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Different Dopaminergic Mechanisms for Amfonelic Acid, Amphetamine and Apomorphine

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SCHECHTER, M. D. *Different dopaminergic mechanisms for amfonelic acid, amphetamine and apomorphine.* PHARMAC. BIOCHEM. BEHAV. 13(4) 497–500, 1980.—Rats were trained to discriminate between the stimulus properties of intraperitoneal 0.16 mg/kg apomorphine and saline in a two-lever, food-motivated operant task. Employing the selected lever and the extended schedule performance measurements to indicate the generalization effect and perserverance of that effect, respectively, neither *d*-amphetamine nor amfonelic acid produced apomorphine-like discriminative properties. In contrast, administration of 0.1 or 0.2 mg/kg n-propylnoraporphine was observed to produce responses and perseverance on the apomorphine-appropriate lever that was similar to that seen after 0.16 mg/kg administration. The results of this behavioral experimentation are consistent with the notion that *d*-amphetamine, amfonelic acid and apomorphine may produce their dopaminergic effects by different mechanisms of action and the possibility of two sites of action for n-propylnoraporphine is discussed.

Drug-induced stimuli Apomorphine Amphetamine Amfonelic acid N-propylnoraporphine Dopamine

IT has previously been reported that apomorphine is capable of producing a discriminative stimulus complex in rats [3,4] and it appears that the action underlying the ability of apomorphine to produce discriminative control of rat behavior is consistent with its dopamine-mimicking activity at specific dopamine binding sites. In support of this hypothesis, numerous neuroleptics that block post-synaptic dopaminergic receptors have been shown to antagonize the discriminative stimulus properties of apomorphine [2]. Using this paradigm in which a rat is trained to make one response after drug administration and a second response after saline injection, rats trained to discriminate the effects of another drug with dopaminergic activity, *d*-amphetamine, were able to generalize (transfer) their discriminative responding to apomorphine [15,17], whereas rats trained to discriminate apomorphine were unable to transfer to *d*-amphetamine [7].

The present study endeavored to observe possible generalization to other known dopaminergic drugs in apomorphine-trained rats. Thus, *d*-amphetamine, amfonelic acid and N-propylnoraporphine were tested in rats trained to discriminate between 0.16 mg/kg apomorphine and saline in a 2-lever, food-motivated discrimination procedure which employed a relatively new measurement technique known as extended schedule performance [16] in which not only is the rats' first lever selection measured but also their perseverance on that lever is considered to indicate the "strength" of their selection.

METHOD

Subjects

The subjects were 6 male ARS/Sprague-Dawley rats weighing 200 ± 10 g at the beginning of experimentation. They were housed in individual living cages and their weights were adjusted (by daily rationing of rat chow) to approximately 85 ± 5% of their free-feeding values as determined by daily weighing of a control free-feeding rat purchased from the supplier (Zivic-Miller, Allison Park, PA) at the same time. Water was continuously available.

Apparatus

The experimental space was a standard rodent Skinner test cage (Lafayette Instrument Co.) equipped with 2 operant levers placed 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 levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and house light. Solid-state programming equipment (LVB Corp.) was used to control and record the sessions and was located in an adjacent room.

Training Procedure

The procedure used to train rats to discriminate between apomorphine and saline has been described in detail elsewhere [3]. Daily discrimination training started after initial shaping to lever-press on both levers on a FR10 schedule of food reinforcement. Thirty min prior to placement into the test chamber, the rats were injected intraperitoneally (IP) with either 0.16 mg/kg apomorphine (as base) or an equal volume (1 ml/kg body weight) of saline. Depending on whether the rat was administered apomorphine or saline, it obtained reinforcement by pressing either the "apomorphine lever" (AL) or the "saline lever" (SL), respectively. After

TABLE 1
EXTENDED SCHEDULE TRANSFER OF APOMORPHINE DISCRIMINATION TO OTHER DOPAMINERGIC DRUGS

Treatment	Dose (mg/kg)	AL responses prior to 10 presses on SL (±SD)	# AL selections/ # trials conducted	SL responses prior to 10 presses on AL (±SD)
Apomorphine	0.16	116.4 (47.3)	12/12	0
Saline	—	0	0/12	106.0 (76.8)
Amphetamine	1.0	11.9 (5.6)	5/12	51.4 (56.2)
	0.5	9.9 (10.6)	3/12	65.0 (84.9)
Amfonelic acid	0.8	15.9 (21.4)	4/12	85.8 (160.2)
	0.4	3.9 (2.9)	1/12	86.7 (95.9)
NPA	0.2	118.8 (81.1)*	11/12	3.7 (4.5)
	0.1	83.4 (47.3)*	10/12	4.1 (3.6)

*Not significantly different from the number of AL responses prior to 10 presses on SL after administration of 0.16 mg/kg apomorphine (t-test of means).

every 10th press (FR10) on the appropriate lever, a 45 mg Noyes pellet was delivered through the food receptacle. Responses on the incorrect lever (i.e., on the SL after apomorphine administration or on the AL after saline administration) were recorded but produced no programmed consequence. To randomize the possible influence of position preference upon discriminative performance, the lever assignments were AL left, SL right for half of the rats and AL right, SL left for the other half and these assignments remained constant throughout the experimentation. The number of responses made on either lever before the first food pellet (FFP) was obtained and, thus, before 10 responses were made on the correct lever, was recorded. The FFP, therefore, reflects the accuracy of the rats' lever selection and the number with which the FFP exceeds 10 equals the number of incorrect responses made before the first reinforcement.

Every week, each rat was run once a day, on 5 consecutive days, in a session of 15 min duration. Daily apomorphine (A) and saline (S) injections were given according to 2 weekly alternating sequences: A-S-S-A-A and S-A-A-S-S. The training criterion was reached when the FFP of the animals did not exceed 12 on 10 consecutive training sessions.

Extended Schedule Discrimination

Once all rats attained the training criterion, testing and training sessions of 15 min duration, with alternating administration of freshly prepared 0.16 mg/kg apomorphine and saline, were continued on Mondays, Wednesdays and Fridays. This procedure endeavored to insure and maintain behavioral discrimination to the trained drug conditions and it was intended that if a rat was observed to fall below the criterion of FFP≤12 on these 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 well-trained rats were injected IP with 2 doses each of d-amphetamine, amfonelic acid or (±)-N-propylnoraporphine (NPA) and, 30 min later, they were placed into the experimental chamber and were allowed to lever press, in extinction, until 10 responses were made on the lever that was *not* the first lever pressed 10 times (the "selected" lever). Thus, for example, when a rat pressed the AL 10 times that lever was designated the "selected lever" and the rat was allowed to continue pressing, without reinforcement, until it pressed the SL 10 times. The number of lever presses made on the AL prior to 10 presses on the SL was recorded. Likewise, if the SL was the selected lever, the rat was allowed to continue pressing until 10 responses were made on the AL.

Each test (novel) drug dose was administered in a random order on 2 occasions with each test session preceded by one saline and one apomorphine training session. In this way, the animal's experience on days preceding test days was counter-balanced with respect to any possible after-effects that might have been produced. In addition, on 2 test sessions each 0.16 mg/kg apomorphine and saline were administered and the rats were tested in extinction to observe their perseverance to the selected lever during trained conditions. All administrations were made at a constant volume (1 ml/kg body weight) without the experimenter (technician) knowing the substance administered.

Drugs and Dosage Rationale

Apomorphine hydrochloride (Merck and Company, Rahway, NY) was used at the same dose that was previously employed to train rats in a similar behavioral task [3]. Dextroamphetamine sulfate (Sigma Chemical Co., St. Louis, MO) was previously tested for transfer effects in a dose range of 0.1–1.0 mg/kg in rats trained to discriminate apomorphine [7]. Amfonelic acid (Sterling-Winthrop Research Institute, Rensallaer, NY) has been reported to be equipotent to d-amphetamine in producing rotational behavior in 6-hydroxydopamine lesioned rats [9]. Lastly, (±)-N-propylnoraporphine (NPA, Win 28,926, Sterling-Winthrop Research Institute, Rensallaer, NY) has previously been shown to be an effective dopaminergic agonist at the doses used in a behavioral study similar to the one employed here [15].

RESULTS

The 6 rats trained to discriminate 0.16 mg/kg apomor-

phine from saline required a median number of 42 training sessions (21 sessions with each condition) in order to meet the criterion of FFP≤12 in 10 consecutive sessions. For the duration of the extended schedule transfer experiments, the discriminative accuracy to the training conditions (during interspersed maintenance sessions) persisted for all rats. Table 1 represents the results of testing rats in extinction after 0.16 mg/kg apomorphine, saline, 0.5 and 1.0 mg/kg *d*-amphetamine, 0.4 and 0.8 mg/kg amfonelic acid, and 0.1 and 0.2 mg/kg NPA administration. Apomorphine administration resulted in 100% AL selection and produced a mean of 116.4 responses on the AL before 10 responses were made on the SL. The SL was first pressed 10 times by all rats after saline administration and they continued pressing the SL for a mean of 106.0 responses before pressing the AL 10 times.

Amphetamine, at the highest dose (1 mg/kg) used, produced 41.7% responding on the AL and significantly less perseverance on the AL than on the SL. Likewise, the highest dose of amfonelic acid (0.8 mg/kg) produced 33.3% AL selections and greater perseverance on the SL than on the AL. In contrast, doses of NPA of 0.1 and 0.2 mg/kg produced both AL selections and perseverance measurements that were not significantly different from those produced by the training dose of apomorphine.

DISCUSSION

By employing the extended schedule [16] rats trained to discriminate between 0.16 mg/kg apomorphine and saline showed a mean perseverance on the apomorphine-appropriate lever of 116.4 responses after apomorphine administration and a mean perseverance on the saline-appropriate lever of 106.0 responses after saline administration. Thus, it appears that the discriminative cue "strength" produced by the drug (apomorphine) state was not significantly different from that of the non-drug (saline) state and drug transfer "overinclusiveness" [14] did not appear to exist in this experimentation. Drug transfer "overinclusiveness" refers to the suggestion that transfer tests reveal results indicating that drugs are similar when, in fact, they differ since in a transfer test the animal subject is asked to indicate which of the two trained conditions is most similar to the test drug state and since the animal must make one of two choices, the drug state (being a "stronger" state) is most often chosen.

Apomorphine-trained rats were only able to partially generalize to the effects of *d*-amphetamine, confirming a previous report in which rats were trained with the same apomorphine dose administered subcutaneously [7]. In rats trained to discriminate *d*-amphetamine, a high dose of apomorphine was reported to transfer [15,17], whereas lower doses produced only partial transfer [7,8]. This type of asymmetrical generalization has been reported when rats trained to discriminate fentanyl will transfer to apomorphine, whereas rats trained to discriminate apomorphine will not generalize to fentanyl [4].

Amfonelic acid is a non-amphetamine stimulant which has been reported to induce stereotyped behavior that is qualitatively similar to that produced by *d*-amphetamine [1] but phenomenologically dissimilar to apomorphine-induced stereotypy [9]. Furthermore, in rats with unilateral 6-hydroxydopamine lesions in the substantia nigra, amphetamine and amfonelic acid produce ipsilateral turning [20,22] whereas, apomorphine produces contralateral turning [9]. It has been proposed that drugs with predominantly presynaptic dopaminergic action cause ipsilateral rotations while these with post-synaptic dopaminergic effects induce contralateral circling [21].

N-propylnoraporphine, apomorphine with an n-propyl group substituted for a methyl group, has been reported to be 2 to 40 times more potent than apomorphine in producing stereotypy in rats [5,19], 8 to 20 times more potent in inducing rotational behavior in 6-hydroxydopamine nigra-lesioned rats [10,13] and equipotent in stimulating the *in vitro* activity of adenylate cyclase [12]. The mechanism of action of these effects is presumed to be by agonistic effects on postsynaptic dopaminergic receptors as evidenced by the fact that haloperidol blocks the stereotypy produced by both agents [18], and it is this action that has led to NPA being successfully used in clinical trials in Parkinsonian patients [6]. In the present study, NPA was found to produce apomorphine-like discriminative responding. A previous behavioral study [15] showed that NPA produced amphetamine-like discriminative responding and since the present study indicates that amphetamine produces only partial apomorphine-like discrimination, it appears that NPA may have two distinct mechanisms of action, i.e., NPA is partly direct or apomorphine-like (dopamine post-synaptic agonism) and partly indirect or amphetamine-like (releasing dopamine). This possibility has been advanced by Menon *et al.* [11] and the indirect effect of NPA has been evidenced in Parkinson's disease patients who have taken NPA and developed tachyphyllaxis which is reversed by L-dopa [6].

In summary, the data are consistent with the possibility that apomorphine, amfonelic acid and *d*-amphetamine in the dose ranges employed may produce dopaminergic effects by different mechanisms. Apomorphine may act solely as a postsynaptic dopamine agonist; amphetamine, by release of newly synthesized dopamine and action upon norepinephrine neurons; amfonelic acid, by releasing dopamine from granular stores and NPA, by both releasing newly synthesized dopamine and by a direct agonist activity on dopamine receptors.

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