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Effects of Selective Dopamine Receptor Agonists in Rats Trained to Discriminate Apomorphine From Saline[1]

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WOOLVERTON, W. L., J. B. KAMIEN AND L. I. GOLDBERG. *Effects of selective dopamine receptor agonists in rats trained to discriminate apomorphine from saline.* PHARMACOL BIOCHEM BEHAV 22(4) 577–581, 1985.—Rats (N=12) were trained to discriminate apomorphine (0.25 mg/kg, IP) from saline in a two-lever, food-reinforced (FR 30) drug discrimination paradigm. When the discrimination was acquired, various doses of apomorphine as well as several other dopamine receptor agonists were injected before test sessions. Apomorphine (0.03–0.25 mg/kg, IP) produced a dose-related increase in the percent of responses that occurred on the drug lever during test sessions. The selective DA_2 receptor agonist piribedil (0.25–8.0 mg/kg, IP) produced a dose-related increase in drug lever responding that was similar to that seen with apomorphine. On the other hand, administration of the selective DA_1 receptor agonist SKF 38393 (1.0–32 mg/kg, IP) resulted in principally saline lever responding, even at doses that substantially reduced the rate of responding. Administration of dopamine (1.0–8.0 mg/kg, IP), which does not readily cross the blood-brain barrier, also resulted in principally saline lever responding. These results suggest that the discriminative stimulus properties of apomorphine are based on its action at a receptor that is similar to the DA_2 receptor that has been characterized in the periphery and that this receptor is centrally located.

Apomorphine Piribedil SKF 38393 Dopamine receptors Drug discrimination Rat

IN recent years it has become apparent that there are multiple receptors for dopamine (DA). Several classification schemes for up to four distinct receptors have been proposed based upon *in vivo* and *in vitro* work both in the central nervous system and the periphery [4, 9, 16, 25]. Most of the hypotheses concerning multiple DA receptors in the CNS are derived from *in vitro* findings and should be corroborated with research designed to evaluate functional roles for these sites in the intact organism.

Behavioral studies with drugs that act on these receptors are one way to approach this issue. However, the intact behaving organism is a complex system and many behavioral methods lack the specificity necessary to demonstrate the existence of multiple CNS receptors. In recent years, drug discrimination methods have been used to study the specific CNS receptor actions of drugs in the intact organism. For instance, these methods have provided evidence for multiple CNS opiate receptors that is consistent with the proposed receptors of Martin *et al.* [11,17]. This approach has also been used to provide *in vivo* evidence for central receptors for traditional neurotransmitters such as acetylcholine and serotonin [1,21]. The data from these studies strongly suggest that the drug discrimination paradigm is a highly selective behavioral method for studying multiple CNS receptors in the intact organism.

The purpose of the present experiment was to use the drug discrimination paradigm to examine the issue of multiple CNS receptors for DA. Drugs with actions selective for different dopamine receptors were tested in animals trained to discriminate apomorphine from saline. In addition, DA, which fails to cross the blood-brain barrier, was tested in an attempt to delimit the site of drug action to the CNS. The results suggest that the apomorphine discriminative stimulus is based upon a drug action at a central receptor that is similar in terms of its profile of agonist actions to the DA_2 receptor that has been characterized in the periphery [9].

METHOD

Subjects

The subjects were twelve male Sprague-Dawley rats (Holtzman Co., Madison, WI) that were maintained at 80±5% of their initial free-feeding body weights (240–310 g). They were individually housed in stainless steel cages in a room maintained at 24°C and on a 12 hour (6 a.m.–6 p.m.) light-dark cycle. In addition to the 45 mg food pellets (P. J.

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Noyes Co., Lancaster, NH) delivered during the experimental sessions, diet was supplemented with Teklad 4% Mouse and Rat Diet (Winfield, IA). Water was continuously available except during experimental sessions.

Apparatus

Two identical operant chambers for rats (Ralph Gerbrands Co., model D-1) were used as experimental chambers. In each chamber, two response levers were mounted on one wall and a food receptacle was mounted between them. Each chamber was illuminated during experimental sessions by a single six watt light located on the wall opposite the levers. Extraneous noise was diminished by enclosing each chamber in an insulated picnic chest and by operating ventilation fans mounted on the outside of each chest. Electromechanical equipment, located in the adjacent room, controlled stimulus events and recorded lever presses.

Procedure

The rats were randomly divided into two groups of six with each group assigned to one of the two experimental chambers. In one chamber the right lever was designated the saline (S, non-drug) lever and the left lever, the drug (D) lever. In the second experimental chamber the reverse condition existed.

Experimental sessions, which lasted fifteen minutes, were conducted six days a week. The rats were initially trained to press the saline lever. Ten minutes after a 1.0 ml/kg (IP) saline injection the house light was illuminated and food was available for every response on the saline lever. Responding on the drug lever was counted but had no other programmed consequence. When a rat was responding reliably on the saline lever (within 2 sessions), training sessions were begun using 0.25 mg/kg apomorphine (IP) ten minutes before the session. During these sessions, rats were required to press the drug lever for food delivery whereas responding on the saline lever was counted but had no other programmed consequence. After lever pressing had been shaped on both levers, a training schedule was used in which saline pretreatment sessions and apomorphine pretreatment sessions were conducted in a double alternation sequence (i.e., D,D,S,S,D,D, . . .). Although this sequence was used in each rat, it was offset by a day on a random basis so that the condition in effect for one rat on a given day was not predictive of the condition for successive rats. In addition, the daily order in which rats were tested was randomized. These manipulations controlled for the possibility of odor cues exerting discriminative control of behavior [7]. Gradually, the response requirement on either lever was increased to thirty responses per food pellet (Fixed Ratio 30, FR 30).

The training sequence was continued until a rat emitted at least 80% of its responses before the first reinforcer on the correct lever for at least 7 of 8 consecutive experimental sessions. At this point the discrimination was considered to be acquired and every third session became a test session. Test sessions were identical to training sessions except that a novel solution was usually administered before a test session and food was available for responding under a FR 30 schedule on either lever. Training sessions were conducted on the remaining four experimental days of each week to maintain and affirm the discrimination. If a rat's responding fell below the training criterion, the rat was returned to the training sequence until it again achieved criterion performance.

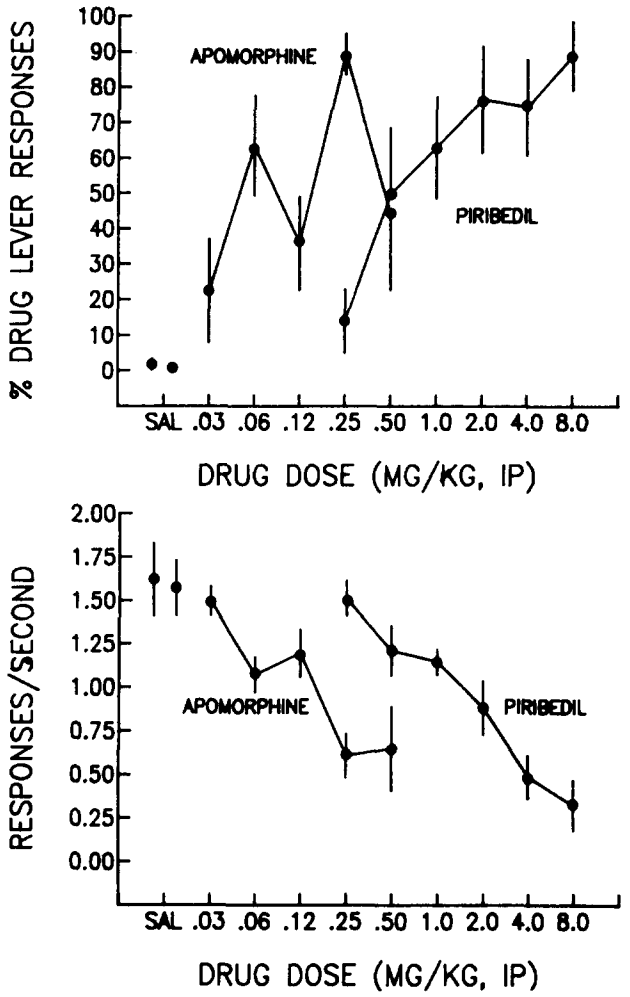

FIG. 1. Effects of piribedil in rats trained to discriminate apomorphine (0.25 mg/kg) from saline. Upper graph: Percent of responses during test sessions that occurred on the apomorphine appropriate lever as a function of dose. Lower graph: Response rate during test sessions as a function of dose. Each point represents the mean and the vertical lines are ±SEM. (N=5–7 per point.)

Data Analysis

For each test session, the percent of total responses that occurred on the drug lever as well as the overall response rate for both levers (responses/sec) were calculated for each rat. For each drug dose the mean and standard error of the mean (SEM) were calculated for the group for both of these measures. The data from all animals tested were included in response rate analyses. However, the data from a given test dose was included in the analysis of percent drug lever responses only if at least 50% of the animals completed at least 30 responses on one or the other lever.

Drugs

Apomorphine HCl (Merck and Co., Rahway, NJ), piribedil monomethane-sulfonate (Les Laboratoires Servier, Neuilly-sur-Seine, France) and dopamine HCl were dis-

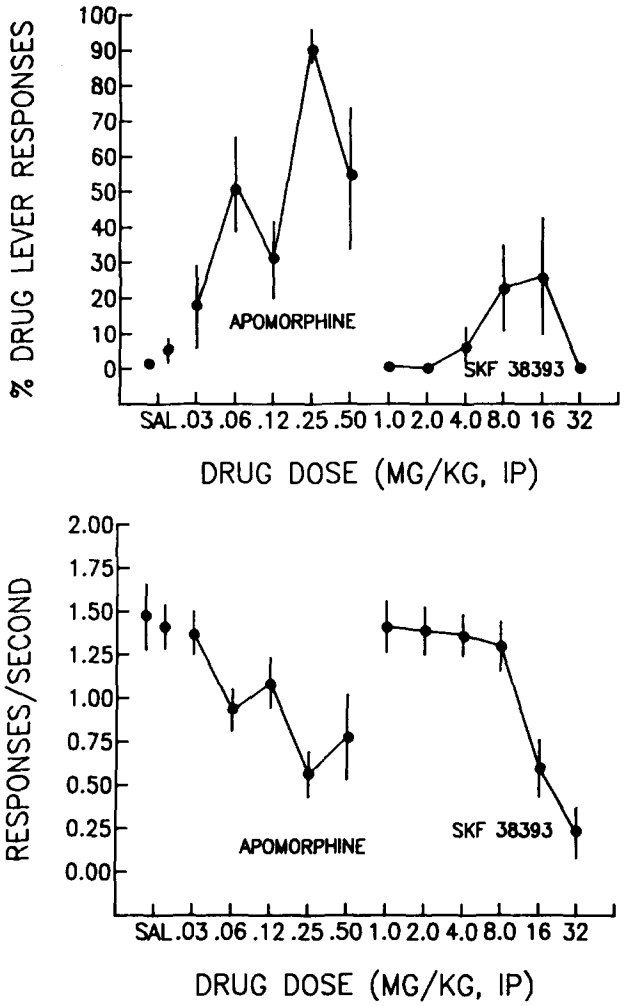

FIG. 2. Effects of SKF 38393 in rats trained to discriminate apomorphine (0.25 mg/kg) from saline. Upper graph: Percent of responses during test sessions that occurred on the apomorphine appropriate lever as a function of dose. Lower graph: Response rate during test sessions as a function of dose. Each point represents the mean and vertical lines are ±SEM. (N=6–9 per point.)

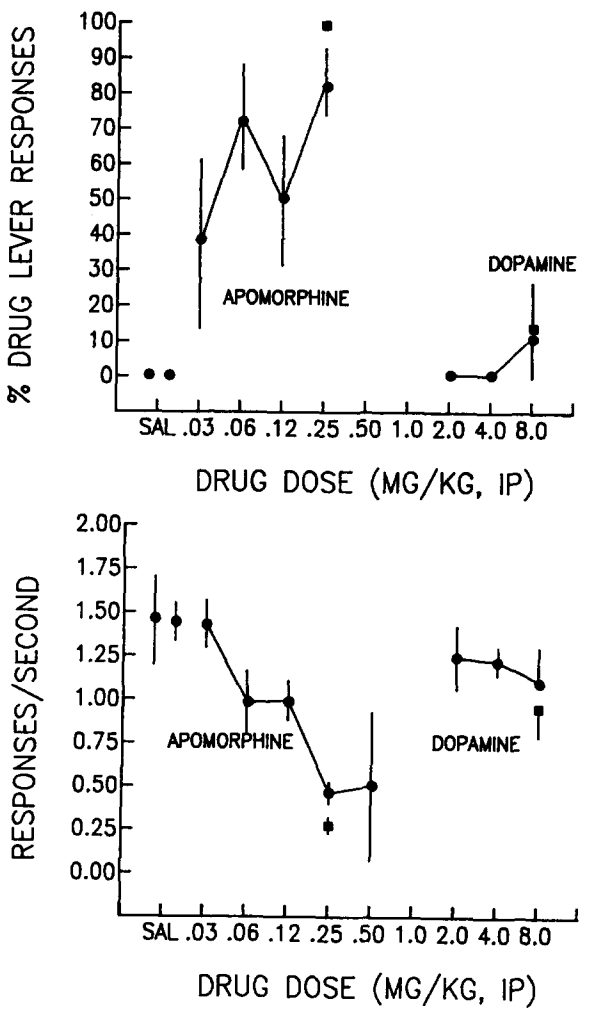

FIG. 3. Effects of dopamine in rats trained to discriminate apomorphine (0.25 mg/kg) from saline. Upper graph: Percent of responses during test sessions that occurred on the apomorphine appropriate lever as a function of dose. Lower graph: Response rate during 15 minute test sessions as a function of dose. Data from sessions with 10 minute pretreatment times are represented by circles and those with 1 minute pretreatment times are represented by squares. Each point represents the mean and vertical lines are ±SEM. (N=3–4 per point.)

solved in 0.9% saline. Apomorphine and dopamine were prepared immediately before use and were protected from light. These drugs were injected IP in a volume of 1.0 ml/kg body weight. Because of solubility limitations, SKF 38393 (7,8-dihydroxy-l-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; Smith, Kline and French Laboratories, Philadelphia, PA) was prepared in a concentration of 4.0 mg/ml in saline using a few drops of lactic acid. For doses higher than 4.0 mg/kg, the injection volume was increased appropriately using the 4.0 mg/ml solution.

A range of doses of each dopamine receptor agonist was administered before test sessions. Doses were tested in a random order and ranged between one that had no effect on response rate and one that virtually eliminated responding in test sessions. The exception was dopamine which was tested up to a dose (8.0 mg/kg) that was 50 times higher, on a molar basis, than the training dose of apomorphine but did not produce comparable effects on response rate. In most cases, drug doses were tested twice, once with each training condition in effect on the preceding session. The pretreatment time was generally the standard 10 minutes used in training sessions. However, because of the possibility of rapid inactivation of DA, the highest dose (8.0 mg/kg) was tested with a 1 minute pretreatment time as well.

RESULTS

The rats required 54 (6.1 SEM) training sessions under FR 30 conditions to meet the criterion for discrimination. In test sessions, the training dose of 0.25 mg/kg apomorphine usually resulted in greater than 90% drug lever responding and reduced response rates to less than 50% of saline rates (Figs. 1–3). For lower doses of apomorphine, the percent of drug lever responses was directly related to dose, except for a

slight decrease that was seen at 0.12 mg/kg. Overall response rate was inversely related to dose. After the highest dose of apomorphine (0.5 mg/kg), approximately 50% of the responses occurred on the drug lever.

Percent drug lever responding was also directly related to dose when piribedil (0.25–8.0 mg/kg) was injected before test sessions (Fig. 1). Although there was some variation between rats in their sensitivity to piribedil, all but one animal emitted more than 90% of its responses on the drug lever at least at one test dose. The effects on overall response rate were inversely related to dose. For both measures, piribedil was approximately $^1/_4$–$^1/_8$ as potent as apomorphine.

In contrast, SKF 38393 (Fig. 2) and dopamine (Fig. 3) engendered principally saline lever responding in test sessions. For SKF 38393, this was true even at doses that substantially reduced response rate (16 and 32 mg/kg). SKF 38393 was approximately $^1/_{64}$ as potent as apomorphine in reducing response rate. In the case of dopamine, saline lever responding predominated up to a dose (8.0 mg/kg) that was more than 50 times higher than the training dose of apomorphine, on a molar basis. This was the case regardless of whether dopamine was administered 10 minutes (Fig. 3, circles) or 1 minute (Fig. 3, squares) before the test session. In contrast, when apomorphine was given 1 minute before the session, greater than 90% of the responses were on the drug lever.

DISCUSSION

The present experiment confirms earlier reports that apomorphine can function as a discriminative stimulus in rats [3, 12, 14, 23]. In addition, the dose-response relationship for apomorphine was similar to that which has been reported previously. The potency differences between apomorphine and piribedil (approximately 4–8 fold) approximated those differences found in other behavioral studies [5,27] as well as the potency difference between these drugs at the DA_2 receptor [8]. Thus, our behavioral results were quantitatively comparable to what has been previously reported for apomorphine and piribedil. SKF 38393 was approximately $^1/_{64}$ as potent as apomorphine in reducing response rate. This potency difference is larger than that reported for these compounds for inducing contralateral circling in rats with unilateral lesions of the nigrostriatal pathway where SKF 38393 was $^1/_2$–$^1/_4$ as potent as apomorphine [26]. The reason for this difference is unclear, although it may be related to the supposedly supersensitive DA receptors found in the unilaterally lesioned rat.

More important, perhaps, than these quantitative comparisons are the qualitative differences between these compounds in substitution tests. Piribedil substituted for apomorphine as a discriminative stimulus in a dose-related manner, while SKF 38393 and dopamine engendered predominantly saline lever responding. These differences parallel what has been reported for these compounds in terms of their actions at peripheral dopamine receptors. Apomorphine is a full agonist at the DA_2 receptor, a receptor on post-ganglionic sympathetic neurons, and functions as a partial agonist at the DA_1 receptor in the renal vascular bed [6,15]. Piribedil has been reported to be a DA_2 receptor agonist with no DA_1 activity [8,10] while SKF 38393 is a DA_1 agonist without DA_2 activity [13,20]. Dopamine has actions at both DA receptors but does not readily cross the blood-brain barrier. Thus, the profile of agonist action in rats trained to discriminate apomorphine from saline is similar to that of the DA_2 receptor and suggests that the apomorphine discriminative stimulus is based upon CNS actions of the drug at a receptor that is similar to the DA_2 receptor.

An important consideration in the present experiment is whether the range of doses of SKF 38393 tested was adequate for CNS activity. Several factors argue that this was the case. SKF 38393 reduced the rate of lever pressing in a dose-related manner, an effect that is usually found to be centrally-mediated. Secondly, other investigators have found doses within the dose range tested here to induce various behavioral effects in rats, including grooming and oral dyskinesias [19,22]. Finally, doses of SKF 38393 between 1.0 and 2.0 mg/kg induced rotation in rats with unilateral nigrostriatal lesions [26]. Thus, it is likely that a behaviorally relevant range of doses was tested in the present experiment.

The classification of multiple receptors for dopamine is an area of much controversy. In addition to the DA_1 and DA_2 receptors characterized by Goldberg and Kohli [9], Kebabian and Calne [16] have classified DA receptors as D_1 and D_2. The D_1 receptor, found in the striatum, is positively linked to adenylate cyclase while the D_2 receptor is found in the pituitary and influences prolactin release. Although the receptors postulated in these two classification schemes are similar in many respects, there are significant differences between them [9]. However, it should be pointed out that piribedil has D_2 receptor activity [2] without D_1 activity [18,24] while SKF 38393 has D_1 activity without D_2 activity [26]. It remains for future research to determine which of these classification schemes most accurately reflects the situation in the intact CNS. However, the major point is that since apomorphine is principally a DA_2 (D_2) receptor agonist, these results provide evidence for the existence of a DA_2 (D_2)-like receptor in the CNS of the rat. These results are consistent with our earlier findings that suggest the existence of a DA_2-like receptor in the CNS of the rhesus monkey [28].

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