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The Effects of Dopaminergic Agonists and Antagonists on the Frequency-Response Function for Hypothalamic Self-Stimulation in the Rat

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NAKAJIMA, S. AND N. B. O'REGAN. *The effects of dopaminergic agonists and antagonists on the frequency-response function for hypothalamic self-stimulation in the rat.* PHARMACOL BIOCHEM BEHAV **39**(2) 465–468, 1991.—The function of dopaminergic synapses in generating the reinforcing effect of brain stimulation was examined in 8 rats. The animals were implanted with bipolar electrodes and trained to press a bar for lateral hypothalamic stimulation. The frequency of stimulation pulses was systematically changed, and a frequency-response curve was plotted for each rat after intraperitoneal injection of a test agent. Dopamine agonists and antagonists selective to either D1 or D2 subtypes of receptors were used. The curve was shifted to a high-frequency range by either SCH 23390 (D1 antagonist) or raclopride (D2 antagonist). SKF 38393 (D1 agonist) failed to shift the curve, and quinpirole and CV 205-502 (D2 agonists) shifted the lower part of the curve to a low-frequency range. The results suggest that an activation of D2 receptors generates a reinforcing effect, and that the effect is expressed only if D1 receptors are activated to an optimal level.

CV 205-502 Dopamine receptor D1 D2 Quinpirole Raclopride Reinforcement SCH 23390 Self-stimulation SKF 38393

A number of studies have shown that SCH 23390, a dopaminergic antagonist selective to D1 receptors, attenuates the effects of a wide range of reinforcers such as food, water, saccharin solution, electrical brain stimulation, and intravenous infusion of opiates and stimulants (7, 9, 11, 14, 18). Raclopride, a selective D2 receptor antagonist, also reduces operant responding for food, heroin, and brain stimulation (12,13). These studies seem to suggest that the neural system that produces reinforcement of operant behaviour in general involves both D1 and D2 dopamine receptors. In accord with this idea, Hoffman and Beninger (5,6) have found that quinpirole, a selective D2 agonist, gives rise to conditioned place preference. Also, piribedil, a dopamine agonist with high D2 affinity, produces a reinforcing effect: rats and monkeys infuse piribedil solution intravenously by making operant responses (20,21). A selective D1 agonist, SKF 38393, however, presents an inconsistency. Not only does it fail to act as a positive reinforcer in intravenous self-administration and in a conditioned place preference experiment (5,20), but it also suppresses responding for food reward and gives rise to a conditioned taste aversion (5,17).

The purpose of the present study was to examine the effects of D1 and D2 agonists and D1 and D2 antagonists, respectively, in the same animals in the same testing situation so that their effects could be compared directly. Rats were trained to press a bar for hypothalamic stimulation, and a frequency-response curve was plotted for each animal after injection of a test agent. The frequency-response curve, also called the reward summation function, is a plot of response rate against the frequency of stimulation pulses. In general, a high frequency stimulation gives rise to a high response rate, whereas a low-frequency stimulation produces a low rate. If a dopamine antagonist reduces the rewarding effect of stimulation, the curve will move toward a higher frequency range; if an agonist enhances the rewarding effect, the curve will move to a lower frequency range. Debilitating motor effect will reduce the maximum without shifting the curve laterally. Typical shifts by amphetamine and pimozide have been demonstrated by Gallistel and Karras (4).

METHOD

Male hooded rats of the Long-Evans strain were obtained from Charles River Canada Ltd. Each rat was implanted with a bipolar electrode into the lateral hypothalamic area. The electrodes were made of two strands of stainless steel wire, twisted together, and insulated throughout except for the tip cross-sections (Plastic Products Co.). The diameter of the wire was approximately 0.08 mm. The stereotaxic coordinates were, with the incisor bar raised 5.0 mm above the interaural line, 0.5 mm posterior to bregma, 1.7 mm lateral to the midline, and 8.0 mm below the cortical surface. After a recovery period of 7 days or more, the animals were trained to press a bar in a Skinner box. The bar was 5 cm wide, protruded 2.5 cm into the box at the height of 2.5 cm above the floor, and required approximately 35 g pressure to press. Each bar-press produced a 0.5 s train of square-wave pulses, 0.3 ms duration, 100 pulses per second (pps). Two resistors, one 100 kohm and the other 1 kohm, were connected to the stimulator output in series to the animal so that

relatively constant current intensity was maintained regardless of the animals' impedance. The intensity of stimulation current was monitored with an oscilloscope connected in parallel to the 1 kohm resistor. For each animal, the intensity was adjusted to produce a response rate approximately 50% of maximal rate. Each daily session lasted for 20 minutes. Eight rats learned bar-pressing responses and demonstrated a stable rate of responding—less than 15% of fluctuation in the daily number of responses.

Frequency-response curves were plotted while the stimulation intensity was kept at the half-maximal level as above. The pulse frequency was first raised to 200 pps, and then reduced through 120, 80, 50, 30, 20, 12 to 8 pps, allowing the animals to respond for 3 min at each step. The mean response rate was determined from the rate in the last 2 min of the 3-min period at each frequency step. If the response rate fell below 10 responses per min at any frequency, the frequency was raised again to 200 pps, and a descending series was repeated. The mean of 5 descending series was taken to plot a curve for each animal. One of the animals showed head-turning response at high frequencies, and the duration of the stimulation pulse train was reduced to 0.1 s. This animal then started responding at a much faster rate (about 200/min at 200 pps) than the previous half-maximal rate, but the stimulation intensity was kept unchanged.

In the subsequent test trials, each animal was intraperitoneally injected with a dopaminergic agent, and tested 30 min after injection taking approximately 30 min to complete two descending series. The agents used in the present study were, SKF 38393 hydrochloride (0.1, 0.2, 0.4 and 0.8 mg/kg) as a D1 agonist, SCH 23390 maleate (0.04 mg/kg) as a D1 antagonist, quinpirole hydrochloride (0.5, 1.0 and 2.0 mg/kg) and CV 205-502 (0.5 mg/kg) as D2 agonists, and raclopride tartrate (0.04 mg/kg) as a D2 antagonist. SCH 23390 and CV 205-502 were first dissolved in methanol (SCH 23390, 1.0 mg/ml; CV 205-502, 7.0 mg/ml) and then diluted with physiological saline (0.9% NaCl). All other agents were dissolved in physiological saline. A minimum of 24 hours was given between injections.

At the conclusion of the experiment, the animals were intracardiacly perfused with physiological saline followed by formalin. The brains were excised and fixed further in formalin. Frozen sections were cut at 40 μm and stained with thionin to identify the location of the electrodes.

RESULTS

The frequency-response curves in the no-injection condition were smooth sigmoid shape in all animals, including the one that responded at a high rate. Injection of physiological saline did not alter the general shape or location of the curves in any rat. In order to compare the effects of dopaminergic agents on animals with different baseline rates, the response rates during injection tests in each animal were expressed in percentage of the maximal response rate found in the no-injection session in the same animal. These maximal rates ranged from 63.6 to 92.6 per min in 7 rats and 202.6 per min in the 8th (with 0.1-s duration of pulse train).

As shown in Fig. 1, the D1 antagonist SCH 23390 (0.04 mg/kg) shifted the curve to the right. With this dose, the animals clearly showed their disinterest in stimulation at the lower frequencies: they moved away from the bar and often started grooming themselves as if stimulation frequency was reduced. These shifts were unrelated to the baseline rates. That is, regardless of their high or low baseline response rate during no-injection sessions, all animals decreased their response rates for the midrange stimulation after the antagonist injection as if the frequency of stimulation were reduced. The D1 agonist, SKF

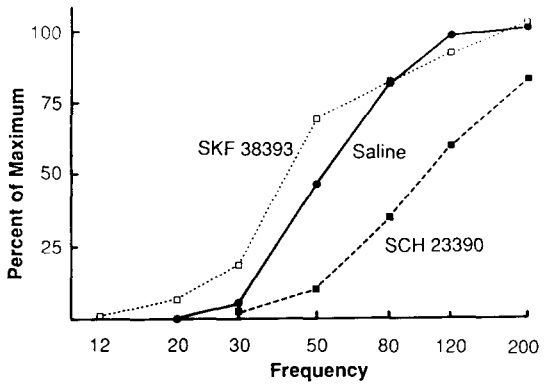

FIG. 1. Frequency-response curves for D1 dopamine receptor agonist (SKF 38393) and antagonist (SCH 23390). The highest number of responses made in no-injection sessions is taken as 100%. The frequency (pulses per s) is plotted on a logarithmic scale.

38393, shifted the frequency-response curve to the left—to a lower frequency range—with a dose of 0.4 mg/kg. With this dose, two rats stopped responding for 200 pps stimulation, while the remaining 6 showed near-maximal response rates (the percent score for SKF 38393 at 200 pps in Fig. 1 is based on those 6 rats). All animals showed high rates of responding for 120–80 pps stimulation. Response facilitation was most clearly observed at 50 pps; the animals responded vigorously as if they were responding to a higher frequency of stimulation. With 0.2 mg/kg dose, a similar facilitation was observed in 3 rats, but the remaining 5 did not show any effect. It had no effect in any rat with 0.1 mg/kg dose. With 0.8 mg/kg, 7 out of 8 rats completely stopped responding, and even priming stimulation did not elicit bar-pressing.

The effects of D2 selective agonists and antagonist are shown in Fig. 2. The D2 antagonist, raclopride (0.04 mg/kg), shifted the curve to the right with little sign of motor interference; the shift was not dependent on the preinjection response rate. There was no behavioral change other than the reduction in response rate. The animals paused longer and more frequently as the stimulation frequency was reduced, just as they did in the no-injection sessions. The only difference was that the stimulation frequency at which they started to pause was one step higher than in the control session. The agonists, quinpirole and CV 205-502, enhanced responding only at lower frequencies. At higher frequencies, quinpirole (1.0 mg/kg) produced an exaggeration of motor effect. Head twisting, which normally appeared only with an extremely high intensity or a prolonged duration of stimulation, began to appear after quinpirole injection even at the half-maximal intensity. One of the animals twisted the entire body after every bar-pressing so much that the response rates at the frequencies above 50 pps were actually lower than those at 8 pps. All other animals responded faster for 120–50 pps stimulation than they did for 200 pps and further continued responding for lower frequencies of stimulation. With a dose of 0.5 mg/kg, about the same degree of motor interference as with 1.0 mg/kg dose was observed at high frequencies, and similar increases in response rates were recorded for low frequencies. With a higher dose (2 mg/kg), testing became impossible in 5 rats because of severe motor interference. CV 205-502 (0.5 mg/kg) produced more intense motor interference at higher frequencies. As the frequency was reduced, the animals continued responding at rates higher than in control session. Furthermore, they continued responding even after the stimulator was turned

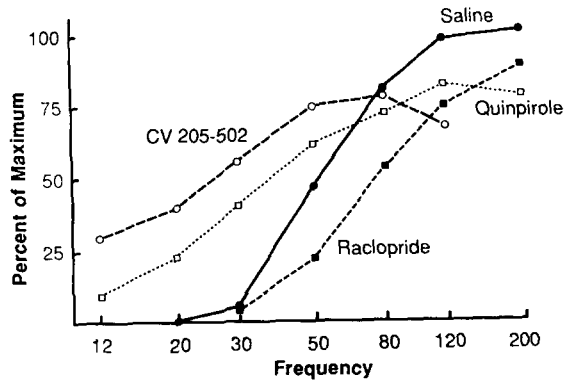

FIG. 2. Frequency-response curves for D2 dopamine receptor agonists (quinpirole and CV 205-502) and antagonist (raclopride).

off; the response rate at this point ranged from 5 to 26 per min. The responding was not simply the result of hyperactivity; the responses were well organized and steadily paced.

The median threshold and the quartile threshold were calculated by linear interpolation in each rat for each treatment as listed in Table 1. The median threshold is the frequency of stimulation that produced 50% of the maximal response rate, and often referred to as the locus of rise in the frequency-response curve. The threshold was determined in all 8 rats under 7 treatment conditions (saline, SCH 23390, 3 dose levels of SKF 38393, raclopride, and CV 205-502) and subjected to analysis of variance (single-factor repeated measures). The effect of treatment was statistically significant, $F(6,42)=14.09$, $p<0.01$. Table 1 also shows the standard error of the mean and the results of post hoc comparisons. Since not all animals achieved 50% level under 3 dose levels of quinpirole, these data were compared with the saline scores by separate t-tests, but none of the comparisons came out to be significant. The quartile threshold is the frequency of stimulation that produced 25% of the maximal response rate. This value was calculated because many rats showed more shift at the relatively low end of the frequency range than at the higher end after D2 agonist injections. All 8 rats provided the quartile thresholds under all treatment conditions except for CV 205-502 and quinpirole 2.0 mg/kg. Analysis of variance showed statistical significance of the treatment effect, $F(7,49)=9.11$, $p<0.01$. Again, the standard error of the mean and the results of post hoc comparisons are listed in Table 1. With quinpirole 2.0 mg/kg only 3 rats could be tested because of severe motor interference, and with CV 205-502 only 3 rats (not the same 3 as above) showed response rate below 25% of the maximum. For these data, separate t-tests were performed but no statistical significance was found. Histological examination of the brain sections showed that the electrodes were located in the lateral hypothalamic area lateral to the ventromedial nucleus.

TABLE 1

THE LOCUS OF RISE (50% INTERSECTION) AND THE QUARTILE THRESHOLD (25% INTERSECTION) EXPRESSED AS THE LOGARITHM OF STIMULATION FREQUENCY

	Agents	Dose (mg/kg)	Locus of Rise (SEM)	Quartile Threshold (SEM)
Control	Saline		1.73 (0.05)	1.64 (0.05)
D1 antagonist	SCH 23390	0.04	2.01 (0.06)*	1.90 (0.06)*
D1 agonist	SKF 38393	0.1	1.77 (0.06)	1.67 (0.06)
	SKF 38393	0.2	1.73 (0.07)	1.63 (0.07)
	SKF 38393	0.4	1.64 (0.07)	1.54 (0.08)
D2 antagonist	Raclopride	0.04	1.88 (0.07)*	1.77 (0.07)*
D2 agonist	Quinpirole	0.5	1.56 (0.07)	1.35 (0.12)*
	Quinpirole	1.0	1.67 (0.10)	1.26 (0.08)*
	Quinpirole	2.0	1.39 (0.15)	1.31 (0.12)
D2 agonist	CV 205-502	0.5	1.34 (0.08)*	1.23 (0.03)

*Significantly different from the saline scores at $p<0.01$.

DISCUSSION

The D1 antagonist SCH 23390 shifted the frequency-response curve to the right, relative to the curve for saline control. A shift in the locus of rise to the right means that, in order to maintain the half-maximal response rate in each animal, the pulse frequency of stimulation had to be increased. A parallel shift of the entire curve indicates that the agent affected the reinforcer in the same manner regardless of the animal's response rate. SCH 23390 may have produced some additional motor interference, but the effect on reinforcement itself was unmistakable. In a previous study (14), SCH 23390 has been shown to shift a frequency-response curve to the right in a straight runway situation. Furthermore, SCH 23390 reduced bar-pressing for brain stimulation in a dose-related manner leading to a complete suppression of responding with a dose of 0.112 mg/kg (as maleate, or 0.08 mg/kg as free base) (12). These findings, along with the present results, strongly suggest that a blockade of D1 receptors attenuates the reinforcing effect of brain stimulation.

The D1 agonist SKF 38393 shifted the frequency-response curve to the left but not enough to bring about a statistical significance. A higher dose of the agonist completely suppressed responding rather than facilitating it. The present results are consistent with the findings in self-administration, place preference, taste aversion, and feeding (5, 6, 17, 20). It seems that injected D1 agonist could hardly make an additional contribution to the reinforcing effect beyond the level already provided by dopamine which is released from presynaptic endings as a result of brain stimulation. Moreover, an excess of SKF 38393 may possibly give rise to an aversive process.

The D2 antagonist raclopride shifted the frequency-response curve to the right. The presence of dose-related reduction and ultimate suppression of self-stimulation with a relatively high dose of raclopride (0.08 mg/kg, IP) have been reported previously (13). All of these findings lead to a conclusion that a blockade of D2 receptors attenuates the efficacy of brain stimulation as a reinforcer. The effects of the two D2 agonists, quinpirole and CV 205-502, seem to involve two components. The first component is a reduction of maximal level in a high-frequency range. The most obvious reason for the reduction is an exaggeration of head-body turning and twisting induced by a drug-stimulation interaction. These responses may have resulted from a facilitation of motor system, an increase in arousal level, induced stereotypy, or any combination of them. They were clearly incompatible with bar-pressing and reduced the response rate particularly in a high-frequency range. Whether there was an attenuation of reinforcing effect or emergence of an aversive effect could not be determined from the present data.

The second component is an increase in response rate at the lower end of frequency range. One possible explanation for this phenomenon is that the D2 agonists enhanced the reinforcing effect of brain stimulation. If a neural circuit responsible for gen-

erating the reinforcing effect of brain stimulation contains dopaminergic synapse with D2 receptors, the injected D2 agonists will have the same postsynaptic effect as presynaptic dopamine released by stimulation, thus increasing the reinforcing effect of stimulation. In addition, the agonists will act on the D2 receptors even when the presynaptic neurons are not being stimulated, creating a noncontingent reinforcement. Facilitation of operant responding by noncontingent reinforcers has been observed in various situations. They increase the resistance to extinction (15) and reinstate extinguished responses (3). Noncontingent reinforcement by apomorphine (a nonselective dopamine agonist) increases responding to a nonreinforcing bar in a characteristic repetitive manner (4), sometimes described as a "stereotypy of a learned response (16)." Thus it is possible that the animals in the present experiment continued responding for extremely low frequencies of stimulation, and even in the complete absence of stimulation, because the D2 agonists produced noncontingent reinforcement. One could entertain an alternative interpretation that the D2 agonists worked as nonspecific stimulants. This interpretation, however, cannot account for the observation that quinpirole shows a reinforcing effect in conditioned place preference test (5,6).

The present results suggest that two subtypes of dopamine receptors interact with each other in reinforcing an operant behaviour: activation of D2 receptors generates a reinforcing effect, but this process is possible only if D1 receptors are activated to a certain optimal level. The modulatory effect of D1 receptors on D2 receptors is normally provided by presynaptic dopamine, and additional D1 agonist action is useless. This synergistic relation is very similar to what has been found with regard to stereotype behaviour (1, 2, 10). Other situations in which D1 receptors play a similar permissive or enabling role have been reported (19). The exact location of interaction, with regard to the reinforcing effect, has to be determined by using an intracranial injection technique (8).

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