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The Effect of Amantadine and (+)-Amphetamine on Motility in Rats after Inhibition of Monoamine Synthesis and Storage

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Abstract. The effect of amantadine and (+)-amphetamine on motility was investigated by subcutaneous administration to rats. Amantadine 50 and 100 mg/kg induced a moderate increase of motility. (+)-amphetamine 1, 2.5 and 5 mg/kg showed a more potent locomotor stimulant effect. A stereotyped licking was found during the hypermotility induced by (+)-amphetamine 5 mg/kg.

Pretreatment with the catecholamine synthesis inhibitor α-methyl-p-tyrosine inhibited the effect of (+)-amphetamine but not that of amantadine. Reserpine potentiated the effects of both amantadine and (+)-amphetamine. Tetrabenazine antagonized the hyperactivity produced by (+)-amphetamine but potentiated amantadine induced hyperactivity. The locomotor stimulant effect of amantadine seems to differ in certain respects from that of amphetamine.

Key words: Amantadine — (+)-Amphetamine — Motility — Rats — Catecholamines.

Introduction

The antiviral agent amantadine has been shown initially by Schwab *et al.* (1969) to have antiparkinson activity and weak central stimulant side effects. In mice and rats the drug has been found to increase spontaneous motor activity (Vernier *et al.*, 1969; Strömberg *et al.*, 1970; Strömberg and Svensson, 1971; Fibiger *et al.*, 1971; Maj *et al.*, 1972a, b).

Scatton *et al.* (1970) has shown amantadine to increase the synthesis and release of dopamine using in vitro experiments with isolated rat brain slices. Farnebo *et al.* (1971) has presented evidence for release of dopamine (DA) and noradrenaline (NA) from nerve terminals in rats using different in vitro and in vivo techniques. In nialamide pretreated mice amantadine was found by Strömberg *et al.* (1970) to accelerate accumulation of O-methylated basic metabolites of DA and NA. On the basis of these findings it has been concluded that amantadine, like amphetamine, releases catecholamines from extragranular binding sites and that the antiparkinson effect of amantadine might be explained by this mechanism.

In a short communication data have been presented showing that amantadine increases motility in rats after pretreatment with the catecholamine synthesis inhibitor α-methyl-p-tyrosine (αMT), reserpine and

tetrabenazine (Buus Lassen, 1971). Similar results were found by Maj *et al.* (1972b). In the present paper further investigations into the effect of amantadine on motility in rats have been performed. In these experiments amphetamine was included for comparison.

Method

Animals. Female rats of the Wistar strain weighing 100—120 g were used. Four animals were placed in a transparent perspex cage (floor area 36×22 cm, height 20 cm) 18 h before the start of the experiment. The experiments were performed at an ambient temperature of 22—24°. The rats were provided with food and water ad libitum before and during the experimental sessions.

Test Procedure. Behavior was observed and motility measured for 6 h periods using an Animex motimeter based on the principle of tuned oscillator coil systems (Svensson and Thieme, 1969). The activity measurement was started immediately after administration of the test drugs. Four identical experiments were performed with each treatment.

Drugs. All drugs were injected subcutaneously in a volume of 5 ml/kg. Amantadine and αMT methylester (H 44/68) were administered as hydrochlorides and (+)-amphetamine as the sulphate. The doses given refer to the salts. Reserpine was given as a dilution of the commercial injection preparation (Serpasil®) in physiological saline, and the other substances were dissolved in physiological saline.

Statistics. Student's *t*-test was used for statistical evaluation.

Results

Treatment of rats with amantadine and amphetamine increased the motility significantly compared to saline treated controls. The results of the activity measurements are given in Table 1. Amantadine 25 mg/kg did not change motility within the first 4 h after administration, but a small decrease of activity was found 4—6 h after the injection. Amantadine 50 mg/kg induced hyperactivity for about 2 h and 100 mg/kg for about 4 h. Amphetamine 1 mg/kg increased motility slightly more than amantadine 100 mg/kg for the first 2 h period, but in the 4—6 h period the activity was slightly depressed. Doses of 2.5 and 5 mg/kg amphetamine produced much higher levels of hypermotility.

Injection of physiological saline induced a transient increase in locomotion, sniffing and grooming. After amantadine 25 mg/kg the behavior was almost identical to this except that a few rearings were also observed. Several behavioral items were observed after amantadine 50 and 100 mg/kg: increased locomotion, rearing, sniffing, head twitches and spells of rapid grooming of the head with the forelegs. Weak tremor was also registered at 100 mg/kg.

Amphetamine 1 mg/kg produced amantadine-like behavior, but 2.5 mg induced more rapid locomotion and very frequent rearing and sniffing at the walls of the cage. Amphetamine 5 mg/kg induced the same behavioral effect the first half hour, but later continuous locomotion on the floor of the cage and simultaneous licking on the walls and food pellets were the predominant behavioral items.

Table 1. The effect of physiological saline, amantadine and (+)-amphetamine on motility in rats

Treatment		Motility in counts per 2 h					
		0—2 h		2—4 h		4—6 h	
Substance	Dose mg/kg s.c.	m ± SD	P	m ± SD	P	m ± SD	P
Physiological saline	—	2118 ± 556		1449 ± 444		1489 ± 825	
Amantadine	25	2708 ± 951	NS	945 ± 57	NS	454 ± 157	0.05
	50	4748 ± 1499	0.02	1896 ± 492	NS	807 ± 220	NS
	100	5719 ± 2176	0.02	2449 ± 497	0.02	2595 ± 1014	NS
(+)-amphetamine	1	6911 ± 1745	< 0.001	2902 ± 1972	NS	575 ± 89	0.04
	2.5	11477 ± 2523	< 0.001	4883 ± 2172	0.02	968 ± 450	NS
	5	12185 ± 1616	< 0.001	8156 ± 1800	< 0.001	1477 ± 914	NS

The activity after treatment with amantadine and amphetamine was compared with the activity of the saline-treated controls. NS means $P > 0.05$.

Table 2. The effect of physiological saline, amantadine and (+)-amphetamine on motility in rats after pretreatment with αMT

Treatment		Motility in counts per 2 h					
		0—2 h		2—4 h		4—6 h	
Substance	Dose mg/kg s.c.	m ± SD	*P*	m ± SD	*P*	m ± SD	*P*
Physiological saline	—	1053 ± 271		541 ± 143		569 ± 75	
Amantadine	50	3787 ± 1219	0.003	2432 ± 379	< 0.001	766 ± 398	NS
	100	5967 ± 2314	0.006	6096 ± 2436	< 0.004	2364 ± 1722	NS
(+)-amphetamine	5	1195 ± 729	NS	408 ± 227	NS	524 ± 213	NS

αMT 250 mg/kg was administered s.c. 2 h before treatment. The activity of amantadine-, and (+)-amphetamine treated rats was compared to the activity of saline-treated controls. NS means $P > 0.05$.

Table 3. The effect of physiological saline, amantadine and (+)-amphetamine on motility in αMT-treated rats

Treatment		Motility in counts per 2 h					
		0—2 h		2—4 h		4—6 h	
Substance	Dose mg/kg s.c.	m ± SD	*P*	m ± SD	*P*	m ± SD	*P*
Physiological saline	—	742 ± 230		432 ± 198		471 ± 177	
Amantadine	50	4223 ± 527	< 0.001	1890 ± 646	0.005	819 ± 480	NS
	100	6647 ± 487	< 0.001	3504 ± 677	< 0.001	1783 ± 266	< 0.001
(+)-amphetamine	5	1093 ± 733	NS	311 ± 146	NS	302 ± 171	NS

αMT 250 mg/kg was administered s.c. twice 2 and 18 h before treatment. The activity of amantadine- and (+)-amphetamine-treated rats was compared to the activity of saline-treated controls.

NS means $P > 0.05$.

Table 4. The effect of physiological saline, amantadine and (+)-amphetamine on motility in rats after pretreatment with reserpine

Treatment		Motility in counts per 2 h					
		0—2 h		2—4 h		4—6 h	
Substance	Dose mg/kg s.c.	m ± SD	P	m ± SD	P	m ± SD	P
Physiological saline	—	322 ± 156		442 ± 290		592 ± 232	
Amantadine	25	3201 ± 2117	0.04	1582 ± 428	0.005	1555 ± 550	0.02
	50	10915 ± 4288	< 0.001	11648 ± 5289	< 0.001	4857 ± 4386	NS
(+)-amphetamine	5	17290 ± 223	< 0.001	13432 ± 4692	< 0.001	5365 ± 4035	NS

Reserpine 7.5 mg/kg was administered s.c. 18 h before physiological saline, amantadine or amphetamine. The activity of amantadine- and amphetamine-treated rats was compared to the activity of saline-treated animals.

NS means $P > 0.05$.

Amantadine 50 and 100 mg/kg increased the motility in rats pretreated once or twice with αMT 250 mg/kg. In contrast, the locomotor stimulation induced by (+)-amphetamine 5 mg/kg was inhibited by αMT (Table 2 and 3).

The reserpine induced symptoms were only partly antagonized by amantadine and (+)-amphetamine. Ptosis and hunched back posture was not abolished, but the reduced motility was changed in different ways by the two drugs. Amantadine produced forward locomotion and sniffing on the floor. (+)-amphetamine induced forward and backward locomotion with simultaneous licking and gnawing on food pellets. Reserpine pretreatment potentiated the locomotor stimulant effect of amantadine and (+)-amphetamine (Table 1 and 4).

Pretreatment with tetrabenazine enhanced the amantadine induced hypermotility (Table 5). The different behavioral items observed after amantadine alone were present 1—2 h after amantadine administration. After this period the behavior changed to continuous locomotion and sniffing in the bottom of the cage. Injection of amphetamine 5 mg/kg to tetrabenazine treated rats increased the activity significantly more than the saline injection during the first 2 h period (Table 5). However, the stimulant effect of amphetamine was weaker in tetrabenazine treated rats than in untreated rats (Table 1).

Discussion

In the present investigation amantadine produced a moderate increase of motility in rats. This is in agreement with the findings of Fibiger *et al.* (1971) and Maj *et al.* (1972a, b).

Pretreatment with αMT inhibited the amphetamine-induced hypermotility but did not alter the locomotor stimulant effect of amantadine. Antagonism of the stimulant effect of amphetamine by αMT has been shown by several authors (Weisman *et al.*, 1966; Randrup and Munkvad, 1966; Hansson, 1967; Dingell *et al.*, 1967; Sulser *et al.*, 1968; Dominic and Moore, 1969; Stolk and Rech, 1970; Scheel-Krüger, 1971).

Scheel-Krüger (1971) has suggested that, in rats, central stimulants can be divided into two groups according to their interactions with catecholamines. The excitation elicited by amphetamine appears to be associated with release of catecholamines from a small, newly synthesized, reserpine resistant pool (Carlsson *et al.*, 1966) while the stimulant effects of pipradrol and methylphenidate may be dependent on release of catecholamines stored in a large reserpine sensitive pool. In this study the locomotor stimulant effect of amantadine was not inhibited by reserpine or αMT. This suggests that amantadine produces stimulation by a mechanism different from pipradrol or amphetamine.

Table 5. The effect of physiological saline, amantadine and (+)-amphetamine on motility in rats after pretreatment with tetrabenazine

Treatment		Motility in counts per 2 h					
		0—2 h		2—4 h		4—6 h	
Substance	Dose mg/kg s.c.	m ± SD	*P*	m ± SD	*P*	m ± SD	*P*
Physiological saline	—	358 ± 219		217 ± 57		376 ± 167	
Amantadine	12.5	871 ± 986	NS	1555 ± 2283	NS	1450 ± 1916	NS
	25	2170 ± 1162	0.02	10920 ± 2859	0.003	6442 ± 3893	0.02
	50	3066 ± 705	< 0.001	11518 ± 3431	0.005	9702 ± 3985	0.003
(+)-amphetamine	5	1200 ± 437	0.01	535 ± 305	NS	268 ± 114	NS

Tetrabenazine 50 mg/kg was administered s.c. 0.5 h before treatment. The activity of amantadine- and (+)-amphetamine-treated rats was compared to the activity of saline-treated controls.

NS means $P > 0.05$.

Amantadine antagonizes the cataleptic effect of spiroperidol (Maj *et al.*, 1972a) and αMT (Papeschi, 1972) in rats. The antagonism of spiroperidol-induced catalepsy was not influenced by αMT. The anti-cataleptic effect of amantadine is therefore probably not related to catecholamine release. Anticholinergic drugs have an anti-cataleptic effect (Bossier and Simon, 1965; Timsit, 1967) but amantadine has been found to have essentially no anticholinergic activity (Grelak *et al.*, 1970; Zetler, 1970). Anticholinergic drugs have a locomotor stimulant effect (Malatray and Simon, 1972) but the behavioral effect of amantadine itself is probably not due to an anticholinergic effect, as an enhanced hyperactivity was found in some rats pretreated with atropine and scopolamine.

Tetrabenazine was found to partially block amphetamine-induced hypermotility in agreement with the results of Rech and Stolk (1969), and Weissman (1972).

In rats with unilateral lesions in the nigrostriatal dopamine pathway, amantadine, like amphetamine, induces rotation to the denervated side (Strömberg and Svensson, 1971; Farnebo *et al.*, 1971). The dopamine receptor stimulating drug apomorphine produces rotation to the intact side (Ungerstedt, 1971).

The regulation of motor activity is complex and presumably dependent on both DA and NA (Svensson, 1969; Svensson and Waldeck, 1969; Svensson and Waldeck, 1970; Leng and Webster, 1971a, b; Van Rossum, 1970). Amantadine-induced hyperactivity is antagonized by DA- and NA-antagonists (Maj *et al.*, 1972a; Buus Lassen, 1971) indicating an activation of both dopaminergic and noradrenergic receptors.

Amantadine shares some of the pharmacological properties of both amphetamine and apomorphine while lacking other characteristic effects of these substances. Thus, amantadine behaves more like apomorphine than amphetamine in producing hyperactivity in rats treated with either αMT or tetrabenazine, but more like amphetamine than apomorphine in producing characteristic rotational behavior in rats with unilateral nigrostriatal lesions and in accelerating DA and NA synthesis and release. On the other hand amantadine, even in high doses, fails to elicit the stereotyped sniffing, licking and gnawing seen after administration of either amphetamine (Randrup *et al.*, 1963) or apomorphine (Ernst, 1967).

These findings indicate that the behavioral effects of amantadine in rats may depend on a combination of mechanisms involving certain catecholamine systems. It appears quite possible that amantadine may exert both a certain DA-receptor stimulating effect as well as DA and NA release. The rotational behavior elicited by amantadine in rats with unilateral nigrostriatal lesions indicate that dopamine release must predominate over dopamine receptor stimulation in the striatum. The failure

of amantadine to elicit stereotyped behavior suggests that this substance activates a special dopamine system or simultaneously activates a noradrenaline system with behavioral modulating effect.

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