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Does Tolerance Develop to Low Doses of d- and l-Amphetamine on Locomotor Activity in Rats?[1]

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WESTON, P. F. AND D. H. OVERSTREET. *Does tolerance develop to low doses of d- and l- amphetamine on locomotor activity in rats?* PHARMAC. BIOCHEM. BEHAV. 5(6) 645–649, 1976. – An observational study of the behavioural effects of chronic regimens of d- and l-amphetamine was designed to investigate possible mechanisms underlying any parallel behavioural changes: (1) Accumulation of p-hydroxynorephedrine in noradrenergic nerve terminals; (2) Altered sensitivity of dopaminergic receptors. The study revealed that locomotor activity seen with low doses of both isomers (2.0 mg/kg d- and 6.0 mg/kg l-) decreased with chronic once daily treatments. However, this was accompanied by an increase in directed sniffing activity and the behaviour came to resemble that seen with higher doses of amphetamine (8.0 mg/kg d- and 16.0 mg/kg l-). Nonsignificant decreases in locomotor activity and increases in directed sniffing to apomorphine administration were observed during chronic amphetamine treatment. These findings suggest that (1) p-hydroxynorephedrine, a metabolite of d- but not l- amphetamine, does not play an important role in these alterations in behaviour with chronic treatment and (2) the tolerance to amphetamine observed under these conditions is due to an increased, rather than decreased, sensitivity of the rats to amphetamine.

d- and l-Amphetamine, Locomotor activity Stereotypy Tolerance Supersensitivity

CONSIDERABLE differences of opinion exist regarding whether tolerance develops to the general activating effects of amphetamines. Some workers have reported a decrease in activity with chronic treatment [2, 6, 7], while others have reported no change [14, 13, 22] or even increases [10,18]. The lack of definition of the animal's behavioural states at different times during each recording interval in many of these experiments has obscured the significance of these results. It was thought, therefore, that an observational study of the effects of amphetamine during chronic treatment could clarify some of the issues surrounding its action on locomotor activity.

While some difficulties surround the interpretation of tolerance to amphetamine's effects on locomotor activity, it is clear that tolerance to some of its effects does develop. For example, tolerance to the anorexigenic and hyperthermic effects of amphetamine has been demonstrated by several laboratories [9, 11, 13, 19]. Some workers have proposed that a metabolite of d-amphetamine, p-hydroxynorephedrine, may build up in noradrenergic nerve terminals during chronic treatment and be responsible for the reduced effects of subsequent administrations [3,13] but others have disputed its importance in tolerance to d-amphetamine [9,19]. Since l-amphetamine is not metabolized to p-hydroxynorephedrine [8,12], it was decided that the role of this metabolite in tolerance to amphetamine's effects on locomotor activity could be assessed by comparing the behavioural changes in rats chronically treated with high and low doses of d- and l-amphetamine.

More recently it has been suggested that supersensitivity to amphetamine develops during chronic treatment [10,18] and that an increased sensitivity of dopaminergic receptors may be involved [10]. This hypothesis was examined by observing the effects of apomorphine before and during chronic d- and l-amphetamine treatment and the effects of d-amphetamine before and after chronic apomorphine treatment.

METHOD

Animals

The animals for all experiments were male hooded Wistar rats from the Flinders University animal house. Their respective ages, weights and experimental histories will be described in the appropriate sections for the various experiments. They were all housed under conditions of continuous light with free access to food and water except for a brief time preceding each observation session.

Activity Recording

All observations of the animals' behaviour were made in

[1] This research formed part of a thesis submitted by the first author in partial fulfillment of the Honours B. Sc. degree in the School of Biological Sciences, Flinders University. Reprint requests should be addressed to Dr. D. H. Overstreet.

glass rectangular open field chambers (50 x 32 cm) in a quiet room under a fluorescent red light. Observation sessions were generally one min in duration.

Observation of the animal's behaviour was facilitated by the use of a manually operated Esterline-Angus Event Recorder, which permitted the paralled assessment of five behavioural parameters: (1) locomotor activity – movement about the chamber requiring the extension of the hind limbs, as distinct from other forms involving movement of fore limbs; (2) directed sniffing – sniffing activity directed above the animal or at the floor or walls of the chamber; (3) oral activity – licking or gnawing a region of the observation chamber; (4) grooming – use of mouth, fore or hind limbs to groom any region of the body; (5) inactivity – period during which no movement, including sniffing, could be observed. The time spent in any one of the above activities was calculated as a percentage of the one-min observation session.

Preliminary Studies

In preliminary experiments doses of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg d-amphetamine sulfate and 2.0, 4.0, 6.0, 10.0, and 16.0 mg/kg l-amphetamine sulfate were administered to 120-day old, 210–330 g rats, and the behaviour was observed at 15-min intervals for one hour. On the basis of these studies it was decided that 2.0 mg/kg d- and 6.0 mg/kg l- produced similar behavioural effects, which were different from those produced by 8.0 mg/kg d and 16.0 mg/kg l-. The peak effect for the majority of the animals occurred at 30 min. Consequently, these parameters were used in subsequent experiments.

Experiment 1

Five groups of eight animals (120–150 days old, 210–440 g) were selected from a larger group of 60 on the basis of their consistent locomotor response to 2.0 mg/kg apomorphine hydrochloride, administered IP three times over a one-week period, with at least one drug-free day separating consecutive injections. Group 1 was subsequently given chronic treatment with isotonic saline; Group 2, with 2.0 mg/kg d-amphetamine sulfate; Group 3, with 6.0 mg/kg l-amphetamine sulfate; Group 4, with 8.0 mg/kg d-amphetamine sulfate; and Group 5, 16.0 mg/kg l-amphetamine sulfate. All injections were given IP daily in a volume of 1 ml/kg. Doses refer to the weights of the respective salts. Fresh solutions of amphetamine were made up at four-day intervals and stored in a refrigerator.

The rat's behariour was observed for a one-min period 30 min after the 1st, 5th, 8th, 12th, 15th and 19th injections of the above treatments. On several days e.g., prior to the 3rd, 10th and 17th administrations, 2.0 mg/kg apomorphine hydrochloride was given IP 30 min before the observation session and the appropriate agent in the chronic regimen was given after the effects of apomorphine appeared to wane.

In order to assess whether cross-tolerance to the isomers of amphetamine could be demonstrated, the groups formerly receiving d-amphetamine were given l-amphetamine as their 21st treatment, while those formerly receiving l-amphetamine were given the d-isomer.

Experiment 2

Eight male hooded Wistar rats, approximately 120 days old and weighing between 204 and 325 g at the start of the experiment, were used to determine if chromic treatment with apomorphine altered the behavioural effects of amphetamine. Each animal received three injections of 2.0 mg/kg d-amphetamine sulfate. The first and the second were separated by 6 drug-free days; the second and third, by 6 consecutive daily treatments with 2.0 mg/kg apomorphine. The rats were observed for 1-min periods immediately before and 30, 60, and 90 min after the injections of d-amphetamine. All animals were permitted 30 min to habituate to the chamber prior to the first recording and were left in the chamber until the 90-min recording was completed.

Data Analysis

Medians were used to summarize that data, ranges were included as indices of variability, and nonparametric statistical methods [20] were used to test for significance.

RESULTS

Acute and Chronic Amphetamine

Table 1 summarizes the effects of acute and chronic treatment with d- and l-amphetamine upon two measures, locomotor activity and directed sniffing. It can be seen that the low doses of both isomers produced a comparable, significant increase in locomotor activity after the first injection. However, this stimulation rapidly disappeared such that by the 5th treatment and thereafter the groups were not significantly different from the control group receiving isotonic saline. In addition, within group comparisons revealed that the activity after the first injection of both isomers was significantly higher than that after the subsequent injections. The groups receiving the higher doses of the two isomers seldom showed any measurable locomotor activity throughout the chronic treatment period. Another feature of these data was the large individual variability in all groups, as reflected by the large ranges.

Following both doses of both isomers there was a large degree of directed sniffing, which was somewhat greater with the higher doses. In addition, there was a tendency for the rats receiving the low dose regimens to show increased amounts of sniffing as the chronic treatment period progressed. Because of the large individual differences within the various groups, however, none of the trends could be confirmed by statistical analysis. Every amphetamine-treated group always showed significantly more directed sniffing than the control group.

A final feature of Table 1 was the similarity in effects when the treatments of the amphetamine groups were altered; i.e., when l-amphetamine was substituted for d-amphetamine and vice versa.

Effects of Apomorphine Before and During Chronic Amphetamine

Table 2 summarizes the effects of apomorphine on two measures, locomotor activity and directed sniffing, before and during chronic treatment with d- and l-amphetamine. There was a large variability in the degree to which apomorphine stimulated locomotor activity. Thus, although there was a tendency for the effects of apomorphine on locomotor activity to decrease during chronic amphetamine treatment there were no significant trends within any group. The stimulation of directed sniffing by apomorphine

TABLE 1

THE EFFECTS OF ACUTE AND CHRONIC HIGH AND LOW DOSES OF D- AND L-AMPHETAMINE ON LOCOMOTOR ACTIVITY AND DIRECTED SNIFFING IN RATS

		Percentage of one-min Observation Session (Medians, N = 8) Treatment Group*				
Behaviour	Treat. No.	2 mg/kg D	6 mg/kg L	8 mg/kg D	16 mg/kg L	Saline
Locomotor	1	22.50(64)†	30.00(44)†	0.00(38)	0.00(46)	0.00(7)
Activity	5	7.00(11)	4.00(28)	0.00(0)	0.00(21)	0.00(3)
	8	5.50(28)	7.00(13)	0.00(92)	0.00(0)	1.25(23)
	12	2.00(47.5)	5.25(31.5)	0.00(64)	0.00(25)	0.00(0)
	15	0.50(11.5)	0.50(32)	0.00(56.5)	0.00(11)	0.00(16)
	19	1.50(22.5)	7.75(62.5)	12.00(75)	0.00(0)	2.50(14.5)
	21‡	3.25(25)	7.50(37)	0.00(15)	0.00(76.5)	0.00(19)
Directed	1	79.50(87)†	89.00(46)†	100.00(0)†	100.00(0)†	0.00(4)
Sniffing	5	78.85(100)†	100.00(49)†	100.00(16)†	100.00(0)†	0.00(2)
	8	64.00(100)†	100.00(47)†	100.00(0)†	100.00(0)†	10.50(42)
	12	100.00(44)†	100.00(55)†	100.00(0)†	100.00(13)†	0.00(0)
	15	100.00(75)†	100.00(0)†	100.00(0)†	100.00(0)†	0.00(17)
	19	92.50(96)†	100.00(38)†	100.00(78)†	100.00(0)†	12.00(46)
	21‡	100.00(72)	100.00(99)†	100.00(8)†	100.00(0)†	1.75(100)

*D = d-amphetamine sulfate, L = l-amphetamine sulfate. The amphetamine salts were dissolved in isotonic saline and injected IP in volumes of 1 ml/kg 30 min before observation.
†Significantly different from saline group of same day according to Mann-Whitney U Tests [20].
‡On this day the treatments were altered such that l-amphetamine was substituted for d-amphetamine and vice versa.

TABLE 2

EFFECTS OF APOMORPHINE ON LOCOMOTOR ACTIVITY AND DIRECTED SNIFFING IN RATS PRIOR TO AND DURING CHRONIC TREATMENT WITH D- AND L-AMPHETAMINE

		Percentage of one-min Observation Session (medians, N = 8) Treatment Group				
Behaviour	Treat. No.	2 mg/kg D	6 mg/kg L	8 mg/kg D	16 mg/kg L	Saline
Locomotor	0*	30.0(43)	36.0(50)	20.0(44)	30.0(49)	37.0(59)
Activity	2†	20.5(39)	23.0(54)	0.0(43)	22.0(35)	32.5(66)
	9†	8.5(67)	22.0(54)	6.0(75)	19.0(47)	37.5(70)
	16†	5.5(55)	13.5(46.5)	2.0(54)	23.0(62)	21.0(45)
Directed	1*	72.5(38)	86.5(50)	82.0(53)	98.0(38)	90.5(31)
Sniffing	2†	100.0(0)	100.0(0)	100.0(0)	100.0(89)	100.0(0)
	9†	100.0(12)	100.0(80)	100.0(0)	100.0(84)	100.0(0)
	16†	100.0(12)	100.0(100)	100.0(100)	100.0(100)	100.0(0)

*Scores are based on the medians of the mean responses of individual animals following three injections of 2.0 mg/kg apomorphine hydrochloride. Numbers in parentheses are ranges.
†Scores are based on the medians for individual animals following a single injection of 2.0 mg/kg apomorphine hydrochloride.

was more consistent, but there were no differences in the degree of directed sniffing during chronic treatment with either dose of d- and l-amphetamine. Thus, Chronic treatment with amphetamine did not produce any significant changes in the animals' response to 2 mg/kg apomorphine.

Effects of d-Amphetamine Before and After Chronic Apomorphine

Locomotor activity was less following the second and third administrations of d-amphetamine than the first, as illustrated in Table 3, but the differences were not statistically significant. However, there was a significantly greater amount of directed sniffing at 60 and 90 min after the second d-amphetamine treatment. This increase was still apparent following chronic treatment with apomorphine. Thus, an increase in the effects of amphetamine was observed when the two injections were separated by 6 drug-free days and this increase was unaltered by an intervening period of chronic apomorphine treatment.

TABLE 3

EFFECTS OF CHRONIC TREATMENT WITH APOMORPHINE ON THE RESPONSE OF RATS TO D-AMPHETAMINE

		Percentage of one-min Observation Session (Medians, N = 8) Time after injection (min)			
Behaviour	Treat.* No.	0	30	60	90
Locomotor	1	0.00(24)	17.75(44)	5.50(38)	5.50(36)
Activity	2	0.00(0)	3.00(53)	1.50(27)	0.00(6)
	3	0.00(0)	11.00(41)	6.50(31)	1.50(6)
Directed	1	15.50(66)	88.50(43)	67.50(74)	47.75(72)
Sniffing	2	3.25(19)	95.50(23)	98.50(33)	99.50(99)†
	3	0.00(0)	100.00(15)	100.00(2)	100.00(25)†

*Of 2.0 mg/kg d-amphetamine sulfate. The first and second treatments were separated by 6 drug-free days; the second and third, by 6 consecutive daily treatments with 2.0 mg/kg apomorphine hydrochloride.

†Significantly different, $p<0.05$, from response after first treatment according to Wilcoxon two-tailed test for related samples [20].

DISCUSSION

The present results suggest that tolerance to the hyperactivating effects of d- and l-amphetamine can be observed under certain circumstances; namely, for relatively low doses only and with the use of outward locomotion as the behavioural parameter. These findings further indicate that the confusion in the literature regarding tolerance to the hyperactivating effects of amphetamine [2, 6, 11, 13] may be related to the degree to which the measuring instruments record outward locomotion versus more restricted, stereotyped movements such as sniffing. Had a very sensitive recording instrument been used in the present experiment, it is very likely that no tolerance to amphetamine would have been observed, since directed sniffing appeared to increase throughout chronic treatment with the low doses of both isomers while outward locomotion decreased. It is important to realize, therefore, that automatic recording devices which fail to distinguish between outward locomotion and stereotypy are of little value in clarifying the actions of amphetamine.

Since directed sniffing increased while outward locomotion decreased during chronic treatment with low doses, it is perhaps ill-advised to refer to these changes as tolerance. A more appropriate term might be sensitization, since the behavioural pattern changes from locomotor activity, normally seen with low doses, to stereotypy, normally seen with higher doses. Thus, the present results essentially support the findings of previous workers who have suggested that chronic treatment with amphetamine results in a supersensitivity to subsequent doses of amphetamine [10,18].

The mechanisms underlying this supersensitivity to amphetamine upon chronic treatment require further study, but certain conclusions are possible on the basis of the present and recent related experiments. Firstly, since the behavioural changes for d- and l-amphetamine were comparable, it is unlikely that p-hydroxynorephedrine, a metabolite of the former only [8,12] plays any role in these. Other recent reports indicate that this metabolite does not play a significant role in the development of tolerance to the hyperthermic effects of amphetamine [19]. Therefore, other mechanisms must be considered.

Recent evidence suggests that both the locomotor activity and the stereotypy induced by d- and l-amphetamine are primarily the result of the release of dopamine [4, 5, 17], refuting earlier contentions that release of noradrenaline is more important in the action of d-amphetamine [21]. Thus, supersensitivity to amphetamine during chronic treatment may be related to neurochemical changes in the dopaminergic system. In particular, if it can be confirmed that amphetamine produces a prolonged decrease in the turnover of dopamine [18], it might be argued that this neurochemical effect will result in a functional, partial denervation of dopaminergic neurons, with the consequent development of postsynaptic or postjunctional supersensitivity [15,23].

If this hypothesis were correct, it would be predicted that the animals chronically treated with amphetamine would exhibit an increased sensitivity to apomorphine, a directly acting dopamine agonist [1]. However, the present results provided only slight evidence for this (See Table 2), possibly because the dose of 2.0 mg/kg was too high. To explore the possibility further other doses of apomorphine (0.15, 0.20, 0.30, 0.50, and 1.00 mg/kg) were given to the animals after at least 9 weeks of chronic treatment. No consistent changes in directed sniffing were observed in any of the five groups, thus failing to demonstrate a supersensitivity to apomorphine. This finding does not confirm an earlier report [10] and emphasizes the need for additional work in this area. It is possible that differences in recording techniques and/or species of animal may partially account for the lack of agreement between the present and previous [10] findings.

A final point that must be emphasized is that the mechanisms underlying behavioural changes during chronic treatment with amphetamine are undoubtedly numerous and may vary among different animals. A striking feature of the present findings is the large individual differences among animals within a particular treatment group (Tables 1, 2, 3). These individual differences have been observed by

other workers using chronic regimens of amphetamine [16], but may not have been given sufficient attention by other workers. It may prove fruitful to use very large samples of animals and relate individual differences in response to amphetamine to neurochemical differences.

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