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EFFECTS OF DISULFIRAM AND DIETHYLDITHIOCARBAMATE ON SPONTANEOUS LOCOMOTOR ACTIVITY AND BRAIN CATECHOLAMINE LEVELS IN MICE*

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Abstract—Intraperitoneal (i.p.) injections of diethyldithiocarbamate (DDC) or disulfiram (DS), both of which inhibit dopamine-β-hydroxylase, did not alter brain levels of dopamine but caused a dose-dependent reduction of both the brain content of norepinephrine and the spontaneous locomotor activity of mice. These latter effects were not causally related, since pretreatment with a monoamine oxidase inhibitor prevented the DDC- and DS-induced depletion of norepinephrine but did not alter the ability of these drugs to depress spontaneous locomotor activity. Exposure of the mice to a 4° environment did not alter DS-induced depletion of brain norepinephrine or behavioral depression. When administered in the diet, DS reduced the brain content of norepinephrine but did not depress motor activity; DDC did not alter either parameter. The results indicate that the central depressant effects of DDC and DS are not exclusively due to the ability of these drugs to alter the absolute levels of brain catecholamines.

VARIOUS drugs have been utilized in an effort to elucidate a functional role for catecholamines in the central nervous system. Studies with reserpine initially suggested that a deficiency of brain catecholamines leads to behavioral depression.[1] However, since reserpinealters steady state brain levels of norepinephrine, dopamine, histamine[2] and 5-hydroxytryptamine,[3] it is difficult to ascribe the behavioral effects of this drug exclusively to the depletion of a single amine. α-Methyltyrosine (αMT) has been utilized to study the consequences of depleting the brain of only catecholamines; this drug does not alter brain levels of 5-hydroxytryptamine.[4] αMT-induced behavioral depression appears to be causally related to the ability of this drug to depress steady state levels of brain norepinephrine or dopamine or both.[5–7] Since it blocks catecholamine biosynthesis at the tyrosine hydroxylase step,[8] αMT depletes the brain of both catecholamines. Therefore, it is not possible to associate αMT-induced behavioral effects exclusively with a depletion of norepinephrine or dopamine.

The present study was initiated in order to examine the behavioral consequences of depleting the brain of only norepinephrine. Disulfiram (DS) and its reduced metabolic product, diethyldithiocarbamate (DDC), were used for this purpose. These agents inhibit dopamine-β-hydroxylase and thereby deplete the brain of norepinephrine without lowering (in some instances actually increasing) the brain content of

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dopamine.[9-11] It will be demonstrated that the behavioral depressant effects of DS and DDC are not exclusively due to their ability to deplete the brain of norepinephrine.

METHODS

Male albino mice (Spartan Farms) weighing 25–30 g were used throughout this study. After various treatments, spontaneous locomotor activity was determined in circular actophotometer cages (Woodward Research Corp.). Two mice were placed in each cage. After a 10-min period of acclimation, motor activity was recorded for 10 min. Similarly treated mice were sacrificed by decapitation and four brains were pooled and analyzed for norepinephrine and dopamine as described by Moore and Rech.[12] DS, suspended in 1% methylcellulose, or sodium DDC (Fisher Scientific Co.), dissolved in water, was injected i.p. In the dietary study, mice (eight per cage) were placed on a ground diet (Wayne Lab-blox) containing various concentrations of αMT, DDC or DS.

All statistical tests were carried out using Student's *t*-test.

RESULTS

The time course of the effects of a single intraperitoneal injection of 200 mg/kg of DDC or DS on spontaneous locomotor activity and brain levels of dopamine and norepinephrine is shown in Fig. 1. Both compounds produced a prompt depression of

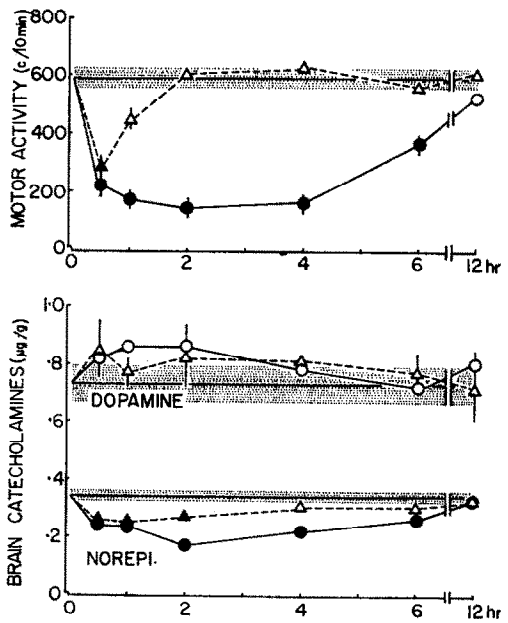

FIG. 1. Time course of effects of disulfiram and diethyldithiocarbamate on motor activity and brain catecholamines. The horizontal lines and shaded areas represent means and standard errors of values obtained from control (untreated) animals. Each point and vertical line represent the mean value ± 1 standard error as obtained from twenty-four animals injected with (○—○) disulfiram (200 mg/kg) or with (△ - - - △) diethyldithiocarbamate (200 mg/kg). Values for motor activity represent twelve determinations, two mice per determination; catecholamine values represent six determinations, four brains per determination (see Methods). Solid symbols represent values differing significantly from those of untreated animals ($P < 0{\cdot}01$).

motor activity. The effects of DDC were brief; by 1 hr the motor activity counts were not significantly different from those of untreated (control) animals. DS, on the other hand, produced a prolonged depression. These behavioral effects were reflected in the changes in the brain content of norepinephrine; DDC produced a short and DS a long-lasting reduction in the brain levels of this amine. Neither agent altered the brain content of dopamine.

Dose–effect curves for DDC and DS are depicted in Fig. 2. The points represent values obtained 30 min after the injection of DDC and 2 hr after DS. Optimum effects

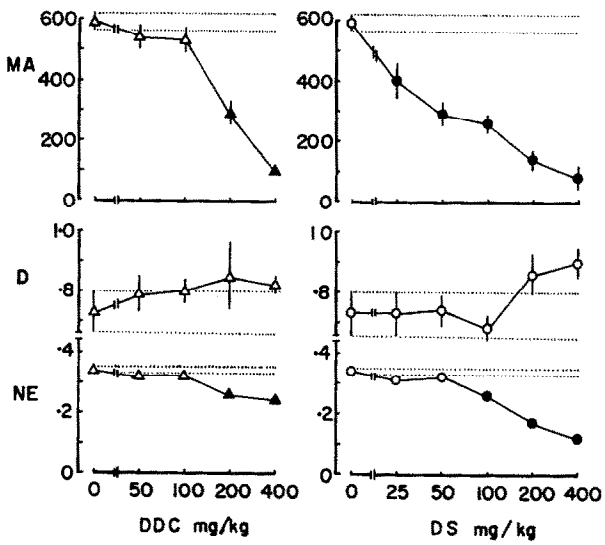

FIG. 2. Dose effects of disulfiram and diethyldithiocarbamate on motor activity and brain catecholamines. Each point and vertical line represent the mean ± 1 standard error obtained 2 hr after the injection of disulfiram (○——○) and 30 min after diethyldithiocarbamate (△——△). Where no vertical line is depicted, the standard error is less than the radius of the point. Values for spontaneous locomotor activity (MA), expressed as counts/10 min, represent twelve determinations (twenty-four animals); dopamine (D) and norepinephrine (NE), expressed as μg/g, represent six determinations (four brains per determination). Solid symbols represent those values which differ significantly from those of untreated animals ($P < 0{\cdot}01$).

were observed at these times (see Fig. 1). Although there was a tendency for the higher doses of both drugs to increase the brain content of dopamine, the effects were not significant at the 1 per cent level. There was, however, a dose-dependent depression of spontaneous locomotor activity and of brain levels of norepinephrine. After DDC, the behavioral depression roughly correlated with the depletion of norepinephrine, i.e. only at the 200 and 400 mg/kg doses were both effects significant. A similar correlation did not occur with DS. For example, 25 and 50 mg/kg of this agent significantly depressed motor activity but did not alter the brain content of norepinephrine. Significant lowering of norepinephrine levels did not occur until 100 mg/kg or higher doses of DS were administered.

Figure 3 illustrates the effects of a monoamine oxidase inhibitor (pheniprazine, JB516) on the actions of DDC and DS. Mice were divided into two groups which received i.p. injections of either saline or pheniprazine (10 mg/kg). Twenty-four hr

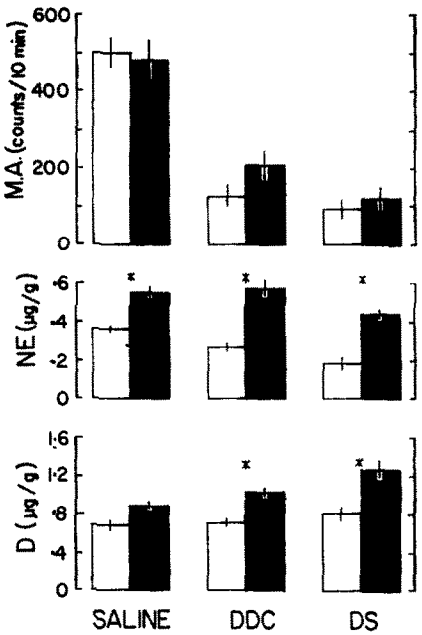

FIG. 3. Effects of pretreatment with a monoamine oxidase inhibitor on the actions of diethyldithiocarbamate and disulfiram. Twenty-four hr after the i.p. injection of saline (open bars) or 10 mg/kg of pheniprazine (solid bars), mice received a single i.p. injection of saline or 200 mg/kg of diethyldithiocarbamate (DDC) or disulfiram (DS). Spontaneous locomotor activity (MA) or brain norepinephrine (NE) or dopamine (D) was determined 30 min after DDC and 2 hr after DS. The height of each bar represents the mean, and the vertical line projected upon it represents the standard error of that mean as obtained from twenty-four mice (twelve separate determinations of MA and six determinations of NE and D). The asterisks denote those values obtained from mice pretreated with pheniprazine which are significantly different from those obtained from saline-pretreated mice ($P < 0{\cdot}01$).
□ Pretreatment with Saline ■ with JB 516.

later, mice from both groups received injections of saline, DDC or DS. Motor activity or brain catecholamine levels were determined 30 min after DDC and 2 hr after DS. In control (saline-treated) mice pheniprazine pretreatment did not alter motor activity but did elevate the brain content of norepinephrine. In saline-pretreated mice injections of both DDC and DS reduced motor activity counts and brain norepinephrine levels. Both DDC and DS significantly increased the dopamine content in brains of animals pretreated with pheniprazine. Although pheniprazine prevented DDC- and DS-induced depletion of norepinephrine, it did not alter the ability of these two agents to depress spontaneous locomotor activity.

Goldstein and Nakajima[13] reported that a cold environment enhanced the ability of DS to deplete brain stores of norepinephrine. Accordingly, an effort was made to determine if cold enhanced the DS-induced depression of spontaneous locomotor activity. Table 1 summarizes the results of one such experiment. After the i.p. injection of DS, mice were placed in individual cages in an environment of 4° or of 23°. Two or 4 hr later, motor activity and brain catecholamine levels were determined in these animals. Spontaneous locomotor activity and the brain content of catecholamines were not altered by cold exposure (control, 4°). Motor activity and brain norepine-

TABLE 1. EFFECTS OF DISULFIRAM (200 mg/kg) ON MOTOR ACTIVITY AND BRAIN CATECHOLAMINE LEVELS OF MICE MAINTAINED AT 23° AND AT 4°

Treatment	Time (hr)	Motor activity (counts/10 min)	Norepinephrine (μg/g)	Dopamine (μg/g)
Control,* 23°		603 ± 26	0·34 ± 0·01	0·73 ± 0·07
Control,* 4°	2	556 ± 32	0·34 ± 0·02	0·74 ± 0·03
	4	561 ± 30	0·33 ± 0·01	0·68 ± 0·04
Disulfiram, 23°	2	131 ± 31†	0·18 ± 0·01†	0·88 ± 0·03
	4	150 ± 49†	0·16 ± 0·01†	0·81 ± 0·04
Disulfiram, 4°	2	100 ± 18†	0·15 ± 0·01†	0·78 ± 0·02
	4	148 ± 26†	0·17 ± 0·02†	0·87 ± 0·03

* Control represents animals which received i.p. injections of the DS vehicle (1% methylcellulose). Values represent means ± 1 standard error for twelve determinations of motor activity and six determinations of brain catecholamines.
† Significantly different from control, 23° ($P < 0·01$).

phrine levels were markedly reduced after DS; there was no difference between the values obtained from mice maintained at 4° and at 23°. Several variations of this study were performed. For example, mice were placed in the cold environment for 1–6 hr prior to receiving DS, but there was no evidence of enhanced behavioral depression or catecholamine depletion in cold-exposed mice. It was noted that DS was more toxic to cold-exposed animals. For example, in a study similar to the one summarized in Table 1, one of thirty-six mice subjected to DS and cold died in 2 hr; four of thirty-six died in 4 hr. This dose of DS caused no deaths in animals maintained at room temperature.

Two hr after an i.p. injection of the insoluble suspension of DS, particles of the drug were found in the peritoneal cavity. These insoluble particles cause irritation[14, 15]

TABLE 2. EFFECTS OF A 24-hr DIET OF α-METHYLTYROSINE (αMT), DIETHYLDITHIOCARBARMATE (DDC) AND DISULFIRAM (DS)*

Diet	Control	αMT	DDC	DDC	DS	DS
% Additive	0	0·3	1	3	1	3
Food intake (g/g body wt.)	0·18 ± 0·02	0·18 ± 0·02	0·13 ± 0·01	0·11 ± 0·01†	0·10 ± 0·01†	0·05 ± 0·01†
% Change in body wt.	+ 1·5	− 0·3	− 2·2	− 3·5	− 3·5	− 8·2
Drug intake (mg/kg)		530	1320	3150	1010	1540
Motor activity (counts/10 min)	504 ± 18	212 ± 16†	490 ± 30	486 ± 30	511 ± 42	527 ± 34
Brain norepinephrine (μg/g)	0·35 ± 0·01	0·20 ± 0·01†	0·34 ± 0·01	0·34 ± 0·01	0·29 ± 0·01†	0·25 ± 0·01†
Brain dopamine (μg/g)	0·71 ± 0·05	0·42 ± 0·04†	0·75 ± 0·05	0·74 ± 0·03	0·79 ± 0·06	0·91 ± 0·04†

* Values for each diet represent the mean ± 1 standard error obtained from thirty-two mice; sixteen separate determinations of motor activity, eight determinations of norepinephrine and dopamine, and 4 determinations of each of the remaining parameters measured.
† Those values that are significantly different from control diet ($P < 0·01$).

and undoubtedly influence the behavior of mice. In order to avoid this complication, a study utilizing the dietary administration of DDC and DS was undertaken. Table 2 summarizes the results of this study. αMT, which has previously been shown to depress motor activity and brain catecholamine levels when administered in the diet,[16, 17] is included for comparison. The results in Table 2 confirm these effects of αMT. Neither DDC nor DS, as 1 per cent and 3 per cent additions to the diet, influenced spontaneous locomotor activity. DDC did not alter brain amine levels, whereas DS significantly reduced the brain content of norepinephrine and, at the higher concentration, increased the dopamine content. The reduction of food intake and body weight seen with the high concentration of both DDC and DS prohibited the addition of more drug to the diet. Nevertheless, with the concentrations used, large amounts of the drugs were consumed. Therefore, in contrast to the effects with αMT, marked changes in motor activity cannot reasonably be obtained by administering DDC or DS in the diet.

DISCUSSION

A single injection of DS into mice caused a prolonged reduction of brain norepinephrine levels; dopamine levels were not significantly altered. These results are in accord with previous reports on the effects of this drug in rats.[9, 18, 19] On the other hand, the administration of DDC produced only a brief reduction in the brain content of norepinephrine. The short duration of action of DDC was not unexpected, since Stromme[15] reported that this compound is very rapidly metabolized. DS is reduced *in vivo* to DDC and as such is also quickly metabolized. However, since DS is injected as an insoluble suspension, it remains as a depot in the peritoneal cavity and thereby provides a continuous and prolonged source of the active metabolite, DDC.

The central depressant effects of DDC and DS have been noted previously but not quantified,[20, 21] although Krantz and Seiden[22] recently demonstrated that DDC disrupts conditioned avoidance performance in rats. Gjessing[23] noted that DS did not alter the psychic state, but caused some "tiredness" in humans. In the present study, both DDC and DS caused a dose-dependent depression of spontaneous locomotor activity. Motor activity and brain content of norepinephrine fell in a parallel fashion after the administration of DDC. A similar correlation did not occur after DS. That is, low doses of DS caused significant lowering of motor activity without altering the steady state levels of brain norepinephrine. In an effort to relate DDC- and DS-induced depression of spontaneous locomotor activity with the depletion of brain norepinephrine, studies were initiated utilizing monoamine oxidase inhibitor pretreatments and cold exposure.

It was previously demonstrated that pretreatment with monoamine oxidase inhibitors reduced both the behavioral depressant and norepinephrine-depleting actions of αMT in rats[12] and mice.[17] Results of the present study indicate that a similar correlation does not occur with DDC or DS. That is, pretreatment with a monoamine oxidase inhibitor blocked DDC- and DS-induced depletion of norepinephrine but did not alter the drug-induced central depression. Carlsson *et al.*[20] also reported that a monoamine oxidase inhibitor (nialamide) did not reverse DDC-induced central nervous system depression, although quantitative data were not presented.

It should be noted that both DDC and DS significantly elevated the brain content of dopamine only in animals pretreated with a monoamine oxidase inhibitor. The lack of accumulation of large amounts of dopamine in the brain after the inhibition of dopamine-β-hydroxylase is probably due to the metabolism of this amine by monoamine oxidase (see also reference 19).

Goldstein and Nakajima[13] reported that cold exposure enhanced the norepinephrine-depleting action of DS in rats. Based upon "visual observations," these authors also reported[21] that DS-induced sedation is much more pronounced in animals exposed to cold. They also state that the animals show signs of "loss of balance". In the present study these gross symptoms were observed in a few cold-exposed, DS-treated animals just prior to death. It would appear therefore that these extreme effects are secondary to the toxicity of DS. The few animals which exhibited these symptoms were not included in the present results. Despite a variety of experimental designs, we were unable to demonstrate any alteration in DS-induced depletion of brain norepinephrine or behavioral depression in mice which were subjected to a cold environment. Differences in species or procedures (for example, Goldstein and Nakajima[13] used adrenalectomized and shaved rats) may explain the negative results obtained in the present study. In addition, there are marked differences in the responses of various strains of rats to cold; in some strains cold exposure does not enhance DS-induced depletion of brain norepinephrine (B. Bhagat, personal communication).

A study utilizing the dietary administration of DDC and DS was initiated in order to avoid complications that might arise from peritoneal irritation resulting from the parenteral administration of these drugs. A 24-hr diet of DDC did not alter spontaneous locomotor activity or the brain content of catecholamines. The lack of effect of DDC was expected, since it is acid labile and as such is rapidly destroyed after oral administration.[15] DS is more acid stable and, although it significantly reduced brain levels of norepinephrine, it was without effect on motor activity. It should be noted that after the injection of 100 mg/kg of DS (Fig. 2), the brain content of norepinephrine was reduced to values that were equivalent to those observed after the 24-hr diet of 3 per cent DS (Table 2). However, only animals receiving the i.p. injection of DS exhibited a significant reduction of motor activity. Peritoneal irritation may be one factor in the observed reduction of spontaneous locomotor activity, but it is not an exclusive one because Carlsson *et al.*[20] noted central depression after subcutaneous injections of DDC.

The results of this study suggest that the behavioral effects of DDC and DS are not directly related to the ability of these drugs to alter steady state levels of brain catecholamines. This does not mean that the depletion of brain norepinephrine does does not play some role in the central effects of these drugs. It has been demonstrated that DS-induced depression of shuttle box avoidance performances of rats is enhanced by pretreatment with reserpine. However, the time course of the enhanced effects is not related temporally to the reduced brain levels of catecholamines (K. E. Moore and R. H. Rech, unpublished observations). The present investigation demonstrates that the central depressant effects of DDC and DS are not exclusively related to the ability of these drugs to alter the absolute level of brain catecholamines and that some other mechanism must be sought to explain the behavioral effects of these drugs.

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