Adrenergic Control of Motor Activity: Effects of PNMT Inhibition Upon Open Field Behavior in the Rat

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KATZ, R. J., B. B. TURNER, K. ROTH AND B. J. CARROLL. *Adrenergic control of motor activity: Effects of PNMT inhibition upon open field behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 9(4) 417–420, 1978.—Two inhibitors of brain PNMT were given to rats in doses which caused equivalent reductions in enzyme activity. Each drug produced similar and dose-related decreases in ambulation, rearing and defecation during open field behavioral testing. Central adrenergic (epinephrine containing) systems may be necessary for normal open field behavior. This may reflect an interaction with other monoamines, particularly dopamine.

Catecholamines   Dopamine   DCMB   Defecation   Epinephrine   Motor activity   Open field
PNMT   Psychomotor   Rearing   SKF64139

THE CATECHOLAMINES (CA), norepinephrine (NE) and dopamine (DA) are known to play critical roles in motor integration and psychomotor activation. For example, l-DOPA (l-dihydroxyphenylalanine), the precursor to CA, produces a syndrome of motor activation after systemic injection [32] and central stimulation of NE and DA postsynaptic receptors increases motor activity [3, 13, 15, 16, 21, 23, 29, 34, 35]. CA depleting agents have an opposite (i.e., motor inhibitory) effect [1, 31, 34, 35].

Several reports have suggested the existence of a third functional CA neurotransmitter in the CNS. The biosynthetic enzyme for epinephrine (E) has been localized in hindbrain cell bodies [10, 19, 27] and both cell bodies and terminal varicosities have been visualized through immunohistofluorescent microscopy [10,14]. Also, epinephrine itself has been demonstrated centrally [2]. Relatively few studies have specifically addressed the role of E in motor activity.

Several studies dating from the early part of the century reported sedation, depression, and stupor after central E administration (summarized in [22]). However, these studies invariably employed physiologically supranormal amounts of transmitter; more recent studies using lower doses and improved infusion techniques suggest E neurons may be motor excitatory [5, 12, 23], note however [11]. If E neurons are necessary for motor activity, it follows that inhibition of E synthesis should cause motor depression. In a preliminary communication we reported that inhibitors of PNMT, the synthesizing enzyme for E, produced an acute lowering of spontaneous motor activity [17]. The present experiments extend these observations by testing two inhibitors of brain PNMT for their effects on open field behavior.

METHOD

Animals

Ninety adult male Sprague-Dawley rats (200–275 g) (Charles River, Portage, MI) randomly chosen from our colony were used for behavioral testing. An additional 23 rats were used for biochemical determinations. All animals were maintained upon ad lib food (Teklad 4.0% fat diet S-0836) and water, and automatically programmed 12 hr light/12 hr dark cycles.

Apparatus

Animals were tested in a 1.22 m² white Plexiglas field containing sixteen 30.5 cm² squares for the assessment of locomotion. The open field was washed between tests. A background masking noise of approximately 20 dB was provided by an air circulation system. Two 75 W fluorescent lights located 3 m from the apparatus provided a difuse low level of illumination.

Drugs

Lilly DCMB [7] (2,3-dichloro-α-methylbenzylamine), 0, 12.5 and 25.0 mg/kg and SKF 64139 [18] (7,8-dichloro, 1, 2, 3, 4-tetrahydroisoquinoline HCl) 0, 10 and 40 mg/kg were injected intraperitoneally (1 ml/kg) in a 0.9% sodium chloride vehicle solution 1 hr prior to testing or biochemical assay. DCMB is known to lower central epinephrine without affecting other catecholamines [8]. SKF64139 also has this effect and in other tests has been shown to cause slight increases in serotonin only at doses considerably in excess of those em-

ployed in the present design [18]. Other studies have shown the latter to be free of effects upon monoamine oxidase or catechol-o-methyltransferase at the doses presently employed [18]. It should be noted that based upon studies in our laboratory of the dose response relationship of these compounds (Turner *et al.*, in preparation), the low and high doses of each drug were chosen to produce equivalent enzyme inhibition across compounds.

Behavioral Procedure

One hour after initial injection of drug or vehicle animals were individually placed in the open field. Except for the actual transportation of animals from their cages to the open field, this was the only handling they experienced during the experiment. Grid crossings, rearings, and defecation were recorded for 4 consecutive 3 min periods. All animals from all conditions were tested on more than 1 occasion and received each treatment. However, a minimum of 48 hr intervened between any repeated tests. Repeated testing produced (equivalent) lowering of initial activity across conditions.

Biochemical Procedure

At 1 hr animals matched for drug treatment, weight, and supplier with behaviorally tested animals were sacrificed by decapitation. Brains were removed and dissected upon a chilled glass plate. A 5×8 mm rectangular punch was used to standardize dissection of the pons-medulla which includes the C_1 and C_2 adrenergic nuclei as defined by Hokfelt *et al.* [14]. Brain tissue was immediately frozen in liquid nitrogen and stored at −30°C prior to assay. PNMT was assayed according to the procedure of Pendleton [24] with several modifications including the use of ^{3}H-methyl-S-adenosylmethionine (New England Nuclear Corp.) as the methyl donor together with unlabelled carrier and the elimination of acid extraction of the labelled product. Triplicate determinations were performed on all samples. Proteins were measured by the method of Lowry [20]. Additional details of the assay procedure have been published elsewhere [33].

Statistics

All comparisons were by Analysis of Variance. All results are presented as mean and standard error. Analysis of variance was by a two-factor mixed design ANOVA with repeated measures on one factor [6]. Factors analyzed were dosage and time, and the BMOD2Y program for analysis of variance of the health sciences computing facility of the University of California was used for the actual analyses. Individual analyses were performed for each behavior. Also, randomized design ANOVA was used for defecation scores.

RESULTS

Biochemical results. The PNMT inhibitors each reduced central enzyme activity in a dose related manner. DCMB (12.5 mg/kg) and SKF 64139 (10 mg/kg) produced 48% and 47% inhibition in comparison to control animals and DCMB (25.0 mg/kg) and 64139 (40 mg/kg) produced 60 and 62% inhibition in comparison to control animals. Actual brainstem activity for control animals was 37.0 ± 3 picomoles/1 mg protein/hr. All other actual values may be calculated from this.

Behavioral results. Following injection informal observations of animals over the 1 hr preceding behavioral testing indicated virtually all (≈80%) experimental animals engaged in vigorous stereotyped sniffing and gnawing of the wood bedding of their cages. Few (< 10%) control animals showed this behavior, and no other behavioral changes were obvious in the observation period. This change in behavior has been observed previously [17]. In the present design drug treated animals continued to show high levels of sniffing during experimental observations. Gnawing and biting and repetitive motor activity were not observed however it must be noted that the open field was a flat surface, and typically lacked objects with which these behaviors could be expressed.

Effects of these drugs upon motor activity (grid crossings), rearing, and defecation are presented in Figs. 1, 2 and 3. In Fig. 1, a dose related decrease in ambulation was found for both compounds. Effects of both drugs and session interval were significant, $F_{(4,85)}$ drug−14.1, p 0.001; $F_{(3,255)}$ session interval -13.25, p 0.001, while there was no significant interaction between these variables, $F_{(12,255)}$ 1.6, p 0.1. Moreover, equivalent amounts of PNMT inhibition produced similar behavioral effects. Figure 2 presents similar findings for rearing. Again, the drug effects were dose related, and roughly correlated behaviorally for biochemically equivalent effects. Both main effects were again significant, $F_{(4,85)}$ drug−9.81, p 0.001; $F_{(3,225)}$ session interval−11.1, p 0.001. Again, no interaction was present, $F_{(12,255)} - 1.0$, $p = 0.4$. Figure 3 presents defecation scores. Both drugs caused a dose-related decrease in defecation. Despite equivalent degrees of PNMT inhibition, DCMB had a more powerful effect on defecation. The apparent correlation of inhibition and dependent variable was therefore less clear in this last case, $F_{(4,85)}$ drug−5.92, p 0.005.

DISCUSSION

Relatively few studies have specifically addressed either the role of epinephrine or of inhibitors of PNMT upon behavior. Nonetheless, our results are consistent with results obtained from central injection of epinephrine in the mouse
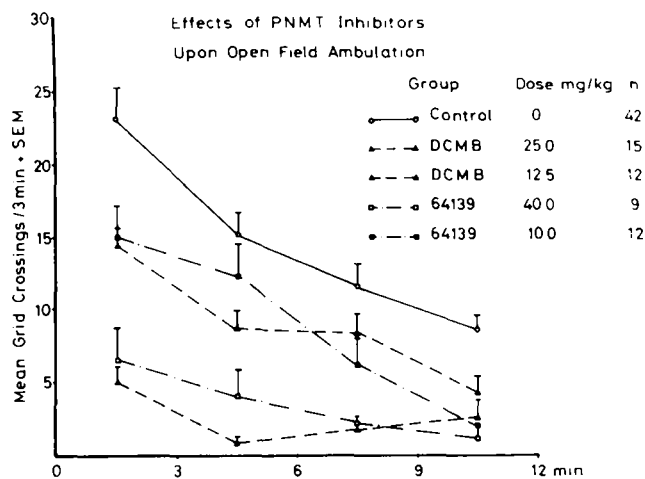

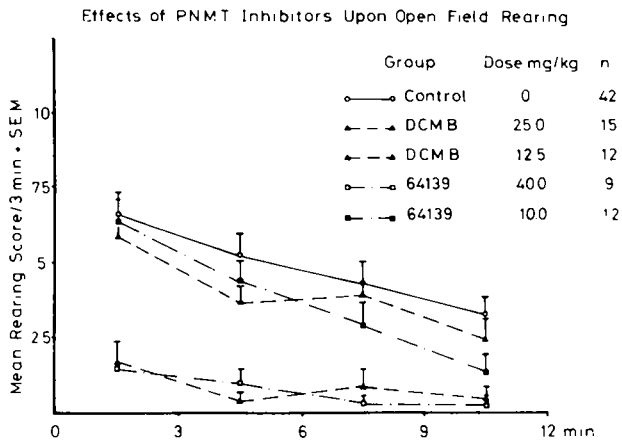FIG. 1. SKF 64139 and Lilly DCMB reductions in open field behavior (ambulation). All data as mean ± standard error.

Effects of PNMT Inhibitors Upon Open Field Rearing

Group	Dose mg/kg	n
Control	0	42
DCMB	25.0	15
DCMB	12.5	12
64139	40.0	9
64139	10.0	12

FIG. 2. SKF 64139 and Lilly DCMB reductions in open field behavior (rearing). All data as mean and standard error.

Effects of PNMT Inhibitiors
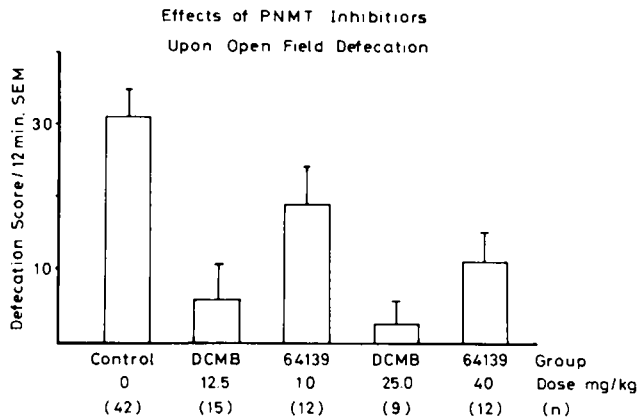Upon Open Field Defecation

Control	DCMB	64139	DCMB	64139	Group
0	12.5	1.0	25.0	40	Dose mg/kg
(42)	(15)	(12)	(9)	(12)	(n)

FIG. 3. SKF 64139 and Lilly DCMB reductions in open field behavior (defecation). All data as mean and standard error.

stereotypy. Clearly an assessment of drug effects upon activity must carefully account for the testing paradigm employed, and in the present circumstances we emphasize two separate sets of behavioral observations.

The similarity of behavioral changes after different drug treatments which produced similar changes in brain PNMT further supports this system as the mediator of the two observed behavioral syndromes. It would appear from our results that adrenergic systems do participate in the control of motor activity. This might also be expected in part from studies reporting moderate PNMT concentrations in areas associated with motor control, e.g., within the substantia nigra and basal ganglia [19]. Low but measurable amounts of enzyme have also been reported in other CNS areas associated with motor control, e.g., in the cerebellum and red nucleus of several primate species, including man [19].

We feel that two interpretations of the changes in open field behavior are possible. By the first, the action of E is direct, e.g., as a necessary component of a final common path for behavioral expression. The second interpretation assigns a modulatory role to E, i.e., E containing neurons may affect another system which in turn controls motor activity. The present findings are consistent with either interpretation. However, we feel particular attention should be paid to the latter. On the one hand previous histochemical findings do not report Epi in motor neurons. Moreover, behavioral and anatomical evidence are consistent with an indirect mode of action. We have pointed out that E, in fact, innervates DA containing areas of the extrapyramidal motor system, as well as other motor areas. Our findings suggest an increase in stereotyped sniffing and gnawing after PNMT inhibition. It is possible E governs DA systems by exerting a tonic inhibition upon them. While it is not clear that motor inhibition and stereotypy are always or necessarily inimical at a behavioral level, a number of experiments (e.g., [26,30]) suggest cases of DA mediated stereotypy may be characterized by relative decreases in ambulation and rearing. Certainly, this is consistent with our findings.

It might also be noted that PNMT inhibition facilitates other stereotyped behaviors which are DA mediated at least in part [17]. Perhaps the most direct evidence for this may be found in recent preliminary observations from our laboratory, that adrenergic inhibition facilitated DA dependent DOPA induced behaviors. While continued investigations of the neural systems involved is necessary, our results do suggest a behavioral significance for adrenergic systems and a possible relation to DA.

[12,23] as well as with our own earlier observations [17] in rats. Adrenergic stimulation has previously been shown to increase motor activity and we have shown an acute lowering of activity after adrenergic inhibition. Observations upon behavioral depression after yohimbine, a blocker of adrenergic sensitive adenyl cyclase [9,36] also support a role for epinephrine in the facilitation of motor behavior. A variety of different experiments using adrenergic stimulation and inhibition therefore suggest a facilitatory role for E in motor behavior.

Our specific findings of decreased ambulation and rearing and possibly increased stereotypy suggest that adrenergic systems normally facilitate exploration but inhibit

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