarrelled micropipettes or administered intraperitoneally.

2 Chlormethiazole (CMZ) caused a decrease in spontaneous firing rate when applied with high currents (>40 nA).

3 When applied with lower currents CMZ did not cause changes in firing rate, but enhanced the inhibitory effects of γ-aminobutyric acid (GABA), muscimol and glycine in a dose-dependent manner. The inhibitory actions of acetylcholine were not affected.

4 Excitatory responses to glutamate and acetylcholine were unaffected by applications of CMZ which caused potentiation of GABA, muscimol and glycine. When applied at higher currents CMZ caused a decrease in the response to glutamate.

5 Intraperitoneal administration of CMZ (50–600 μmol kg^{-1}) also enhanced responses to microiontophoretically applied GABA, muscimol and glycine.

6 These results are compared with those reported for other anticonvulsant drugs and possible mechanisms of action of CMZ are discussed.

Introduction

Chlormethiazole (CMZ) has anticonvulsant, sedative and hypnotic properties. It is widely used in the treatment of alcohol withdrawal reactions, particularly in delirium tremens associated with convulsive episodes (Dencker, Wilhelmson, Carlsson & Bereen, 1978). It is effective in status epilepticus in patients failing to respond to benzodiazepines or barbiturates (Harvey, Higenbottam & Loh, 1975). More recently it has been used as a hypnotic (Briggs, Castleden & Kraft, 1980) particularly in psycho-geriatric patients (Harenko, 1975).

Little is known of the mechanism of action of CMZ and no previous studies have been carried out to investigate its effects on neuronal activity in the mammalian central nervous system. A simplistic approach to the actions of anticonvulsants is to suggest that apart from possible direct effects they may either enhance inhibition or depress excitation in the brain. Therefore, we examined the effects of CMZ, applied microiontophoretically, on single unit activity and on neuronal responses to some inhibitory and excitatory neurotransmitters.

Methods

The results presented here were obtained from experiments performed on 56 Wistar rats of either sex, weight range 200–400 g. The rats were anaesthetized with urethane given intraperitoneally (1.5 to 2 g kg^{-1}). The trachea was cannulated to assist respiration. The skin and underlying soft tissue on the back of the head were removed. Miniature rongeurs were inserted into the foramen magnum and part of the occipital region of the skull was cut away. The dura was cut and reflected and warm liquid paraffin was applied to the exposed cerebellum and brain stem to prevent dessication. The head was mounted rigidly in a stereotaxic frame. Rectal temperature was maintained at 38 ± 1°C with a thermostatically controlled heating pad.

Glass micropipettes (5- or 7-barrelled) were made from fibred glass tubing and pulled to a tip diameter of 5 to 8 μm. Two barrels were filled with 3 M NaCl, one for recording, one for current controls, and the remaining barrels were filled with the following drug

solutions for microiontophoresis: γ-aminobutyric acid (GABA), 0.1 M pH 4.5; glycine, 0.1 M pH 4.5; glutamic acid, 0.1 M pH 8.5; acetylcholine bromide, 0.1 M pH 5.0; muscimol, 0.001 M in 0.165 M NaCl pH 5.0 (all from Sigma, London); chlormethiazole edisylate, 0.05 M in 0.165 M NaCl pH 2.0 (Astra Pharmaceuticals). The pH of each solution was adjusted, where necessary, with 0.1 M HCl or 0.1 M NaOH.

Conventional equipment was used to make extracellular recordings of the activity of neurones in the medullary reticular formation, 0.5 to 2 mm lateral to the midline and 1 to 5 mm rostral to the obex. Neurones were selected for study if they showed spontaneous activity and were sensitive to GABA or muscimol. Retaining currents were applied to all barrels between ejection periods and automatic current compensation was used routinely. (A current equal in value but opposite in polarity to the sum of the currents in the drug-containing barrels was passed through a barrel containing a 3 M NaCl solution.) On some neurones the effects of current were also tested separately.

Results

Effects of CMZ alone

CMZ was applied microiontophoretically to 125 neurones. When applied with low currents (0–30 nA) for short periods (less than 2 min) CMZ had no effect on spontaneous firing rate. When applied continuously for longer periods to test its effects on responses to other compounds, as described below, no effects were seen on the majority of cells but a small and slowly developing decrease in firing rate was observed on some 40% of cells. On many of these occasions this was difficult to distinguish from normal variation in spontaneous firing rate. When applied with higher currents (greater than 30 nA), CMZ often caused a rapid, dose-dependent and reversible decrease in firing rate.

Effects of CMZ on inhibitory responses to GABA and muscimol

During ejection of CMZ with currents which did not markedly affect the spontaneous firing rate, the de-

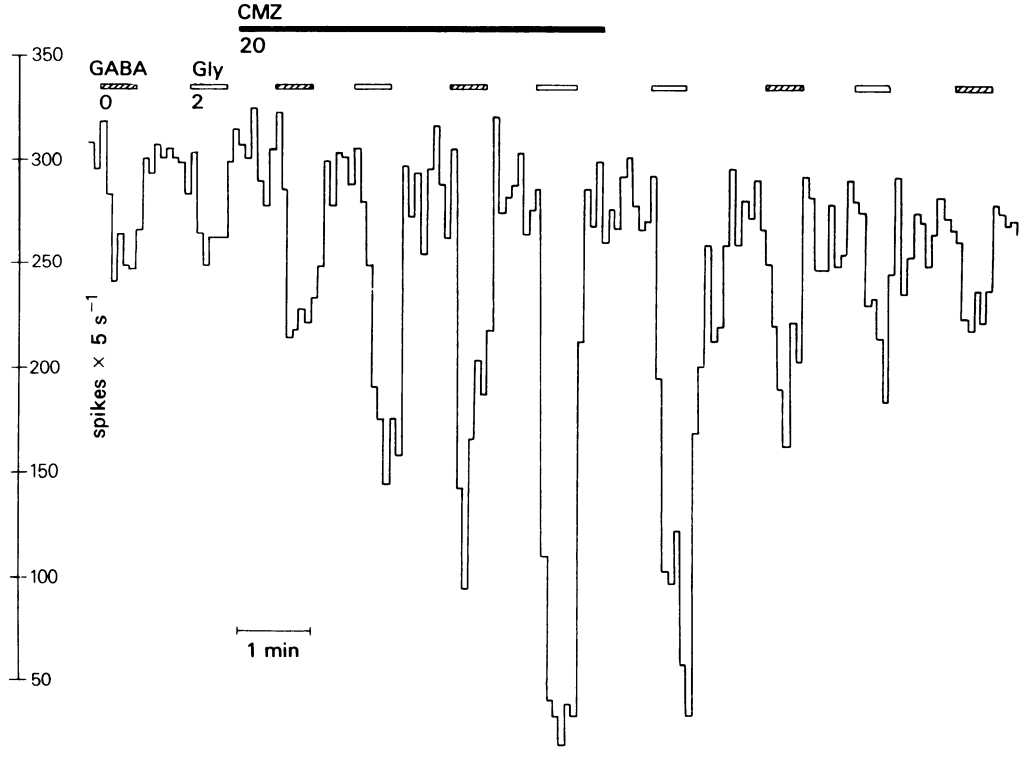

Figure 1 The effects of chlormethiazole (CMZ) on responses to GABA and glycine (Gly). Inhibitory responses to both substances were enhanced by CMZ (20 nA). In this and Figures 2 and 3 the ordinate scale shows mean firing rate in successive 5 s epochs and the abscissa scale time (scale indicated by the bar). Drugs were applied microiontophoretically during the periods indicated by bars, with the application currents indicated in nA.

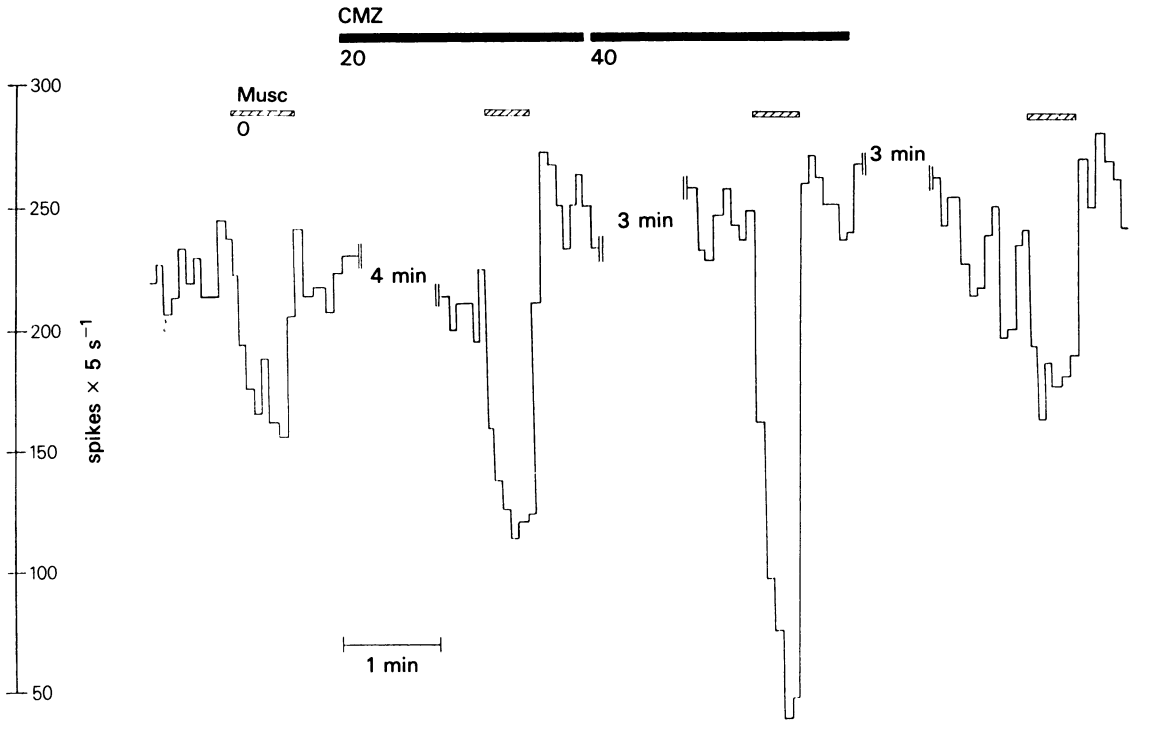

Figure 2 The effect of chlormethiazole (CMZ) on responses to muscimol (Musc). The application of CMZ (20 nA) caused potentiation of the muscimol response after 4 minutes. When the ejecting current was increased to 40 nA the degree of potentiation was also increased, after a further 3 minutes. Responses to muscimol returned to control levels some 3 minutes after CMZ application was terminated.

pressant actions of microiontophoretically applied GABA on neuronal firing rate were repeatably and reversibly potentiated (Figure 1). The potentiation became apparent within 20 s. It reached maximum in 1–8 min, usually in less than 3 min, and recovery was complete within 10 min after termination of the application of CMZ. This effect was seen on 49 out of 52 cells (94%) and where tested was dose-dependent with respect to CMZ.

Responses to microiontophoretically applied muscimol were potentiated in a similar, repeatable, reversible, dose-dependent manner (Figure 2). Responses to muscimol were potentiated in 45 out of 48 neurones tested (94%).

Responses were arbitrarily considered potentiated if the enhanced response, measured as percentage change in firing rate at equilibrium, obtained during application of CMZ was 15% or more greater than control, pretreatment responses. The maximum potentiation observed was some 400%, but it was usually less marked (mean ± s.e.mean for GABA, 72 ± 8.9%, and for muscimol 76 ± 9.7%).

Effects of CMZ on inhibitory responses to glycine

CMZ, applied with currents which did not markedly affect the spontaneous firing rate, also caused potentiation of the inhibitory response to microiontophoretically applied glycine (Figure 1). Potentiation of responses to glycine was seen on 53 out of 64 neurones (83%) and where tested was seen to be dose-dependent with respect to CMZ. The maximum potentiation of responses to glycine was 525% (mean ± s.e.mean, 86 ± 18.9%).

The effects of CMZ on responses to GABA and glycine were compared on 22 cells. No particular trend was observed; on 5 cells a greater potentiation of the response to GABA was seen, on 7 cells a greater potentiation of the response to glycine, and on 9 cells an approximately equal potentiation of responses to both GABA and glycine. On one cell, effects on the response to glycine were negligible, while the response to GABA was potentiated by 90%.

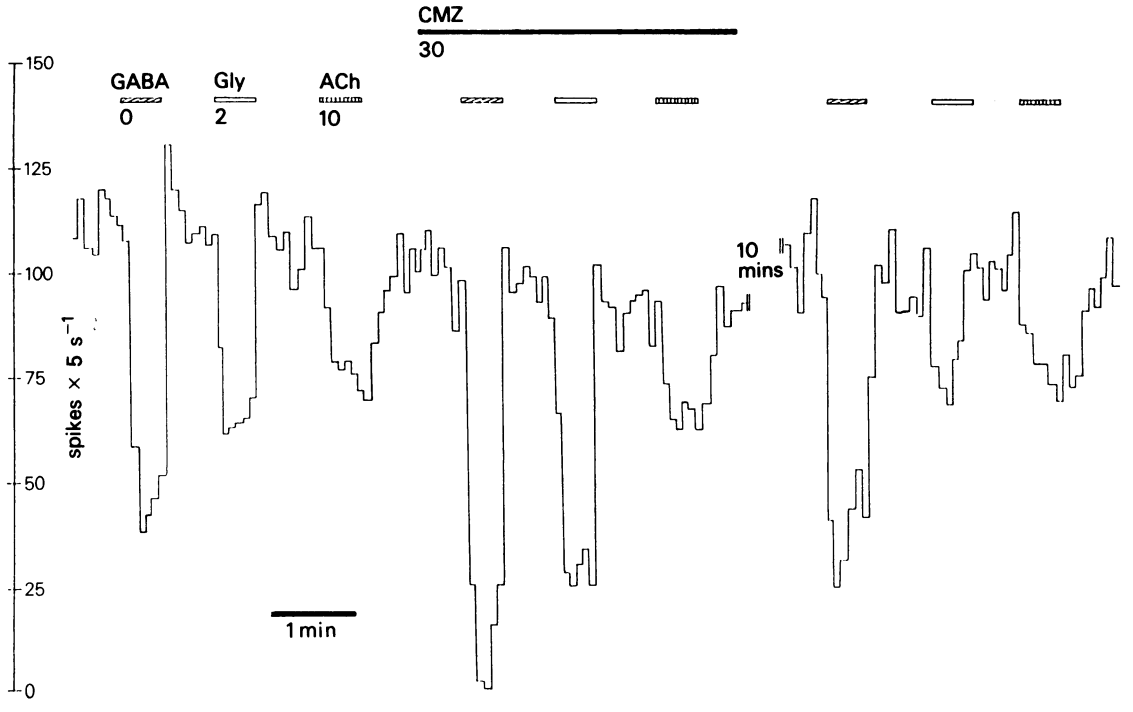

Figure 3 The effects of chlormethiazole (CMZ) on responses to GABA, glycine (Gly) and acetylcholine (ACh). Inhibitory responses to GABA and Gly were potentiated during application of CMZ but the response to ACh was unaffected.

Effects of CMZ on responses to acetylcholine

A decrease in firing rate in response to acetylcholine was seen on only four cells. On each occasion the inhibitory response to acetylcholine was unaffected by CMZ ejected at currents which caused a clear potentiation of inhibitory responses to GABA and glycine (Figure 3).

On cells on which CMZ caused potentiation of inhibitory responses to GABA, muscimol and glycine, excitatory responses to microiontophoretically applied acetylcholine were unaffected in 13 out of 14 cells, although on one cell the response to acetylcholine was decreased by some 50%.

Effects of CMZ on excitatory responses to glutamate

When CMZ was applied with currents which were

Table 1 The effects of chlormethiazole (CMZ), applied microiontophoretically with low currents (< 30 nA), on responses to microiontophoretically applied agonists

Agonist	*Potentiation*	*No effect*	*Decrease*
GABA	49 (94%)	2 (4%)	1 (2%)
Muscimol	45 (94%)	3 (6%)	0 (0%)
Glycine	53 (83%)	9 (14%)	2 (3%)
Acetylcholine (inhibition)	0 (0%)	4 (100%)	0 (0%)
Acetylcholine (excitation)	0 (0%)	13 (93%)	1 (7%)
Glutamate	0 (0%)	14 (82%)	3 (18%)

Numbers represent the number of cells on which each effect was seen.

Table 2 The effects of intraperitoneally injected chlormethiazole (CMZ) on responses to microiontophoretically applied agonists

Cell No.	*CMZ* (μmol kg^{-1})	*GABA*	*Muscimol*	*Glycine*
		(% potentiation of response)		
1 (a)	50	21		
1 (b)	350	44		
2	100	37		0
3	100	38		139
4	100	166		46
5	100		237	386
6	100	45		
7	100	64		
8	100		45	
9 (a)	300		42	
9 (b)	600		95	

Each cell was studied in a different animal. In cells 1 and 9, a second injection of CMZ was given.

shown, on the same cell, clearly to potentiate responses to GABA or muscimol and/or glycine, excitatory responses to microiontophoretically applied glutamate were unaffected on 14 out of 17 cells tested. On the remaining 3 cells a slight decrease in the response to glutamate was observed in the presence of CMZ. However, if CMZ was then ejected with higher currents (greater than 30 nA) the excitatory responses to glutamate were decreased by 15–30%. This effect of CMZ at higher ejection currents was also observed on a further 4 cells. However, the higher currents required to produce this effect usually caused some decrease in the spontaneous firing rate.

The results from experiments in which CMZ was applied microiontophoretically are summarized in Table 1.

Effects of CMZ given parenterally

CMZ (50–600 μmol kg^{-1}) was administered intraperitoneally in experiments on nine different animals, while the firing rate of a single cell was continually monitored. Only one cell was studied in each animal. At low doses (50 and 100 μmol kg^{-1}) CMZ caused only transient changes in firing rate, and such changes were also observed after control injections of 0.165 M NaCl solution. With higher doses of CMZ changes in firing rate were sometimes more marked and the recording was often lost, presumably as a result of changes in blood pressure. Results from such experiments are not included here.

In successful experiments, responses to microiontophoretically applied GABA, muscimol and glycine were potentiated to a similar degree to that seen when CMZ was applied microiontophoretically. Potentiation became maximal between 5 and 15 min after the injection and in no case was recovery observed. The effects of CMZ on responses to GABA, muscimol and glycine are summarized in Table 2. In two experiments, two successive injections of CMZ were given, and in both cases the enhancement of the responses to GABA agonists was greater when the dose of CMZ was increased.

Discussion

CMZ has been shown in this study to potentiate the inhibitory effects of microiontophoretically applied GABA, muscimol and glycine. The lack of effect on inhibitory responses to acetylcholine on cells where GABA- or glycine-induced inhibition was potentiated suggests that this action is a selective one.

Potentiation of GABA-mediated inhibition has also been shown for benzodiazepines and for barbiturates (Macdonald & Barker, 1979, Alger & Nicoll, 1982), for sodium valproate (Gent & Phillips, 1980; Kerwin, Olpe & Schmutz, 1980) and for phenytoin (Ayala, Johnston, Lin & Dichter, 1977; Gent & Haigh, unpublished observations). With all of these substances glycine-mediated inhibition was not affected. Thus it seems that potentiation of inhibitory neurotransmission is a feature of many anticonvulsants but potentiation of both GABA- and glycine-mediated inhibition is unique to CMZ.

CMZ does not display any affinity for GABA binding sites, (S–O Orgren, personal communication), and is thus unlikely to potentiate responses to GABA by causing subthreshold activation of GABA receptors. Benzodiazepines enhance the effects of GABA, but CMZ has been shown not to displace [^{3}H]-diazepam to any significant extent compared with benzodiazepines (Muller, Schlafer & Wollert, 1978). There is no evidence in the literature that CMZ affects either uptake or degradation of GABA or glycine.

Both CMZ and anticonvulsant barbiturates have been shown to inhibit [^{3}H]-α-dihydropicrotoxinin binding (Leeb-Lundberg, Snowman & Olsen, 1981). It has been suggested that picrotoxin antagonizes an endogenous process which facilitates or prolongs the opening of the chloride ionophore (Simmonds, 1980). The possibility that CMZ may potentiate GABA by an interaction with such a process may be relevant to the present study as there is some evidence to suggest that GABA and glycine may share the same chloride ionophore (Barker & McBurney, 1979). Although picrotoxin is usually considered a specific GABA antagonist, there is evidence in the literature to indicate a lack of specificity (Krnjevic, Randic & Straughan, 1966; Bernardi, Marciani, Morocutti & Giacomini, 1976) and Davidoff & Aprison (1969) found picrotoxin to block responses to glycine. Thus it is possible that our results may be explained by an action at the picrotoxin recognition site.

CMZ was also shown to diminish excitatory responses to microiontophoretically applied glutamate, although higher ejection currents were needed than those required to potentiate responses to GABA or glycine. Similar actions have been found for barbiturates (Macdonald & Barker, 1979).

CMZ administered intraperitoneally caused potentiation of responses to GABA, muscimol and glycine. As with microiontophoretically applied CMZ, the degree of potentiation varied considerably. However, results from these experiments do indicate similar effects with each type of administration, suggesting that the concentration achieved in the local environment of neurone is of the same order in each case. The doses of CMZ used (50 to 600 μmol kg^{-1}) span the anticonvulsant ED_{50} values for CMZ against bicuculline, picrotoxin, isoniazide and metrazole (S-O Ogren, personal communication). Although there is no apparent relationship between dose and response in different animals, the increased potentiation of responses with increased doses of CMZ in the same animal indicates that this effect is dose-dependent.

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References

ALGER, B.E. & NICOLL, R.A. (1982). Pharmacological evidence for two kinds of GABA receptors on rat hippocampal pyramidal cells studied in vitro. *J. Physiol. (Lond.)*, **328**, 125–141.

AYALA, G.F., JOHNSTON, D., LIN, S. & DICHTER, H.N. (1977). The mechanism of action of diphenylhydantoin on invertebrate neurons. II. Effects on synaptic mechanisms. *Brain Res.*, **121**, 259–270.

BARKER, J.L. & McBURNEY, R.N. (1979). GABA and glycine may share the same conductance channel on cultured mammalian neurones. *Nature*, **277**, 234–235.

BERNARDI, G., MARCIANI, M.G., MOROCUTTI, C. & GIACOMINI, P. (1976). The action of picrotoxin and bicuculline on rat caudate neurones inhibited by GABA. *Brain Res.*, **102**, 379–384.

BRIGGS, R.S., CASTLEDEN, C.M. & KRAFT, C.M. (1980). Improved hypnotic treatment using chlormethiazole and temezepam. *Br. med. J.*, **280**, 601–604.

DAVIDOFF, R.A. & APRISON M.H. (1969). Picrotoxin antagonism of the inhibition of neurones by glycine. *Life Sci.*, **8**, 107–112.

DENCKER, S.J., WILHELMSON, G., CARLSSON, E. & BEREEN, F.J. (1978). Piracetam and chlormethiazole in acute alcohol withdrawal: a controlled clinical trial. *J. Int. med. Res.*, **6**, 395–400.

GENT, J.P. & PHILLIPS, N.I. (1980). Sodium di-n-propylacetate (valproate) potentiates responses to GABA and muscimol on single central neurones. *Brain Res.*, **197**, 275–278.

HARENKO, A., (1975). A comparison between chlormethiazole and nitrazepam as hypnotics in psychogeriatric patients. *Curr. Med. Res. Opin.*, **2**, 657–663.

HARVEY, P.K.P., HIGENBOTTAM, T.W. & LOH, L. (1975). Chlormethiazole in treatment of status epilepticus. *Br. med. J.*, **2**, 603–605.

KERWIN, R.W., OLPE, H.-R. & SCHMUTZ, M. (1980). The effect of sodium-n-dipropylacetate on GABA-dependent inhibition in the rat cortex and substantia nigra in relation to its anticonvulsant activity. *Br. J. Pharmac.*, **71**, 545–551.

KRNJEVIC, K., RANDIC, M. & STRAUGHAN, D.W. (1966). Pharmacology of cortical inhibition. *J. Physiol. (Lond.)*, **184**, 78–105.

LEEB-LUNDBERG, F., SNOWMAN, A. & OLSEN, R.W. (1981). Interaction of anticonvulsants with the barbiturate-benzodiazepine-GABA receptor complex. *Eur. J. Pharmac.*, **72**, 125–129.

MACDONALD, R.L. & BARKER, J.L. (1979). Enhancement of GABA-mediated postsynaptic inhibition in cultured mammalian spinal cord neurones; a common mode of anticonvulsant action. *Brain Res.*, **167**, 323–336.

MULLER, W.E., SCHLAFER, U. & WOLLERT, U. (1978). Benzodiazepine receptor binding: the interactions of some non-benzodiazepine drugs with specific (H)-diazepam binding to rat synaptosomal membranes. *Naunyn-Schmiedebergs Arch. Pharmac.*, **305**, 23–26.

SIMMONDS, M.A. (1980). A site for the potentiation of GABA-mediated responses by benzodiazepines. *Nature*, **284**, 558–560.

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