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# Changes in behavioural responses to the combined administration of D1 and D2 dopamine agonists in normosensitive and D1 supersensitive rats

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**Abstract.** The selective D1 receptor stimulant SKF 38393 dose-dependently increased grooming time in rats without affecting locomotor activity or eliciting stereotyped behaviour. The selective D2 receptor agonist LY 171555 induced a dose-dependent increase in rat motility, a marked decrease in grooming time and a low occurrence of stereotyped behaviour. Concurrent administration of the two selective agonists induced high-degree stereotyped responses and reductions in locomotor and grooming behaviours. Rats withdrawn from repeated treatment with the selective D1 receptor blocker SCH 23390 (0.05 mg/kg twice daily for 21 days; 7 days of washout) did not exhibit any change of locomotor and grooming responses to threshold doses of LY 171555 and SKF 38393 given alone or in combination. On the contrary, a significantly greater occurrence of high-degree stereotyped responses to the combination of the two selective agonists was observed. The data support the view that D1 and D2 receptors have a cooperative role in the generation of stereotypies and suggest that D1 receptor supersensitivity needs D2 stimulation to be revealed.

**Key words:** LY 171555 – SKF 38393 – Combined D1 and D2 stimulation – SCH 23390 – D1 supersensitivity – Locomotor activity – Grooming – Stereotyped behaviour – Rat

Several lines of evidence suggest that in the intact rat the full expression of some dopamine-mediated responses depends on the cooperation of distinct D1 and D2 receptor systems, as indicated by the observation that stereotyped responses appear only following the simultaneous stimulation of both receptors (Mashurano and Waddington 1986; Arnt et al. 1987). Other studies in rats with quinolinic acid-induced striatal lesions have shown that dose-dependent ipsilateral turning behaviour is elicited by the D2 agonist LY 171555 but not by the selective D1 agent SKF 38393; the latter, however, is able to increase the total number of turns induced by LY 171555 (1 mg/kg) (Barone et al. 1986). Moreover, rats treated with alpha-methyl-*p*-tyrosine, which extensively depletes endogenous dopamine, fail to respond to the selective agonists given alone, whereas the behavioural responses are restored when the agonists are administered in combination (Braun and Chase 1986), suggesting that the effects induced by selective dopaminergic drugs in intact animals may be due to the concurrent stimulation of the complementary receptor by the endogenous neurotransmitter.

Recent observations from our laboratory (Vaccheri et al. 1987) have shown that the mixed D2/D1 agonist apomorphine elicits a significantly augmented stereotyped response in rats withdrawn from the repeated administration of the selective D1 receptor blocker SCH 23390, a treatment which causes a selective and significant increase of the number of D1 but not D2 receptors (Creese and Chen 1985; Gandolfi et al. 1988).

This observation suggests that an increase in D1 receptor number could be sufficient for the expression of a behavioural supersensitivity to a mixed agonist.

In order to support this view further, the present study has been undertaken to reproduce the abovementioned finding by challenging rats withdrawn from chronic SCH 23390 treatment with a combination of selective D1 and D2 agonists, namely SKF 38393 and LY 171555, respectively. Previous reports have shown that normal rats treated with increasing doses of the selective D2 receptor agonist LY 171555 exhibit a dose-dependent linear increase in locomotor activity; stereotyped behaviour was rarely observed even at the highest doses (Breese and Mueller 1985; Braun and Chase 1986; Arnt et al. 1987). On the other hand, the selective D1 receptor agonist SKF 38393 induces, in control rats, non-stereotyped grooming behaviour without significantly influencing locomotor activity (Molloy and Waddington 1984).

In the present study, the two abovementioned behavioural responses, as well the occurrence of stereotyped behaviour, were evaluated following separate or combined administration of the two selective dopamine agonists both in control and in D1-supersensitive rats.

## Materials and methods

### *Animals*

Male Sprague-Dawley rats (200–250 g) from Nossan (Correzzana, Italy) were used. The animals were housed four per cage under controlled conditions of light (from 7:00 a.m. to 7:00 p.m.), temperature (22 ± 2° C) and humidity (60%) and were allowed free access to standard laboratory diet and tap water. They were submitted to the behavioural trials only once and were always tested between 9:00 a.m. and 3:00 p.m.

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*Drugs*

LY 171555 (Lilly, Indianapolis, IN) and SCH 23390 (Schering-Plough, Bloomfield, NJ) were dissolved in saline; SKF 38393 (RBI, Wayland, MA) was dissolved in distilled water.

*Apparatus*

Eight individual 38 × 30 × 25 cm actometric cages were employed. A DC current (65 V, 25 μA) was continuously delivered to the stainless steel grid floor of the cage and every closure of the circuit performed by rat feet was recorded as one motility count by an electronic counter: in this way only horizontal displacements of the animal across the cage were recorded, whereas rearings or tramples did not by themselves activate the circuit unless associated with animal locomotion. The cages had the front panel and the top cover made of transparent plexiglas, in order to observe the animal's behaviour, and were located into a soundproof and dimly illuminated room.

*Behavioural responses*

The following responses were measured.

*Locomotor activity*. The motility counts were recorded every 10 min for 120 min.

*Grooming behaviour*. The total time (min) of grooming episodes performed by the animals in 1 h was recorded by observers unaware of the treatments.

*Stereotyped behaviour*. The stereotypy scores were assigned by an observer unaware of the treatments every 10 min for 2 h according to the following rating scale: 0 = no change in normal behaviour; 1 = intermittent sniffing; 2 = continuous sniffing; 3 = intermittent licking or biting; 4 = continuous licking or biting.

*Procedure*

The rats were always accustomed to the the experimental cages for 1 h before drug administration. Immediately after the drug injection the animals were again put into the experimental cages and the behavioural responses were taken as follows: locomotor activity: between 20 and 120 min after injection; grooming time: between 0 and 60 min after injection; stereotyped behaviour: between 30 and 90 min after injection. Two experiments were performed: i) study of the dose-effect relationships of each selective dopamine receptor agonist given alone or in combination with a fixed dose of the other; ii) challenge of rats withdrawn from chronic administration of the selective D1 blocker SCH 23390 with either selective dopamine receptor agonist given alone or in combination with the other. The experimental designs of the two experiments were as follows:

*Study of dose-effect relationships of the two selective dopamine receptor agonists given alone or in combination.* The selective D1 agonist SKF 38393 was administered to different groups of rats at the doses of 0, 2.5, 5 or 10 mg/kg IP; half of the animals from each group were treated with the selective D2 stimulant LY 171555 (0.25 mg/kg IP) immediately before receiving SKF 38393 and the remaining rats were pretreated with saline. Likewise, different groups of rats received the selective D2 agonist LY 171555 (0, 0.25, 0.5 or 1 mg/kg IP) immediately after saline or SKF 38393 (5 mg/kg IP).

*Withdrawal from SCH 23390 chronic treatment.* Twenty-four rats were administered with SCH 23390 (0.05 mg/kg IP) twice daily for 3 weeks and 24 animals with saline according to the same schedule. After a 7-day washout period, the rats from each pretreatment group were assigned to four treatments as follows: saline, SKF 38393 (2.5 mg/kg IP), LY 171555 (0.25 mg/kg IP) and the combination of SKF 38393 (2.5 mg/kg IP) + LY 171555 (0.25 mg/kg IP).

*Statistical analysis*

Grooming time values and log-transformed motility counts were evaluated according to 2 × 4 (first experiment) or 2 × 2 × 2 (withdrawal experiment) ANOVAs followed by post hoc comparisons of means. Individual stereotypy data were represented by the highest score exhibited by the animal during the observation period. Such data were statistically analysed by comparing the occurrence of high (3 and 4) scores by means of Fisher's exact probability test.

## Results

*Study of dose-effect relationships of the two selective dopamine receptor agonists given alone or in combination*

Table 1 summarizes the effects of SKF 38393 (2.5–10 mg/kg IP), given either alone or in combination with LY 171555 (0.25 mg/kg IP), upon the three behavioural responses considered. Table 2 summarizes the effects of LY 171555 (0.25–1 mg/kg IP), given either alone or in combination with a fixed dose of SKF 38393 (5 mg/kg IP).

*Motility*. SKF 38393 did not affect rat locomotor activity at any of the three doses tested. Likewise, when the selective D1 receptor agonist was given in combination with a fixed dose of LY 171555, only the stimulant effect of the latter was observed (Table 1). On the other hand, LY 171555 dose-dependently stimulated locomotor activity when given alone (Table 2). When the rats were concurrently treated with the fixed dose of SKF 38393, the lowest dose of LY 171555 still enhanced rat motility approximately to the same extent as in saline-pretreated rats, whereas the two higher doses were followed by a fall of motility values in comparison to the corresponding groups pretreated with saline (Table 2). Such results are to be seen in the light of the corresponding changes in stereotyped responses (see below).

*Grooming behaviour*. SKF 38393 dose-dependently prolonged the time that the saline-pretreated rats spent grooming. The concurrent treatment with the selective D2 agonist LY 171555 sharply reduced grooming time at any dose of SKF 38393 (Table 1). Similar drastic fall of grooming activity was observed when increasing doses of LY 171555 were given to rats concurrently treated with either saline or a fixed dose of SKF 38393 (Table 2).

*Stereotyped behaviour*. SKF 38393, when given alone, did not elicit stereotyped behaviour at any dose, whereas the number of rats exhibiting high-degree stereotyped responses

**Table 1.** Dose-response relationships of SKF 38393 alone (upper part) or combined with a fixed dose of LY 171555 (lower part) on locomotor activity (mean ± SEM of motility counts recorded from 20 to 120 min after drug injection), grooming behaviour (mean ± SEM of second spent grooming during 60 min after drug injection) and stereotyped behaviour (number of rats exhibiting scores of 3–4 during the period 30–90 min after drug injection). The animals were habituated to the experimental cages for 1 h before behavioural trials

| Concurrent treatment | Response | SKF 38393 doses (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Control | 2.5 | 5 | 10 |
| Saline | Motility | 190 ± 15 | 175 ± 13 | 165 ± 12 | 167 ± 42 |
| | Grooming | 397 ± 74 | 457 ± 51 | 521 ± 69 | 684 ± 71[a] |
| | Stereotypy | 0/13 | 0/6 | 0/12 | 0/12 |
| LY 171555 0.25 mg/kg | Motility | 534 ± 80[b] | 570 ± 144[b] | 493 ± 125[b] | 594 ± 107[b] |
| | Grooming | 87 ± 30[b] | 153 ± 53[b] | 188 ± 66[b] | 60 ± 32[b] |
| | Stereotypy | 0/ 6 | 2/6 | 5/ 6[a,b] | 5/ 6[a,b] |

[a] Significantly ($P < 0.05$) different from the respective control
[b] Significantly ($P < 0.05$) different from the corresponding group concurrently treated with saline

**Table 2.** Dose-response relationships of LY 171555 alone (upper part) or in combination with a fixed dose of SKF 38393 (lower part) on locomotor activity (mean ± SEM of motility counts recorded from 20 to 120 min after drug administration), grooming behaviour (mean ± SEM of seconds spent grooming during 60 min after drug administration) and stereotyped behaviour (number of rats exhibiting scores of 3–4 during the period 30–90 min after drug administration). The animals were habituated to the experimental cages for 1 h before behavioural trials

| Concurrent treatment | Response | LY 171555 doses (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Control | 0.25 | 0.5 | 1.0 |
| Saline | Motility | 190 ± 14 | 534 ± 80[a] | 808 ± 56[a] | 1479 ± 211[a] |
| | Grooming | 397 ± 74 | 87 ± 28[a] | 125 ± 36[a] | 111 ± 23[a] |
| | Stereotypy | 0/13 | 0/6 | 3/ 6[a] | 9/14[a] |
| SKF 38393 | Motility | 165 ± 12 | 493 ± 125[a] | 155 ± 42[b] | 382 ± 66[a,b] |
| | Grooming | 521 ± 69 | 188 ± 66[a] | 47 ± 19[a] | 97 ± 39[a] |
| | Stereotypy | 0/12 | 5/6[a,b] | 11/11[a,b] | 12/12[a,b] |

[a] Significantly ($P < 0.05$) different from the respective controls
[b] Significantly ($P < 0.05$) different from the corresponding group concurrently treated with saline

dose-dependently increased when SKF 38393 was given in combination with a fixed dose of LY 171555 (Table 1). On the other hand, the two highest doses of the selective D2 receptor agonist induced occurrence of high-degree stereotyped responses in 50–64% of the rats pretreated with saline, whereas the same doses caused a 100% occurrence of stereotyped behaviour in rats concurrently treated with a fixed dose of SKF 38393; even the lowest dose of LY 171555, which did not elicit any high-degree stereotyped response in control rats, caused the appearance of stereotyped behaviour in 83% of rats concurrently treated with the selective D1 receptor agonist (Table 2).

*Withdrawal from SCH 23390 chronic treatment*

Table 3 summarizes the behavioural responses to SKF 38393 (2.5 mg/kg IP) and LY 171555 (0.25 mg/kg), given either alone or in combination, exhibited by rats withdrawn from repeated treatment with the selective D1 receptor antagonist SCH 23390 (0.05 mg/kg IP, twice daily for 3 weeks) or saline.

*Motility.* In both saline- and SCH 23390-pretreated rats the administration of the selective D2 receptor agonist LY 171555 caused a slight increase in locomotor activity, which, however, did not reach statistical significance, unlike what obtained in the acute experiment. The selective D1 receptor agonist SKF 38393 did not affect locomotor activity in either pretreated groups. On the other hand, when both controls and SCH 23390-pretreated rats received the combination of the two agonists, a statistically significant increase of motility counts was obtained.

*Grooming behaviour.* In both saline- and SCH 23390-pretreated rats the selective D1 receptor agonist SKF 38393 caused a significant increase in grooming time, without any difference between the two pretreatments, whereas both LY 171555 alone and LY 171555 plus SKF 38393 were followed by a significant decrease in the response.

*Stereotyped behaviour.* In both saline- and SCH 23390-pretreated groups the threshold dose of LY 171555 did not elicit any high-degree stereotyped response, and no significant increase of stereotyped responses was caused in saline-pretreated rats by the concurrent administration of SKF 38393. On the contrary, when the SCH 23390-pretreated rats received both LY 171555 and the selective D1 receptor agonist SKF 38393, a 100% occurrence of high-degree stereotyped responses was obtained.

**Table 3.** Effects of SKF 38393 and LY 171555 given alone or in combination to rats repeatedly treated with saline (upper part) or SCH 23390 (0.05 mg/kg IP, twice daily) (lower part). The behavioural trials were performed after a 7-day washout period. Motility: mean ± SEM of motility counts recorded from 20 to 120 min after drug administration. Grooming: mean ± SEM of time (s) spent grooming during 60 min after drug administration. Stereotypy: number of rats exhibiting scores of 3–4 during the period 30–90 min after drug administration. The animals were habituated to the experimental cages for 1 h before behavioural trials

| Chronic pretreatment | Challenge | Motility | Grooming | Stereotypy |
|---|---|---|---|---|
| Saline | Saline-Saline | 198 ± 26 | 321 ± 39 | 0/6 |
| | Saline-LY 171555 | 332 ± 78 | 127 ± 23[a] | 2/6 |
| | Saline-SKF 38393 | 215 ± 44 | 564 ± 62[a] | 0/6 |
| | SKF 38393-LY 171555 2.5–0.25 mg/kg | 668 ± 234[a] | 102 ± 21[a] | 2/6 |
| SCH 23390 | Saline-Saline | 187 ± 40 | 286 ± 40 | 0/6 |
| | Saline-LY 171555 0.25 mg/kg | 310 ± 64[a] | 89 ± 15[a] | 0/6 |
| | Saline-SKF 38393 2.5 mg/kg | 204 ± 37 | 631 ± 55[a] | 0/5 |
| | SKF 38393-LY 171555 2.5–0.25 mg/kg | 481 ± 153[a] | 117 ± 37[a] | 7/7[a, b] |

[a] Significantly ($P < 0.05$) different from the respective saline-saline challenge
[b] Significantly ($P < 0.05$) different from the saline-pretreated group challenged with the combination of the two agonists

## Discussion

The first part of the present study has shown that the separate administration of the D2 and D1 specific agonists dose-dependently increased rat locomotor activity and grooming behaviour, respectively, as already reported by others (Molloy and Waddington 1984; Breese and Mueller 1985). The higher doses of LY 171555 also caused the occurrence of some stereotyped behaviours; this response, in accordance with the finding by Braun and Chase (1986), consisted of brief and intermittent bursts of licking or biting interrupted by elevated locomotor activity (score 3). Continuous licking and biting (score 4) accompanied by reduced locomotion, as it occurs with high doses of the mixed D1/D2 agonist apomorphine (Ernst 1967), were usually absent. On the contrary, the concurrent administration of low doses of both agonists caused a pattern of responses consisting of maximal stereotyped behaviour, less marked locomotion and disappearance of grooming activity. These results confirm those previously obtained by others (Mashurano and Waddington 1986; Braun and Chase 1986; Arnt et al. 1987) who reported intense stereotyped behaviour following the simultaneous stimulation of both receptors. Indeed, the purpose of the first part of our investigation was to detect threshold doses of either agonists to employ in the second part of the study, when rats chronically treated with a selective D1 receptor blocker were challenged with the combination of the two agonists.

As far as the second part of our study is concerned, some discrepancies with the results of the acute experiment emerged with respect to the locomotor response. The threshold dose of the selective D2 receptor agonist LY 171555 caused a significant increase in motility counts only when given in combination with the D1 stimulant SKF 38393, whilst in the acute study it elicited per se a significant enhancement of motility already at a dose of 0.25 mg/kg. A possible explanation of this discrepancy could depend on the prolonged handling to which the rats withdrawn from chronic treatments had been submitted. Owing to this, drug injection may have represented a less stressful event than in the acute animals, where a possibly greater release of endogenous catecholamines triggered by the novelty of situation could have attenuated the difference between the treatment with LY 171555 alone and that with the combination of the two stimulants.

The rats withdrawn from chronic treatment with the selective D1 blocker SCH 23390 exhibited a locomotor response to the combined administration of D2 and D1 agonists which was not significantly different from that shown by the parallel group withdrawn from chronic administration of saline. Thus, D1 receptor supersensitivity is not reflected by any relevant change in locomotor response to D1/D2 stimulation. Indeed, SCH 23390-withdrawn rats showed a less marked motility response to LY 171555 plus SKF 38393 challenge in comparison to saline-withdrawn group and this seems to be the counterpart of the changes observed in stereotyped response (see below).

The magnitude of SKF 38393-induced grooming activity did not significantly change after withdrawal of chronic administration of the selective D1 receptor blocker SCH 23390, and this finding suggests that the selective increase in D1 receptor number (Gandolfi et al. 1988) brought about by our schedule of SCH 23390 chronic treatment was not accompanied by any enhancement of the behavioural response to the selective D1 receptor stimulation. These findings are at variance with others (Hess et al. 1986) which indicate potentiated behavioural responses induced by SKF 38393 in rats repeatedly treated with SCH 23390; however, the abovementioned authors employed a different schedule of SCH 23390 treatment (0.5 mg/kg SC once daily); moreover, the behavioural trials of that study suffer from some methodological drawbacks (i.e. lack of appropriate controls) which make questionable that the observed effects are specifically related to dopamine agonist administration, as the authors themselves acknowledge.

Interestingly, while in acute experiments only the dose of 10 mg/kg SKF 38393 significantly increased the grooming behaviour, in chronic experiments even 2.5 mg/kg SKF 38393 prolonged the grooming time both in saline and in SCH 23390-pretreated rats. This agrees with the observation that SKF 38393 potentiated LY 171555-induced hypermotility only in chronic experiments (see above) and suggests that the effects of SKF 38393 are better revealed when the rats are habituated not only to the experimental cages, as already suggested by Molloy and Waddington (1984), but also to handling and drug injection.

The main finding of the chronic experiment was the significantly greater occurrence of high-degree stereotyped responses to the combination of threshold doses of SKF 38393 and LY 171555 exhibited by SCH 23390-withdrawn rats, whereas neither D1 nor D2 selective challenges were followed by any change in the magnitude of the respective specific responses. Therefore our data support the view that D1 and D2 receptors have a cooperative role in the generation of high-degree stereotyped behaviour and that a behavioural correlate of D1 receptor supersensitivity may be obtained only by means of concurrent D2 stimulation.

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