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Effect of α-Methyltryptamine on Spontaneous Activity in Mice[1]

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RUSTERHOLZ, D. B., J. P. LONG AND D. E. NICHOLS. *Effect of α-methyltryptamine on spontaneous activity in mice*. PHARMAC. BIOCHEM. BEHAV. 10(2) 223–227, 1979.—A standard dose of 10 mg/kg (48 μmole/kg) of (±)-α-methyltryptamine (AMT) induced a significant and long lasting increase in spontaneous activity in mice. Pretreatment of mice with either pimozide or α-methyl-para-tyrosine methyl ester HCl (AMPT) prevented the activity increase induced by AMT. In similar trials, methysergide or para-chlorophenylalanine (PCPA) were also found to antagonize the development of hyperactivity following a standard dose of AMT. The results suggest that both endogenous dopamine and serotonin may participate in the hyperactivity induced by AMT.

α-Methyltryptamine Hyperactivity Catecholamine-serotonin interaction Para-chlorophenylalanine

ALTHOUGH numerous investigators have studied the behavioral and biochemical effects of "classical" hallucinogens such as LSD or mescaline, relatively little effort has been directed toward the study of α-methyltryptamine (AMT). Early studies by Murphree *et al.* [20] and Szara [25] demonstrated this latter compound to be an effective hallucinogenic agent in man. One of the pronounced behavioral effects of AMT is its ability to elicit increases in spontaneous motor activity in mice [28] and chicks [8]. It has been noted that the dextrorotatory isomer is more active than the levo. The stereochemistry of (+)-α-methyltryptamine has been shown to correspond to that of (+)-amphetamine [23]. *In vitro* studies have shown that AMT has moderate serotonin agonist properties in the rat fundus [2,27] as well as potent competitive MAO inhibitory properties [10,11]. Recent reports [13,21] have suggested that certain hallucinogenic agents may exert effects on both serotonergic and dopaminergic systems in the central nervous system (CNS). Since increases in motor activity have frequently been related to dopamine receptor stimulation [15, 23, 24], we have attempted to investigate some possible mechanisms by which AMT may stimulate locomotor activity.

METHOD

Male Swiss-Webster mice (Biolabs) weighing 20–50 g were housed in groups of six and maintained on Teklad 4% fat lab chow and water ad lib. Mice were allowed several days to become accustomed to their surroundings before use. Lighting was on a 12 hr on (06:00), 12 hr off (18:00) cycle. Experiments were carried out in a quiet, well-lighted laboratory area.

Mice were placed in groups of three into a white plastic 18×25×15 cm recording cage covered with a clear Plexiglas sheet with ventilation holes atop a selective activity meter (Columbus Instruments, Model S). Fluorescent lighting in the room provided illumination. Spontaneous activity was then recorded continuously for six hours. Mice were first allowed three hours to acclimate to the recording cage prior to test drug injections. During this period both pretreated and control groups achieved a low baseline level of activity before the time of test drug administration. Pretreatments were given at various times prior to the test drug as indicated in Fig. 1. Activity counts were summed over 20 min intervals and differences between treatment and control counts were analyzed using a grouped *t* test. Total counts for all treatments were summed over the 3 hr recording period and analyzed using a one-way analysis of variance. Comparisons were made using the method of Scheffé [1].

Racemic AMT was prepared by lithium aluminum hydride reduction of the corresponding nitrovinylindole derivative. The free base was converted into the HCl salt and recrystallized from isopropanol-ether. Other drugs used were *para*-chlorophenylalanine (Aldrich), pimozide (Janssen), methysergide maleate (Sandoz), and alpha-methyl-*para*-tyrosine methyl ester HCl (prepared from alpha-methyl-*para*-tyrosine by the method of Corrodi and Hanson [6]). Drugs were dissolved in saline and administered intraperitoneally. A small amount of HCl or acetic acid was used to dissolve *para*-chlorophenylalanine and pimozide, respectively.

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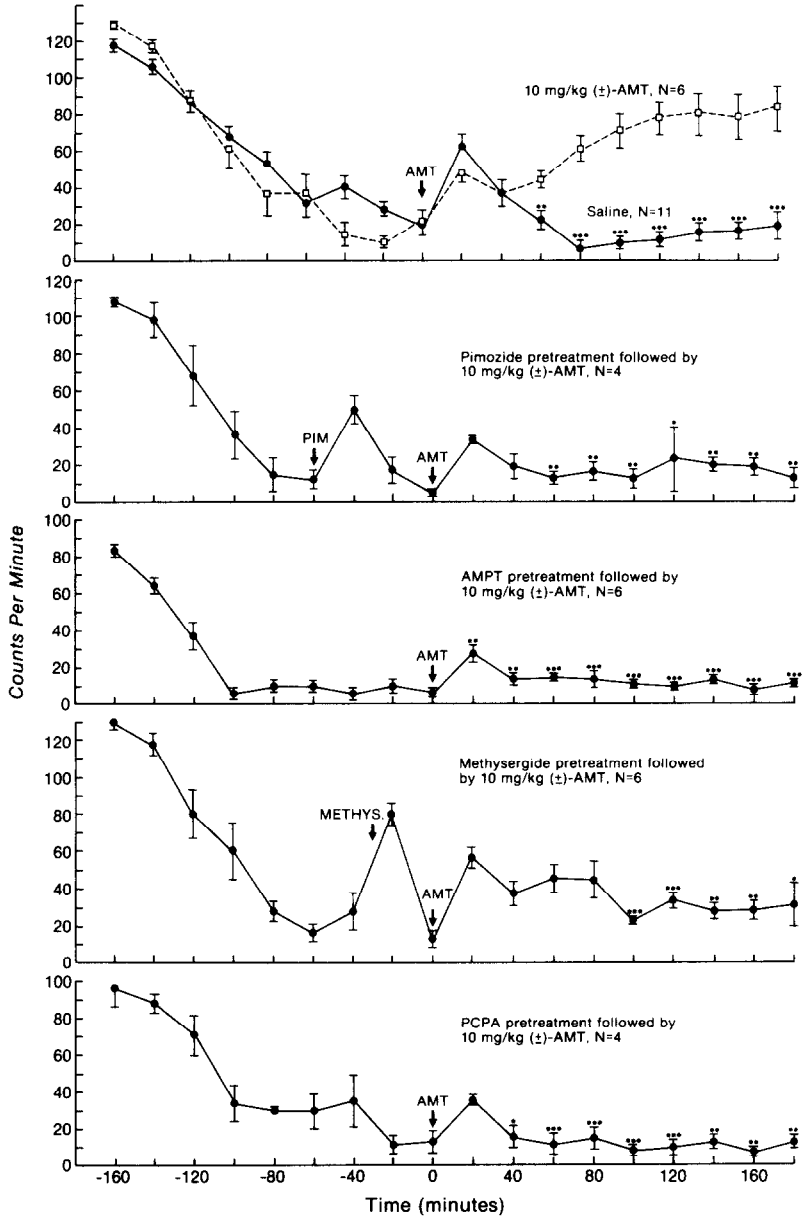

FIG. 1. Spontaneous activity profiles of mice treated with 10 mg/kg (±)-AMT, saline, or pretreatment plus 10 mg/kg (±)-AMT. Mice were grouped three to a cage and placed on the spontaneous activity meter three hours prior to time zero when the saline or AMT injections were given. Pimozide (0.5 mg/kg) was given one hour prior to time zero. α-Methyl-para-tyrosine methyl ester HCl (AMPT) (250 mg/kg) was administered four hours before time zero. Methysergide maleate (1 mg/kg) was given thirty minutes prior to time zero, and PCPA (300 mg/kg) was given 48 and 24 hours prior and 100 mg/kg was given four hours prior to the injections at time zero. Statistical significance of the differences between the AMT only treated group and the pretreatment plus AMT or saline control groups was computed for each interval using a grouped t test; *denotes $p<0.05$; **$p<0.01$; ***$p<0.001$. The n value refers to number of groups of three mice. Vertical bars indicate the standard error of the mean.

TABLE 1

THE EFFECTS OF VARIOUS TREATMENTS ON SPONTANEOUS ACTIVITY IN MICE

Treatment	Activity Counts Mean ± SE Baseline −20–0[a]	80–100[b]	Total 0–180[c]		N
Saline	19.1 ± 5.0	11.0 ± 2.4	4097 ± 424.8		11
(±)-AMT (48 μmole/kg)	21.3 ± 6.3	72.2 ± 9.0	11,887 ± 1365.4	*[h]	6
Pimozide[d] + saline	1.3 ± 0.4	4.4 ± 2.4	1566 ± 260.8	N.S.[h]	5
AMPT + saline	6.0 ± 1.8	8.8 ± 5.4	1514 ± 269.8	N.S.[h]	6
Methysergide[f] + saline	16.2 ± 5.9	10.7 ± 2.8	3535 ± 371.4	N.S.[h]	6
PCPA[g] + saline	8.0 ± 4.0	7.0 ± 1.7	2812 ± 222.2	N.S.[h]	5
Pimozide + AMT	5.3 ± 1.4	12.9 ± 5.6	3554 ± 685.6	*[i]	4
AMPT + AMT	6.4 ± 2.6	10.6 ± 1.6	2379 ± 157.8	*[i]	6
Methysergide + AMT	13.4 ± 4.4	22.1 ± 2.4	6370 ± 841.8	*[i]	6
PCPA + AMT	13.3 ± 6.6	7.7 ± 1.3	2501 ± 296.0	*[i]	4

[a]Mean counts/20 min immediately prior to time zero. [b]Mean counts/20 min 100 min after treatment at time zero. [c]Total counts/3 hr following time zero. [d] 0.5 mg/kg given 1 hr prior. [e]250 mg/kg given 4 hr beforehand. [f]1 mg/kg given 30 min beforehand. [g]300 mg/kg given 48 and 24 hr prior and 100 mg/kg given 4 hr prior. [h]Significance as compared to saline control total counts using a one-way ANOVA and Scheffé's method of comparison. [i]Significance as compared to (±)-AMT total counts using Scheffé's method; N. S. denotes not significant; $*p<0.01$.

RESULTS

As reported by others [8,28], AMT produces a significant and long-lasting increase in spontaneous activity (Fig. 1). Following saline injection, activity becomes slightly elevated, but declines to baseline within about 60 minutes. Following injection of AMT (48 μmole/kg; 10 mg/kg) activity was not different from controls for about one hour. Following this latency period, mice became increasingly active for the duration of the experiment (Table 1). The increased activity consisted primarily of locomotor behavior with occasional grooming. Stereotyped behavior or tremor was not apparent. Autonomic symptoms such as diarrhea, salivation or piloerection were not observed.

In order to assess the importance of dopaminergic and serotonergic mechanisms in the hyperactivity response induced by AMT, a number of pharmacological treatments were employed. Administration of the dopamine receptor antagonist pimozide (0.5 mg/kg) one hour prior to the administration of AMT completely abolished the increase in activity seen following AMT alone.

Pretreatment of animals with the tyrosine hydroxylase inhibitor alpha-methyl-*para*-tyrosine methyl ester HCl (AMPT) has been shown to decrease brain dopamine levels [6] and is known to antagonize the behavioral effects of catecholamine releasing drugs such as (+)-amphetamine. In the present study, we studied the possibility of such an action by pretreatment of the mice with AMPT (250 mg/kg) four hours prior to injection of AMT. As can be seen in Fig. 1 and the Table, AMPT pretreatment completely antagonized AMT-induced hyperactivity.

The previously reported ability of methysergide to block AMT-induced activity increases in chicks [8], and the 5-HT agonist properties of AMT in vitro [2,27], prompted an examination of the possible role of serotonin in the hyperactivity syndrome. Injection of methysergide (1 mg/kg) thirty min prior to AMT was found to antagonize significantly the activity increase produced by AMT. Significant antagonism occurred about 90–100 min following administration of AMT. To ascertain the possible involvement of endogenous 5-HT, mice were pretreated with *para*-chlorphenylalanine (PCPA). Mice were given two doses of PCPA (300 mg/kg) at 48 and 24 hours prior to AMT administration, and 100 mg/kg again four hours prior to AMT administration. This treatment also abolished the increase in activity induced by AMT. Activity was not significantly elevated in mice given only PCPA (Table 1).

DISCUSSION

A number of recent reports have suggested that there may be an interactive involvement of both dopamine and serotonin pathways in the control of locomotor activity in rodents [5, 7, 12, 24]. Further, direct injection studies in the nucleus accumbens have implicated stimulation of dopamine receptors in this structure in the mediation of motor activity increases [15,22]. However, manipulation of serotonin pathways has usually indicated an inhibitory role for serotonin in the control of motor behavior [5, 7, 12, 14].

Elucidation of the mechanism(s) of action for AMT may prove very difficult. Vasko *et al.* [29] have suggested that AMT causes release of 5-HT, in much the same way that amphetamine induces catecholamine release. Gorkin *et al.* [10] have shown that AMT is a more effective inhibitor of serotonin oxidation by MAO, than of tyramine or tryptamine oxidation. Grieg *et al.* [11] found that AMT more effectively inhibits MAO oxidation of 5-HT than of NE. Lessin *et al.* [17] found that AMT was able to reverse reserpine ptosis. Since AMT is potentiated by irreversible MAO inhibitors, but is not a substrate for MAO, these workers also argued for an indirect action of AMT. The delayed onset which we observed is consistent with these earlier studies, and seems to argue for either an indirect action or production of some active metabolite.

The present results with methysergide suggest that a serotonergic mechanism may be involved in the action of

AMT. Pretreatment with methysergide significantly attenuated the activity increase produced by AMT. Likewise, the AMT-induced activity increase was also blocked by the PCPA pretreatment, arguing for an indirect effect. Although PCPA is known to reduce 5-HT levels via the inhibition of tryptophan hydroxylase, in the doses given here it may also depress catecholamine levels by inhibiting tyrosine hydroxylase. Miller *et al.* [19] observed a 30% reduction in the norepinephrine content of rat brains one day after a single injection of 400 mg/kg of PCPA. Although it is possible that decreased catecholamine levels may be involved in the attenuation of AMT-induced hyperactivity, and admittedly confound the interpretation of the results, we believe that depletion of 5-HT, which is the major result of PCPA administration, is primarily involved in the effectiveness of this pretreatment. Thus, it seems that AMT may accomplish two things at serotonin neurons. First, it may release endogenous stores of 5-HT. Subsequently, as a potent competitive MAO inhibitor, it would retard the destruction of accumulated serotonin.

Increases [5, 9, 18], decreases [3, 4, 26] and no change [16] in locomotor activity in rodents have been reported following treatment with PCPA. The difference in the behavioral outcome in experiments such as these may be attributed to differences in species, the treatment regimen or the testing procedure. Brody [3] observed that PCPA-treated rats showed lowered activity except when the animals were stimulated by changes in the testing environment. Fibiger and Campbell [9] have suggested that the effect of serotonin on exploratory activity may be quite different from, or even opposite to, its effect on more chronic measures of behavioral arousal. Under the conditions employed in this study, PCPA pretreatment appeared to produce a slight, nonsignificant depression in activity (Table 1). Furthermore, the stimulation due to handling and injection at time zero did not bring about a sustained increase in activity beyond the normal transient activity increase induced by this manipulation in all treatment groups.

In this study pimozide and AMPT were also effective in preventing locomotor increases induced by AMT, suggesting that endogenous levels of dopamine play a role in the hyperactivity response. Whereas dopaminergic effects might be mediated by AMT itself, it also seems possible that increased serotonergic function could bring about changes in motor activity *via* the secondary involvement of dopamine pathways. For example, increases in 5-HT content in selected areas of the brain might serve to disinhibit certain dopamine pathways. This view would be consistent with the idea that serotonergic systems serve at the level of general arousal, rather than in control of specific behavioral effects.

While the exact sequence of events following administration of AMT is certainly not clear, it does appear likely that both serotonin and dopamine are involved in the action of this drug. In view of the MAO inhibitory properties of AMT, its hallucinogenic effects, and the possibility for release of both serotonin and dopamine, it is perhaps not surprising that definitive studies on this substance have not appeared.

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