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ENHANCEMENT OF DRUG-INDUCED MOTOR ACTIVITY BY AN INHIBITOR OF PHENYLETHANOLAMINE-*N*-METHYLTRANSFERASE

RICHARD J. KATZ, BERNARD J. CARROLL and LAURA LEIBLER

Mental Health Research Institute, Department of Psychiatry, University of Michigan, Ann Arbor, Mich 48109 (U.S.A.)

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SUMMARY

The behavioral effects of DCMB (2,3-dichloro-α-methylbenzylamine, Lilly), an inhibitor of brain phenylethanolamine-*N*-methyltransferase (PNMT; EC 2.1.1.28), were studied in rats and mice. The drug decreased spontaneous motor activity and induced stereotypic gnawing in both species. The motor activity response of rats to *d*-amphetamine was enhanced after DCMB, and unusual stereotypic head bobbing developed. The stereotypic motor responses of mice to morphine were increased after DCMB. These findings suggest that adrenaline neurons (which project to the extrapyramidal system) are involved in normal motor activity and in the suppression of some forms of stereotypy.

The syndromes of motor hyperactivity caused by drugs have been studied extensively in rodents. A critical role for the catecholamines, especially noradrenaline (NA) and dopamine (DA), has been demonstrated in the effect of *d*-amphetamine on rats [10,11] and in the action of morphine on mice [1,5]. In each case motor activation was decreased or eliminated by pretreatment with α-methylparatyrosine, which inhibited the synthesis of catecholamines [1,5,10,11]. Since it has been shown that there are neurons in the brain stem which contain another catecholamine, adrenaline (A), as the transmitter [3,4,7], the question of their possible involvement in these motor responses needs to be examined. We have studied the effects of inhibiting the synthesis of adrenaline in brain without affecting the synthesis of NA or DA. The inhibitor of phenylethanolamine-*N*-methyl transferase (PNMT) used for this purpose was DCMB (2,3-chloro-α-methylbenzylamine: 3, 6, 7).

MATERIALS AND METHODS

Thirty adult male Sprague—Dawley rats (300—480 g) and 200 adult male

Swiss-Webster mice (25—35 g) (Charles River, Portage, Mich) were maintained upon ad libitum food and water, and day—night cycles of 12 h. Some animals were tested more than once, with at least 7 days intervening between tests.

Motor activity was measured on field sensitive monitors (Stoelting, Chic.). Four monitoring platforms were used simultaneously and were calibrated to within 5% sensitivity of each other on a selective mode for the registration of gross body movements. The assignment of platforms to treatment groups was rotated systematically throughout the series of tests. Rats were tested individually in polypropylene containers 48 × 27 × 20 cm (Scientific Products, Series 140). Mice were tested in groups of 5 in smaller containers (36 × 33 × 17 cm; Scientific Products, Series 50). Fresh pine chip bedding was added to cover the floor of each container.

Automated data recording was supplemented by visual inspection of amphetamine injected animals at 1, 3 and 7 h. All subjects were observed at 1 and 3 h and a total of 28 subjects were observed at 7 h. Observations included a representative sample from all conditions. Observations were also made of all morphine treated animals at 20 and 60 min and of 24 cages of subjects at 180 min. Informal observations of all conditions were also made throughout the experiment.

Motor activation was induced in rats by d-amphetamine sulphate 2 mg/kg and in mice by morphine sulphate 50 mg/kg. These doses were selected to produce moderate but not maximal activity [1,10]. DCMB or vehicle (0.9% saline) was injected 3 h before *d*-amphetamine (rats) and 1 h before morphine (mice). The doses of DCMB were 11, 22 and 44 mg/kg. The highest dose (44 mg/kg) was found to cause 90% inhibition of rat brain PNMT activity within 1 h [6]. In our laboratory this dose of DCMB produced a substantial but somewhat lower degree of inhibition (≈60%) (Turner et al., unpublished observation). An additional control group received saline pretreatment and a second saline injection instead of psychostimulant administration. All drugs were injected intraperitoneally with injection volumes of 1 ml/kg (rats) or 10 ml/kg (mice). Drugs were prepared within 1 h of use. *d*-Amphetamine was injected at 6:00 pm. Morphine was given between 10:00 am and 2:00 pm.

During each test 2 recording platforms were used for animals pretreated with DCMB and 2 for control animals. At least 8 tests were conducted to obtain each data point. For each test at least one recording platform was assigned to animals receiving saline pretreatment followed by *d*-amphetamine sulphate 2 mg/kg (rats) or morphine sulphate 50 mg/kg (mice). The activity of this group was used as the reference against which the activity of the other groups tested concurrently was compared. The raw activity scores were transformed to percentages of the reference group in order to reduce the large variation between days. Statistical comparisons were performed by Friedman's analysis of variance [8] using the percentage-transformed activity scores.

RESULTS

DCMB did not produce motor activation by itself. Both mice and rats which

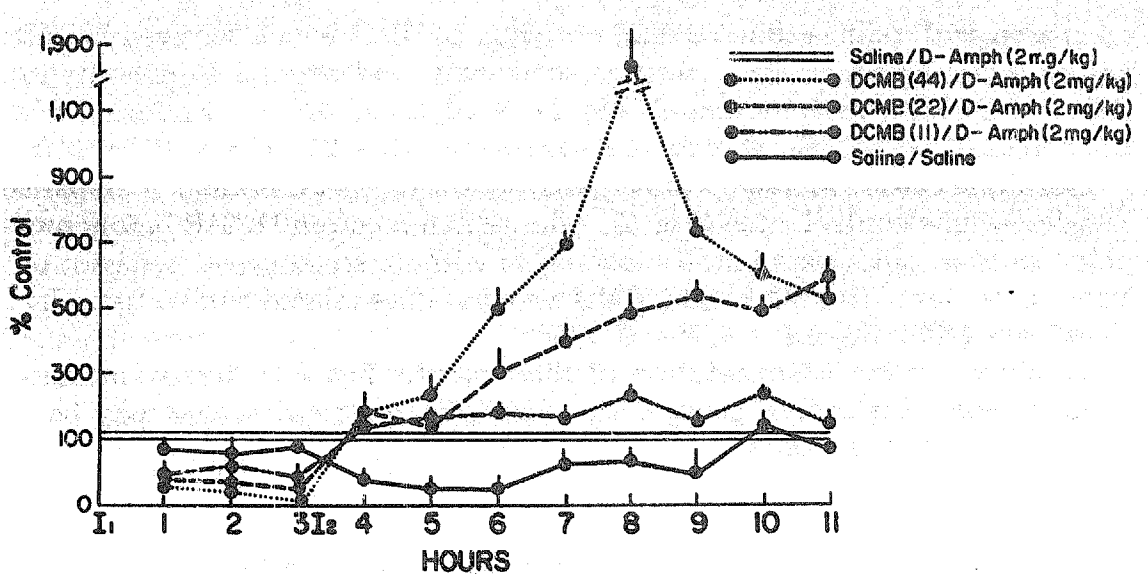

Fig. 1. Effect of pretreatment with DCMB (11—44 mg/kg) on motor activation of rats following d-amphetamine. Percentage-Transformed Scores + % Standard errors. I_1, Saline or DCMB; I_2, Saline or Amphetamine.

received pretreatment with DCMB were less active than saline-pretreated control animals. Gnawing of wood chips on the cage floor was seen after DCMB injection in both rats and mice but not in saline-treated animals. Mice treated with DCMB-morphine frequently showed a hunched back posture while running and rats treated with DCMB-amphetamine showed abnormal behaviors in the form of slow head bobbing (40—60 times/min) and intermittent 'frozen' posturing. Head bobbing was not registered on the sensors.

d-Amphetamine caused increased motor activity for at least 4 hours when

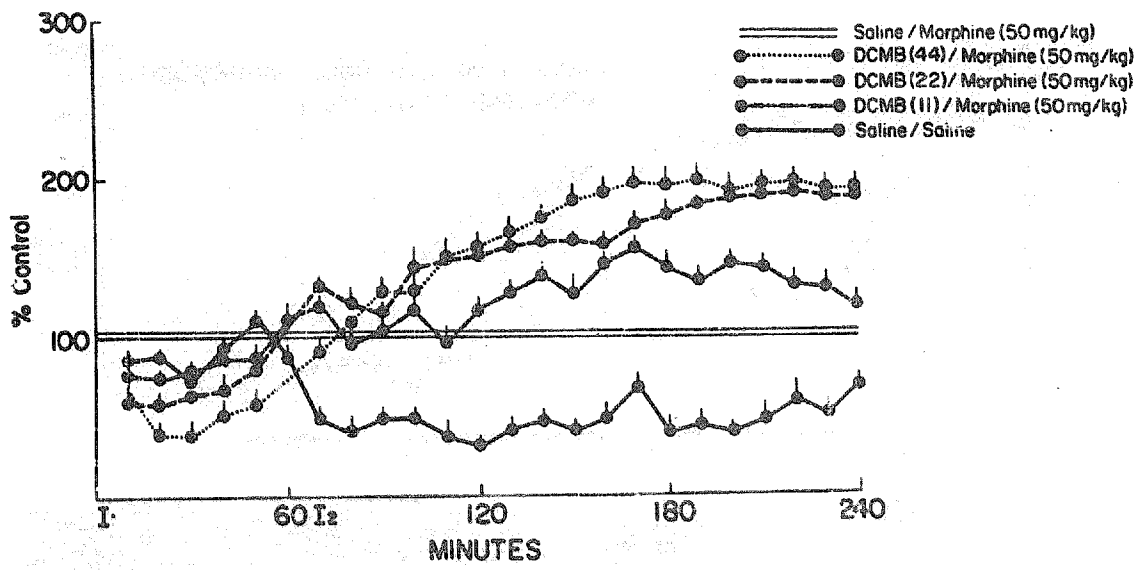

Fig. 2. Effect of pretreatment with DCMB (11—44 mg/kg) on motor activation of mice following morphine. Percentage-Transformed Scores + % Standard errors. I_1, Saline or DCMB; I_2, Saline or Morphine.

compared with saline-saline control rats (Fig. 1). Rats which received DCMB before d-amphetamine showed increased scores, and beyond 3—4 hours this effect of DCMB was dose-related (Fig. 1). Analysis of variance indicated that this effect of DCMB was statistically significant ($X_r^2 = 21$; df = 4; $P < 0.01$).

Morphine caused increased motor activity for at least 3 hours in comparison to saline-saline control mice (Fig. 2). Mice which received DCMB before morphine showed potentiated motor activation with no stereotyped behavior or bobbing evident. This effect of DCMB also was dose-related and statistically significant ($X_r^2 = 38$; d.f. = 4, $P < 0.01$).

To allow further interpretation of these results Fig. 3 indicates untransformed scores for critical groups, all other untransformed scores may be calculated from these data.

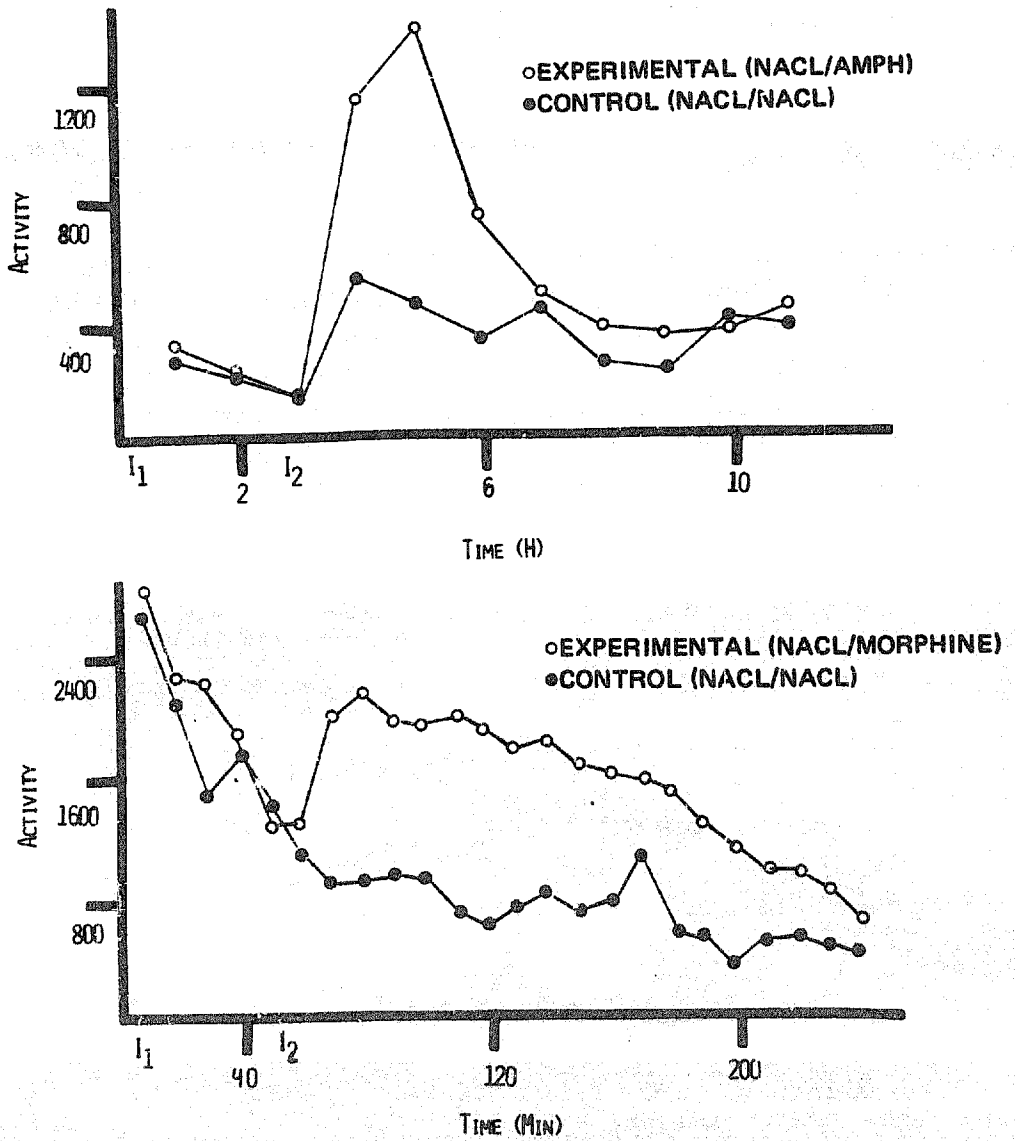

Fig. 3. Untransformed scores for 4 critical groups. I_1, Saline; I_2, Saline or Amphetamine or Morphine.

DISCUSSION

The inhibitor of PNMT did not produce activation per se, and, in fact, appeared to lower ambient locomotor activity. In other recent comparative studies of several PNMT inhibitors (Katz, Roth, Carroll and Turner in preparation) we have seen further evidence of decreased activity in an open field situation. The present data and our other observations suggest a possible excitatory contribution by adrenergic neurons to motor activity.

The interactions of DCMB and motor stimulants, however, point to an enhanced behavioral activation after adrenergic inhibition. Looking first to the effects of DCMB upon amphetamine, we have observed a long lasting and dose-related increase in behavioral stimulation after this drug combination, in comparison with amphetamine alone. Observations of both amphetamine and DCMB-amphetamine syndromes indicated qualitative differences in activity, with the DCMB-amphetamine group showing extensive head bobbing in addition to motor activity. The present results resemble the effects of the dopamine-β-hydroxylase (DBH) inhibitor FLA63 upon amphetamine syndromes [2]. Pretreatment with this compound also increased certain forms of stereotypy (sniffing, licking, biting). Since the inhibition of DBH necessarily reduced functional adrenaline stores, both findings may represent a common neurochemical mechanism, with adrenaline possibly inhibiting the normal occurrence of stereotypy. It should be noted, however, that the stereotypy observed in our experiment was rather specific in nature, i.e., no oral-buccal stereotypy was observed after DCMB-amphetamine, nor were any repetitive movements of the extremities. The head bobbing was regular and stereotypic, and did not resemble exploratory sniffing behavior. DCMB also enhanced the sterotypic running caused by morphine in mice. With regard to all these effects of DCMB it should be noted that moderate PNMT activity has been found in substantia nigra, globus pallidus, caudate nucleus and putamen [7]. Our results suggest that this adrenergic input to the extrapyramidal system could be important for the inhibition of stereotypic behaviors.

It might be questioned whether DCMB exerted a physiological or pharmacological effect upon behavior, i.e., whether DCMB may have altered drug disposition and metabolism, rather than directly affecting brain amine systems. A number of factors argue against this, however. On the one hand, increased activity was found in two species with two dissimilar drugs. If there were some interference with drug metabolism, it would have to occur for both opiates and sympathomimetics, which are inactivated through different routes. Secondly, the motor syndromes induced by amphetamine and DCMB followed by amphetamine showed clear behavioral differences. Finally, we have recently observed similar facilitation effects upon behavior by DCMB given prior to L-DOPA. Since DOPA is an amine precursor, this further renders a physiological mode of action likely.

Our results suggest an involvement of adrenergic systems both in locomotion and stereotypy. Much work is needed to clarify the anatomy and physiology of

this involvement, but these results do indicate behavioral functions for central adrenergic systems.

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