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Discriminative Stimulus Properties of d-Amphetamine and Related Compounds in Rats

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HUANG, J. T. AND B. T. HO. *Discriminative stimulus properties of d-amphetamine and related compounds in rats.* PHARMAC. BIOCHEM. BEHAV. **2**(5) 669–673, 1974. – The discriminative stimulus properties of amphetamine were demonstrated in rats trained to discriminate between 0.8 mg/kg of d-amphetamine sulfate and saline. During the discriminative training, animals were shaped on a DRL 15-second schedule to respond to one of two levers for a food reward when they were given d-amphetamine, and to respond to the other lever when they were treated with saline. Tests for the discriminative stimulus properties consisted of 10-min extinction sessions in which the reinforcement delivery was disconnected. Animals receiving low doses (0.2–0.4 mg/kg) of d-amphetamine exhibited mostly saline-like responses, but at a dose of 0.8 mg/kg they produced more than 80% responses on the amphetamine lever. Doses higher than 2.4 mg/kg caused an initial stereotyped behavior and the animals showed a period of latency before responding on the amphetamine lever. In order to elucidate the structural characteristics of d-amphetamine involved in the production of the discriminative stimulus properties, a number of amphetamine derivatives and related compounds were administered to these animals. l-Amphetamine, ephedrine, norephedrine, 4-methoxyamphetamine and methylphenidate all produced the discriminative stimulus properties similar to d-amphetamine, but doses of 2–10 times greater than d-amphetamine were necessary. Mescaline, STP and DOET did not produce the d-amphetamine-like responses. These results suggest that most psychomotor stimulants, although having different structures, are likely to produce discriminative stimulus properties similar to d-amphetamine.

Discriminative stimulus properties
Generalization to amphetamine cue
d-Amphetamine derivatives and related compounds

UTILIZATION of amphetamine as a discriminative stimulus has been reported in several papers [1, 4, 10, 12, 14]. Rats trained on a differential reinforcement of low response rate (DRL) schedule [1,14] or shock-escape procedure [12] are capable of making correct response choices between saline and d,l-amphetamine [1,14] or d-amphetamine [12]. We adapted the DRL schedule to determine the effects of structural modification of amphetamine on the responding control by d-amphetamine-induced discriminative stimulus. This method was chosen because the procedure involving electric shock as motivation generally required higher doses of the drug than that involving positive reinforcement. It is hoped that by testing various amphetamine derivatives for the production of d-amphetamine-like responses, the relevant molecular structures of the derivatives involved in the generation of the interoceptive cue produced by d-amphetamine can be revealed.

METHOD

Chemicals

All chemicals in their salt forms were dissolved in 0.9% saline. 4-Methyl-2,5-dimethoxyamphetamine (STP), 4-ethyl-2,5-dimethoxyamphetamine (DOET), 4-methylamphetamine, 4-methoxyamphetamine, and 2,5-dimethoxyamphetamine were synthesized in our laboratories (Ho and Tansey, to be published). Other chemicals were purchased from commercial sources.

Animals

Twenty naive male Sprague-Dawley rats (350–450 g) purchased from Texas Inbred Mice Co., Houston, Texas, were individually housed with access to water ad lib. Throughout the study a 24-hr food deprivation schedule maintained individual animals at 80 ± 5% of normal body weight.

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Apparatus

Five operant chambers (Scientific prototype, Model A-100) each equipped with two operant levers (Scientific prototype, Model PLS-100) were used for behavioral training and testing. Each chamber was enclosed in a sound attenuating chamber (Scientific prototype, Model SPC-300) equipped with a fan to circulate fresh air and with a 7-W house light to provide illumination. Reinforcement consisted of single 45-mg Noyes pellets (standard formula). All behavioral contingencies and data collection were controlled by solid state programming equipment (Grason-Stadler 1200 series). Cumulative recorders (Gerbrands, Model G3) were also used during extinction test sessions.

Preliminary Training

Animals were deprived of food until stabilized at about 80% normal body weight, and allowed to get familiar with the cage and lever responding, and then trained for 4 days on a daily 30-min continuous reinforcement (CRF) schedule to respond on each lever. Differential reinforcement of low response rate (DRL) schedules were then followed. The first four days animals were on daily 30-min sessions under DRL 5-sec schedule, with two days on left lever and two days on right lever. The procedure was repeated with DRL 10-sec followed by DRL 15-sec schedules. Thereafter, the animals were trained on DRL 15-sec on 30-min sessions throughout all experiments.

Discriminative Training

After the 4-day training on DRL 15-sec schedule, the discriminative training was begun. The animals were injected intraperitoneally with d-amphetamine sulfate (0.8 mg/kg) or saline (1 ml/kg) 15 min before each 30-min training session. For 2 days following amphetamine administration only responses made on the left lever were reinforced, while on the other 2 days when saline was given reinforcement was contingent only upon pressing the right lever.

Extinction Testing

On the fifth day of a week following the 4-day discriminative training the animals were injected with d-amphetamine or saline, and then placed in the operant chambers with the reinforcement delivery disconnected (extinction). The degree of discrimination between d-amphetamine and saline was reflected in the percentage of responses made on the lever appropriate to the state of the animal during 10-min testing in the absence of reinforcement feedback. After 6–8 extinction test sessions a criterion to establish the discriminative control occurred when all animals responded more than 80% on the correct lever. Other derivatives of d-amphetamine were then administered for testing generalization to the d-amphetamine state. Most of the animals were used more than once but not on the same extinction test.

When animals received high doses (4 and 8 mg/kg) of amphetamine, the stereotyped behavior temporarily prevented their lever performance. They were placed in the operant chambers at 15 and 30 min, 1, 2, 3, and 4 hr time intervals, and only when the lever responding resumed to complete a 10-min extinction test was the data recorded.

Generalization Testing

Extinction sessions were again performed on every fifth day of a week with the derivatives of d-amphetamine and related compounds listed in Table 1 for the purpose of testing the degree of generalization of the d-amphetamine-induced interoceptive cue. Each compound in saline was injected i.p. to the animals 15-min prior to the test session, and results are expressed in Table 1 as the number of correct responses divided by the total number of responses. With each group of animals four daily (M–Thur) 30 min retraining sessions in the order of saline, d-amphetamine, d-amphetamine, saline was intervened prior to the next extinction test with another compound. On the first day of retraining with d-amphetamine a 10-min extinction test was performed to check the animal's correct response to d-amphetamine. This schedule was used throughout experiments. The order of administering test compounds was randomized; also each animal did not receive a compound twice.

RESULTS

The results presented in Fig. 1 show the discriminative control by d-amphetamine established in rats. The rats responded on the appropriate lever more the 85% on the 7th 10-min extinction test; that is, after the 28 discriminative training sessions.

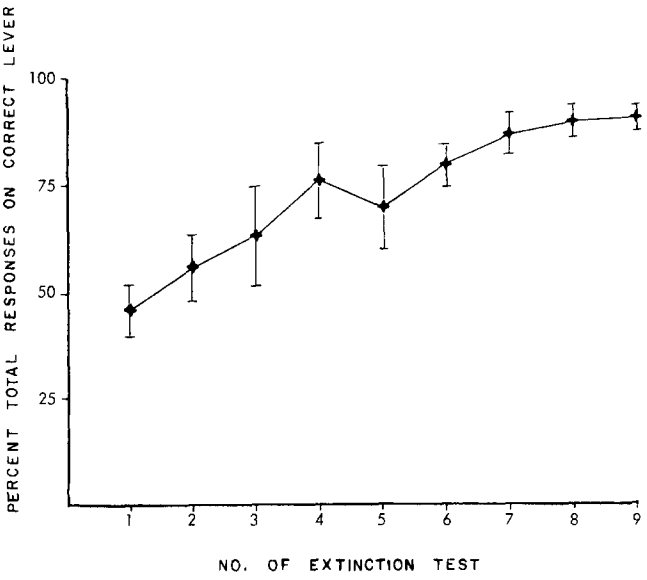

FIG. 1. Acquisition of discriminative control of responding by d-amphetamine sulfate. Each extinction test was performed after a four-day retraining on discriminative response. Each point represents the mean (±S.E.) of 20 rats. For details of method used, see *Method.*

Figure 2 shows the generalization gradient of discriminative control by d-amphetamine. When receiving a dose of 0.2 mg/kg of d-amphetamine, the animals produced saline-like responses. An increase of the d-amphetamine lever choice was observed at 0.4 mg/kg, and over 80% responses on the d-amphetamine lever were reached at the dose of 0.8 mg/kg or above.

Fifteen min after receiving d-amphetamine at doses larger than 2.4 mg/kg, the animals exhibited a severe stereotyped behavior with sniffing, gnawing or licking the bottom of the cage or lever. Because of their failure to press the lever, there was no way to evaluate any discriminative stim-

TABLE 1

DISCRIMINATIVE PROPERTIES OF d-AMPHETAMINE AND RELATED COMPOUNDS

Compound*	Dose (mg/kg)	% Amphetamine lever choices† 5 min	10 min
d-Amphetamine	0.8‡	89.0 ± 4.7	88.1 ± 7.2
l-Amphetamine	0.8	57.0 ± 11.9[c]	60.0 ± 8.7[c]
	1.6	86.0 ± 9.9	85.7 ± 9.1
Methamphetamine	0.8	87.9 ± 7.2	87.8 ± 4.7
	1.6	90.0 ± 5.2	92.1 ± 7.0
4-Methoxyamphetamine	1.0	36.0 ± 5.3[c]	34.1 ± 4.7[c]
	2.0§	78.0 ± 6.6	76.0 ± 4.6
3-Methoxyamphetamine	2.0	30.9 ± 8.6	32.1 ± 8.1
	4.0[a]	36.7 ± 4.6	39.6 ± 7.8
2,5-Dimethoxyamphetamine	1.0	7.0 ± 3.0[c]	4.2 ± 1.6[c]
	2.5	49.6 ± 6.4[c]	56.4 ± 7.5[c]
	4.0	11.0 ± 4.0[c]	9.8 ± 2.4[c]
4-Methylamphetamine	1.0	42.0 ± 14.5[c]	46.0 ± 11.9[c]
	2.0§	50.0 ± 15.1[c]	52.0 ± 16.7[c]
4-Hydroxyamphetamine	1.0	36.0 ± 5.2[c]	34.1 ± 4.7[c]
	2.0	22.6 ± 5.8[c]	25.1 ± 6.8[c]
STP	0.25	21.0 ± 5.3[c]	17.2 ± 3.0[c]
	0.8	39.0 ± 8.7[c]	39.8 ± 4.8[c]
	1.0[a]	35.7 ± 7.3[c]	38.1 ± 9.4[c]
DOET	0.25	28.0 ± 7.3[c]	30.0 ± 12.0[c]
	0.5[a]	38.0 ± 8.8[c]	29.0 ± 8.3[c]
Ephedrine	2.0	46.0 ± 7.3[c]	49.0 ± 5.1[c]
	4.0	58.0 ± 9.6[c]	61.0 ± 11.4[c]
	8.0	80.9 ± 10.4	82.4 ± 8.9
Norephedrine	1.0	15.5 ± 4.3[c]	16.8 ± 7.0[c]
	8.0	75.0 ± 6.1	70.0 ± 2.1
Mescaline	12.5	28.7 ± 4.9[c]	33.0 ± 9.0[c]
	25	34.3 ± 4.0[c]	34.0 ± 6.4[c]
Tyramine	1.0	15.1 ± 6.0[c]	19.5 ± 7.3[c]
	4.0	15.4 ± 3.6[c]	14.2 ± 4.8[c]
Methylphenidate	0.5	31.9 ± 7.3[c]	31.9 ± 7.5[c]
	1.0	40.0 ± 7.7[c]	45.6 ± 6.3[c]
	2.5	89.9 ± 7.5	88.1 ± 6.8
Cocaine	7.5	92.7 ± 8.4	90.0 ± 10.3
Saline	1.0‡[b]	19.5 ± 5.5	15.0 ± 4.1

*All chemicals were in the form of hydrochloride salt except d-amphetamine and l-amphetamine which were in the sulfate form.

†Rats, after being trained for discriminative responding to d-amphetamine, were injected intraperitoneally with various doses of the chemicals, and the 10-min extinction test was performed 15 min

after the injection. Correct response was judged by pressing the amphetamine lever during 5 and 10 min periods. Each value represents the mean (± S.E.) of five animals. Most of the animals were used more than once but not on the same generalization test.

‡All rats made more than 50 lever responses during 10 min.

§Two of the five animals made less than 10 lever responses during 10 min.

[a]One of the five animals made less than 10 lever responses during 10 min.

[b]ml/kg.

[c]Significantly different from d-amphetamine, $p<0.05$; all other values are not significantly different from d-amphetamine. Statistical comparisons were performed by Student's t-test, the criteria for significance being $p<0.05$.

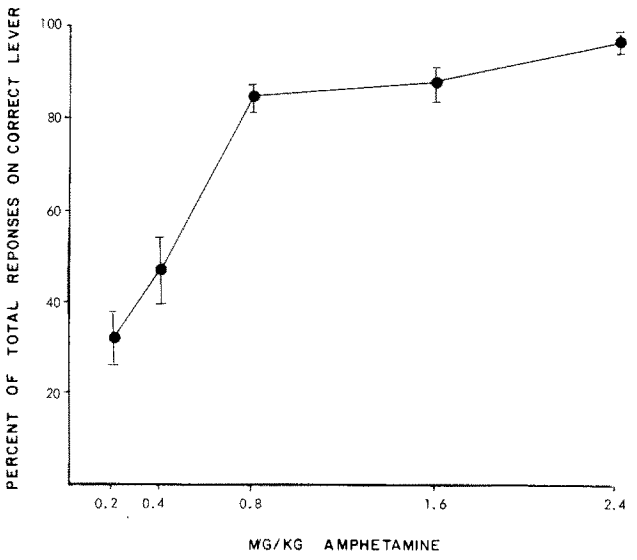

FIG. 2. Generalization gradient of discriminative control by d-amphetamine (0.8 mg/kg was the training dose). Each point represents the mean (±S.E.) of five rats.

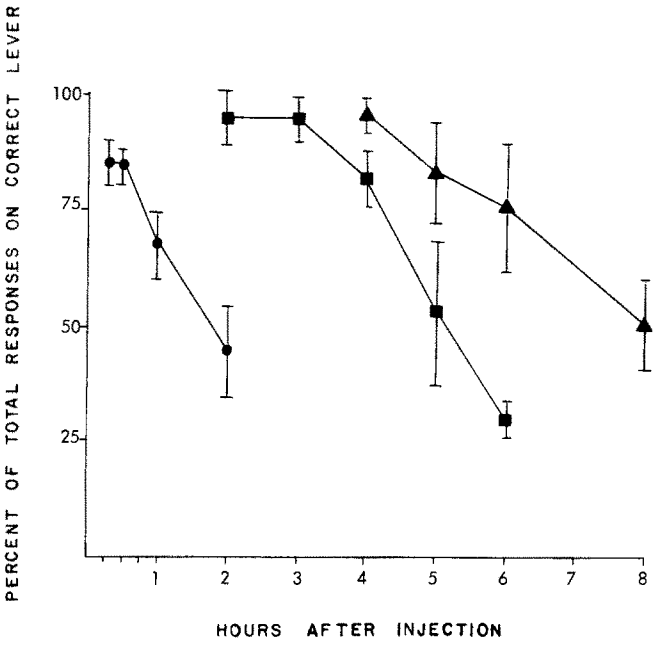

FIG. 3. The onset and duration of discriminative control by various doses of d-amphetamine. Each point represents the mean (±S.E.) of each separate group of four rats; during the same day of testing none of the animals was used for more than one extinction test. For details of method used, see *Method.* Key: •, 0.8 mg/kg; ■, 4 mg/kg; ▲, 8 mg/kg.

ulus produced. However, after this interfering stereotyped behavior had subsided, responding on the amphetamine lever occurred. Delays in responses were 2 and 4 hr with 4 and 8 mg/kg of d-amphetamine respectively as shown in Fig. 3.

When other amphetamine derivatives and related compounds were tested for discriminative stimulus properties, methamphetamine produced the same lever choice response as did the same dose of d-amphetamine, whereas it required twice as large a dose of l-amphetamine to produce the d-amphetamine-like response (Table 1). Methylphenidate exhibited a similar discriminative stimulus property to d-amphetamine only at a dose three times higher than d-amphetamine. The amphetamine-like cueing effect was also observed by a higher dose of 4-methoxyamphetamine. However, 4-methylamphetamine produced only 50% of the amphetamine lever responses at 2 mg/kg, and when a higher dose was given, the animals stopped responding and stayed at the corner of the cages with no stereotyped behavior being observed. For ephedrine and norephedrine to exert amphetamine-like cueing effect, a dose which was about 10 times higher than d-amphetamine was necessary. STP and other amines did not show a lever choice response like d-amphetamine within the dose range that animals would respond to the lever.

DISCUSSION

Results of the present study clearly demonstrate that d-amphetamine at 0.8 mg/kg can serve as an effective discriminative stimulus. The development of discriminative control by other drugs such as alcohol [3] and nicotine [6] utilizing the lever choosing method has also been documented.

The interference of lever pressing by the amphetamine-induced stereotyped behavior has been reported by Rundrup and Munkvad [8], who trained rats to avoid electric shock by pressing the lever to shut off electricity. The same phenomenon was observed in our animals trained to respond to positive reinforcement. Animals receiving higher doses of d-amphetamine exhibited a delay in lever pressing due to stereotyped behavior, then assumed responding as accurately as when given the training dose (0.8 mg/kg) of d-amphetamine (Fig. 3). However, a smaller dose (0.2 mg/kg) of d-amphetamine which was not enough to evoke stereotyped behavior, also failed to produce the same discriminative cueing effect as that observed with 0.8 mg/kg.

Maickel *et al.* [5], using the same strain and weight range of rats as those in this study, showed the concentration of amphetamine in rat's brain at 30 min with an i.p. dose of 0.25 mg/kg was the same as at 4 hr with 4 mg/kg. Thus, in the present study the delay in responding at high doses persisted until the concentration of d-amphetamine in the brain was reduced to a level which the rat was able to perform.

The present study showed that d-amphetamine-induced discriminative control can generalize to a number of amphetamine derivatives, most of these compounds having CNS stimulant properties. Compounds that were found to be ineffective in producing the amphetamine lever response are also known to exert less CNS stimulant action. A higher dose of l-amphetamine required in producing the lever choice responses similar to that produced by d-amphetamine correlated with the l-isomer being a less potent CNS stimulant than d-amphetamine. The requirement of twice as large a dose of l-amphetamine to produce d-amphetamine shock-escape responses has also been reported [12]. Taylor and Snyder [13] reported d-amphetamine was twice as potent as l-amphetamine in producing compulsive gnawing behavior. These results suggest that the discriminative cue of d-amphetamine is due to the action of d-amphetamine on the CNS instead of on the peripheral nervous system. Production of this discriminative cue has been demonstrated by the intraventricular administration of 100 μg of d-amphetamine [9].

Structural modification of d-amphetamine resulted in compounds having different discriminative stimulus properties. Our previous study [2] showed that the β-phenethylamine moiety is an essential part of the structure of d-amphetamine for the production of the discriminative cue in rats. In this study it was shown that both the nature and position of substitution on the aromatic ring appear to be determining factors for producing the d-amphetamine-like responses. When either a methyl or hydroxy group were substituted in the 4-position, compounds resulted which did not exhibit the stimulus properties of d-amphetamine. However, 4-methoxyamphetamine produced the same stimulus condition as d-amphetamine but 3-methoxyamphetamine did not cause the d-amphetamine-like responses. Alteration of the side-chain of d-amphetamine also produced changes in the amphetamine-like responses. Both norephedrine and ephedrine, resulting from hydroxylation of the side chain, were weaker stimuli than d-amphetamine.

Hallucinogens such as mescaline, DOET, and STP showed responses to lever choice different from d-amphetamine. Schechter and Rosecrans [11] reported that mescaline and psilocybin, but not d-amphetamine, could produce the discriminative cue of LSD. They have further demonstrated that mescaline or LSD could not produce the d-amphetamine cue [12]. Our results confirm their findings on mescaline and further suggest that d-amphetamine acquires a discriminative cue different from most of hallucinogens.

The present study suggests that compounds of similar pharmacological property can produce the same discriminative cueing effect. Two psychomotor stimulants, methylphenidate and cocaine, were demonstrated to produce lever choice responses similar to that produced by d-amphetamine (Table 1). Recently, we have observed the generalization of the amphetamine responses by a high dose (2 mg/kg) of nicotine (Huang and Ho, submitted for publication). Nicotine has been shown to have similar behavioral effects similar to d-amphetamine [7,15].

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