D1–D2 Interaction in Feedback Control of Midbrain Dopamine Neurons

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Dopamine (DA) D1-like receptors are present in pathways implicated in feedback control of midbrain DA neurons. However, stimulation of these receptors either produces no effect on DA cells, or the effect is inconsistent. It is possible that the expression of a D1 feedback effect requires co-activation of D2-like receptors. To test this hypothesis, we recorded extracellularly the spontaneous activity of nigral DA cells in a low cerveau isolé rat preparation. SKF38393 and dyhydrexidine, two D1 agonists, were administered systemically to animals pretreated with different doses of the D2 agonist quinpirole. Supporting the hypothesis, the two D1 agonists consistently inhibited DA cells in animals given high doses of quinpirole (≥40 μg/kg, i.v.). However, no significant D1 effect was observed in animals pretreated with only low doses (≤20 μg/kg) of quinpirole. Because low doses of D2 agonists preferentially act on DA autoreceptors, and because the D1 inhibition persisted in animals whose DA autoreceptors were blocked by intranigral application of raclopride, our results suggest that the expression of D1 feedback inhibition requires co-activation of D2-like receptors on DA target neurons, instead of DA neurons themselves. These results, together with the finding that chloral hydrate completely blocked the D1 inhibition, may explain why previous studies have failed to show a consistent D1 effect on DA cells and suggest that drugs designed to act specifically on one subtype of DA receptor may, via feedback pathways, influence the action of endogenous DA on other DA receptor subtypes as well.

Key words: DA neuron; feedback pathway; D1; D2; synergistic; SKF38393; dyhydrexidine; substantia nigra; striatonigral; single-unit recording

The activity of midbrain DA neurons is regulated by short and long feedback pathways. The short feedback pathways, mediated by inhibitory D2-like autoreceptors, have been studied extensively. Little is known, however, about the long feedback pathways. By definition, the long feedback pathways involve DA receptors located on neurons postsynaptic to DA terminals. For substantia nigra (SN) DA cells, one such pathway may involve striatonigral GABAergic neurons. These neurons receive direct synaptic input from DA terminals (Freund et al., 1984; Caille et al., 1996) and project to the SN (Bunney and Aghajanian, 1976a), where their terminals make direct synaptic connections with DA cells (Nitsch and Riesenberg, 1988; Bolam and Smith, 1990; Yung et al., 1995; Caille et al., 1996). Consistent with the presence of the striatonigral feedback pathway, the GABA antagonist picrotoxin has been shown to block the inhibition of SN DA neurons induced by the indirect DA agonist amphetamine, and lesions of the striatonigral pathway attenuate the ability of amphetamine to inhibit DA neurons (Bunney and Aghajanian, 1976b, 1978).

However, despite the evidence for their presence, the way long feedback pathways operate is still controversial. One issue concerns the role of D1-like receptors. According to anatomical studies, D1-like receptors should play a major role in the striatonigral pathway because they are the main DA receptors expressed in striatonigral neurons (Yung et al., 1995). Electrophysiologically, however, only D2-like receptor agonists have been shown to have an effect on DA cells. Selective activation of D1-like receptors produces either an inconsistent effect or none at all (Carlson et al., 1987; Huang and Walters, 1992; Sun et al., 1993).

A number of electrophysiological, biochemical, and behavioral studies have shown that the expression of some DA effects requires activation of both D1- and D2-like receptors (e.g., Walters et al., 1987; White, 1987; Bordi and Meller, 1989; Wachtel et al., 1989; Bertorello et al., 1990). It is thus possible that the expression of the D1 feedback effect also requires concurrent activation of D2-like receptors. To test this hypothesis, we made extracellular recordings from SN DA neurons in a low cerveau isolé rat preparation and determined whether co-administration of a D2 agonist could enable a D1 agonist to produce an effect on DA cells.

Some results were reported previously in abstract form (Smith et al., 1994).

MATERIALS AND METHODS

Animal preparation. All procedures were performed in accordance with those outlined in the Guide for the Care and Use of Laboratory Animals published by the United States Public Health Service and approved by the Yale Animal Care and Use Committee. Male Sprague Dawley rats weighing between 160 and 300 gm were used. Previously, general anesthesia, including that induced by chloral hydrate, was shown to suppress the activity of striatal neurons (Kelland et al., 1988) and to block some DA receptor-mediated effects, including the D1 effect on nigral DA neurons in reserpine animals and on subthalamic neurons (Huang and Walters, 1992; Kreiss et al., 1996). Because of this, most experiments were performed in a low cerveau isolé preparation. However, for comparison, a few experiments were performed in chloral hydrate-
Figure 1. Diagram illustrating the position of brainstem transection for the low cereau isolé preparation. A, Sagittal view of a rat brain showing the site of transection between the pons and the medulla oblongata (1 mm posterior to the lambda, indicated by the thick line). B, Coronal view of the transected areas (shaded) through the cerebellum and the brainstem. Thick lines illustrate the initial and final positions of the transection knife (a flattened needle). See Materials and Methods for details. Figures are redrawn from the rat atlas of Paxinos and Watson (1997).

In nine identified DA neurons, SKF38393 (10 mg/kg) was administered intravenously in either a single dose (n = 3) or multiple doses (5 and 5 mg/kg, n = 2; or 2.5, 2.5, and 5 mg/kg, n = 4). Consistent with a previous study (Carlson et al., 1987), the D1 agonist failed to produce a consistent effect on DA cells. Five cells showed an increase in firing rate (34.5 ± 9.9% of baseline, ranging from 16.7 to 70.8%), and four showed a decrease (42.7 ± 19.2% of baseline, ranging from 18.2 to 100%). To determine whether the effects of SKF38393 were mediated by D1-like receptors, the D1 antagonist SCH23390 (20–100 μg/kg) was administered after SKF38393 in five cells. In four cells, SCH23390 showed no effect on either SKF38393-induced excitation (n = 2) or inhibition (n = 2). In one cell, however, the inhibitory effect (18.2% of baseline) of SKF38393 was reversed.

To test further whether the variable effect of SKF38393 is related to D1-like receptor stimulation, the new full D1 agonist DHX was administered to six other SN DA neurons (1 mg/kg in a single dose). No significant change in the firing rate was observed in five of six cells (<10% change of baseline). In one cell, DHX produced an 18% inhibition of basal activity. Overall, the effect of DHX was statistically not significant (4 ± 3% decrease in baseline; p = 0.697; n = 6, paired t test).

Effect of D1 agonists on DA neurons pretreated with a D2 agonist

To test whether activation of D2-like receptors is necessary for the expression of a D1 effect, nine DA cells were exposed to the D2-selective agonist quinpirole (administered i.v.) before the injection of SKF38393. Because quinpirole inhibits DA cells by itself, low doses (11.7 ± 2.2 μg/kg, ranging from 5 to 20 μg/kg) were injected to avoid a complete inhibition. In seven cells, SKF38393 (10 mg/kg, n = 6; 20 mg/kg, n = 1), after quinpirole, produced no significant effect. Firing rate change after SKF38393 was indistinguishable from the spontaneous recovery (Fig. 2A,B). In the remaining two cells, SKF38393 (10 mg/kg, n = 1; 20 mg/kg, n = 1) produced a clear further inhibition (>10% of baseline). When the nine cells were combined, the firing rate was decreased from 56.5 ± 7.3% of baseline immediately before SKF38393 to 52.8 ± 9.4% at the maximum effect of SKF38393 (Fig. 2A). This change was statistically not significant (n = 9; p = 0.757, paired t test).

However, it is possible that a D1 effect depends on activation of D2-like receptors located on DA target neurons rather than D2 autoreceptors. DA neurons are known to be more sensitive than DA target neurons to systemically administered DA agonists (Skirboll et al., 1979; White and Wang, 1986; Piercey et al., 1996a). The doses of quinpirole used in the above experiments may have been too low to activate D2-like receptors significantly on DA target neurons. To test this possibility, high doses of quinpirole were administered to 10 rats. To avoid a complete inhibition, doses of quinpirole were administered to 10 rats. To avoid a complete inhibition, doses of quinpirole were injected slowly at 3–10 min intervals (see Fig. 3B). Perhaps because of the development of tachyphalaxis of autoreceptors (Aghajanian and Bunney, 1973; Piercey et al., 1996a), DA cells remained active after a slow injection of 40–160 (92 ± 15.8) μg/kg. In 9 of 10 cells, subsequent injection of SKF38393 (5 mg/kg, n = 1; 10 mg/kg, n = 6; 20 mg/kg, n = 2) produced a clear further inhibition (>10% of baseline; Fig. 3A,B). In the remaining one cell, SKF38393 (20 mg/kg) produced no significant effect. Overall, the firing rate was decreased by SKF38393 from 39.3 ± 4.7 to 10.9 ± 4.1% of baseline (p < 0.0001; n = 10; paired t test; Fig. 3A,B). Unlike the inhibition induced by quinpirole alone, which was not affected at all by SCH23390 (160 μg/kg; n = 5; Fig. 3C), the inhibition...
induced by SKF38393 was completely reversed by SCH23390 (20–80 μg/kg; n = 6; Fig. 3B), confirming that it was a D1 effect.

To rule out the possibility that the slow speed of injection may play a role in the above observed enabling effect of quinpirole, a low dose of quinpirole (10 μg/kg) was administered slowly to five rats. In four of the five cells examined, SKF38393 (10 mg/kg) produced either no effect (n = 2) or a small increase in firing rate (n = 2; 12.9 and 13.9% of baseline, respectively). In one cell, SKF38393 inhibited the firing by 19% of baseline. A single dose of quinpirole (QUIN, 10 μg/kg, i.v.) inhibited the firing of the cell to ~40% of baseline. Subsequent administration of SKF38393 (SKF, 10 mg/kg, i.v.) did not significantly affect the remaining activity. In this and following figures, arrows indicate the times of drug injection, and the numbers above the arrows represent the doses of the drug injected.

Effect of blockade of D2 autoreceptors on the D1 inhibition of DA neurons

To test more directly the idea that the expression of the D1 effect does not require co-activation of DA autoreceptors, raclopride (2–8 μg), a selective D2 antagonist, was locally applied to the SN 15 min before beginning recordings. In these locally treated cells, large doses of quinpirole (40 μg/kg, n = 6; 160 μg/kg, n = 11; 640 μg/kg, n = 1) could be injected within a short period without completely inhibiting the cell. On average, the firing rate was inhibited by 47.9 ± 5.1%. In 17 of 18 cells tested, SKF38393 (5 mg/kg, n = 3; 7.5 mg/kg, n = 1; 10 mg/kg, n = 13; 20 mg/kg, n = 1) after quinpirole produced a clear further inhibition of firing (>10% of baseline; Fig. 4A,B). In the remaining one cell, the firing rate was reduced by 5%. Overall, SKF38393 significantly decreased the firing rate from 47.9 ± 5.1 to 16.1 ± 5.1% of baseline (n = 18; p < 0.0001, paired t test; Fig. 4A).

Similar experiments were performed in seven other locally raclopride-treated (4–10 μg) DA cells, in which SKF38393 was replaced with DHX (0.5 mg/kg, n = 3; 0.75 mg/kg, n = 1; 1 mg/kg, n = 3). After pretreatment with quinpirole (160 μg/kg),
DHX produced a clear inhibition in all seven cells (from $45.0 \pm 7.0$ to $13.8 \pm 4.1\%$ of baseline; $p < 0.001$, paired $t$ test; Fig. 5).

Effect of chloral hydrate on D1-mediated inhibition of DA neurons

Some D1-mediated effects have been shown to be blocked by the commonly used anesthetic chloral hydrate (Huang and Walters, 1992; Kreiss et al., 1996). To determine whether the D1 inhibition observed in the present study shares a similar property, the effect of SKF38393 was reexamined in animals treated with chloral hydrate. Seven animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). To be sure that transection did not affect the result, four of the animals also had their brainstems transected. Because animals in the latter group did not breathe spontaneously, artificial respiration was performed. The respiration rate was adjusted to maintain an expired CO2 level of 3.5–4.1% as measured by a CO2 analyzer. No difference was found between the results obtained from the two groups. Thus, in all animals treated with chloral hydrate, pretreatment with quinpirole (40 mg/kg, $n = 3$; 80 mg/kg, $n = 3$; 160 mg/kg, $n = 1$, injected slowly as described above; see Fig. 3B) failed to enable SKF38393 (10–20 mg/kg) to produce any further inhibition (Fig. 6).

DISCUSSION

The present study suggests that feedback control of DA neurons involves not only D2- but also D1-like receptors. However, the D1-mediated feedback inhibition requires co-activation of D2-like receptors to be expressed and is blocked completely by a commonly used anesthetic, chloral hydrate.

D1 agonists alone produce variable or no effects on the spontaneous activity of DA neurons

Consistent with a previous study in a locally anesthetized, paralyzed preparation (Carlson et al., 1987), our study shows that systemically administered SKF38393 produces variable effects on SN DA neurons. However, two other similar studies showed no effect of SKF38393 on SN DA cells (Huang and Walters, 1992, Sun et al., 1993). The cause for the discrepancy in findings is unknown. However, if SKF38393 alone has an effect, the effect may not be mediated by D1-like receptors. The present study shows that the effect induced by SKF38393 alone was not reversed by the D1 agonist SCH23390 in most cells examined and not mimicked by the full D1 agonist DHX. In chloral hydrate-anesthetized animals, SKF38393 was reported to have no effect on the firing rate of DA cells. However, it modulates the D2 response (Kelland et al., 1988). In control animals, the ability of the D2 agonist quinpirole to inhibit DA cells is negatively correlated with their basal firing rate. Pretreatment with SKF38393 eliminated the rate depen-
dency of the D2 inhibition, suggesting a modulatory role of the D1 feedback pathway. However, as will be discussed, the D1 pathway, under certain conditions, also has an inhibitory effect on DA neurons.

**Co-activation of D2-like receptors enables a D1 agonist to inhibit DA neurons**

It is well known that the expression of some DA effects requires co-activation of D1- and D2-like receptors (Walters et al., 1987; White, 1987; Bordi and Meller, 1989; Wachtel et al., 1989; Bertonello et al., 1990). The present study suggests that a similar D1–D2 interdependency is involved in feedback control of DA neurons. Our data showed that the D1 agonists SKF38393 and DHX, although having no effect or producing a variable response in control animals, consistently inhibited DA cells in animals pretreated with high doses of the D2 agonist quinpirole.

The proposed D1–D2 interaction may also explain why a D1 agonist is capable of inhibiting DA neurons in reserpine-treated animals (Huang and Walters, 1992; Sun et al., 1993). Chronic treatment with reserpine is known to cause a breakdown of D1–D2 interdependency. As a result, activation of either receptor alone can produce an effect that, otherwise, can only be observed when both D1- and D2-like receptors are activated (e.g., Arnt, 1985; LaHoste et al., 1996). Although the underlying mechanism for this action of reserpine remains unclear, it is possible that reserpine treatment also interrupts D1–D2 interaction in feedback control of DA cells so that a D1 agonist alone can produce an inhibition of DA cells (Huang and Walters, 1992; Sun et al., 1993).

**D1-mediated feedback inhibition requires co-activation of D2-like receptors on DA target neurons, rather than DA neurons themselves**

D2-like receptors are present on both DA neurons (DA autoreceptors) and DA target neurons. Both receptors could be activated by systemically administered quinpirole, and both may play a role in enabling the D1 effect. Our results suggest, however, that the D1-mediated feedback inhibition does not require activation of DA autoreceptors. In the initial experiments, animals were pretreated with only low doses of quinpirole (<20 \( \mu \)g/kg). Although quinpirole produced a significant inhibition in these experiments, no further effect of SKF38393 was observed. Because a low dose of a D2 agonist acts preferentially on DA neurons (Skirboll et al., 1979; White and Wang, 1986; Piercey et al., 1996a), these results provided the first evidence suggesting that the expression of the D1 effect may not depend on activation of DA autoreceptors. In the second set of experiments, animals were pretreated with high doses of quinpirole. However, quinpirole had to be injected slowly to avoid a complete inhibition of DA cells. Although SKF38393 produced a clear inhibition in these cells, it was difficult to determine whether it was the dose of quinpirole injected, the speed of injection, or the degree of inhibition induced by high doses of quinpirole that modified the response of the cell to a D1 agonist. To rule out the possibility that the slow injection may play a role, animals were given, slowly, a low dose of quinpirole (10 \( \mu \)g/kg). In four of five cells tested, SKF38393 failed to produce a clear effect, suggesting that the kinetics of injection of quinpirole is not a critical factor in the emergence of the D1 effect. To test more directly whether activation of DA autoreceptors is needed for the expression of D1 inhibition, raclopride was applied locally to the SN to block DA autoreceptors. In these experiments, DA cells remained active even after a fast injection of a large dose of quinpirole. Although the degree of inhibition induced by quinpirole was less compared with that seen in the second set of experiments, SKF38393 produced an even greater inhibition (see Figs. 3, 4). Thus, neither the speed of quinpirole injection nor the degree of inhibition induced by quinpirole seems to play a role in the observed D1 inhibition. By demonstrating a D1 effect even after autoreceptors were blocked, this last set of experiments provided more direct evidence suggesting that D2-like receptors on DA target neurons are more important in determining the expression of the D1 feedback inhibition.

Clearly, further experiments are needed to pinpoint where exactly D1 and D2 agonists may interact to regulate the activity of DA cells. With the data available, particularly from anatomical studies (see the introductory remarks), one may speculate that striatonigral GABA neurons may play a key role. D1-like receptors are found on both their cell bodies in the striatum and their terminals in the SN. An in vitro study showed that activation of D1-like receptors on GABAergic terminals in the ventral tegmental area increases GABA\(_G\) receptor-mediated GABA input to DA neurons (Cameron and Williams, 1993). Whether this is also the case in the SN has not been determined. An in vivo study in reserpinized animals suggests, however, that systemically admin-
istered SKF38393 may act mainly in the striatum to inhibit DA neurons (Sun et al., 1996).

A D2 agonist may act directly on the same striatonigral neurons to modulate the D1 effect, if D1- and D2-like receptors co-localize in these cells (Lester et al., 1993; Surmeier et al., 1993, 1996). Alternatively, D2 agonists may act on a different population of striatal or nonstriatal neurons to modulate D1-mediated feedback pathways indirectly. Previously, systemic administration of the D2 agonist quinpirole was shown to increase the activity of some striatal neurons (at doses higher than those needed to inhibit DA cells; Piercey et al., 1996b). The D1 agonist SKF38393 alone, on the other hand, produced only a small or no effect (Piercey et al., 1996b). Although it is unclear how the D2 excitatory effect is produced, a recent in vitro study suggests that during D2-induced excitation, a D1 agonist may become capable of further increasing the activity of striatal neurons (Hernandez-Lopez et al., 1997). If, under such conditions, striatonigral neurons are among the cells that are further excited by the D1 agonist, this D1-mediated excitation in the striatum would be translated into an inhibition of nigral DA neurons. Further experiments are needed to determine whether this hypothesis is correct.

**Clinical speculations**

The involvement of both D1- and D2-like receptors in feedback control of DA neurons could have significant clinical implications if drugs selective for DA receptor subtypes were used for treating disorders such as schizophrenia, Parkinson’s disease, and substance abuse. Because of the presence of feedback pathways, these drugs may produce unexpected results. For example, a D2-selective agonist may be used for treating Parkinson’s disease. However, at low doses, D2 agonists may act preferentially on D2 autoreceptors to inhibit the activity of the remaining DA cells and their release and, thus, exacerbate the symptoms. At high doses a D2 agonist may act postsynaptically to increase D2 receptor-mediated function, the residual D1 function, mediated by endogenously released DA, may become further reduced because of the D2-mediated feedback inhibition. Our data suggest that a D1 agonist may lead to a similar reduction in the availability of endogenous DA for action at D2-like receptors.

Most antipsychotic drugs are D2 antagonists. These drugs are known to block feedback inhibition and to increase DA cell activity and DA release. If an antipsychotic drug blocks only D2 receptors, the increased DA release would lead to a selective stimulation of D1 receptors. Similarly, a selective D1 antagonist may increase the availability of endogenous DA for action at D2-like receptors. Although remaining to be verified, the proposed feedback pathway-mediated effects may contribute to the clinical effects of current drugs acting on DA systems.

**REFERENCES**


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