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Responses to Selective D-1 and D-2 Agonists After Repeated Treatment With Selective D-1 and D-2 Antagonists

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GANDOLFI, O., R. DALL'OLIO, A. VACCHERI, P. RONCADA AND N. MONTANARO. *Responses to selective D-1 and D-2 agonists after repeated treatment with selective D-1 and D-2 antagonists.* PHARMACOL BIOCHEM BEHAV 30(2) 463–469, 1988.—This study was aimed at achieving a better understanding of the functional role of D-1 and D-2 receptors in some dopamine-mediated behaviors. Hypermotility, grooming behavior and stereotyped behavior were induced, respectively, by LY 171555 (D-2 agonist), SKF 38393 (D-1 agonist) and apomorphine (mixed agonist). Acute pretreatment either with the D-1 selective antagonist SCH 23390 (0.02 mg/kg) or with the D-2 receptor blocker YM 09151-2 (0.02 mg/kg, IP) blocked all these behaviors, suggesting the existence of functional interactions between D-1 and D-2 receptors. Striatal membranes prepared from rats receiving repeated administrations with SCH 23390 (0.05 mg/kg, twice daily for 21 days) showed an increase in the number of D-1 but not of D-2 receptors. On the contrary the repeated treatments with YM 09151-2 increased only the B_{max} values of D-2 receptors. While the D-1 supersensitive rats showed only enhancement of apomorphine-induced stereotyped behavior, the D-2 supersensitive rats exhibited an increase of both apomorphine-elicited stereotypy and LY 171555-elicited hypermotility. SKF 38393-induced grooming was unaffected by any pretreatments. Moreover when D-2 supersensitive rats were acutely pretreated with SCH 23390, the enhancement of apomorphine-induced stereotyped behavior was abolished. It is concluded that the behavioral expression of D-1 receptor supersensitivity requires the simultaneous activation of D-1 and D-2 receptors.

D-1 Receptors D-2 Receptors SCH 23390 YM 09151-2 SKF 38393 LY 171555 Apomorphine Binding Hypermotility Stereotyped behavior Grooming behavior Supersensitivity

SEVERAL studies suggest that the functional consequences of the pharmacological activation or blockade of brain dopamine (DA) receptors are due to involvement of D-2 receptors. In fact: (1) a good correlation has been reported between the neuroleptic-induced inhibition of apomorphine-elicited stereotyped behavior and the ability of the different compounds to displace the D-2 ligand [^{3}H]-spiroperidol from striatal membranes [12]; (2) a close correlation between the clinical efficacy of several neuroleptics and their ability to displace ligands from D-2 sites have been found [2,24].

In contrast, any behavioral role for D-1 receptors has been questioned or denied [8,25], as the selective D-1 stimulation does not elicit the classical dopaminergic responses (e.g., hypermotility and stereotyped behavior), although an increase of grooming behavior has been obtained in rats following the administration of the selective D-1 receptor agonist SKF 38393 [16].

Recent biochemical studies have shown that a functional interaction between D-1 and D-2 receptors could exist in mammalian brain. In fact SKF 38393 has been shown to increase the efflux of c-AMP from superfused rat striatal tissue slices, this increase being antagonized by the D-2 receptor agonist LY 171555 [26]. Other studies have pointed out that the selective D-1 antagonist SCH 23390 blocks the DA-stimulated c-AMP formation in striatal homogenates, this antagonism being reversed by the D-2 antagonist (−)-sulpiride [20]. Moreover, the D-1 receptor agonist SKF 38393 enhances the ability of spiperone, a D-2 antagonist, to increase rat striatal DOPAC and HVA concentrations and, conversely, reduces the ability of the D-2 agonist LY 171555 to decrease DA metabolite concentrations [22].

Behavioral studies further support the hypothesis of a functional interaction between D-1 and D-2 receptors. D-2 agonists, such as LY 171555, pergolide and RU24213, inhibit, in a dose-dependent manner, SCH 23390-induced catalepsy [15]. Conversely, SCH 23390 blocks in a dose-dependent fashion sniffing and locomotion elicited in the rat by the D-2 agonist RU24213 [18,21]. All these observations taken together suggest that the blockade of D-1 receptors might influence the expression of the D-2 receptor mediated

motor behavior [17]. Furthermore, recent behavioral data indicate that stereotyped response occurs in rats only when both D-1 and D-2 specific agonists are concurrently administered, this suggesting that the simultaneous stimulation of the two receptors is necessary for the full expression of dopaminergic behaviors [3,14].

In the present work the behavioral responses triggered by D-1 and D-2 stimulation were investigated in conditions of drug-induced selective enhancement of D-1 or D-2 receptor sensitivity, in the aim of better understanding the co-operative role of the two receptor subtypes in the expression of dopamine-mediated behaviors. To this purpose, rats withdrawn from repeated treatment with the selective D-1 blocker SCH 23390 or the D-2 selective antagonist YM 09151-2 [19] were challenged with SKF 38393, LY 171555 or apomorphine. Grooming induced by SKF 38393 was taken as a behavior mediated by D-1 receptor activation [16], LY 171555-induced hypermotility as a D-2 receptor-mediated behavior [6], and apomorphine-induced stereotyped behavior as a response triggered by the activation of both D-1 and D-2 receptors [3]. Parallel radioligand binding studies were performed in order to assess (1) the specificity of the antagonist employed, and (2) the possible development of supersensitivity of DA receptor subtypes following repeated administrations of specific blockers. Acute experiments assessed the most suitable doses of the agonists to reveal behavioral dopaminergic supersensitivity.

METHOD

Animals

Male Sprague-Dawley rats (200 to 250 g) from Nossan (Correzzana, Italy) were used in all our experiments. They were housed under standard laboratory conditions with automatic control of light (from 7:00 a.m. to 7:00 p.m.), temperature (22±2°C) and relative humidity (60%). The animals had free access to standard diet and tap water. They were used only once and were always tested between 10:00 a.m. and 3:00 p.m.

Drugs and Chemicals

LY 171555 ((+)-4,4a,5,6,7,8,8a,9-octahydro-6-propyl-2H-pyrazolo-(3,4)-quinoline di HCl) (Eli Lilly Company, Indianapolis, IN), SCH 23390 ((R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-OL maleate) (Schering Corporation, Bloomfield, NJ), and apomorphine (Sigma, St. Louis, MO) were dissolved in saline; SKF 38393 (2,3,4,5-tetrahydro-7,8,-dihydroxy-1-phenyl-1H-3-benzazepine) (RBI, Wayland, MA) was dissolved in distilled water; YM 09151-2 (cis-N-(benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide) (Yamanouchi, Tokyo) was dissolved in distilled water with few drops of hydrocloric acid.

In radioligand binding studies, [^{3}H]-SCH 23390 and [^{3}H]-spiroperidol (NEN, Boston, MA) and (+)-butaclamol (Sigma, ST. Louis, MO) were employed.

Biochemical Studies

High affinity radioligand binding studies. The binding assays for [^{3}H]-spiroperidol and [^{3}H]-SCH 23390 were carried out, with minor modifications, according to Billard *et al.* [4] and Andersen *et al.* [1]. The rats were injected IP with saline, SCH 23390 (0.05 mg/kg, twice daily) or YM 09151-2 (0.02 mg/kg) for 21 consecutive days. After a seventy-two hour washout period, the animals were killed by cervical dislocation, the brain rapidly removed, blotted into ice-cold saline and the striata were dissected, pooled and used for radioreceptor assays. Striata were homogenized with polytron in ice-cold 50 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged twice in the same buffer. The final pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Aliquots (200 μl) of membrane suspensions were incubated at 37°C for 30 min with different concentrations of [^{3}H]-SCH 23390 (ranging from 0.05 to 5 nM) or [^{3}H]-spiroperidol (0.025 to 0.5 nM). The reaction was stopped by rapid filtration under reduced pressure through Watman GF/C filters. The radioactivity remaining on the filters were counted by liquid scintillation spectrometry using ATOMLIGHT, NEN (Boston, MA). The specific binding was determined between the total binding and the binding remaining in the presence of a specific displacer: 1 μM cold SCH 23390 and 1 μM (+)-butaclamol respectively for [^{3}H]-SCH 23390 and [^{3}H]-spiroperidol binding. The kinetic characteristics of the specific bindings were analyzed according to the method of Scatchard [23]. Proteins were measured according to Lowry *et al.* [13].

In in vitro binding studies, 10 different concentrations (ranging from 10^{-8} to 10^{-3} M) of SCH 23390 or YM 09151-2 were added to suspensions of rat striatal membranes and the respective displacement of [^{3}H]-spiroperidol or [^{3}H]-SCH 23390 specific bindings were determined. The IC_{50} values were determined by means of least squares regression analysis of log-logit transformed data. K_i values for the displacement were computed according to the following equation: $K_i = IC_{50}/(1 + [^3H]\text{-ligand}/K_d)$.

Behavioral Studies

LY 171555-induced hypermotility. Rat locomotor activity was evaluated by means of actometric cages consisting of 38×30×25 cm plastic cages with a stainless steel grid floor. DC current (65 V, 25 μA) was continuously delivered to the grid floor and every closure of the circuit performed by rat feet was recorded as one motility count by a counter. In this way only the animal horizontal displacements across the cage were recorded; rearings or tramples did not activate by themselves the circuit unless they were associated with animal locomotion. A transparent top cover and front panel allowed observation of the rat in order (1) to rule out that unusual behaviors were affecting animal motility evaluation; and (2) to assess behavioral responses such as stereotypies and grooming (see below). The animals, after having become accustomed to the cage (1 hr), were administered with LY 171555 (0.1–0.3–1 mg/kg, IP) and their motility counts were taken every 10 min for 2 hr.

Other groups of rats were pretreated 1 hr before with either SCH 23390 (0.02 mg/kg, IP) or YM 09151-2 (0.02 mg/kg, IP) and their motility response to LY 171555 (1 mg/kg, IP) was evaluated as above.

SKF 38393-induced grooming behavior. This response is considered a non-stereotyped D-1 receptor-mediated behavior consisting of "episodes of grooming with the snout being directed vigorously into the body" [16]. The rats were placed into the actometric cages described above and were allowed to explore the experimental environment for 60 min before receiving SKF 38393 (5, 10 and 20 mg/kg) or saline. Immediately following the drug administration, observers unaware of the treatments recorded the total grooming time (min) for 60 min. Animal motility was also simultaneously recorded as described above.

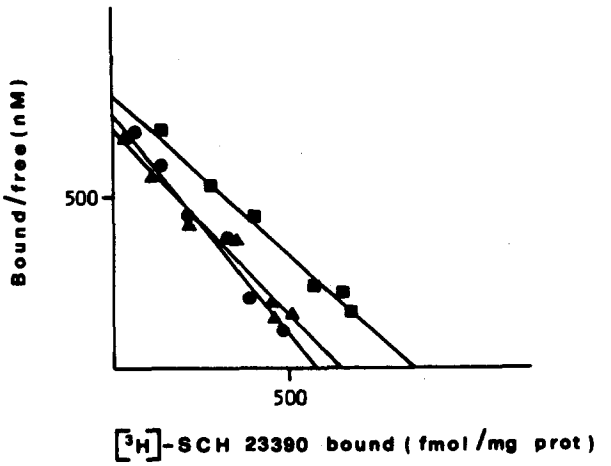

FIG. 1. Scatchard plot of [^{3}H]-SCH 23390 specific binding to suspensions prepared from crude striatal membranes obtained from rats repeatedly treated (21 days) with SCH 23390 (0.05 mg/kg IP, twice daily) (squares) or with YM 09151-2 (0.02 mg/kg IP) (circles) or saline (5 ml/kg IP) (triangles). The specific binding of [^{3}H]-SCH 23390 (0.5 to 5 nM) was determined 72 hr after the last injection. B_{max} (fmol/mg proteins) and K_d (nM) values were respectively: SCH 23390: 860±36*, 1.10±0.04; YM 09151-2: 590±32, 0.80±0.04; saline: 658±28, 0.94±0.03. *$p<0.05$ (Student's t-test) when compared to saline-treated rats.

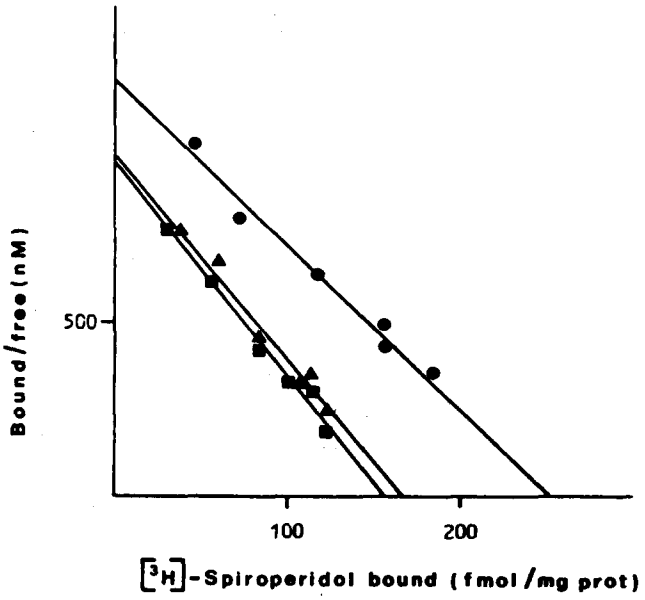

FIG. 2. Scatchard plot of [^{3}H]-spiroperidol specific binding to suspensions prepared from crude striatal membranes obtained from rats repeatedly treated (21 days) with SCH 23390 (0.05 mg/kg IP, twice daily) (squares) or with YM 09151-2 (0.02 mg/kg IP) (circles) or saline (5 ml/kg IP) (triangles). The specific binding of [^{3}H]-spiroperidol (0.025 to 0.5 nM) was determined 72 hr after the last injection. B_{max} (fmol/mg proteins) and K_d (nM) values were respectively: SCH 23390: 158.0±9.0, 0.17±0.01; YM 09151-2: 255.1± 13.0*, 0.21±0.03; saline: 166.6±9.2, 0.17±0.01. *$p<0.05$ (Student's t-test) when compared to saline-treated rats.

Other groups of rats were pretreated 1 hr before with either SCH 23390 (0.02 mg/kg, IP) or YM 09151-2 (0.02 mg/kg, IP) and their grooming response to SKF 38393 (20 mg/kg, IP) was evaluated as above.

Apomorphine-induced stereotyped behavior. The rats were allowed to become accustomed to the above described cages for 1 hr, then they were injected with apomorphine (0.25–0.5 mg/kg, SC) and, 10 min later, observed for their stereotyped behavior. The scores were assigned every 10 min for one hour according to the following rating scale: 0=no change in normal behavior; 1=intermittent sniffing; 2=continuous sniffing; 3=intermittent licking or biting; 4=continuous licking or biting. Motility counts were recorded simultaneously.

Other groups of rats were pretreated 1 hr before with either SCH 23390 (0.02 mg/kg, IP) or YM 09151-2 (0.02 mg/kg, IP) and their stereotyped response to apomorphine (0.5 mg/kg, SC) was evaluated as above.

Behavioral studies following repeated administrations. In a different series of experiments, groups of rats received, for 21 days, repeated administrations of saline, SCH 23390 (0.05 mg/kg, IP, twice daily) or YM 09151-2 (0.02 mg/kg, IP). After a washout period of 7 days, the animals were administered with threshold doses of LY 171555 (0.2 mg/kg, IP), SKF (5 mg/kg, IP) or apomorphine (0.25 mg/kg, SC) and evaluated for locomotion, grooming behavior and stereotyped activity, respectively.

Rats submitted to the same repeated treatment with the D-1 blocker SCH 23390 were acutely injected with the D-2 blocker YM 09151-2 (0.02 mg/kg, IP) 1 hr before being evaluated for their stereotyped response to apomorphine (0.25 mg/kg, SC). Similarly, a group of rats repeatedly treated with YM 09151-2 were acutely injected with SCH 23390 (0.05 mg/kg, IP) 1 hr before receiving apomorphine.

Statistical Evaluation

Motility counts, grooming time values and biochemical data were statistically analyzed by means of ANOVAs followed by single comparisons of the means (Dunnet t-test in behavioral studies; Student's t-test in radioreceptor binding studies). Stereotypy scores were analyzed by means of Mann-Whitney U-test after the overall Kruskal-Wallis nonparametric analysis of variance.

RESULTS

Biochemical Studies

Scatchard analysis of the saturation data (Fig. 1) from striatal membranes prepared from repeatedly-treated rats showed that SCH 23390 increased (30%) the number of D-1 binding sites labelled by [^{3}H]-SCH 23390. No change in the apparent affinity constant (K_d) was observed, ruling out the possibility that the increase in the density was due to a direct effect of the drug still present in the tissue at the time of the assay. In contrast, repeated administrations with YM 09151-2 failed to modify either density or affinity of [^{3}H]-SCH 23390 binding sites (Fig. 1).

Scatchard plot of [^{3}H]-spiroperidol binding in striatal plasma membranes of rat repeatedly administered with YM 09151-2 revealed that this treatment increased (50%) the B_{max} of D-2 binding sites with no change in K_d values (Fig. 2). No change was obtained following repeated treatment with SCH 23390 either in the D-2 receptor number or affinity.

Behavioral Studies

LY 171555 induced dose-dependent hypermotility which appeared about 20 min after the administration of the drug and lasted about 2–5 hr with the highest dose (Fig. 3).

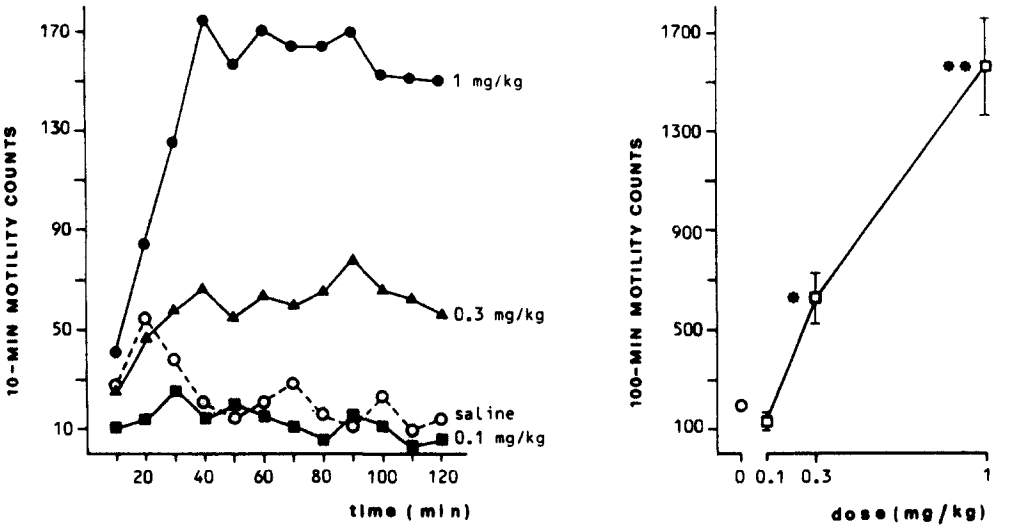

FIG. 3. Hypermotility induced by LY 171555: mean values ±SEM of actometric counts. Left: time course of motility response (10 min actometric counts) to different doses: open circles=saline; squares=0.1; triangles=0.3; solid circles=1 mg/kg IP. Right: dose-response relationship (20 to 120 min actometric counts). Open circle=saline; open squares=LY 171555. $*p<0.05$ and $**p<0.01$ when compared to controls (Dunnet *t*-test).

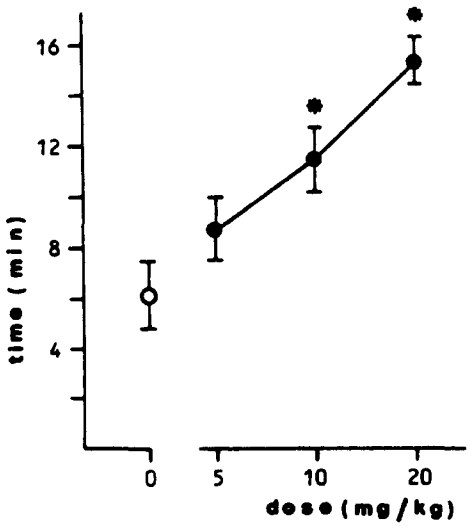

FIG. 4. Grooming induced by different doses of SKF 38393 (solid circles). Mean time (min) ±SEM of grooming during a 1-hr observation period. $*p<0.05$ when compared to saline-injected group (open circle) (Dunnet *t*-test).

SKF 38393 prolonged in a dose-dependent manner the rats' grooming time (Fig. 4), up to a two-fold increase with the highest dose. The locomotor activity simultaneously recorded from the same rats over the observation period failed to show any change, when compared to the saline-treated groups (data not shown).

The blockade of either receptor antagonized all the behavioral responses studied. The selective D-2 receptor blocker YM 09151-2 (0.02 mg/kg, IP) significantly reduced both LY171555-induced hypermotility and SKF 38393-elicited grooming behavior as well as apomorphine-induced stereotyped activity (Table 1). Similar findings were observed following the administration of the selective D-1 receptor blocker SCH 23390 (0.02 mg/kg, IP).

Figure 5A shows the motility counts exhibited by rats submitted to the 21-day administration of YM 09151-2 or SCH 23390 and injected 7 days later with LY 171555. While similar spontaneous locomotor activity during abituation occurred in all pretreated groups (ex-saline=525.22 ±29.95, ex-SCH 23390=499.67±45.77; ex-YM 09151-2= 558.89 ±41.81), a significantly higher locomotor response to the threshold dose of the D-2 selective agonist was detected only in YM 09151-2-pretreated rats. In contrast, the repeated treatment with either the D-1 or the D-2 blocker failed to change the magnitude of the grooming response to SKF 38393 when compared to controls (Fig. 5B).

Both D-1 and D-2 supersensitive rats showed increased stereotyped responses to apomorphine (Fig. 5C). However, when the D-2 dopamine receptors of D-1 supersensitive rats were blocked, apomorphine failed to elicit stereotyped behavior (Table 2). Consistently, D-2 supersensitive rats did not enxhibit stereotyped behavior when their D-1 receptors were blocked by SCH 23390.

DISCUSSION

The findings obtained in the acute experiment of the present investigation have confirmed the results obtained by other investigators [18,21]. Thus the blockade of D-1 receptors by SCH 23390 antagonized not only the SKF 38393-induced grooming behavior and the apomorphine-elicited stereotyped response but also the hypermotility evoked by the D-2 selective agonist LY 171555. Conversely, the pretreatment of rats with the D-2 blocker YM 09151-2 not only counteracted the LY 171555 hypermotility as well as apomorphine-induced stereotyped behavior, but also antagonized the SKF 38393-induced grooming behavior.

Such antagonism did not appear to be caused by altered penetration of the specific agonists into the brain, since pre-

TABLE 1

EFFECT OF PRETREATMENT WITH YM 09151-2 OR SCH 23390 ON DOPAMINE AGONIST-INDUCED BEHAVIORAL RESPONSES

Pretreatment (1 hr before)	LY 171555-Induced Hypermotility (1 mg/kg IP)	SKF 38393-Induced Grooming (20 mg/kg IP)	Apomorphine-Induced Stereotypy (0.5 mg/kg SC)
Saline (5 ml/kg IP)	1581.11 ± 211.53	15.40 ± 0.96	2.06 ± 0.23
YM 09151-2 (0.02 mg/kg IP)	172.29 ± 49.89*	6.22 ± 0.96*	0.79 ± 0.27*
SCH 23390 (0.02 mg/kg IP)	250.88 ± 37.15*	8.87 ± 1.00*	1.31 ± 0.29*

Mean values ± SEM of LY 171555-induced hypermotility (motility counts in 100 min of recording), SKF 38393-induced grooming time (min in 1 hr of observation) and apomorphine-induced stereotyped behavior (mean scores from six 10-min spaced observations according to a 0 to 4 scale).

*$p<0.05$ when compared to the respective controls (Dunnet t-test for motility and grooming data; Mann-Whitney U-test for stereotypy scores).

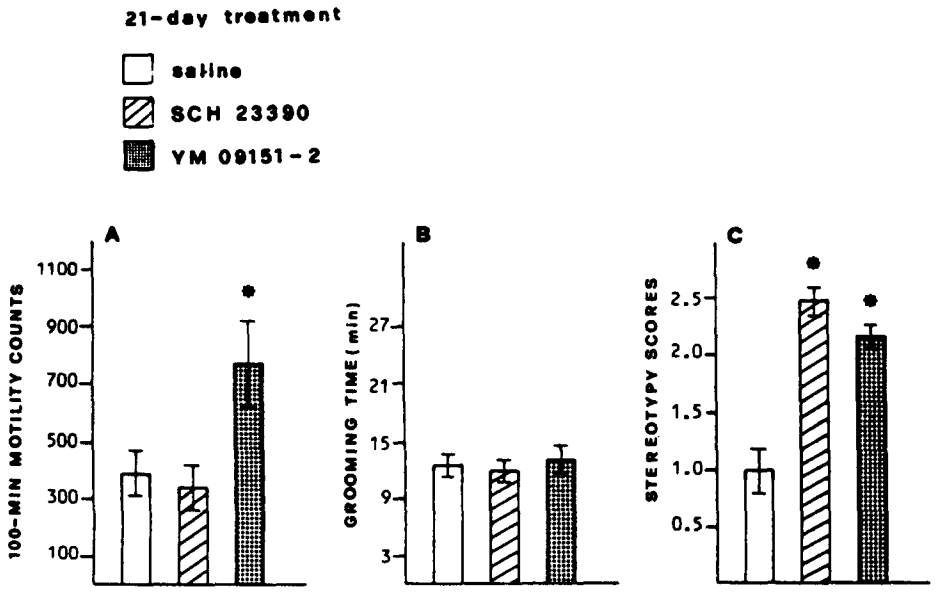

FIG. 5. Behavioral responses of rats challenged 7 days after a 21-day treatment with saline, SCH 23390 (0.05 mg/kg IP, twice daily) or YM 09151-2 (0.02 mg/kg IP). (A) Hypermotility induced by LY 171555 (0.2 mg/kg IP): mean motility counts ±SEM (20 to 120 min after injection). (B) Grooming induced by SKF 38393 (5 mg/kg IP): mean time (min) ±SEM of grooming during a 1-hr observation period. (C) Stereotyped behavior induced by apomorphine (0.25 mg/kg SC): mean scores ±SEM. Individual data were the average scores from three 10-min (from 0 to 30 min) spaced observations. *$p<0.05$ when compared to the respective control (Dunnet t-test for motility and grooming data; Mann-Whitney U-test for stereotypy scores).

treatment of rats with a dose of SCH 23390 that counteracts hypermotility, grooming behavior and stereotyped activity failed to modify the ID_{50} values of [^{3}H]-spiroperidol binding to D-2 receptors, as measured ex vivo (data not shown). Furthermore, the drug specificity is proved by the high selectivity of the above mentioned antagonists: SCH 23390 was 2,000 times more potent on D-1 receptors while YM 09151-2 was 25,000 times more selective on D-2 than on D-1 dopamine receptors (data not shown).

The main findings of our study concerned the data obtained from rats withdrawn from chronic treatments. Repeated treatment with YM 09151-2 increased the number of D-2 but not of D-1 receptors in crude striatal synaptic plasma membranes. As expected, an enhancement in LY 171555-induced hypermotility but not in SKF 38393-induced grooming was obtained. In contrast, while repeated injections with SCH 23390 specifically increased the B_{max} values of D-1 receptors to striatal membranes (see Fig. 1 and [9]), we failed to find any enhancement in the functional correlate of the selective D-1 or D-2 receptor stimulation. These results are at variance with others [10] which indicate a potentiated locomotor response induced by both SKF 38393 or LY 171555

TABLE 2

EFFECT OF ACUTE PRETREATMENT WITH D-2 OR D-1 ANTAGONISTS ON APOMORPHINE-INDUCED STEREOTYPED BEHAVIOR OF D-1 OR D-2 SUPERSENSITIVE RATS

Repeated (21 days) Pretreatment	Acute Treatment (1 hr before)	Stereotypy Scores
Saline	Saline	1.00 ± 0.19
SCH 23390	Saline	2.48 ± 0.12*
YM 09151-2	Saline	2.17 ± 0.11*
SCH 23390	YM 09151-2	0.71 ± 0.11
YM 09151-2	SCH 23390	0.46 ± 0.11

Mean scores ±SEM of the stereotyped response to apomorphine (0.25 mg/kg, SC) given 7 days after discontinuation of a 21-day repeated treatment with SCH 23390 (0.05 mg/kg IP, twice daily) or YM 09151-2 (0.02 mg/kg IP). Rats repeatedly treated with the D-1 blocker were acutely injected (1 hr before) with the D-2 blocker and *vice versa*. Individual data were the average scores from three 10-min (from 0 to 30 min) spaced observations.

*$p<0.05$ when compared to saline-saline group (Mann-Whitney U-test).

in rats repeatedly treated with SCH 23390: however a different paradigm of treatments (0.5 mg/kg SC vs. 0.05 mg/kg IP, twice daily) could explain such inconsistencies.

On the other hand, our study has shown that while D-1 supersensitive rats failed to provide increased responses to the selective agonists, the apomorphine-induced stereotyped behavior was potentiated in either D-1 or D-2 supersensitive rats (see also [27]). This means that apomorphine, by activating simultaneously D-1 and D-2 receptors, stimulates the system in balanced (physiological) way allowing the full expression of the dopaminergic function. In fact, when in the apomorphine-treated rats the system was unbalanced by the blockade of either recognition site, regardless of the level of the other (whether supersensitive or not), the expression of the synaptic function was suppressed. In other words, the behavioral expression of D-1 supersensitivity may be seen only when D-1 and D-2 receptors are simultaneously activated, with the stimulation of the increased number of D-1 receptors modulating to a greater extent the activity of the D-2 receptors. This view is consistent with other observations which indicate that (1) the D-2 agonist bromocriptine requires concurrent stimulation of D-1 receptors so as to allow the full expression of its behavioral stimulant effects [11]; (2) the concurrent stimulation of D-1 and D-2 receptors is necessary for maximal activation of globus pallidus neuron firing [7]; (3) α-methyl-p-tyrosine pretreatment blocked the effect of the selective agonists administered independently, the elicitation of any behavioral response requiring concurrent D-1 and D-2 receptor activation [5].

In conclusion, the present results strongly support the view that at least some behavioral functions of dopamine in the rat appear to be mediated through complex interactions between D-1 and D-2 receptors, these interaction being a requirement for the full expression of DA-receptor mediated function.

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