Pharmacology Biochemistry & Behavior, Vol. 11, pp. 351–354. Printed in the U.S.A.

Actions and Interactions of Amphetamine on Self-stimulation in Rats

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Received 10 October 1977

AULAKH, C. S. AND S. N. PRADHAN. *Actions and interactions of amphetamine on self-stimulation in rats.* PHARMAC. BIOCHEM. BEHAV. 11(3) 351–354, 1979.—The dose-response relationship for d-amphetamine (0.125–2 mg/kg, IP) and its l-isomer (0.125–3 mg/kg, IP) was studied in self-stimulation behavior of rats each with an electrode at posterior hypothalamus (PH, mainly monoaminergic) or area ventralis tegmentum (A10, dopaminergic). The drug effects increased with the dose reaching a peak (at 0.5 mg/kg with d-amphetamine and at 1.0 mg/kg with l-amphetamine) and then decreased. The d-isomer was approximately twice as potent as the l-isomer in enhancing intracranial self-stimulation (ICSS) rate with electrodes at either site. Azaperone (mainly an α-adrenergic blocker) and haloperidol (an antidopaminergic neuroleptic) used in small doses (0.05 and 0.008 mg/kg respectively) which did not affect the baseline responding, blocked amphetamine-induced enhancement of ICSS in both groups of rats. Thus, amphetamine-induced facilitation of ICSS at both PH and A10 areas and its blockade by an α-adrenergic blocker as well as an antidopaminergic neuroleptic show the involvement of both noradrenergic and dopaminergic mechanisms in self-stimulation behavior.

Self-stimulation Posterior hypothalamus Area ventralis tegmentum d- and l-Amphetamine Rat Azaperone Haloperidol

OPINION varies concerning the potency and the mechanism of action of the d- and l-isomers of amphetamine. Phillips and Fibiger [13] reported that d-amphetamine was 7–10 times more potent than its l-isomer in enhancing intracranial self-stimulation (ICSS) rate when the electrode was implanted in the lateral hypothalamus, but the two isomers were equipotent in enhancing ICSS rate with electrode implanted in the substantia nigra. Stephens and Herberg [19] reported that the mean percentage difference in enhancement of self-stimulation by d- and l-amphetamines at the locus ceruleus (noradrenergic) was even 5 times higher than that at the substantia nigra (mainly dopaminergic). These differential effects of the isomers at different electrode sites may be partly accounted for by the fact that ICSS is mediated by dopamine (DA) at the substantia nigra and by norepinephrine (NE) at the lateral hypothalamus or locus ceruleus.

Early studies showed that amphetamines produced their central stimulant effects by releasing NE [5,18], but more recent studies suggest that the release of DA is more important for many of their central actions [4, 11, 20]. The mechanism of action of amphetamine have been further studied by interaction experiments with drugs of known mechanism of action. Thus, adrenergic blockers, whether administered IP [22] or intracerebroventricularly [3] decreased self-stimulation responding to varying degrees, and pretreatment with these agents also reduced the facilitating effect of amphetamine on this behavior, thus showing the involvement of noradrenergic mechanism in this behavior. On the other hand, antidopaminergic neuroleptics (e.g. haloperidol and pimozide, IP) significantly decreased ICSS in rats with electrodes in the nucleus accumbens that contains mainly the dopaminergic cell bodies [14], indicating thereby the involvement of dopaminergic mechanism in self-stimulation. However, these antidopaminergic neuroleptics also decreased the ICSS rate even with the electrode site at the dorsal NE bundle [14] and lateral hypothalamus [21], which is difficult to explain.

The objectives of the present study are to further investigate the effects of the d- and l-amphetamines on self-stimulation behavior particularly with respect to their dose-response relationship, and mechanism of action by using two different electrode sites, posterior hypothalamus (PH, monoaminergic) and area ventralis tegmentum (A10, dopaminergic). Their mechanism of action was further studied in drug interaction experiments using drugs of known mechanism of action.

METHOD

Male albino rats of Wistar-derived Walter Reed strain with initial body weight of 200–250 g were used in this experiment. All rats were housed individually and had free access to food and water. The methods of electrode implantation, experimental procedure, drug treatment and data evaluation were essentially the same as reported earlier [15]. A set of bipolar stainless steel electrodes was stereotaxically implanted in the posterior hypothalamus (PH) or in the area ventralis tegmentum (A10 area, dorsal to the interpeduncular nuclei [7]) in each rat under pentobarbital (50 mg/kg, IP) anesthesia. The stereotaxic coordinates of the PH were 3.5 mm posterior to the bregma, 0.75 mm lateral to the midline and 9 mm in depth from the top of the skull; those of the A10 area were 5.5 mm posterior to the bregma, 0.1 mm lateral to the midline and 8.5 mm in depth from the top of the skull

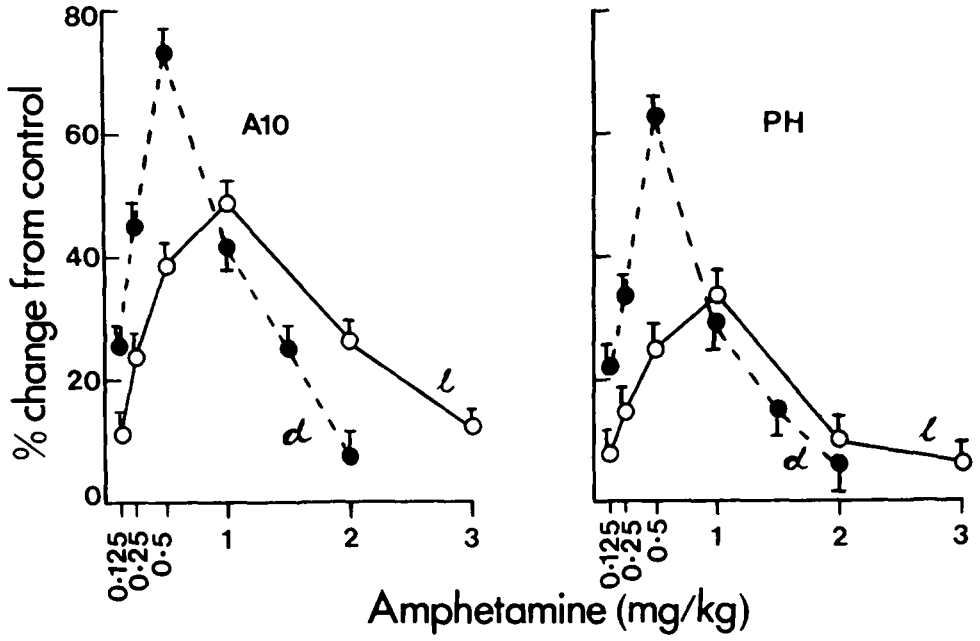

FIG. 1. Dose-response relationship of d- and l-amphetamine in self-stimulation behavior in rats with electrode in the posterior hypothalamus (PH) or area ventralis tegmentum (AI0). Each point in the graph represents mean and standard error of the mean of the data from at least 5 rats.

according to Atlas of Hart [10]. The animals will be designated as PH or A10 rats according to their electrode placement. Verification of electrode tips was done histologically in each representative rat upon completion of the experiment according to the atlas of König and Klippel [12].

After recovery from surgery, the rats were trained to press the bar in a Skinner box to be reinforced with intracranial electrical stimulation. The box (22 × 27 × 47 cm) made of plastic was equipped with a bar at the center of a side wall 6 cm above the floor. The bar was connected with programming and recording equipment and each bar press provided a stimulus train of 0.4 sec duration, stimulus being a sine wave of 60 Hz. The threshold current level for a rat was set to be the minimum current which would generate 1 to 10 responses/min in excess to that observed in the absence of the current. The current intensity (measured as rms) remained constant at slightly above the threshold level throughout the sessions, and varied from 10 μA to 25 μA from rat to rat. Bar-pressing responses were recorded every 10 min during daily 60-min sessions.

Rats were subjected to a daily session 6 days a week. When the responding of each rat reached a stable level (i.e. bar-pressing rate in a session was within 10% of the daily average for 5 to 6 days), treatment with drugs was initiated. Five rats were used at each dose level in dose response study and there were four rats in each group during interaction study. Two isomers (d- and l-) of amphetamine sulfate, azaperone (an α-adrenergic blocker), and haloperidol (an antidopaminergic neuroleptic) were used in this experiment. Amphetamine (d- and l-isomers) was dissolved in saline, whereas azaperone and haloperidol were dissolved in tartaric acid.

All the drugs were administered IP. For interaction study, amphetamine (d- or l-isomer) was injected at 0 min just before the onset of session and an interacting drug was injected 10 min prior to amphetamine. A dose of a drug was injected only when the control data from two seccessive daily sessions reached the previous baseline level and was within 10% of the average.

The data from the drug sessions were reported as the percent change from controls. The data of similar category from several rats, one from each rat, at a particular dose of a drug was used to calculate the mean, the standard error of the mean (± SE) wherever necessary; the difference between two means was also estimated and Student's t was calculated to determine the level of significance.

RESULTS

Dose-Response Relations

In this study, ICSS responding varied from 4000 to 6000/hr for AI0 rats and from 2000 to 4000/hr for PH rats. The behavioral effects over wide dose ranges for d-amphetamine (0.125–2 mg/kg) and its l-isomer (0.125–3 mg/kg) in both PH and AI0 rats are shown in Fig 1. Although there were variations in the baseline rates and in response to a drug dose, a rough dose-response relationship could be observed in the same rat. In general, the drug effects increased with the dose reaching a peak and then decreased. Peak effects were observed in both groups of rats at 0.5 mg/kg doses with d-amphetamine and at 1 mg/kg doses with the l-isomer. Both isomers showed greater facilitation of responding at all doses in AI0 rats compared to PH rats. On comparing the potency of the two isomers in enhancing self-stimulation at AI0 or PH electrode sites, d-isomer was found to be approximately twice as potent as l-isomer over 0.125–0.5 mg/kg dose range. However, at higher doses their potency did not appear to differ.

TABLE 1

INTERACTIONS OF D- AND L-AMPHETAMINE WITH DIFFERENT DRUGS ON SELF-STIMULATION

Effect* of amphetamine†	Rat group	Interacting drug Drug & dose†	Effect*	Effect* of drug combination†‡
D-Amphetamine				
63 ± 1 (4)	PH	Azaperone, 0.05	−1 ± 2 (4)	18 ± 7 (4)
63 ± 1 (4)	PH	Haloperidol, 0.008	−3 ± 1 (4)	14 ± 5 (4)
73 ± 2 (4)	AIO	Azaperone, 0.05	−3 ± 1 (4)	16 ± 3 (4)
73 ± 2 (4)	AIO	Haloperidol, 0.008	−4 ± 1 (4)	8 ± 2 (4)
L-Amphetamine				
24 ± 1 (4)	PH	Azaperone, 0.05	−1 ± 2 (4)	7 ± 1 (4)
24 ± 1 (4)	PH	Haloperidol, 0.008	−3 ± 1 (4)	6 ± 2 (4)
38 ± 2 (4)	AIO	Azaperone, 0.05	−3 ± 1 (4)	15 ± 1 (4)
38 ± 2 (4)	AIO	Haloperidol, 0.008	−4 ± 1 (4)	9 ± 1 (4)

*percent change from the controls; mean ± S.E.; figures in parentheses indicate the number of experiments in each group

†Doses were in mg/kg; amphetamine was used in 0.5 mg/kg doses. Each rat was given the same dose of an interacting drug whether used alone or in combination with amphetamine

‡Significantly different from the effects of amphetamine alone $p<0.01$

Drug Interaction

The interactions of amphetamine (d- and l-isomers) with different drugs are presented in Table 1. d-Amphetamine (0.5 mg/kg) and l-amphetamine (0.5 mg/kg) produced a significant increase in self-stimulation responding. Azaperone and haloperidol, when administered alone in doses of 0.05 and 0.008 mg/kg respectively, did not affect baseline responding, but significantly decreased amphetamine-induced enhancement of self-stimulation at both PH and AI0 sites.

DISCUSSION

The present study suggests that d-amphetamine is more potent than the l-isomer in increasing ICSS rate with electrodes aimed either at PH or AI0 area. This observation is consistent with that of Goodall and Carey [8] who used the lateral hypothalmus and substantia nigra as the electrode sites. Our data from both PH and AI0 rats shows that d-isomer was approximately twice as potent as the l-isomer in facilitating ICSS rate. The potency ratios between d- and l-amphetamine in 2 rat groups of the present experiment are less than the reported 5:1 ratio at the anterior midbrain tegmentum [17] and 7–10:1 ratio at the lateral hypothalamus [13] in self-stimulation behavior, and 6:1 ratio in intravenous self-administration [16]. However, they are in agreement with the potency ratios of d- and l-isomers for a number of behavior, such as 2.07–2.49 for self-stimulation [8], 2.38–2.98 for self-administration [23], and 2.75 for anorexia [2].

Besides these differential behavioral effects, the two amphetamine isomers also showed difference in their neurochemical effects. Thus, d-isomer was shown to be 10 times more potent than the l-isomer in blocking norepinephrine (NE) reuptake by noradrenergic synaptosomal segments, whereas both isomers were equipotent in blocking dopamine (DA) reuptake by dopaminergic synaptosomal segments [6]. In the contrary, while these two isomers were almost equipotent in inhibiting uptake of ^{3}H-NE by the cerebral cortex and mesencephalic synaptosomes, d-amphetamine was 4–5 times more potent than l-isomer in inhibiting uptake of ^{3}H-DA by striatal synaptosomes [9,20].

In the present experiment, the dopaminergic AI0 site was more sensitive to either isomer of amphetamine in facilitating ICSS rate, compared to PH site which is generally monoaminergic. In contrast, Phillips *et al.* [14] demonstrated the greater ICSS facilitation at a noradrenergic site (dorsal noradrenergic bundle) as compared to a dopaminergic site (nucleus accumbens). Both d- and l-amphetamines also facilitated ICSS at the lateral hypothalamus to a greater extent compared to the substantia nigra [8]. However, these differences in ICSS response may be attributed to electrode placements at different noradrenergic and dopaminergic sites.

In interaction studies, the facilitating effects of both d- and l-amphetamines were significantly reduced in both PH and AI0 rats following pretreatment with an α-noradrenergic blocker (azaperone), which is in agreement with the findings of Wise *et al.* [22] with IP doses of α-adrenergic blockers, and those of Bailey and Pradhan [3] with their intraventricular injections. On the other hand, pretreatment with antidopaminergic neuroleptic (haloperidol) also blocked the amphetamine-induced ICSS enhancement in the PH and AI0 rats. In a previous study from our laboratory, it was shown that azaperone (α-adrenergic blocker) and haloperidol (antidopaminergic neuroleptic) used in small doses which did not affect baseline responding also reduced cocaine-induced facilitation of self-stimulation behavior at both PH and AI0 sites [1]. However, it is obvious that α-noradrenergic blockers as well as antidopaminergic neuroleptics were both effective in blocking amphetamine-induced enhancement of ICSS rate at both the PH as well as AIO electrode sites, thus further showing the involvement of both noradrenergic and dopaminergic mechanisms in self-stimulation at such areas.

REFERENCES

1. Aulakh, C. S., B. Ghosh and S. N. Pradhan. Actions and interactions of cocaine on self-stimulation in rats. *Psychopharmacology* **63:** 75–79, 1979.
2. Baez, L. A. Role of catecholamines in the anorectic effects of amphetamine in rats. *Psychopharmacologia* **35:** 91–98, 1974.
3. Bailey, P. T. and S. N. Pradhan. Interactions of adrenergic stimulants and blockers on self-stimulation behavior in rats. *Res. communs chem. Pathol Pharmac.* **11:** 533–552, 1975.
4. Carlsson, A. Amphetamine and brain catecholamines In: *Amphetamines and Related Compounds*, edited by E. Costa, and S. Garattini, New York: Raven Press, 1970, pp. 289–300.
5. Carr, L. A. and K. E. Moore. Norepinephrine: Release from brain by d-amphetamine in vivo. *Science* **164:** 322–323, 1969.
6. Coyle, J. T. and S. H. Snyder. Catecholamine uptake by synaptosomes in homogenates of rat brain: Stereospecificity in different areas. *J. Pharmac. exp. Ther.* **110:** 221–231, 1969.
7. Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine neurons in the central nervous system. *Acta physiol Scand. Suppl.* **247:** 1–55, 1965.
8. Goodall, E. B. and R. J. Carey. Effects of d- versus l-amphetamine, food deprivation, and current intensity on self-stimulation of the lateral hypothalmus, substantia niagra and medial frontal cortex of the rat. *J. comp. physiol. Psychol.* **89:** 1029–1045, 1975.
9. Harris, J. E. and R. J. Baldessarini. Uptake of ^{3}H-catecholamines by homogenates of rat corpus striatum and cerebral cortex: Effects of amphetamine analogues. *Neuropharmacology* **12:** 669–678, 1973.
10. Hart, B. L. The rat brain in section: A stereotaxic atlas. In: *Experimental Neuropsychology*, edited by S. Coopersmith, W. H. Freeman and Company, San Francisco, 1969, pp. 93–102.
11. Hollister, A. S., G. R. Breese and B. R. Cooper. Comparison of tyrosine hydroxylase and dopamine-beta hydroxylase inhibition with the effects of various 6-hydroxydopamine treatment on d-amphetamine-induced motor activity. *Psychopharmacology* **36:** 1–16, 1974.
12. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brainstem*. The Williams and Wilkins Company, Baltimore, 1963.
13. Phillips, A. G. and H. C. Fibiger. Dopaminergic and noradrenergic substrates of positive reinforcement: differential effects of d- and l-amphetamine. *Science* **179:** 575–577, 1973.
14. Phillips, A. G., S. M. Brooke and H. C. Fibiger. Effects of amphetamine isomers and neuroleptics on self-stimulation from the nucleus accumbens and dorsal noradrenergic bundle. *Brain Res.* **85:** 13–22, 1975.
15. Pradhan, S. N. and C. Bowling. Effects of nicotine on self-stimulation in rats. *J. Pharmac. exp. Ther.* **176:** 229–243, 1971.
16. Risner, M. E. Intravenous Self-administration of d- and l-amphetamine by dog. *Eur. J. Pharmac.* **32:** 344–348, 1975.
17. Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. *Fedn. Proc.* **23:** 836–850, 1964.
18. Stein, L. and C. D. Wise. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. *J. comp. Physiol.* **67:** 189–198, 1969.
19. Stephens, D. N. and L. J. Herberg. Catecholamines and Self-stimulation: Pharmacological differences between near and far-lateral hypothalamic sites. *Brain Res.* **90:** 342–351, 1975.
20. Thornburg, J. E. and K. E. Moore. Dopamine and norepinephrine uptake by rat brain synaptosomes: relative inhibitory potencies of l- and d-amphetamine and amantadine. *Res. communs. chem. pathol. Pharmac.* **5:** 81–89, 1973.
21. Wauquier, A. and C. J. E. Niemgeers. Intracranial self-stimulation in rats as a function of various stimulus parameters II. Influence of haloperidol, pimozide and pipamperone on medial forebrain bundle stimulation with monopolar electrodes. *Psychopharmacologia* **27:** 191–202, 1972.
22. Wise, O. D., B. D. Berger and L. Stein. Evidence of α-noradrenergic reward receptors and serotonergic punishment receptors in the rat brain. *Biol. Psychiat.* **6:** 3–21, 1973.
23. Yokel, R. A. and R. Pickens. Self-administration of optical isomers of amphetamine and methylamphetamine by rats. *J. Pharmac. exp. Ther.* **187:** 27–33, 1973.