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Dithiocarbamate central effects in relation to their possible influence on drug metabolizing enzymes

We have previously shown that administration of diethyldithiocarbamate to rats causes behavioural changes (Mayer & Ebyl, 1971). We now report the effects of two other dithiocarbamate derivatives, dimethyldithiocarbamate and disulfiram, upon the exploratory activity of rats, and also the effect of diethyldithiocarbamate upon drug metabolism as indicated by the disappearance of pentobarbitone from plasma. Spironolactone was used to induce the drug metabolizing enzymes.

The exploratory activity of female albino rats (Wistar, 150–200g) was tested. A plastic box (37 × 21 × 25 cm) equipped with an automatic register for measuring the frequency of standing-up reactions, and having the floor divided into 8 painted equal squares was used. The number of squares crossed by a rat (by at least one half of its body) was counted by a trained observer. No significant difference was found between the data so obtained and those obtained automatically with apparatus described by Mayer & Eybl (1971). The stereotyped movements of the rats perceptible after the amphetamine pretreatment and defined as continuously sniffing, licking and head-moving (the amphetamine stereotypes) were registered at the same time. The sleeping time of male mice (Lysolaje strain) was registered in minutes as the period of lost righting reflex, and calculated as the geometric mean value. Plasma concentrations of pentobarbitone in male mice were estimated according to Tietz (1970).

Sodium diethyldithiocarbamate (DDC, Lachema Brno), sodium dimethyldithiocarbamate (DMDC, Lachema Brno), pentobarbitone (Spofa), and commercial solutions of amphetamine sulphate (Psychoton) were administered intraperitoneally

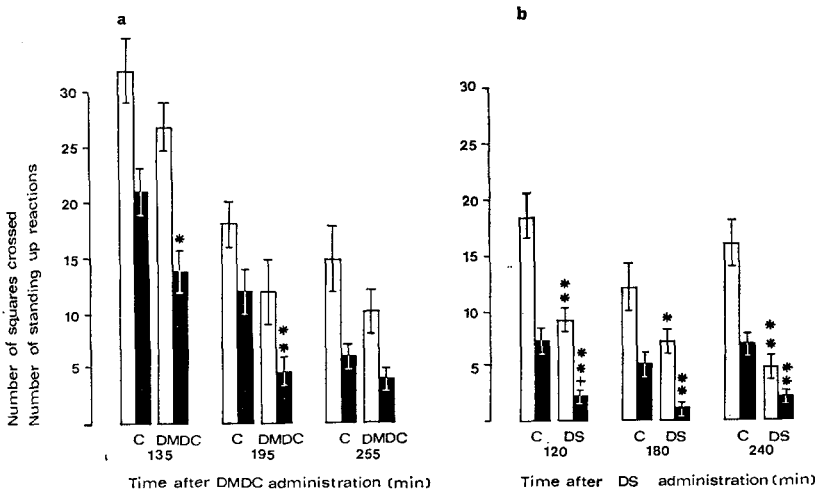

FIG. 1. Exploratory activity of rats. a. The animals received intraperitoneally distilled water (c) or dimethyldithiocarbamate (DMDC) 150 mg kg^{-1}. b. Animals received intraperitoneally distilled water (c) or disulfiram (DS) 150 mg kg^{-1}. The measurements were repeated in the same animals at intervals indicated on the abscissa. The mean value $\pm$ s.e. and the significance of differences from the corresponding control group calculated by Student's *t*-test are given, $*P<0{\cdot}05$, $**P<0{\cdot}01$. Open columns = number of squares crossed; black columns = number of standing up reactions.

diluted with distilled water, while tetraethylthiuramdisulphide (disulfiram, Antabuse, Dumex) was given suspended in 1% methylcellulose. Spironolactone (Boehringer Mannheim) suspended in 1% methylcellulose was given by stomach tube twice daily for 3 consecutive days.

DMDC, 150 mg kg^{-1} resulted in decreasing the exploratory activity of rats (Fig. 1a). The level of the activity was always lower in the pretreated group than in the corresponding control group, and decreased in the course of repeated measurements in the same animals.

DMDC pretreatment of animals that were given amphetamine, 5 mg kg^{-1}, 90 min later (Fig. 2a) resulted in the decrease of exploratory activity and in a simultaneous increase in time spent by animals in performing amphetamine stereotypies. The rats pretreated with DMDC made these movements during the whole period when the large locomotor movements were absent, namely, at intervals of 105 and 165 min after amphetamine administration. In the rats treated with amphetamine only, on the other hand, about the same time was devoted to performing both the large locomotor and small stereotyped movements.

Disulfiram, 150 mg kg^{-1} produces effects similar to DMDC and its depressive action (Fig. 1b) was observed at all times. In this experiment, animals familiar with the experimental environment, were used. Probably for this reason the exploratory activities when measured for the third time had a higher value (the phenomenon of dishabituation).

The interaction of disulfiram with amphetamine (Fig. 2b) was assessed using amphetamine at 3 mg kg^{-1}. In this way it was possible to demonstrate hypermotility of the rats 30 and 90 min after the administration of amphetamine. Pretreatment with disulfiram decreased the activity; the potentiating effect on amphetamine stereotypies was not significant.

DDC, 100 mg kg^{-1} administered to male mice 60 min before pentobarbitone (60 mg kg^{-1}, i.p.) approximately doubled the sleeping time (Table 1). Repeated spiro-

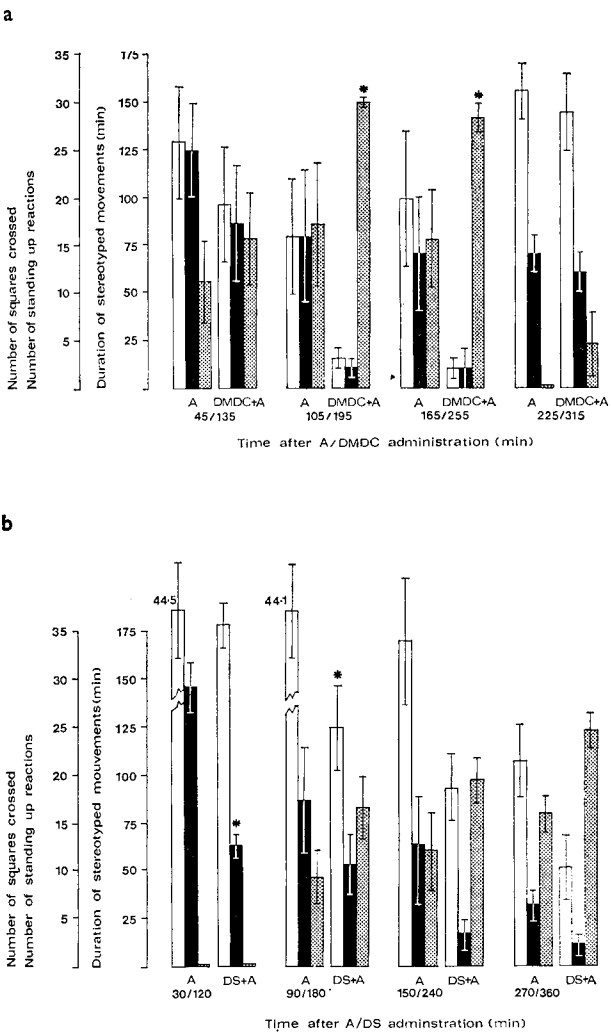

FIG. 2. Exploratory activity and duration of the stereotyped movements provoked by amphetamine in rats. a. The animals received intraperitoneally either amphetamine (A) 5 mg kg^{-1} or dimethyldithiocarbamate 150 mg kg^{-1} and 90 min later amphetamine (DMDC + A). b. The animals received i.p. either amphetamine (A) 3·5 mg kg^{-1} or disulfiram 150 mg kg^{-1} and 90 min later amphetamine (DS + A), The measurements were repeated in the same animals at intervals indicated on the abscissa. The mean value ± s.e. and the significance of differences from the corresponding amphetamine group calculated by Student's *t*-test are given, $*P<0{\cdot}05$. Open and black columns as Fig. 1; stippled columns = duration of stereotyped movements.

nolactone pretreatment (6 times 100 mg kg^{-1}) shortened the barbiturate sleeping time, while DDC partially antagonized this effect.

The plasma content of pentobarbitone in male mice (Table 2) was estimated 45 min after pentobarbitone administration (60 mg kg^{-1}, i.p.). DDC administered 24 h and 90 min before the barbiturate did not change the pentobarbitone plasma content in the still sleeping mice. The pentobarbitone plasma content in mice treated with spironolactone was half that of the controls. DDC pretreatment completely eliminated this effect of spironolactone.

Table 1. *Effect of diethyldithiocarbamate and spironolactone on the pentobarbitone-induced sleeping time of male mice.* Diethyldithiocarbamate (150 mg kg^{-1}) was injected i.p. 60 min before pentobarbitone (60 mg kg^{-1}, i.p.) and spironolactone was administered orally (100 mg kg^{-1}) twice daily, for 3 successive days before pentobarbitone.

Pretreatment	Saline	Diethyldithiocarbamate	Spironolactone	Spironolactone + diethyldithiocarbamate
sleeping time (min)	67·8 (63·7–72·2)	115·0** (113·6–116·5)	13·1** (10·1–16·8)	40·6*† (32·7–50·–)

The geometric mean value ± s,e, are given
Differs from saline group, $*P<0.05$, $**P<0.01$
Differs from spironolactone group, $\dagger P<0.01$

Table 2. *Effect of diethyldithiocarbamate and spironolactone on the content of pentobarbitone in plasma of male mice.* Pentobarbitone (60 mg kg^{-1}) was administered i.p., and its plasma content estimated 45 min later. Diethyldithiocarbamate (200 mg kg^{-1}) was injected i.p. 24 h and 90 min before pentobarbitone, and spironolactone (100 mg kg^{-1}) was administered orally twice daily for 3 successive days before the pentobarbitone administration.

Pretreatment	Saline	Diethyldithiocarbamate	Spironolactone	Spironolactone + diethyldithiocarbamate
$\mu g\ g^{-1}$	47·5±2·6	53·0±11·6	22·5±6·0**†	53·7±4·3

The mean value ± s.e. are given
Differs from saline group, $**P<0.01$
Differs from spironolactone + diethyldithiocarbamate group, $\dagger P<0.01$.

Additionally we followed the plasma pentobarbitone content 70 min after pentobarbitone administration (60 mg kg^{-1}, i.p.) in female mice, which are considered to metabolize the drugs less rapidly (Gillete, 1971). In this experiment the plasma content in DDC pretreated female mice corresponded to 37·8 ± 11·2 $\mu g\ g^{-1}$ while in the non-pretreated awakened controls to 7·9 ± 2·8 $\mu g\ g^{-1}$ ($P<0.05$).

We conclude from our experiments that dithiocarbamates have a depressive action on exploratory activity of rats. So far our results correspond to those of Krantz & Seiden (1968), Moore (1969), Kleinrok, Zebrowska & Wielosz (1970) and Maj, Sowińska & Baran (1971), and reflect the sedative-hypnotic action of the carbamate derivative, urethane.

We have demonstrated for both DMDC and disulfiram here, and for DDC previously (Mayer & Eybl, 1971), that in the animals stimulated by amphetamine, dithiocarbamates enhance stereotyped behaviour but at the same time inhibit coordinated locomotory activity. Our results suggest amphetamine effects a pass from a hypermotility phase affecting both components of exploratory activity to a phase of small stereotyped movements and the absence of large ones.

The elevated dopamine content in brain produced by the inhibitory effect of dithiocarbamate on dopamine-β-hydroxylase is believed to be the reason for the potentiation of amphetamine stereotypies (Randrup Munkvad, 1967; D'Encarnacao, D'Encarnacao & Tapp, 1969). FLA 63 (an inhibitor of dopamine-β-hydroxylase), potentiates this behaviour, especially after reserpine pretreatment (Corrodi, Fuxe & others, 1970). Inhibition of this enzyme by dithiocarbamates may be due to chelation

of copper (Friedman & Kaufman, 1963). Moreover, the copper-DDC complex penetrates into the cns more readily than the copper ion itself which results in an accumulation of copper in the cns (Koutenský, Eybl & others, 1971; Iwata, Watanabe & others, 1970). This is probably the reason why DDC potentiates morphine analgesia (Iwata, Watanabe & Matsui, 1970). and why it increases the toxicity of $CuCl_2$ (Koutenský, Eybl & others, 1971).

A direct action of amphetamine on the 5-HT receptors has been reported (Stein & Wise, 1970), however, dithiocarbamates did not significantly influence the 5-HT level of brain (Maj, Grabowska & Kwiek, 1970).

Our experiments show that DDC slows the disappearance of pentobarbitone from plasma and abolishes the decrease of pentobarbitone in plasma caused by spironolactone. The stimulation of barbiturate metabolism by spironolactone has been reported (Solymoss, Krajny & others, 1970; Gerald & Feller, 1970; Feller & Gerald, 1971), on the contrary, DDC inhibited barbiturate metabolism.

SKF 525 A, a drug metabolism inhibitor, potentiates both kinds of methamphetamine-induced hyperactivity, differentiated as the amphetamine stereotypies and total hypermotility (Babbini, Montanaro & others, 1971). Hence, the amphetamine-potentiating effect of dithiocarbamates could be due to inhibition of the biotransformation of amphetamine.

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