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BRIEF COMMUNICATION

Amfonelic Acid: Similarity to Other Dopamine Agonists

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SCHECHTER, M. D. *Amfonelic acid: Similarity to other dopamine agonists.* PHARMACOL BIOCHEM BEHAV **26**(2) 413–416, 1987.—Rats were trained to discriminate between the stimulus properties of intraperitoneally administered 0.8 mg/kg amfonelic acid and its vehicle in a two-lever, food-motivated operant task. Once trained, rats showed a dose-related decrease in discriminative performance with lower amfonelic acid doses and analysis of the dose-response curve indicated an ED50 of 0.11 mg/kg. Administration of 0.08–0.6 mg/kg *d*-amphetamine produced a pattern of responding similar to that observed with amfonelic acid, with an ED50 of 0.10 mg/kg and a non-parallel dose-response curve. Likewise, the discriminative stimulus properties of amfonelic acid were shown to generalize to both *d,l*-cathinone and cocaine but not to apomorphine. The results suggest that amfonelic acid, as well as other non-amphetamine stimulants, acts by a different mechanism of action than does amphetamine and biochemical studies are reviewed to further evidence this observation.

Drug discrimination Amfonelic acid Amphetamine Dopamine Cocaine Cathinone Apomorphine

AMFONELIC acid (AFA) is a central nervous system stimulant which, like amphetamine, produces increased locomotor activity and stereotyped behavior in animals and symptoms in humans which closely resemble paranoid schizophrenia [1,8]. The effects of these two drugs have been evidenced to be mediated by central dopamine neurons [8]. Although these drugs produce similar behavioral effects, their biochemical actions are dissimilar in that amphetamine acts to preferentially release newly synthesized dopamine whereas AFA acts to preferentially release older, stored dopamine [9].

The drug discrimination procedure is a sensitive and specific behavioral paradigm that has been employed by numerous investigators to evidence the dopaminergic activity of various central stimulants. In all of these studies, at least one other stimulant drug was tested to indicate its ability to produce the interoceptive cue that was produced by the trained drug, a process referred to as either generalization, substitution, or transfer [10]. AFA has been reported to transfer from rats trained to discriminate the interoceptive cues produced by *d*-amphetamine [2] but not transfer from those trained with apomorphine [7]. However, AFA has never been used to train animals to make a discriminative response. The purpose of the present experimentation was to train rats to discriminate between AFA and its vehicle and to determine dose-effect relationships for it and for other dopaminergically-mediated drugs.

METHOD

Eight experimentally-naive male ARS/Sprague-Dawley rats were used, and their body weights were maintained at approximately 80% of free-feeding weight by partial food deprivation. Behavioral training and testing was conducted in standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN) each equipped with two operant levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the two levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and 9 W house-light. Solid-state programming equipment (Med. Associates, E. Fairfield, VT) was used to control and record the sessions and was located in an adjacent room.

Discrimination Training

Drug discrimination training was based upon procedures described in detail elsewhere [7,10]. Briefly, all rats were trained to respond on both levers for food (45 mg Noyes pellet) reinforcement under an FR10 schedule of reinforcement. After lever-pressing was established, each daily session was preceded by an intraperitoneal (IP) injection of either 0.8 mg/kg AFA or its saline vehicle (0.9% sodium chloride with one drop of Tween 80 per 10 ml). All injections were made 30 min prior to placement into the operant box and training sessions were 15 min in duration. Responding on one of the levers was reinforced after administration of AFA, whereas responding on the other lever was reinforced after administration of saline. Drug (D) or saline (S) was administered on a pseudo-random schedule: D,S,S,D,D; S,D,D,S,S. The lever first pressed 10 times was designated as the

TABLE 1
RESULTS OF GENERALIZATION STUDIES USING AMFONELIC ACID-TRAINED RATS

Treatment	Dose (mg/kg)	No. Trials	Quantal*	Quantitative (±SD)†	ED50‡ (mg/kg)
Saline	—	34	4.2	27.4 (5.7)	
Amfonelic acid	0.8	34	90.6	82.7 (4.9)	0.114
	1.6	2	93.8	83.4 (9.3)	
	0.4	2	68.8	57.6 (3.0)	
	0.2	2	62.5	56.6 (5.4)	
	0.1	2	50.0	54.8 (0.2)	
d-Amphetamine	0.6	2	100.0	97.1 (0.6)	0.102
	0.3	2	81.3	72.3 (17.8)	
	0.15	2	62.5	55.1 (27.4)	
	0.08	2	43.8	48.0 (12.8)	
d,l-Cathinone	0.6	2	100.0	78.8 (1.8)	0.071
	0.3	2	93.8	71.9 (4.7)	
	0.15	2	75.0	63.5 (3.6)	
Apomorphine	0.24	2	56.3	53.7 (2.6)	
	0.16	2	68.8	57.2 (5.9)	
	0.08	2	68.8	55.9 (8.3)	
Cocaine	5.0	2	87.5	64.9 (7.1)	0.677
	2.5	2	81.3	58.7 (4.8)	
	1.25	2	62.5	55.5 (12.4)	

*Percentage of rats selecting the lever appropriate for amfonelic acid.
†Total number of lever presses on amfonelic acid-correct lever divided by total response made prior to 10 responses on either lever, times 100.
‡ED50 generated by Litchfield-Wilcoxon method [5] as applied to quantal measurements.

"selected" lever and the training criterion was reached when the animal selected the appropriate lever, according to the drug or non-drug state imposed, on eight of ten consecutive sessions.

Stimulus Generalization Studies

After the rats attained the discriminative training criterion, testing and training sessions of 15 min duration with alternating administrations of either 0.8 mg/kg AFA or saline were continued on Mondays, Wednesdays and Fridays. It was intended that if a rat was observed to make more than two incorrect lever selections in any of 10 consecutive maintenance sessions, the data on that rat's performance would be deleted from the results. This, however, did not occur. On Tuesdays and Thursdays, the rats of each group were injected IP with either one of several different doses of AFA then used for initial training or other dopaminergic agents, and 30 min later they were placed into the experimental chamber. They were allowed to lever press, without receiving reinforcements, until ten presses were made on either lever. To preclude training at an AFA dose different than employed to train the animals or to another agonist, the rats were immediately removed from the experimental chamber once the total responses on one lever reached 10 presses. Each of the test doses of drug was tested in each animal on two occasions with each test preceded both by an AFA and a saline maintenance session.

Measurements and Statistical Treatment

The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination. In addition, the total number of lever presses on both levers made before ten presses on either lever were counted constitutes the quantitative measurement, i.e., the number of responses on the drug-correct lever divided by total responses made prior to ten responses, times 100. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [5] which employs probit vs. log-dose effects and generates ED50's and tests for parallelism.

RESULTS

The eight rats required a mean (±SD) of 21.7 (12.5) training sessions to attain discriminative criteria, i.e., to reach the first of ten consecutive sessions in which 8 of 10 lever selections were correct according to the drug state imposed. The range was 5–38 sessions. Subsequently, the training dose of (0.8 mg/kg) AFA produced 90.6% of first choice responses (selected lever; quantal measurement) upon the AFA-appropriate lever during maintenance sessions, whereas saline vehicle administration produced 4.2% of selections upon this lever, or 95.8% of selections upon the saline-correct lever (Table 1). Administration of one higher and 3 lower doses than that used to train the rats indicated decreased AFA-appropriate lever selection with decreasing dose. The ED50 was found to be 0.114 (95% confidence limits: 0.044–0.294) mg/kg.

Administration of 0.6 mg/kg *d*-amphetamine in two trials, each preceded by an AFA and a saline maintenance session, produced 100% lever selection on the AFA-correct lever. Decreasing doses of *d*-amphetamine produced decreased quantal and quantitative responding upon the drug-correct lever and the ED50 for *d*-amphetamine in AFA-trained rats was 0.102 (95% confidence limits: 0.051–0.206) mg/kg. Application of a test for parallelism between the dose-response curve for AFA and *d*-amphetamine indicated that the slopes of the two curves were significantly different within 95% confidence limits, i.e., calculated $t=2.998 >$ critical $t=2.571$ [5].

Likewise, the highest dose (0.6 mg/kg) of *d,l*-cathinone produced total substitution for AFA and lower doses produced decreased discriminative performance generating an ED50 of 0.071 (0.036–0.143) mg/kg. Cocaine, at dose of 1.25, 2.5, and 5.0 mg/kg, also was observed to produce dose-responsive generalization with an ED50 of 0.677 (0.218–2.101) mg/kg. Both the *d,l*-cathinone dose-response slope (calculated $t=0.776 <$ critical $t=2.776$) and the cocaine dose-response slope (calculated $t=2.550 <$ critical $t=2.776$) were parallel to the AFA dose-response curve. Administration of 0.08–0.24 apomorphine to AFA-trained rats produced intermediate results ranging from 56.3 to 68.8% responding on the drug-appropriate lever.

DISCUSSION

The results of this experimentation show that AFA administration can function as a discriminative stimulus controlling lever selection in rats. In addition, decreasing doses of AFA were observed to produce dose-related decreases in discriminative performance both in terms of quantal and quantitative measurements.

The discriminative stimulus properties of *d*-amphetamine were shown to be similar to those of AFA in that one dose of the former controlled 100% of AFA lever selection. A previous study [2], in which rats were trained to discriminate 1.0 mg/kg *d*-amphetamine from saline, reported that AFA would substitute for the training drug in a similar behavioral paradigm. Whereas that study indicated that AFA was 1.5 times as potent as *d*-amphetamine, these two drugs were shown to be equipotent in the present study. In addition, analysis of the dose-response curves for AFA and *d*-amphetamine indicates that the lines were not parallel. This suggests that these two dopaminergic agonists might be acting by different mechanisms of actions, since it is generally recognized that when drugs produce parallel dose-response curves they have similar mechanisms/sites of action [4]. In addition, the slope of the dose-response line for AFA in this study employing AFA-trained rats appears to be markedly less steep than a similar AFA dose-response curve generated in a previous study using *d*-amphetamine-trained rats [2]. This further indicates the possibility that different mechanisms are involved in the interoceptive cueing properties of these two drugs.

Although both drugs increase locomotor activity and induce stereotyped behavior in laboratory animals and humans [1,8] by facilitating neuronal release of dopamine, biochemical studies indicate that there are differences in the mechanisms by which this release is effected. Thus, blockade of tyrosine hydroxylase with α-methylparatyrosine blocks the central actions of *d*-amphetamine, whereas reserpine does not prevent them [3]. In contrast, reserpine blocks the central actions of AFA, while α-methylparatyrosine does not block these effects [1]. These observations led to the hypothesis that amphetamine acts to preferentially release newly synthesized dopamine whereas AFA acts to preferentially release older, stored dopamine [6,8].

Administration of *d,l*-cathinone, a drug with discriminative properties similar to *d*-amphetamine [10], was shown to substitute, in a dose-responsive manner, for the discriminative stimulus properties of AFA. Indeed, cathinone was 1.6 times as potent as AFA and produced a dose-response curve parallel to the training drug. Likewise, AFA was shown to generalize to cocaine and the former drug was approximately six times as potent. The differential effect of reserpine pretreatment upon the ability of non-amphetamine stimulant, such as AFA, and cocaine to produce hyperactivity and stereotyping and amphetamine has been previously noted [8].

Apomorphine administration was seen to produce only intermediate results in AFA-trained rats with doses of 0.08–0.24 mg/kg producing 56.3–68.8% of lever selections on the drug-appropriate lever. Apomorphine-trained rats have previously been reported to only partially generalize to *d*-amphetamine and AFA [7].

In summary, the present experimentation indicates that AFA can function as a discriminative stimulus in the rat, that it produces this effect by the mediation of dopaminergic systems and suggests that it may act by a mechanism like other non-amphetamine stimulants.

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REFERENCES

1. Aceto, M. D., J. Botton, M. Levitt, R. Martin, H. D., Bentley and P. T. Speight. Pharmacologic properties and mechanism of action of amfonelic acid. *Eur J Pharmacol* **10**: 344–354, 1970.
2. Aceto, M. D., J. A. Rosecrans, R. Young and R. A. Glennon. Similarity between (+)-amphetamine and amfonelic acid. *Pharmacol Biochem Behav* **20**: 635–637, 1984.
3. Chiueh, C. C. and K. E. Moore. *d*-Amphetamine-induced release of "newly synthesized" and "stored" dopamine from the caudate nucleus *in vivo*. *J Pharmacol Exp Ther* **192**: 642–653, 1975.
4. Levine, R. R. *Pharmacology: Drug Actions and Reactions*, 2nd edition. Boston: Little, Brown, and Co., 1978, pp. 169–170.

5. Litchfield, J. T. and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* **96:** 99–113, 1949.
6. Miller, H. H. and P. A. Shore. Effects of amphetamine and amfonelic acid on the disposition of striatal newly synthesized dopamine. *Eur J Pharmacol* **78:** 33–44, 1982.
7. Schechter, M. D. Different dopaminergic mechanisms for amfonelic acid, amphetamine, and apomorphine. *Pharmacol Biochem Behav* **13:** 497–500, 1980.
8. Shore, P. S. Actions of amfonelic acid and other non-amphetamine stimulants on the dopamine neuron. *J Pharm Pharmacol* **28:** 855–857, 1976.
9. Speciale, S. G., Jr., F. Karoum and R. J. Wyatt. Different effects of amphetamine and amfonelic acid on peripheral and central catecholamine metabolism. *Eur J Pharmacol* **62:** 297–307, 1980.
10. Young, R. and R. A. Glennon. Discrimination stimulus properties of amphetamine and structurally related phenalkylamines. *Med Res Rev* **6:** 99–130, 1986.