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Rapidly induced dopaminergic supersensitivity: D1/D2 receptor participation and its prevention by an MAO-inhibitor

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Abstract

The dopaminergic system requires combined dopamine D1/D2 receptor stimulation to express its activity; a phenomenon called synergism D1/D2. Dopamine receptors develop supersensitivity following dopamine de-afferentation and/or reserpine treatment. Acute supersensitivity occurs with reserpine treatment. The breakdown of D1/D2 synergism has been proposed implicating the genesis of this kind of supersensitivity. We sought to determine the best conditions for inducing acute dopaminergic supersensitivity evaluated by apomorphine-induced stereotyped behaviour, to examine whether D1/D2 synergism breakdown occurs in this reserpine-induced acute supersensitivity model, and whether it can be prevented by the monoamino-oxidase (MAO) inhibitor selegiline. Reserpine (2.0 mg/kg) was injected 3 h before apomorphine (0.6 mg/kg) induced stereotypy. D1/D2 synergism was investigated using specific antagonists (D1-SKF 83566 2.5 mg/kg, D2-haloperidol 2.0 mg/kg) and selegiline (10 mg/kg) was used to analyze the influence of dopamine "de-novo" synthesis. All antagonist treatments suppressed stereotypy and selegiline prevented supersensitivity. These data suggest that reserpine-induced acute dopaminergic supersensitivity is not due to the breakdown of D1/D2 synergism and such supersensitivity can be prevented by recently synthesised dopamine.

Keywords: D_1/D_2 synergism; Plasticity; Reserpine; Dopamine; MAO inhibitor; Stereotypy

1. Introduction

The dopaminergic system has major influence on motor systems whose regulation depends on the interaction of dopamine D1 and D2 receptors subfamilies. Dopamine D1 class and D2 class receptors can be distinguished by their second messenger coupling and ligand binding (Kebabian and Calne, 1979; Sokoloff and Schwartz, 1995; Stoof and Kebabian, 1981; Strange, 1997). In rats, the administration of drugs with agonist effects on both receptors may result in the expression of a diversity of motor behaviours. For instance, apomorphine (a full direct agonist) and amphetamine (an indirect agonist) may elicit motor activity and/or stereotyped behaviour according to the dose used (Buus Lassen, 1976; Felicio et al., 1987; Nasello et al., 2003; Rubovits and Klawans, 1972; Tieppo et al., 1995, 1997, 2000). Stereotypy can be defined as the performance of an invariant sequence of movements in a repetitive manner. In rats, for example, this is often manifested as a continuous repetition of certain behavioural elements including sniffing, locomotor activity and rearing, and at higher doses or with chronic administration, possibly, licking and gnawing (Fray et al., 1980). Furthermore, when selective dopamine D1 or D2 receptor agonists are administered, stereotypy is observed only following combined dopamine D1/D2 receptors stimulation. In many behavioural, electrophysiological, and gene-activity processes the concomitant stimulation of dopamine D1 class and D2 class receptors is required, the so-called dopamine D1/D2 receptor synergism (Braun and Chase, 1986; Christensen et al., 1984; Gershanik et al., 1983; Mailman et al., 1984; Marshall et al., 1997; Molloy and Waddington, 1985; Walter et al., 1987).

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The participation of dopamine D1/D2 receptor synergism may differ depending on the kind of dopaminergic behaviour studied. For instance, alterations in dopamine D1/D2 receptor synergism may account for enhanced stereotypy and reduced climbing in mice lacking dopamine D2L receptor (Fetsko et al., 2003).

It has been well established that the dopaminergic system of rats expresses supersensitivity, developing super-responsiveness, following denervation, chemical blockade or neurotransmitter depletion (Burt et al., 1977; Felicio et al., 1987; Seeman et al., 2005; Tieppo et al., 1995). The characteristics of supersensitivity depend on the agent and/or period of time elected for its induction. Supersensitivity can be induced in the brain during development, and this kind of supersensitivity is reversible (Felicio et al., 1987; Sandstrom et al., 2003). In long-term deprivation of dopaminergic transmission following 19 (Chipkin et al., 1987) or 21 days (LaHoste and Marshall, 1992) of repeated treatments with D1 or D2 antagonists, a binding site up-regulation was observed. As a consequence, the D1 selective drug increased only the number of D1 binding sites, and the D2 selective drug increased only the number of D2 binding sites. There was no change in D1 or D2 receptor affinities. While it has been suggested that receptor up-regulation is present after antagonist treatments, it does not account for the profound changes in sensitivity after dopamine depletion (LaHoste and Marshall, 1992, 1994).

LaHoste and Marshall (1992), data support the view that there are two types of D2 receptor-mediated supersensitivity: a modest one associated with increased striatal D2 density and a more profound one that is independent of D2 density and may be related to the breakdown of D1/D2 synergism. Although chronic D2 antagonism and dopamine denervation are equivalent in their effects on striatal D2 density, their data indicate that they are not equivalent in their effects on D1/D2 synergism. Both types of treatment increase D2 density with a latency of about 2–3 weeks (Burt et al., 1977; Neve et al., 1982; Stauton et al., 1981), whereas denervation produces the additional effect of causing a breakdown in the normal D1/D2 synergism, an effect that occurs early (4–5 days, Arnt, 1985b). In short-term treatments that induce dopamine depletion, such as 4 or 5 days of reserpine, D1/D2 receptor synergism was not demonstrated (Arnt, 1985a; Hu et al., 1990; LaHoste and Marshall, 1992; LaHoste et al., 1993; Needham et al., 1993; Rubinstein et al., 1988a,b). An up-regulation of dopamine D1 receptor transduction mechanism was demonstrated (Missale et al., 1989). Supersensitivity can also appear following acute treatments, such as 5 h of reserpine (White et al., 1988). Some authors proposed a dopamine D1/D2 receptor synergism breakdown implicating the genesis of this type of dopaminergic supersensitivity (Marshall et al., 1997), but others have not corroborated this fact (Gershanik et al., 1983; Rubinstein et al., 1988a,b; White et al., 1988).

Clinical manifestations in diseases affecting the dopamine system include deficits in emotional, cognitive and motor function. Although the parallel organization of specific corticostriatal

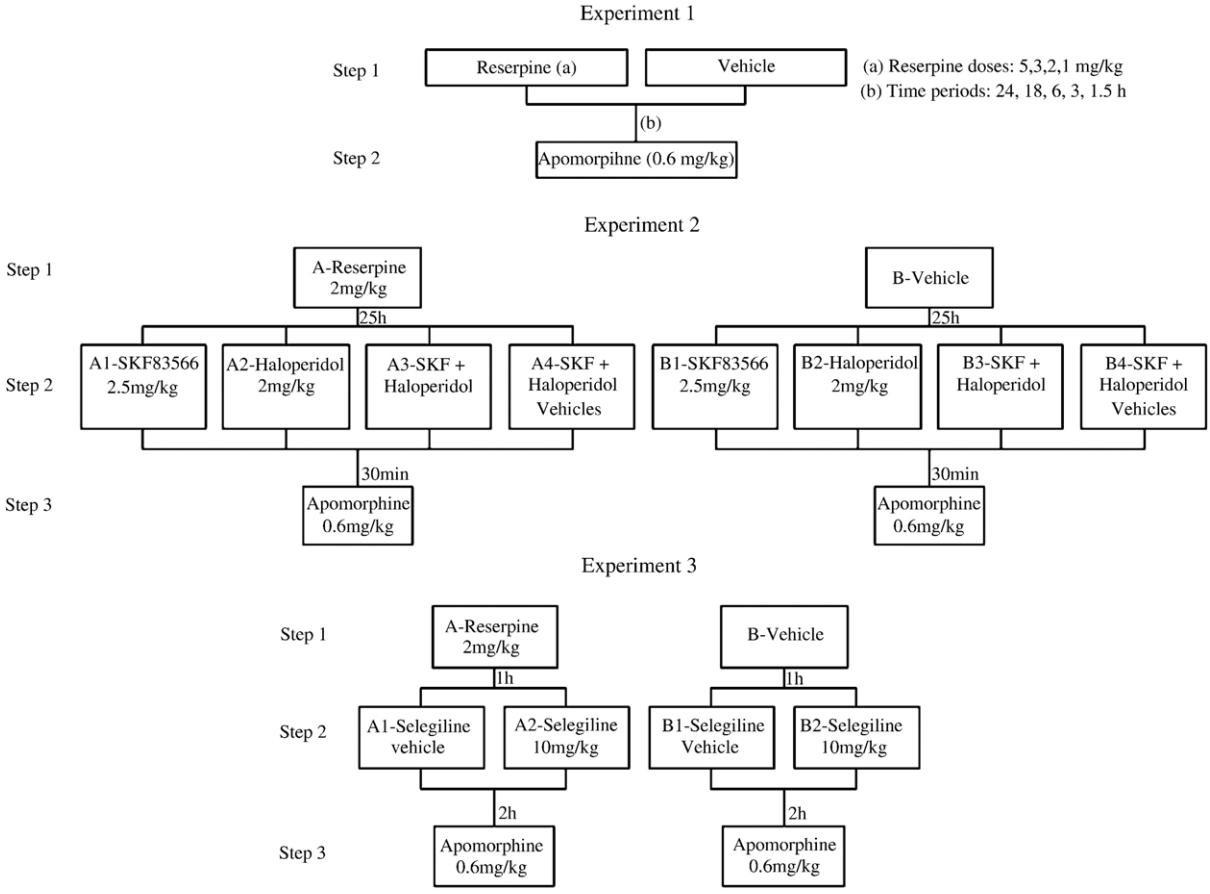

Fig. 1. Flow chart of the experiments.

pathways is well documented, mechanisms by which dopamine might integrate information across different cortical/basal ganglia circuits are less understood (Haber et al., 2000). Dopaminergic system plasticity may be also involved in many side effects of therapeutic drugs: for instance, dyskinetic reactions in Parkinsonian patients treated with L-DOPA, some motor effects of long-term treatments with anti-psychotic drugs and reserpine withdrawal psychosis (Baldessarini, 1996; Samuels and Taylor, 1989). Other drugs, whose therapeutic use is not related to the central nervous system, can also cause dopaminergic alterations producing central nervous system side effects. For instance, amilodipine, nicardipine, diltiazem and verapamil recommended for cardiovascular diseases (Dogrul and Yesilyurt, 1999).

These data show the need for a better understanding of the mechanisms underlying dopaminergic supersensitivity. Our initial goal was to develop a simple and rapid model to study dopaminergic plasticity. We were able to determine the minimal dose and time of a single reserpine injection needed to produce acute dopaminergic supersensitivity and verified whether dopamine D1/D2 receptor synergism was still present at the onset of supersensitivity. For the analysis of dopamine D1/D2 receptor synergism, we used specific dopamine D1 and D2 receptor antagonists, i.e. SKF 83566 (Fritts et al., 1998) and haloperidol (Schwartz et al., 1998) respectively; in case apomorphine-induced stereotypy did not appear when one of the dopamine receptors was blocked, it was assumed that the stimulation of both receptors was still needed to restore locomotor activity. The breakdown of dopamine D1/D2 receptor synergism could thus not explain acute dopaminergic supersensitivity.

The similarity of subchronic reserpine treatment with 6-OHDA treatment suggests that it is not the loss of dopamine terminals per se that results in breakdown in synergism, but rather the loss of some terminal product associated with storage vesicles (LaHoste and Marshall, 1992). Reserpine prevents the storage of dopamine in synaptic vesicles. The synthesis of the neurotransmitter still continues but is not released in functional amounts because MAO metabolises it. Using an MAO-inhibitor, the newly synthesized dopamine is preserved and released. The question that arises is whether such small quantities would stimulate the dopaminergic system and prevent the development of supersensitivity. Selegiline, an MAO B inhibitor with predominant striatum action (Knoll, 1983, 1992; Magyar and Szende, 2004), was used to verify this hypothesis by restricting the mechanisms involved in this adaptive process and preventing as much as possible the side effects of dopaminergic supersensitivity. The sensitivity of the dopaminergic system was evaluated throughout the measurement of stereotyped behaviour induced by apomorphine treatment.

2. Materials and methods

2.1. Animals

341 adult male Wistar rats, weighing 200–280 g, were used ($n \sim 10$ per group). The animals were kept under a controlled 12 h light–dark cycle (lights on at 06:00 h) with food and water available ad libitum, in polypropylene cages (41 × 34 × 16 cm), 6 by cage, containing approximately 1.0 L of medium-grade pine flakes, throughout the experiments. All behavioural tests were done from 11:00 am to 2:00 pm. Animals were tested individually in wire cages (16 × 30 × 18 cm); they were placed into them 3 h before the experiments. Stereotypy was measured by two independent observers. The data were checked afterward and the inconsistent values were unconsidered. Degree of inconsistency was less than 1%. Animals used in this investigation were maintained in compliance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and our institutional ethics committee approved it.

2.2. Drugs

The following drugs were used in the present study: reserpine (Sigma), apomorphine chlorhydrate (Merck) and selegiline (Tocris), SKF 83566 hydrochloride (±)-7-Bromo-8-Hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (Tocris) and Haloperidol (Janssen).

Apomorphine, SKF83566, selegiline and haloperidol were dissolved in saline. Reserpine was diluted in 20% ascorbic acid solution.

2.3. Experimental procedure (Fig. 1)

2.3.1. Experiment 1

For the first experiment, 216 adult male rats were used. Apomorphine (0.6 mg/kg, s.c.) induced stereotyped behaviour was evaluated in the following experiments: 1) reserpine at various doses (5.0, 3.0, 2.0, 1.0 mg/kg) 24 h before apomorphine treatment; 2) reserpine 2.0 mg/kg at various pre-treatment times (18, 12, 6, 3, 1.5 h) before apomorphine. Reserpine was administered i.p. The control groups for the

Table 1
Experiment 1: doses of reserpine, time before apomorphine (0.6 mg/kg), medians and respective ranges of total scores of stereotypy at 90 min of observation, and their respective statistical differences

Doses of reserpine (mg/kg)	Period of time before apomorphine (h)	Total Score of Stereotypy (Ranges)		Statistical difference (*P*)
		Reserpine	Vehicle	
5	24	29 (24–34) *n*: 10	22.5 (17–26) *n*: 10	<0.0001 [a]
3	24	33 (22–39) *n*: 10	25 (19–28) *n*: 10	0.0015 [a]
2	24	26.5 (19–32) *n*: 10	19 (14–24) *n*: 10	0.0003 [a]
1	24	26.5 (19–35) *n*: 10	25 (19–28) *n*: 10	0.2475 [a]
2	18	27.5 (25–36) *n*: 10	22 (19–25) *n*: 10	0.0001 [a]
2	12	33 (28–38) *n*: 15	25.5 (22–35) *n*: 10	0.0002 [a]
2	6	33 (24–39) *n*: 8	24.5 (17–30) *n*: 10	0.001 [a]
2	3	43 (40–54) *n*: 12	31 (24–36) *n*: 10	<0.0001 [a]
2	1.5	31 (27–45) *n*: 10	29.5 (14–34) *n*: 10	0.1230

[a] Significantly different.

different experiments received a vehicle solution of reserpine, ascorbic acid 20%, depending on the time and injection volume.

2.3.2. Experiment 2

For the second experiment, 83 adult male Wistar rats were distributed in 2 groups (A and B)

A — Experimental group: reserpine (2 mg/kg, i.p.)
B — Control group: ascorbic acid (20%, i.p.)

After 2.5 h, these groups were subdistributed in 8 groups: A1–B1, A2–B2, A3–B3, and A4–B4:

A1–B1 received SKF 83566 (2.5 mg/kg, i.p.) and 30 min later, apomorphine (0.6 mg/kg, s.c.)
A2–B2 received Haloperidol (2 mg/kg, i.p.) and 30 min later, apomorphine (0.6 mg/kg, s.c.)
A3–B3 received both SKF83566 and Haloperidol and 30 min later, apomorphine (0.6 mg/kg, s.c.)
A4–B4 received saline and 30 min later, apomorphine (0.6 mg/kg, s.c.)

2.3.3. Experiment 3

For the third experiment, 42 adult male Wistar rats were distributed in 2 groups (A and B)

A — Experimental group: Reserpine (2 mg/kg, i.p.)
B — Control group: Ascorbic acid (20%, i.p.)

After 1 h, these groups were subdistributed in 4 groups: A1–B1, A2–B2:

A1–B1 received selegiline vehicle, saline, and after 2 h, apomorphine (0.6 mg/kg, s.c.)

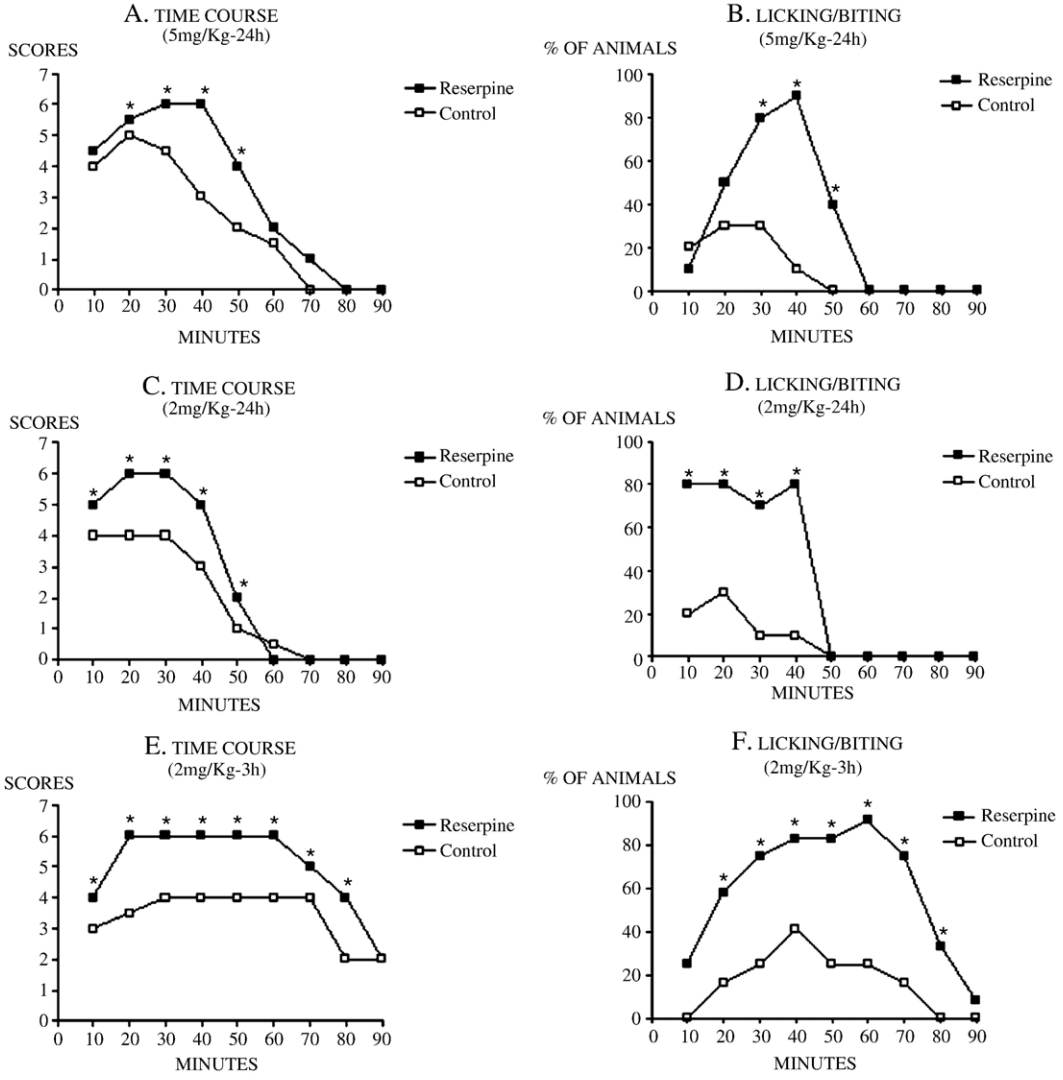

Fig. 2. Time course of stereotypy evaluated by Setler's method (A, C, E) and Time course of Fray's method analysis (B, D, F) induced by apomorphine (0.6 mg/kg s.c.) after pre-treatment with reserpine (A and B: RES 5 mg/kg-24 h i.p., C and D: RES 2 mg/kg-24 h i.p., E and F: RES 2 mg/kg-3 h i.p.), or reserpine vehicle. A, C and E: data are medians. B, D and F: data are percentage of animals that showed the licking/biting Fray's behaviour. *$P < 0.05$ Control *vs* Reserpine (for exact P values see results).

A2–B2 received selegiline (10 mg/kg, s.c.) and after 2 h, apomorphine (0.6 mg/kg, s.c.)

Stereotypy was quantified for 10 s every 10 min for 90 min immediately after apomorphine. Setler's scoring system modified by Troncone was used as follows: 0 — asleep or still; 1 — active; 2 — predominantly active but with bursts of stereotyped sniffing and rearing; 3 — constant stereotyped activity such as sniffing, rearing, or head bobbing, but with locomotor activity still present; 4 — constant stereotyped activity maintained in one location; 5 — constant stereotyped activity but with bursts of licking and/or gnawing and biting; 6 — continual licking of cage grids; 7 — continual biting of cage grids (Setler et al., 1976; Troncone et al., 1988). In addition, stereotyped behaviour was also evaluated by Fray's method (Fray et al., 1980). This method consists of observing the presence for more than 3 s or absence of the following behaviours: locomotion, rearing, sniffing, licking and gnawing. The data were recorded simultaneously with the previously described scores. The stereotypy was measured by two independent observers (Tieppo et al., 1995, 1997, 2000, 2001).

2.4. Statistical analysis

The results were analysed by Kruskal–Wallis's non-parametric analyses of variance followed by Mann–Whitney's *U*-tests for Setler's scores and Fischer's test for Fray's method data. The probability of $P<0.05$ was considered a significant difference for all comparisons made.

3. Results

3.1. Experiment 1

The different doses of reserpine, the different periods of time before apomorphine, medians and respective ranges of total

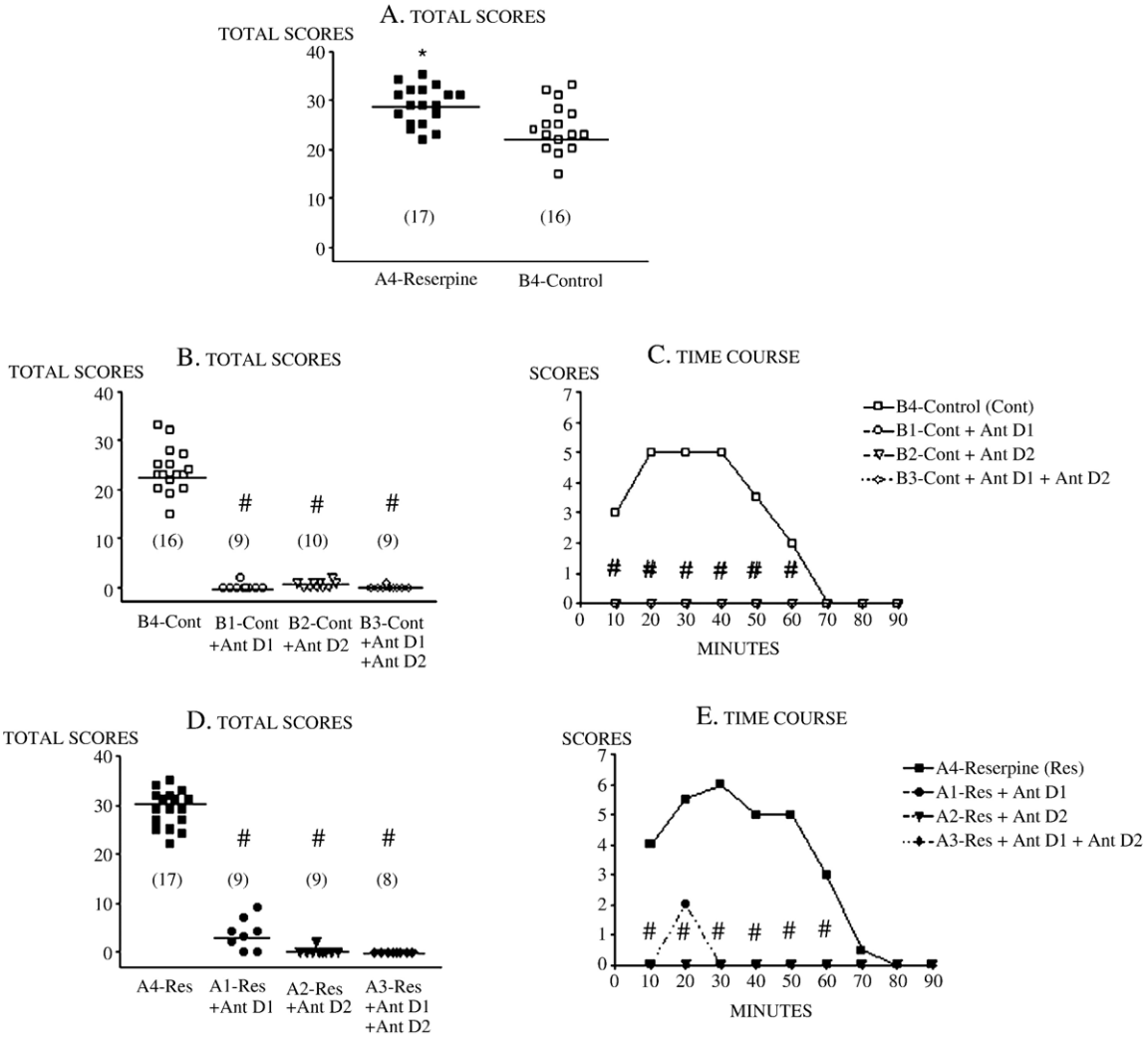

Fig. 3. Total stereotyped behaviour (A, B, D) and time course of stereotypy (C and E) evaluated by Setler's method induced by apomorphine (0.6 mg/kg s.c.) after 3 h pre-treatment with reserpine (2 mg/kg i.p.) or reserpine vehicle, and 2 h 30 min after dopamine antagonists treatment, SKF83566 (2.5 mg/kg s.c.) and haloperidol (2 mg/kg s.c.), or their vehicle. A, B and D: data are individual total scores during 90 min of observation and their respective median. C and E: data are medians. *$P<0.04$ Reserpine *vs* control; #$P<0.0001$ Control *vs* Cont+Ant D1; Control *vs* Cont+Ant D2; Control *vs* Cont+Ant D1+Ant D2 and Reserpine *vs* RES+Ant D1; Reserpine *vs* RES+Ant D2; Reserpine *vs* RES+Ant D1+Ant D2 (for exact *P* values see results).

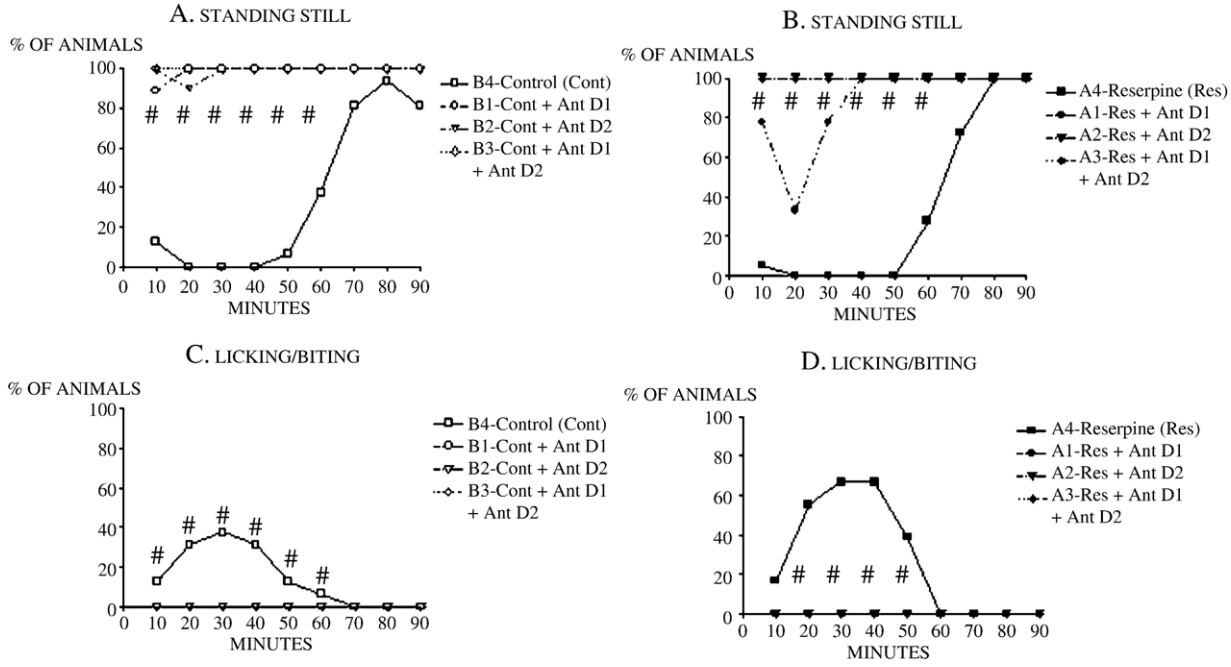

Fig. 4. Time course of Fray's method analysis of apomorphine-induced stereotyped behaviour (0.6 mg/kg s.c.) after 3 h of pre-treatment with reserpine (2 mg/kg i.p.) or reserpine vehicle, and after 2 h 30 min of dopamine antagonists treatment, SKF83566 (2.5 mg/kg s.c.) and haloperidol (2 mg/kg s.c.), or their vehicle. Data are percentage of animals that showed the respective behaviour: A: standing Still (control groups), B: standing still (reserpine groups), C: licking/biting (control groups), D: licking/biting (reserpine groups). #$P<0.05$ Control *vs* Cont+Ant D1; Control *vs* Cont+Ant D2; Control *vs* Cont+Ant D1+Ant D2 and Reserpine *vs* RES+Ant D1; Reserpine *vs* RES+Ant D2; Reserpine *vs* RES+Ant D1+Ant D2 (for exact *P* values, see results).

scores during 90 min of observation, and their respective statistical difference are depicted in Table 1.

Reserpine injection 5 mg/kg and 2 mg/kg, 24 h before apomorphine challenge, increased stereotyped behaviour when compared to control (Fig. 2A and C). In Fig. 2A (5.0 mg/kg of reserpine, 24 h), the differences were significant at 20, 30, 40 and 50 min (*$P<0.02$). When using reserpine 2.0 mg/kg, 24 h, the differences were significant at 10, 20, 30, 40 and 50 min (*$P<0.05$; Fig. 2C). In Fray's method the differences in licking/biting-stereotyped behaviour were significant at 30, 40 and

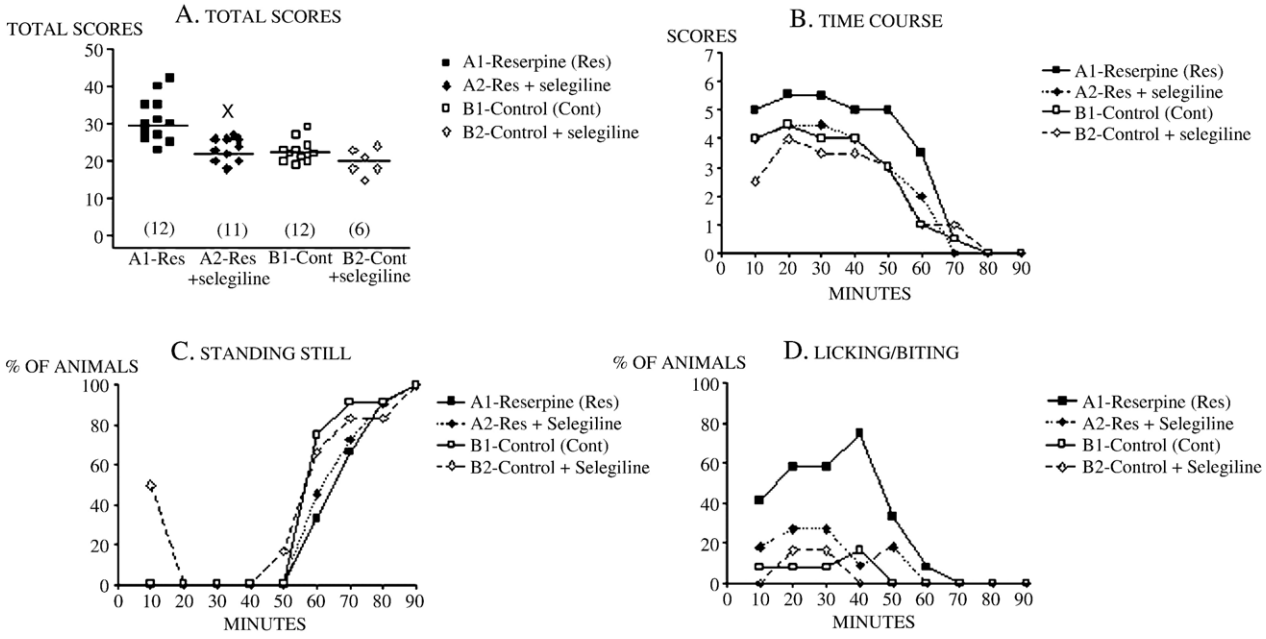

Fig. 5. Stereotyped behaviour (A), Time course of stereotypy (B) evaluated by Setler's method and Time course of Fray's method analysis (C and D) of apomorphine-induced stereotyped behaviour (0.6 mg/kg s.c.) after 3 h of pre-treatment with reserpine (2 mg/kg i.p.) or reserpine vehicle, and after 2 h of pre-treatment with selegiline (10 mg/kg s.c.) or selegiline vehicle. A: data are individual total scores during 90 min of observation and their respective median. B: data are medians. C and D: data are percentage of animals that showed the respective behaviour. X $P<0.03$ Reserpine *vs* reserpine+selegiline (for exact *P* values, see results).

50 min (*$P<0.04$) for 5 mg/kg of reserpine 24 h before apomorphine (Fig. 2B); at 10, 20, 30 and 40 min (*$P<0.02$) for 2 mg/kg reserpine 24 h before apomorphine (Fig. 2D).

In rats pre-treated with reserpine 2.0 mg/kg 3 h before apomorphine treatment, stereotyped behaviour increased significantly. The differences were significant at 10, 20, 30, 40, 50, 60, 70 and 80 min (*$P<0.02$; Fig. 2E). In Fray's method, the differences in licking/biting-stereotyped behaviour were significant at 20, 30, 40, 50, 60, 70, 80 min (*$P<0.04$) for 2 mg/kg reserpine 3 h before apomorphine (Fig. 2F).

3.2. Experiment 2

Reserpine 2 mg/kg 3 h before apomorphine increased stereotyped behaviour when compared to saline (*$P<0.002$; Fig. 3A). In the time-response curve the difference was significant at 30 and 50 min (*$P<0.04$, data not shown). All antagonist treatments were able to suppress the stereotypy induced by apomorphine (#$P<0.0001$; Fig. 3B and D). The time-response course of antagonist was the same for control (Fig. 3C) and treated animals (Fig. 3E). The differences were significant at 10, 20, 30, 40, 50 and 60 min (#$P<0.0001$; Fig. 3C and E). The akinesia (standing still observed in Fray's method) could not be reversed by apomorphine in any of the treated groups with antagonists (#$P<0.0001$; Fig. 4A and B) and no stereotyped behaviour (licking/biting) was observed (Fig. 4C and D). The differences were significant at 10, 20, 30, 40, 50 and 60 min (#$P<0.002$) for control animals (Fig. 4C) and for reserpine treated rats at 10, 20, 30, 40 and 50 min (#$P<0.04$; Fig. 4D).

3.3. Experiment 3

Reserpine 2 mg/kg 3 h before apomorphine increased stereotyped behaviour when compared to saline ($P=0.0002$; Fig. 5A). In the time response curve, this difference was significant at 10, 20, 40, 50 and 60 min ($P<0.04$; Fig. 5B). In reserpine treated rats, lower stereotypy was observed in rats treated with selegiline (X $P=0.0008$; Fig. 5A). In the time response curve, the difference between reserpine treated rats that received or not selegiline was significant at 10, 20 and 40 min (X $P<0.04$; Fig. 5B). There was no statistical difference between control rats treated or not with selegiline ($P=0.0934$; Fig. 5A). In the time-response curve, the difference between saline rats, treated or not with selegiline, was significant at 10 min ($P<0.003$; Fig. 5B). In the analysis of rats treated with selegiline, reserpine treated animals presented higher stereotyped behaviour but these two groups did not differ statistically ($P=0.0934$). In the time-response curve, the difference was significant between rats treated with selegiline at 10 min ($P=0.042$; Fig. 5B). Fray's method showed a statistical difference for standing still behaviour at 10 min comparing rats treated with selegiline ($P<0.03$; Fig. 5C) and control rats treated or not with selegiline ($P=0.0294$; Fig. 5C). Reserpine treated compared to saline treated rats, showed statistical difference in licking/biting behaviour at 20, 30, 40 min ($P<0.03$; Fig 5D). A significant difference was observed between reserpine treated rats that did or did not receive selegiline at 40 min (X $P=0.008$). There was no significant difference among the other groups.

4. Discussion

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility (Missale et al., 1998).

Over the past 30 years, numerous studies have focused on dopaminergic systems. Several pathological conditions are reverted either by dopamine agonists or antagonists. For instance, Parkinson's disease (extrapyramidal motor system alteration, reverted by agonists), schizophrenia (mesolimbic and mesocortical system alteration, reverted by antagonists), Tourette's syndrome (motor symptoms not related with extrapyramidal system, reverted by antagonists), and hyperprolactinemia (hormonal alteration reverted by agonists) have been linked to a deregulation of dopaminergic transmission. In Parkinson's disease patients treated with L-Dopa, the dopamine D1 receptor was identified as the agent responsible for diskinesia (Gerfen, 2003), contrary to the findings of Turjanski et al. (1997). In contrast, blockade of dopamine receptors can induce extrapyramidal effects similar to those resulting from dopamine depletion, and high doses of dopamine agonists can cause psychoses. The therapies for disorders resulting from dopamine imbalances are associated with severe side effects (Missale et al., 1998). The present scenario indicates a need for a better understanding of the dopaminergic system.

The dopaminergic system adapts plastically to changes in neuronal inputs. Dopamine D1 and D2 receptors have multiple mechanisms to overcome persistent loss in dopamine neurotransmission. The *Ras* Homolog Enriched in Striatum (Rhes) is highly expressed in brain regions receiving dopaminergic input. Its expression is decreased when dopamine is removed from the striatum. Harrison and LaHoste (2006), suggest Rhes may inhibit dopamine-mediated supersensitivity in striatum. A decrease in Rhes mRNA after dopamine removal may contribute to the supersensitivity response. Chronic dopamine antagonist treatment – 21 days of haloperidol 1 mg/kg – increased dopamine D1 and/or D2 receptors density in the striatum, but had no effect on the synergistic interaction between dopamine D1 and D2 receptors. In contrast, 5 days of reserpine (1 mg/kg) treatment did not alter striatal dopamine D1 or D2 receptors density, yet caused a breakdown in synergism (LaHoste and Marshall, 1992).

Therefore, it seemed essential to find the minimal dose and time to induce acute dopaminergic supersensitivity. Thus, a useful model is presented herein that yielded prompt results in order to find the causes of this supersensitivity and acquire a better understanding of the dopaminergic plasticity.

Our present results show that the dopaminergic system displays acute supersensitivity following reserpine-induced

neurotransmitter depletion. Pre-treatment with reserpine (2.0 mg/kg) 1.5 h before apomorphine injection or 1.0 mg/kg of reserpine, 24 h before apomorphine did not significantly modify stereotyped behaviour expression. Therefore, the phenomenon could be fully induced with 3 h pre-treatment of 2.0 mg/kg of reserpine (Fig. 2E). Dopamine D2 receptor supersensitivity following 6-hydroxidopamine or reserpine treatments is very similar to that observed when the dopamine D2 receptor agonists are administered to previously untreated rats concomitantly with a maximal stimulating dose of a dopamine D1 receptor agonist (LaHoste and Marshall, 1992). In this regard, it has been proposed that the breakdown in dopamine D1/D2 receptor synergism was implicated in the genesis of dopamine acute supersensitivity (for a review see Marshall et al., 1997). Since reserpine-induced akinesia in mice can be reversed only by combined administration of selective dopamine D1 or D2 receptor agonists 3 h after reserpine treatment (Gershanik et al., 1983), it seems that supersensitivity precedes synergism breakdown. To verify this proposition, dopamine D1 and D2 receptor selective antagonists were administrated alone or in combination. The akinesia induced by reserpine could not be reversed by apomorphine in any treated groups with antagonists, i.e. dopamine D1 receptor antagonist (SKF83566), dopamine D2 receptor antagonist (haloperidol) or the combination of both of them, suggesting the maintenance of dopamine D1/D2 receptor synergism (Figs. 3C,E and 4A and B). Our results are similar to those observed by Gershanik et al. (1983), who used specific agonists to study dopamine D1/D2 receptor synergism in rats treated with reserpine (5 mg/kg; 3 h of pre-treatment). Neither dopamine D1 receptor agonist nor the dopamine D2 receptor agonist, administered alone, overcame reserpine akinesia, but together they restored locomotor activity. The main difference between the treatment used by Marshall et al. (1997) and the present study was that the former adopted a longer period of time of reserpine administration, which may have elicited different plastic responses.

The reserpine-induced supersensitivity was due to a depletion of dopamine in synaptic vesicles, but the synthesis of the neurotransmitter was not blocked. Almost all dopamine synthesised is metabolised by MAO. MAO inhibition with selegiline will permit, predominantly in the striatum, the dopamine synthesised "de-novo" to be released to the synaptic cleft and stimulate the post-synaptic receptors. Spooren et al. (1999), described that the deprenyl (selegiline)-treated 6-OHDA lesioned rats responded with a reduced number of rotations in response to apomorphine but not to amphetamine as compared to vehicle-treated 6-OHDA-lesioned rats. They also described that a post-lesion treatment with deprenyl reduced the dopaminergic supersensitivity without a concomitant increase in striatal dopamine content. We intended to analyse whether dopamine "de-novo" quantities were sufficient to impair receptor supersensitivity induction, and our results showed the prevention of acute dopaminergic supersensitivity (Fig. 5A, B, C, D). After reserpine treatment, the only dopamine present in the cytoplasm to be released was dopamine "de-novo" synthesised, but MAO oxidised this dopamine. In the presence of MAO inhibitor selegiline, a certain amount of dopamine was not oxidised and was released. In our experiment, regardless of the amount of dopamine released, reserpine-induced supersensitivity was prevented.

5. Conclusion

Collectively, these results suggest that the magnitude of the plasticity of the dopaminergic systems is such that supersensitivity appears with reserpine 2.0 mg/kg and, as quickly as 3 h later, the modifications are fully established. This is not due to the dopamine D1/D2 receptor synergism breakdown and can be reversed by small quantities of dopamine recently synthesised present in the synapse cleft after selegiline treatment.

The acute clinical and therapeutic implications of this velocity of adaptation to the deficiency of dopamine should be considered, for instance, in Parkinson disease and/or extrapyramidal side effects of antipsychotic drug treatments. The nigrostriatal pathway from the substantia nigra to the caudate putamen is the pathway primarily associated with Parkinson's disease. Both dopamine D1 and D2 receptors are found in the striatum. The important role of these two receptors in the striatum is to modulate the function of the direct and indirect pathways involved in the control and initiation of motor activity (Marsden, 2006). An antagonism of striatal postsynaptic dopamine D2 receptors can explain the initial extrapyramidal side effects of antipsychotic drugs (Marsden et al., 1975). All these diseases seem to be associated with initial changes in the dopaminergic system showing the relevance of a better understanding of the acute characteristics of the dopaminergic plasticity.

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