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Biochemical and pharmacological studies on amineptine (S 1694) and (+)-amphetamine in the rat

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The pharmacological activities of amineptine (S 1694) and (+)-amphetamine and their interaction with biogenic amines have been examined in rats. The locomotor activity, stereotyped behaviour and hypothermia induced by amineptine were similar to but not as marked as those produced by (+)-amphetamine, and there was little or no anorectic action. Amineptine does not modify the concentrations of brain noradrenaline or acetylcholine which are respectively reduced and increased by (+)-amphetamine. Moreover, amineptine does not affect significantly the decrease of brain noradrenaline induced by an intraventricular injection of 6-hydroxydopamine, an effect significantly antagonized by (+)-amphetamine. On the other hand, like amphetamine, amineptine significantly reduces the effect of 6-hydroxydopamine on brain dopamine. Both drugs increase the striatal concentrations of homovanillic acid and show a cross tolerance in this action. Therefore they could act similarly on the striatal dopaminergine system. Amineptine thus appears to be a new type of antidepressant with a brain biochemical profile differing from that of other drugs used in depressive disorders.

Amineptine (S 1694) [(dihydro-10,11 dibenzo [a,d] cycloheptenyl-5) amino]-7 heptanoic acid, is a new central stimulant with antidepressant activity (Bourret, Girard & Schott, 1976; Duche, 1976; Lonchamp & Raffi, 1976) having some action in common with (+)-amphetamine (J. Carpentier, personal communication). Like (+)-amphetamine, it causes an increase in locomotor activity, stereotyped movements and hyperthermia in rats, although to a lesser degree, while, unlike (+)-amphetamine, it produces little or no anorectic effect.

Amphetamine produces a variety of effects on brain biogenic amines, particularly catecholamines (Glowinski, Axelrod & Iversen, 1966; Glowinski, 1970; Garattini, Bizzi & others, 1975) and it has been suggested that these may play a role in the various pharmacological effects of amphetamine including increase of locomotor activity, stereotyped behaviour, anorectic and hyperthermic effects (Weissman, Koe & Tenen, 1966; Taylor & Snyder, 1971; Fibiger, Fibiger & Zis, 1973; Samanin, Bernasconi & Garattini, 1975a). We have therefore examined the possible interaction of amineptine with biogenic amines in the brain and compared its actions with those of (+)-amphetamine.

MATERIALS AND METHODS

Female Charles River rats, 180–200 g, were used. Amphetamine and amineptine were given intraperitoneally, the latter in doses of 10, 20 or 40 mg kg^{-1} unless otherwise stated.

Motor activity was recorded in an activity cage (Basile, Italy) placed in an uniformly-illuminated, sound-attenuated room. The rats were placed in the cage for 30 min to allow adaptation. The animals were then injected with (+)-amphetamine sulphate (1·5 mg kg^{-1}) or amineptine. 10 min recording for each rat were made beginning at 30 min after injection which was the time of peak effect of both compounds. All the experiments began at 3·00 p.m.

The stereotyped behaviour was scored according to Costall, Naylor & Olley (1972). The animals were placed in individual observational cages 30 min before drug administration to allow adaptation. After this period, the animals were injected with (+)-amphetamine sulphate (10 mg kg^{-1}) or amineptine. The stereotypy was evaluated at the peak effect which was observed at 60 min from injection. The experiments were performed between 10·00 a.m. and 1.00 p.m., and the stereotypy was scored by two observers unaware of the treatment schedule.

Food intake. The animals were singly caged and trained to take their daily food during 6 out of 24 h (water was freely available). On the day of the experiments, the animals received (+)-amphetamine sulphate (1·25 mg kg^{-1}) or amineptine. Immediately after the rats were placed in a cage containing a

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weighed amount of food and 2 h later the food was re-weighed to the nearest 0·1 g. The difference conconstituted the measure of intake.

Body temperature was measured rectally by thermocouple immediately before the injection and then at 30 min intervals for 90 min after (+)-amphetamine sulphate (15 mg kg^{-1}) or amineptine.

Determination of monoamine concentrations and some metabolites. The animals received intraperitoneally (+)-amphetamine sulphate (15 mg kg^{-1}) or amineptine or an equal volume of saline. They were killed 1 h later and the brains were quickly removed. In one group they were frozen for the fluorimetric estimation of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) according to Giacalone & Valzelli (1969). Noradrenaline and dopamine were measured according to Chang (1964) and Laverty & Taylor (1968) and 3-methoxy-4-hydroxy-phenylglycol sulphate (MOPEG SO_4) was determined after isolation on DEAE Sephadex columns and condensation with ethylenediamine, according to Meek & Neff (1972), with minor modifications.

In the other group the striata were dissected and frozen for estimation of dopamine, homovanillic acid (HVA), acetylcholine and choline. Dopamine was determined according to Laverty & Taylor (1968), HVA according to Korf, Van Praag & Sebens (1971), acetylcholine and choline were measured radiochemically (Saelens, Allen & Simke, 1970).

Effect of (+)-amphetamine or amineptine on the depletion of brain catecholamines or 5-HT induced respectively by 6-hydroxydopamine (6-OHDA) and fenfluramine. The animals received (+)-amphetamine sulphate (5 mg kg^{-1}) or amineptine (20 mg kg^{-1}). 1 h later they were injected intraventricularly with 200 μg of 6-OHDA according to Noble, Wurtman & Axelrod (1967). 6-OHDA was dissolved in 20 μl of saline containing ascorbic acid (0·1 mg ml^{-1}). 30 min before the 6-OHDA administration, the animals received 50 mg kg^{-1} (i.p.) of pargyline, since this procedure is known to increase the effect of 6-OHDA on dopamine (Breese & Traylor, 1971). Control animals received pargyline and were injected with an equal volume of the vehicle. The animals were killed 1 week later and brain noradrenaline and dopamine assayed according to Chang (1964) and Laverty & Taylor (1968).

In another experiment the animals were given (+)-amphetamine (5 mg kg^{-1}, i.p.) or amineptine (20 mg kg^{-1}, i.p.) 30 min later they received 15 mg kg^{-1} (i.p.) of fenfluramine and were killed 2 h later for the assay of brain 5-HT (Giacalone & Valzelli, 1969).

Cross tolerance with (+)-amphetamine. The animals received amineptine or (+)-amphetamine sulphate or both according to the doses and schedules shown in Table 3. At 1 h after the last injection the animals were killed and HVA in the striata was estimated according to Korf & others (1971).

Statistical analysis. The data were statistically analysed by Duncan's new multiple range test.

Drugs. Amineptine and (±)-fenfluramine were a gift from Servier Labs., Paris.

RESULTS

Pharmacological effects

As shown in Table 1, amineptine in rats has some actions similar to (+)-amphetamine but they are much less marked especially those on food intake and stereotyped behaviour. The dose of amineptine required to produce a decrease in food intake comparable to that produced by 1·25 mg kg^{-1} of (+)-amphetamine is 40 mg kg^{-1}. Even at that dose amineptine does not elicit typical stereotyped movements such as the continuous licking, gnawing or biting produced by 10 mg kg^{-1} amphetamine. Only sniffing and rearing, with occasional licking was observed in rats treated with amineptine. Also the effect of amineptine on body temperature is less evident than that of amphetamine.

On the other hand, amineptine produces a marked motor stimulation in rats, with doses that are of the same order of magnitude as those of amphetamine necessary to elicit the same effect.

Table 1. *Various pharmacological effects of (+)-amphetamine and amineptine in rats.*

Groups (mg kg^{-1}, i.p.)		Food intake (g/rat in 2 h ± s.e.)	Motor activity (Counts/10 min ± s.e.)	Stereotypy (mean score ± s.e.)	Body temperature[1] (°C ± s.e.)
Saline	—	9·6 ± 0·8	41 ± 8	0·1 ± 0·1	+0·2 ± 0·1
Amineptine	10	8·3 ± 0·8	243 ± 45*	0·3 ± 0·2	+1·0 ± 0·2*
Amineptine	20	7·4 ± 0·5	465 ± 56*	1·7 ± 0·2*	+1·3 ± 0·1*
Amineptine	40	4·9 ± 0·5*	494 ± 77*	1·8 ± 0·2*	+1·0 ± 0·07*
(+)-Amphetamine**		4·3 ± 0·3*	183 ± 15*	3·8 ± 0·2*	+2·3 ± 0·2*

Each figure is the mean ± s.e. of 6 animals.
* $P < 0.01$ when compared with saline treated animals.
** The doses of (+)-amphetamine sulphate used for food intake, motor activity, stereotypy and body temperature were respectively: 1·25, 1·5, 10 and 15.
(1) The figures indicate the difference in body temperature recorded before and 30 min after the treatment.

Biochemical effects

With the exception of a significant increase in the striatal concentrations of HVA (Table 2), amineptine does not produce marked changes in brain concentrations of 5-HT, 5-HIAA, noradrenaline, MOPEG SO_4 or dopamine. Unlike amphetamine, it does not modify the concentrations of noradrenaline and acetylcholine which are respectively decreased (from a saline control value of 0·52 ± 0·01 to 0·25 ± 0·02 μg g^{-1} tissue, $P < 0·01$) and increased (Table 2)

Table 2. *The effect of amineptine and (+)-amphetamine on striatal levels of dopamine, homovanillic acid (HVA) acetylcholine and choline in the rat.*

Treatment	mg kg^{-1}, i.p.	Striatal concentrations (μg g^{-1} ± s.e.) Dopamine	HVA	Acetylcholine	Choline
Saline	—	6·70 ± 0·24	0·30 ± 0·03	3·91 ± 0·16	9·80 ± 0·36
Amineptine	20	7·30 ± 0·34	0·57 ± 0·01*	3·71 ± 0·23	9·96 ± 0·27
Amineptine	40	7·42 ± 0·40	0·76 ± 0·02*	3·70 ± 0·09	10·55 ± 0·53
(+)-Amphetamine sulphate	15	6·95 ± 0·31	0·63 ± 0·05*	5·15 ± 0·52*	9·46 ± 0·25

Each figure is the mean of 4 or 6 determinations.
Each determination of HVA was performed on a pool of 3 striata.
* $P < 0·01$ with respect to saline treated animals.

by amphetamine. Both drugs produce a comparable increase of the striatal concentrations of HVA (Table 2). At 5 mg kg^{-1}, (+)-amphetamine significantly ($P < 0·01$) antagonizes the decrease of brain noradrenaline (saline + 6-OHDA 0·06 ± 0·01, amphetamine + 6-OHDA 0·22 ± 0·01 μg g^{-1} tissue) and dopamine (saline + 6-OHDA 0·58 ± 0·01, amphetamine + 6-OHDA 0·85 ± 0·06 μg g^{-1} tissue) produced by an intraventricular injection of 6-OHDA while S 1694, 20 mg kg^{-1}, reduced only the effect of 6-OHDA on dopamine (0·83 ± 0·07 μg g^{-1}). Neither drug modified the decrease of brain 5-HT induced by an intraperitoneal injection of (±)-fenfluramine (saline 0·54 ± 0·02, fenfluramine 0·28 ± 0·2 μg g^{-1} tissue, $P < 0·01$).

Cross tolerance with (+)-amphetamine

As indicated in Table 3 a dose of 15 mg kg^{-1} of (+)-amphetamine sulphate does not elicit the usual increase of striatum HVA if given to rats previously treated for 4 days with 5 mg kg^{-1} of drug daily—an effect reported by Jori & Bernardi (1972). Similarly tolerance for the same biochemical effect can be shown for amineptine (40 mg kg^{-1}) when given on the fifth day after 4 days of treatment with amineptine (7·5 mg kg^{-1}). Animals made tolerant to either drug also show cross-tolerance (see Table 3).

Table 3. *Cross tolerance between (+)-amphetamine and amineptine on striatum HVA.*

Pretreatment mg kg^{-1} daily × 4 days		Treatment mg kg^{-1}		Striatum HVA (ng g^{-1} ± s.e.)
Saline		Saline		408 ± 20
Saline		Amphetamine	15	628 ± 50*
Saline		Amineptine	40	632 ± 37*
Amphetamine	5	Amphetamine	15	300 ± 19†
Amineptine	7·5	Amphetamine	15	465 ± 16†
Amineptine	7·5	Amineptine	40	381 ± 16†
Amphetamine	5	Amineptine	40	378 ± 43†

* $P < 0·01$ in respect to rats treated with saline.
† $P < 0·01$ in respect to the corresponding rats not submitted to a pretreatment.
The treatment was given 24 h after the last treatment. HVA determinations were made 1 h after treatment.

DISCUSSION

Although some similarities exist between the pharmacological effects of amineptine and (+)-amphetamine, the former differs particularly in its effects on brain monoamines. Amineptine is practically devoid of anorectic activity since doses of 10 and 20 mg kg^{-1}, which produce a pronounced stimulation of motor activity, do not significantly modify the food intake of rats. On the contrary, comparable low doses of (+)-amphetamine produce both a marked anorexia and a locomotor stimulation. Recently it has been suggested that brain catecholamines play a role in the anorectic effect elicited by amphetamine (Samanin & others, 1975a), although the relative roles of noradrenaline and dopamine remain unclear. An electrolytic lesion placed at the level of the ventral noradrenergic bundle completely antagonizes the anorectic effect of amphetamine (Ahlskog & Hoebel, 1973) suggesting that brain noradrenaline is preferentially involved in this effect. This is supported by the findings. Unlike amphetamine, amineptine neither modifies the concentrations of brain noradrenaline nor antagonizes its decrease as induced by 6-OHDA, an effect that is specifically shown by blockers of noradrenaline uptake like desipramine and nomifensine (Samanin & others, 1975b).

Like amphetamine, amineptine produces a marked increase of striatal HVA, which could indicate an increased release and/or synthesis of dopamine. This would explain the marked increase of locomotor activity induced, an effect that has been attributed to a stimulation of the dopaminergic system in the brain (Pijnenburg & van Rossum, 1973; Thornburg & Moore, 1973; Asher & Aghajanian, 1974; Kelly, Seviour & Iversen, 1975).

The biochemical effect of amineptine on the dopaminergic system also appears to be similar to that of (+)-amphetamine, since, like (+)-amphetamine, amineptine is able to antagonize the effect of 6-OHDA on brain dopamine. These findings

indicate that amineptine can interfere with the uptake mechanism of dopamine into the neurons since drugs such as nomifensine and amphetamine, which block this mechanism, significantly antagonize the effect of 6-OHDA on brain dopamine (Samanin, Bernasconi & Garattini, 1975b).

The relatively low efficacy of amineptine in eliciting stereotyped behaviour is surprising since this effect is commonly attributed to a stimulation of the dopaminergic system in the brain (Scheel-Krüger & Randrup, 1967). It has been suggested that the stereotyped behaviour may be due mainly to a stimulation of dopaminergic neurons in the striatum while those in the n. accumbens appear to be particularly involved in the increase of locomotor activity observed in various situations (Pijnenburg & van Rossum, 1973; Asher & Aghajanian, 1974; Kelly & others, 1975). No information is at present available on the effect of amineptine on the dopaminergic system in the n. accumbens.

The fact that, unlike amphetamine, amineptine does not modify the concentrations of striatal acetylcholine suggests that amineptine, despite its marked effect on striatal HVA, does not markedly stimulate dopamine receptors in the striatum, since the increase of striatal acetylcholine induced by amphetamine appears to be due to stimulation of striatal dopamine receptors (Ladinsky, Consolo & others, 1975). Whether this is due to differences in the distribution or in the biochemical profiles of the two drugs remains to be clarified. However the effect of amineptine on striatum dopamine must have a common mechanism with that elicited by (+)-amphetamine since both drugs show cross tolerance in increasing the major metabolite of dopamine in the striatum.

In conclusion amineptine appears to be a new type of central stimulant which shows interesting differences from (+)-amphetamine because its stimulant effect is clearly dissociated from other effects such as the decrease in food intake. Furthermore amineptine unlike (+)-amphetamine appears mainly to affect the dopaminergic system without exerting a depleting effect on brain noradrenaline. Amineptine may be therefore a useful tool in exploring brain functions.

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