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Effects of Alpha Methyltyrosine and Parachlorophenylalanine on Open Field Behavior in Rats Given Tranylcypromine Stereoisomers and Lithium Carbonate

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SMITH, D. F. AND M. SHIMIZU. *Effects of alpha methyltyrosine and parachlorophenylalanine on open field behavior in rats given tranylcypromine stereoisomers and lithium carbonate.* PHARMAC. BIOCHEM. BEHAV. 5(5) 515–518, 1976. – Parachlorophenylalanine (PCPA) and alpha methyltyrosine (AMT) were used to study the roles of serotonin and catecholamines in hyperactivity produced by the stereoisomers of Tranylcypromine (d-Tc and l-Tc) in male Wistar rats fed a normal diet (control groups) or a diet containing lithium carbonate (lithium groups). Components of locomotor activity were measured in an open field. Lithium decreased ambulation. d-Tc increased ambulation and caused jerky side-to-side movements. PCPA and AMT prevented the effects of d-Tc on ambulation while only PCPA prevented the effects of d-Tc on movement. l-Tc increased ambulation. The effects of l-Tc on ambulation were potentiated by PCPA and prevented by AMT. Rearing was increased by l-Tc and d-Tc in rats given lithium. PCPA and AMT prevented the effects of l-Tc and d-Tc on rearing in lithium groups. The findings suggest that the roles of serotoninergic and catecholaminergic mechanisms differ for components of open field behavior in control rats and rats given lithium.

Hyperactivity Open field Tranylcypromine stereoisomers Lithium Parachlorophenylalanine Alpha methyltyrosine

THE effectiveness of lithium salts in the treatment of manic-depressive disorders has led to interest in the effects of the lithium ion on behavior and brain biochemistry in laboratory animals. Studies on behavior show a reduction in locomotor activity in rats given lithium [5, 7, 12, 18, 20]. Studies on brain biochemistry show an increase in serotonin synthesis, catecholamine uptake and monoamine oxidase activity in rats given lithium [5, 9, 12, 14, 16]. Effects on serotonin and monoamine oxidase appear to be involved in the action of lithium on locomotor activity in rats since inhibition of serotonin synthesis and monoamine oxidase antagonized the behavioral effects of lithium [18].

In contrast to its activity-suppressant effects, lithium potentiates hyperactivity in rats given Tranylcypromine (trans-dl-2-phenylcyclopropylamine sulfate) [4,8]. Studies on behavior in rats given the stereoisomers of Tranylcypromine individually showed different effects of the d form (d-Tc) and l form (l-Tc) on activity [19]; d-Tc appeared to influence primarily serotoninergic mechanisms while l-Tc seemed to affect principally catecholaminergic mechanisms. The present study was carried out to determine further the roles of serotoninergic and catecholaminergic mechanisms in the effects of d-Tc and l-Tc on behavior in control rats and in rats given lithium.

METHOD

Ninety male albino Wistar rats weighing 250–330 g at the time of tests were used. They were randomly divided into 18 equal groups and housed in groups of 5 in 60 x 40 x 20 cm transparent cages in a thermostatically controlled room (23° C) on a 12 hour light-dark cycle (lights on 8 a.m. to 8 p.m.). They had free access to tap water and to a wet mash diet [13] containing 600 mmol Na^+/kg dry weight, 700 mmol K^+/kg dry weight, and either no lithium (9 control groups) or lithium carbonate (9 lithium groups). The concentration of lithium in the diet was increased from 20 to 40 to 60 to 80 mmol/kg dry weight at 4 day intervals and then kept at 80 mmol/kg for 2–3 weeks prior to the tests. The rats were weighed periodically.

Tests were carried out between 1:15 p.m. and 3:15 p.m. in an open field according to the method described previously [20]. The rats were tested individually. Ambulation was measured by the time it took the rat to go from the center of the open field to a wall and by the number of lines crossed while in the open field for 3 min. Rearing was measured by the number of times the rat raised both forepaws simultaneously off the floor while in the open field. In addition, a record was kept of the general appearance of the rat's behavior.

All rats in a cage received the same treatment which was SC injection [5 ml/kg) of either parachlorophenylalanine methylester (PCPA) (200 mg/kg) 74 hr before tests, alpha methyltyrosine methylester (AMT) (2 x 150 mg/kg) 24 and 4 hr before tests, or sterile isotonic saline (vehicle) followed

TABLE 1

SERUM LITHIUM CONCENTRATIONS AND AMBULATION AND REARING DURING 3 MINUTE OPEN FIELD TEST IN CONTROL AND LITHIUM GROUPS TREATED WITH SALINE, *d*-Tc OR *l*-Tc AND PRETREATED WITH SALINE, PCPA OR AMT. VALUES ARE MEANS ± SEM FOR 5 RATS

Pretreatment	Treatment	Group	Serum lithium concentration mEq/l	Ambulation Time to wall sec	Ambulation No. of lines crossed	Rearing No. of Rears
Saline	Saline	Control		40 ± 11	24 ± 7	5.2 ± 1.2
		Lithium	0.80 ± 0.04	136 ± 26	14 ± 3	1.6 ± 1.0
	d-Tc	Control		56 ± 19	85 ± 37	3.2 ± 0.7
		Lithium	0.45 ± 0.04	71 ± 22	56 ± 11	8.2 ± 2.0
	l-Tc	Control		63 ± 30	25 ± 5	5.4 ± 1.9
		Lithium	0.41 ± 0.02	66 ± 31	38 ± 9	7.6 ± 2.3
PCPA	Saline	Control		53 ± 32	12 ± 2	3.6 ± 1.5
		Lithium	0.56 ± 0.08	116 ± 39	24 ± 12	2.8 ± 1.7
	d-Tc	Control		73 ± 28	12 ± 5	3.4 ± 1.7
		Lithium	0.39 ± 0.05	108 ± 38	10 ± 2	1.8 ± 1.2
	l-Tc	Control		13 ± 5	28 ± 11	4.8 ± 1.4
		Lithium	0.36 ± 0.03	17 ± 7	24 ± 4	2.2 ± 1.0
AMT	Saline	Control		79 ± 27	20 ± 4	3.0 ± 1.0
		Lithium	0.74 ± 0.10	146 ± 34	4 ± 1	0.8 ± 0.8
	d-Tc	Control		115 ± 40	22 ± 2	1.2 ± 0.8
		Lithium	0.55 ± 0.04	135 ± 26	22 ± 4	0.8 ± 0.4
	l-Tc	Control		134 ± 32	58 ± 29	1.4 ± 0.7
		Lithium	0.63 ± 0.10	170 ± 9	9 ± 2	0

by IP injection (2 ml/kg) of either d-Tc (10 mg/kg), l-Tc (10 mg/kg) or sterile isotonic saline (vehicle) 2.5 hr before tests.

After tests blood samples were drawn under ether anesthesia from the vena cava of rats in the lithium groups. The concentration of lithium in serum was determined by flame photometry [1].

Statistical analysis of the data was carried out using analysis of variance [11]. Data from open field tests were analysed with respect to groups (control and lithium), treatments (IP injection of d-Tc, l-Tc and saline) and pretreatments (SC injection of PCPA, AMT and saline).

RESULTS

A weight gain of 2–3 g/rat/day occurred in all groups. The concentration of lithium in serum in the lithium groups appears in Table 1. The serum lithium concentration in lithium groups treated with d-Tc and l-Tc were significantly lower than in lithium groups treated with saline ($p<0.01$).

The results of the open field tests and of the statistical analysis of open field data appear in Tables 1 and 2, respectively. The main effects of groups and pretreatments were significant for the time to reach a wall. Further analysis of the main effects showed that administration of lithium as well as pretreatment with AMT significantly prolonged the time it took the rats to reach a wall ($p<0.05$). The treatments × pretreatments interaction for the time to reach a wall was significant. Further analysis showed that l-Tc had opposite effects on the time to reach a wall in groups pretreated with PCPA and AMT; the time was significantly decreased by PCPA ($p<0.05$) and increased by AMT ($p<0.05$) in rats treated with l-Tc.

The main effects of treatments and of pretreatments on the number of lines crossed were significant. Further analysis of these main effects showed that treatment with d-Tc and l-Tc each significantly increased the number of lines crossed ($p<0.05$) while pretreatment with PCPA and AMT each decreased the number of lines crossed significantly ($p<0.05$). The treatments × pretreatments interaction for the number of lines crossed was significant. Further analysis showed that d-Tc significantly increased this component of ambulation in groups pretreated with saline compared to groups pretreated with PCPA and AMT ($p<0.05$).

The main effect of pretreatments on rearing was significant. Further analysis showed that AMT pretreatment significantly decreased rearing ($p<0.05$). The data also showed that rearing was increased significantly by d-Tc and l-Tc in lithium groups pretreated with saline ($p<0.05$), but not in lithium groups pretreated with PCPA and AMT.

The open field behavior of control rats was well-coordinated and silent. After about 15 sec in the center of the open field, control rats walked in a straight path from the center of the field to a wall, along the walls, and reared mainly while in corners. The general appearance of open field behavior in rats given lithium, l-Tc, PCPA and AMT resembled that seen in control rats, except that rats treated with l-Tc after PCPA pretreatment ran immediately, directly and rapidly from the center of the field to a wall at the start of the test. On the other hand, rats treated with d-Tc vocalized, had tremors, and moved with jerky side-to-side movements around in small circles with hind legs abducted in the center of the open field. Pretreatment with PCPA prevented vocalization, tremors and jerky movements in rats treated with d-Tc, while AMT pretreatment failed to prevent this type of behavior induced by d-Tc.

DISCUSSION

As in previous studies [5, 7, 12, 18, 20], lithium

TABLE 2

SUMMARY OF STATISTICAL ANALYSIS (ANOVA) ON OPEN FIELD BEHAVIOR OF CONTROL AND LITHIUM GROUPS TREATED WITH SALINE, *d*-Tc OR *l*-Tc AND PRETREATED WITH SALINE, PCPA OR AMT

Source	df_1/df_2	Ambulation: Time to wall F	Ambulation: No. of lines crossed F	Rearing: No. of rears F
Group (G)	1/76	8.36†	2.62	0.44
Treatments (T)	2/76	0.75	3.44*	0.45
Pretreatments (P)	2/76	10.80†	4.98†	13.00†
G x T	2/72	2.16	0.17	2.18
G x P	2/72	0.03	1.33	2.10
T x P	4/72	2.74*	3.96†	1.58
G x T x P	4/72	0.25	1.64	1.87

*=$p<0.05$; †=$p<0.01$.

administration decreased locomotor activity in rats. It is to be noted that none of the serum lithium levels in the present study were in the range considered to be toxic in rats [15, 21]. The most pronounced effect of lithium was on a measure of ambulation, the time it took the rats to go from the center of the open field to a wall. Previous studies have found effects of lithium on this measure of ambulation [20] as well as other measures of ambulation and on rearing [5, 7, 18, 24].

An unexpected finding in the present study was the lower serum lithium concentration in lithium groups treated with d-Tc and l-Tc. We do not know why the serum lithium concentration was lowered by d-Tc and l-Tc. There were no differences in weight gain between the groups to suggest that the differences in serum lithium concentration might be due to differences in food intake. It is also unlikely that d-Tc and l-Tc could increase urinary lithium excretion enough during the short-time that elapsed (4 hr) between administering the drugs and taking the blood samples to lower the serum lithium concentration significantly. Possibly d-Tc and l-Tc altered the distribution of lithium in the body in addition to an effect on lithium excretion.

AMT and PCPA pretreatments were used to study the roles of serotonin and catecholamines, respectively, in the effects of d-Tc and l-Tc on behavior. AMT in the dose used depletes selectively catecholamines in rat brain [8,23]. The reduction in ambulation and rearing seen in the present study in rats given AMT agrees with previous reports [2,10] and supports the notion that catecholamines play a role in these components of open field behavior [10]. PCPA in the dose used depletes selectively serotonin in rat brain [3,22]. The reduction in ambulation seen in the present study in rats given PCPA agrees with reports of increased resting behavior in rats treated with PCPA [17] and suggests that serotoninergic mechanisms play a role in ambulation in the open field.

Hyperactivity induced by l-Tc consisted in increased ambulation without symptoms of serotonin-dependent hyper- crossed during the open field test, and in jerky side-to-side and circling movements characteristic of serotonin-dependent hyperactivity [6]. Both PCPA and AMT prevented increased ambulation in rats treated with d-Tc, while only PCPA prevented the jerky side-to-side movements induced by d-Tc. These findings suggest that serotoninergic and catecholaminergic mechanisms play a role in increased ambulation induced by d-Tc, while mainly serotoninergic mechanisms influence the type of movement produced by d-Tc.

Hyperactivity induced by l-Tc consisted in increased ambulation without symptoms of serotonin-dependent hyperactivity. The effects of AMT and PCPA on ambulation in rats treated with l-Tc were less clear-cut than their effects on hyperactivity induced by d-Tc. Nevertheless, the data show that the failure of l-Tc to increase ambulation after AMT pretreatment occurred mainly in the lithium group. This suggests that catecholaminergic mechanisms might play a greater role in the effects of l-Tc on ambulation in rats given lithium than in control animals. PCPA potentiated the effects of l-Tc on ambulation measured by the time it took the rats to reach a wall. The rapidity with which PCPA-pretreated rats treated with l-Tc ran to a wall was very striking and consistent. It contrasted sharply with the behavior of PCPA-pretreated rats treated with d-TC. The reason for the opposite effects of PCPA on ambulation in rats treated with d-Tc and l-Tc is unknown. It is interesting to speculate, however, that d-Tc might affect excitatory serotoninergic pathways while l-Tc might influence inhibitory ones [17].

Rearing was increased in lithium groups by d-Tc and l-Tc. In a previous study rearing in rats given lithium was increased only by l-Tc [19]. The discrepancy between the results obtained in the two studies may be related to differences in the doses of the drugs and in the number of times each rat received a drug. In the present study a lower dose of d-Tc and l-Tc was used and each rat was treated only once in order to avoid toxic effects. AMT and PCPA prevented increased rearing induced by d-Tc and l-Tc in lithium groups. This effect of AMT and PCPA suggests that catecholaminergic as well as serotoninergic mechanisms play a role in rearing induced by d-Tc and l-Tc in rats given lithium.

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