On the hypnogenic and anticonvulsant activities of demethyldiazepam and chlordemethyldiazepam: time-effect relations*

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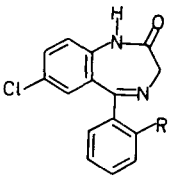
Demethyldiazepam (DMDZ), (IA) is a metabolic product of chlordiazepoxide, diazepam, chlorazepate, medazepam, prazepam and pinazepam (Schwartz, 1973; Greenblatt & Shader, 1974), it has all the pharmacological properties of diazepam and it is more active (Marcucci, Mussini & others, 1971; de Angelis, Traversa & Vertua, 1974a).



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A, R = H. B, R = Cl

We have previously shown that DMDZ and its chlor-derivative possess marked sleep-inducing and anticonvulsant activities (de Angelis, Traversa & Vertua, 1974b). We concluded that the molecule with the highest activity was chlordemethyldiazepam (Cl-DMDZ) (IB), a metabolic precursor of lorazepam (de Silva, Bekersky & Puglisi, 1973).

We have now studied the time-effect relations of DMDZ and Cl-DMDZ in tests evaluating their sleep-inducing effect in combination with hexobarbitone or chlorprothixene, and their antileptazol activity.

Female albino Swiss strain mice, 20–30 g, were fasted for 16 h before and during the experiment, but had free access to tap water. Both compounds were suspended in 1% w/v carboxymethylcellulose and given by mouth.

Experiments were made 30, 60, 150, 300, 600 min and 24 h after administration of the compounds.

Sleep was induced by the intraperitoneal injection of hexobarbitone (100 mg kg^{-1}) according to Kuhn & Van Maanen (1961) and Dervinis, Glassman & Seifter (1958), and also by oral administration of chlorprothixene (25 mg kg^{-1}), according to Randall, Schallek & others (1969).

720 animals were used for the hexobarbitone and 544 for the chlorprothixene test. They were divided into groups of 8:24 groups were used as controls. In both tests, the sleeping time was measured as the interval between the loss and the recovery of the righting reflex.

In the hexobarbitone experiments, the ED50's, defined as the doses of benzodiazepine causing half the mice of a group to lose the righting reflex for a period at least twice that of the control animals, were determined. In the chlorprothixene experiments, the HD50's, defined as the doses of benzodiazepine causing half the mice of a group to lose the righting reflex for at least 3 min, were calculated. Both the ED50's and HD50's and their confidence limits (95%) were calculated according to Litchfield & Wilcoxon (1949). A minimum of five dose levels were used for each value.

Seizures were elicited by the intraperitoneal injection of leptazol (125 mg kg^{-1}) according to Everett & Richards (1944). 896 animals were used for this test.

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Table 1. *Effect of chlordemethyldiazepam and demethyldiazepam preadministration on hexobarbitone (100 mg kg^{-1}, i.p.) (ED50) or chlorprothixene (25 mg kg^{-1}, orally) (HD50) sleeping time in the mouse.*

	Oral ED50 and HD50* confidence limits mg kg^{-1}			
Time before drug (min)	Chlordemethyldiazepam ED50	Chlordemethyldiazepam HD50	Demethyldiazepam ED50	Demethyldiazepam HD50
30	0·37 (0·47–0·29)	0·041 (0·079–0·021)	0·52 (0·81–0·33)	0·138 (0·202–0·094)
60	0·26 (0·33–0·20)	0·030 (0·050–0·018)	0·50 (0·61–0·41)	0·118 (0·197–0·071)
150	0·44 (0·67–0·29)	0·036 (0·042–0·031)	0·65 (1·04–0·41)	0·170 (0·294–0·098)
300	0·68 (1·27–0·36)	0·054 (0·078–0·037)	0·98 (1·11–0·87)	0·170 (0·260–0·110)
600	0·76 (0·94–0·61)	0·066 (0·086–0·051)	1·22 (1·39–1·07)	0·220 (0·451–0·107)
1440	1·18 (1·95–0·71)	0·190 (0·384–0·094)	4·50 (7·88–2·57)	0·710 (0·990–0·510)

* see text.

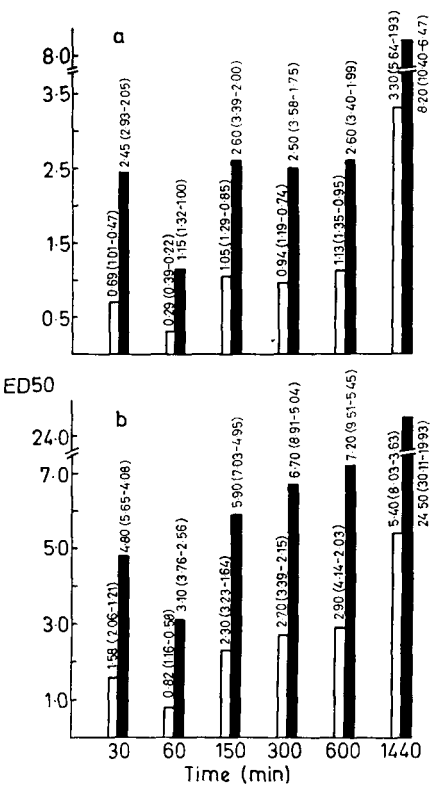

FIG. 1. Antileptazol (125 mg kg^{-1}, i.p.) activity in the mouse of chlordemethyldiazepam and demethyldiazepam administered orally at different times before the convulsant: (a) protection against mortality. (b) protection against convulsions. Ordinate—ED50 (mg kg^{-1}, orally). Abscissa—Time (min) before administration of leptazol. Demethyldiazepam (closed columns). Chlordemethyldiazepam (open columns).

They were divided into groups of 8:12 groups were used as controls. The ED50 values against mortality were calculated as the doses of benzodiazepine preventing death in half the mice after administration of the convulsant. The ED50's against convulsions were calculated as the doses of benzodiazepine preventing convulsions in half the mice after the administration of the convulsant. Also for this test, the ED50's and the confidence limits (95%) were calculated according to Litchfield & Wilcoxon (1949), employing a minimum of six dose levels for each value.

The time-effect relations of the hexobarbitone sleep-inducing activity of the two compounds are in Table 1. From these data it appears that the peak effect of both compounds occurs 60 min after administration. However, while the ED50 of Cl-DMDZ at 60 min differed significantly from the values at 30 and 150 min, the ED50 values of DMDZ at 30, 60 and 150 min were not significantly different. From 60 to 600 min the activity of the compounds decreased slowly. At 24 h both still retained an appreciable effect but the activity of Cl-DMDZ was reduced to a quarter and DMDZ to 1/10th of that observed at 60 min.

Table 1 also summarizes the data obtained after the administration of the two drugs in combination with chlorprothixene. Here too, it appears that both compounds showed maximal activity 60 min after the treatment, but the HD50 values at times 30, 60 and 150 min were not significantly different. Up to 600 min both drugs had a similar behaviour with a slow progressive reduction in activity, which was for both compounds about 1/6th of that found at 60 min.

In the antileptazol test the peak effect of the compounds in protecting against mortality (Fig. 1a) occurred at 60 min. Thereafter, the activity slowly decreased up to 600 min and at 24 h a sharp decrease was evident which was more pronounced for DMDZ.

Fig. 1b, shows the protection afforded against convulsions. The time-effect relations are similar to those for mortality and again the peak effect was at 60 min. Between 150 and 600 min there was a parallel decrease in activity and at 24 h activity was still detectable, that for DMDZ being 1/8th and of Cl-DMDZ 1/6th that at 60 min.

We previously showed that DMDZ and Cl-DMDZ possess marked sleep-inducing and anticonvulsant activities (de Angelis & others, 1974b) and we have now confirmed the previous data and established the time-effect relations of these two compounds.

It is clear that Cl-DMDZ is much more effective than the parent compound and this is in agreement with preliminary clinical trials that outline the particular effectiveness of Cl-DMDZ (Morandini & Andreoli, 1975a,b; Cesco, Giannico & others, 1976, 1977).

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Hallucinogen binding to dopamine/neuroleptic receptors

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Although previous work has established that the hallucinogenic drugs have a high affinity for 5-hydroxytryptamine and (+)-lysergic acid diethylamide receptor sites (Bennett & Snyder, 1976; Fillion, Fillion & others, 1976; Lovell & Freedman, 1976), it is known that the hallucinogen actions are blocked by neuroleptic drugs (Snyder, Faillace & Hollister, 1967; Lloyd, 1970) which are thought to act by dopamine receptor blockade (Andén, Roos & Werdinius, 1964; van Rossum, 1967; Andén, 1968).

In order to examine the suggestion (Pieri, Pieri & Haefely, 1974; Von Hungen, Roberts & Hill, 1974; Stone, 1974) that hallucinogenic drugs can act directly on brain dopamine receptors, we tested the effect of various hallucinogens on the binding of [^{3}H]haloperidol and [^{3}H]apomorphine to brain tissue. It is known that these two ligands bind to sites closely related to, if not identical with, the dopamine receptor (Seeman, Wong & Lee, 1974; Seeman, Chau-Wong & others, 1975; Burt, Creese & Snyder, 1976; Seeman, Lee & others, 1976a,b).

The experiments were done on crude homogenates of calf caudate, using procedures previously described (Seeman & others, 1976a, b). The final concentration in the incubation tube was 3·3 nM for [^{3}H]haloperidol and 1·5 nM for [^{3}H]apomorphine. The stereoselective component of binding was defined as that amount of [^{3}H]haloperidol or [^{3}H]apomorphine bound in the presence of (−)-butaclamol (inactive neuroleptic) minus that bound in the presence of (+)-butaclamol (active neuroleptic); 100 nM butaclamol was used for [^{3}H]haloperidol, and 1 μM butaclamol was used for [^{3}H]apomorphine.

The results (Table 1) indicate that *N,N*-dimethyl tryptamine (DMT), *N,N*-diethyltryptamine (DET) bufotenin and ibogaine were all rather active on the neuroleptic receptor ([^{3}H]haloperidol) in the nM region; methysergide and 2,5-dimethoxy-4-methylamphetamine (STP) were active in the 30–50 nM range, while mescaline and LSD blocked in the 100–500 nM region. The tryptamine derivatives which were potent in blocking the binding of [^{3}H]haloperidol were generally much weaker in blocking the binding of [^{3}H]apomorphine. In contrast (+)-LSD has a very low IC50 against [^{3}H]apomorphine binding (6 nM) but was less active against [^{3}H]haloperidol binding (500 nM).

Table 1. *The effect of hallucinogens on [^{3}H]haloperidol and [^{3}H]apomorphine binding**.

	Butaclamol-specific [^{3}H]haloperidol binding IC50 (nM)	Butaclamol-specific [^{3}H]apomorphine binding IC50 (nM)
DET	0·5	6000
Ibogaine	3	450 000
Bufotenin	4	5000
DMT	18	1000
Methysergide	30	70
Mianserin (GB-94)	30	900
STP	44	300 000
Mescaline	100	14 000
(+)-LSD	500	6
5-Methoxy-*NN*-dimethyltryptamine	—	3000
5-HT	—	6000

* The IC50 values are the concentrations of the drugs which reduced the stereospecific binding of [^{3}H]-haloperidol or [^{3}H]apomorphine by 50%.

The low IC50 for (+)-LSD on [^{3}H]apomorphine binding (6 nM) does suggest that there is a direct action of (+)-LSD on dopamine receptors. The crude relation in Fig. 1 further suggests that the tryptamine derivatives may reciprocally affect both the antagonist as well as the agonist state of the dopamine receptor, if such a two-state hypothesis is correct (Bennett & Snyder 1976).

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