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Short communication

Priming of 6-hydroxydopamine-lesioned rats with L-DOPA or quinpirole results in an increase in dopamine D_1 receptor-dependent cyclic AMP production in striatal tissue

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Abstract

Priming with a dopamine agonist greatly enhances the behavioral effectiveness of dopamine D_1 receptor agonists in rats with 6-hydroxydopamine lesions of the nigrostriatal pathway. The present study investigated the influence of priming on cyclic AMP production in striatal slices. Stimulation of dopamine D_1 receptors with dopamine or the dopamine D_1 receptor agonist, 1-phenyl-6-Cl-7,8-diol-2,3,4,5-tetrahydro-(1H)-3-benzazepine (SKF 81297), increased cyclic AMP production in the lesioned striatum of rats primed with L-3,4-dihydroxyphenylalanine (L-DOPA) by 329% and 405%, respectively, whereas in drug-naive rats the increase was 183% and 187%, respectively. Priming with quinpirole produced similar results. It is suggested that priming with either L-DOPA or a dopamine D_2 receptor agonist results in increased effectiveness of dopamine D_1 signal transduction, apparently not only related to previous stimulation of D_1 receptors. © 1997 Elsevier Science B.V.

Keywords: cAMP; Dopamine D_1 receptor; Dopamine D_2 receptor; Striatum; Parkinson's disease

1. Introduction

In patients with Parkinson's disease, a disease characterized neuropathologically by degeneration of primarily dopamine-containing neurons in the ventral mesencephalon, prolonged treatment with L-3,4-dihydroxyphenylalanine (L-DOPA) or dopamine D_2 receptor agonists such as pergolide and bromocriptine is associated with the gradual development of serious motor fluctuations not observed initially (Marsden and Parkes, 1976). These motor fluctuations have been suggested to result from alterations in dopamine receptor sensitivity (Hossain and Weiner, 1993).

Rats with a unilateral 6-hydroxydopamine lesion of the dopaminergic nigrostriatal pathway provide a useful model to test drugs with anti-Parkinsonian activity (Ungerstedt, 1971). Administration of these agents leads to turning behavior contralateral to the lesioned side. In this experimental model it has been observed that a low dose of a dopamine D_1 receptor agonist failed to elicit turning behavior 17 days after lesioning. Interestingly, the same dose was able to induce turning behavior after a previous (3 days before) exposure to a dopamine receptor agonist stimulating dopamine D_1, D_2 or D_1/D_2 receptors at the same time (priming) (Morelli et al., 1993a). Recent studies have demonstrated that priming is not associated with alterations in dopamine receptor number or affinity but with changes in the transduction mechanism, phosphorylation of DARPP-32 and increased metabolic activity of striatal output neurons (Morelli et al., 1990; Morelli et al., 1993b; Barone et al., 1994). Thus, priming in 6-hydroxydopamine-lesioned rats provides a model for studying the neurochemical processes related to changes in dopamine receptor sensitivity as a consequence of treatment with L-DOPA.

In this study we evaluated whether priming in vivo influences dopamine receptor-mediated cyclic AMP production in rat striatal slices. The behavioral effects of priming are particularly manifested in experiments in which dopamine D_1 receptors are stimulated, therefore, we studied the effects of priming on dopamine D_1 receptor-mediated cyclic AMP production. Fourteen days after a unilat-

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eral lesion of the nigrostriatal dopaminergic system with 6-hydroxydopamine, rats were primed with L-DOPA or with the dopamine D_2 receptor agonist, quinpirole. Three days later cyclic AMP production was measured.

2. Materials and methods

2.1. Animal lesions

Male Wistar rats (300–320 g body weight) were lesioned unilaterally in the medial forebrain bundle at coordinates A 3.4, L 1.5, V 8.0 according to the atlas of Paxinos and Watson (1985), by injecting 6-hydroxydopamine-HCl (8 μg dissolved in 3 μl of saline containing 0.05% ascorbic acid). All rats were treated with 10 mg/kg (i.p.) of desipramine, to prevent damage to noradrenergic neurons.

2.2. Evaluation of turning behavior

Spontaneous ipsilateral turning was measured every day for 4 or 5 days, starting the first day after the lesion. Only rats showing at least 10 ipsilateral rotations in 3 min were used for the experiments.

Two weeks after 6-hydroxydopamine lesioning, the animals were primed with either a single injection of L-DOPA (50 mg/kg i.p.) + carbidopa (100 mg/kg i.p.), quinpirole (0.2 mg/kg s.c.) (primed rats) or injected with saline (drug-naive rats). Rats displayed, in 2 h, more than 300 turns after L-DOPA and more than 200 turns after quinpirole.

2.3. Cyclic AMP evaluation in brain slices

Three days after priming, the rats were decapitated and the striatum was rapidly dissected from the brain. Cyclic AMP production measurements were essentially performed as described by Drukarch et al. (1990) and references therein.

2.4. Materials

6-hydroxydopamine-HCl, L-DOPA, desipramine, dopamine, (−)-sulpiride and (−)-propranolol were purchased from Sigma; 3-isobutyl-1-methylxanthine (IBMX) from Aldrich; [^{3}H]adenine from Amersham; quinpirole, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) and 1-phenyl-6-Cl-7,8-diol-2,3,4,5-tetrahydro-(1H)-3-benzazepine (SKF 81297) from Research Biochemicals International.

2.5. Statistics

Striatal tissue from 4 or 6 (2 or 3 drug-naive versus 2 or 3 primed) rats was used for each experiment. Consequently, each experiment involved 4 different kinds of tissue: lesioned (naive), lesioned (primed), non-lesioned (naive) and non-lesioned (primed). In the figures, the data are the average values (±S.E.M.) from 3 independent experiments. For each of the 3 independent experiments the data are expressed as % of the particular control. Since all experimental conditions in each experiment were run in triplicate, $n = 9$ observations.

The statistical significance of differences was determined by one-way analysis of variance (ANOVA) followed by the Newman–Keuls post hoc test.

3. Results

3.1. L-DOPA priming

Administration of L-DOPA (50 mg/kg i.p.), induced contralateral turning behavior in primed rats, whereas rats that received saline (drug-naive), did not show contralateral turning.

Three days after priming, the rats were killed and striatal slices were exposed to a supramaximal concentration of dopamine (20 μM) (Stoof and Kebabian, 1981), in the presence of 10 μM of the dopamine D_2 receptor antagonist (−)-sulpiride, in order to block the action of dopamine on dopamine D_2 receptors. (−)-Propranolol (5 μM) was added to prevent effects of dopamine on the β-adrenoceptor.

It is shown in Fig. 1 that exposure of slices from the intact striatum of drug-naive rats to dopamine induced a

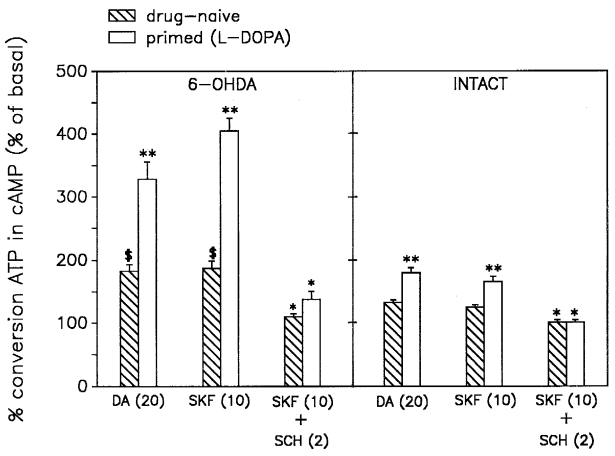

Fig. 1. Effect of priming on cyclic AMP formation in 6-hydroxydopamine-lesioned or intact striatum. Rats were primed with L-DOPA (50 mg/kg i.p.). Cyclic AMP formation was expressed as % conversion of ATP to cAMP (% of basal values). Cyclic AMP formation was measured in the absence of drugs (basal value = 100%), and in presence of 20 μM dopamine + 10 μM sulpiride, 10 μM SKF 81297, or 10 μM SKF 81297 + 2 μM SCH 23390. Data are mean ± S.E.M. values from 9 observations obtained in 3 independent experiments. ** $P < 0.001$ primed 6-hydroxydopamine-lesioned or intact striatum vs. drug-naive 6-hydroxydopamine-lesioned or intact striatum; $ $P < 0.001$ drug-naive 6-hydroxydopamine-lesioned striatum vs. drug-naive intact striatum; * $P < 0.001$ SKF 81297 (10 μM) + SCH 23390 (2 μM) vs. SKF 81297 alone.

significant stimulation (133% of basal) of cyclic AMP production. 6-hydroxydopamine lesioning increased the dopamine-induced cyclic AMP production to 183% of the basal value in drug-naive rats. Priming with L-DOPA led to further potentiation of the dopamine-induced cyclic AMP production in the lesioned striatum to 329% of the basal production. A much smaller, but still statistically significant, potentiation of dopamine-induced cyclic AMP production in the intact striatum of primed rats (180% of basal) as compared to the intact striatum of drug-naive rats (133% of basal) was observed.

To verify whether the effects of dopamine were mediated by dopamine D_1 receptors, slices were exposed to the full, and selective, dopamine D_1 agonist, SKF 81297, either alone or in combination with the dopamine D_1 receptor antagonist, SCH 23390. SKF 81297 was used in a supramaximal concentration of 10 μM (Vermeulen et al., 1994). As also shown in Fig. 1, SKF 81297 induced a significant stimulation (125% of basal) of cyclic AMP production in the intact striatum of drug-naive rats. Exposure of the lesioned striatum of drug-naive rats to SKF 81297 resulted in a cyclic AMP production amounting to 187% of the basal production. Priming with L-DOPA led to a further potentiation of the SKF 81297-induced cyclic AMP production in the lesioned striatum to 405% of basal production. There was a smaller, but still statistically significant, potentiation of SKF 81297-induced cyclic AMP production in the intact striatum of primed rats (166% of basal) as compared to the intact striatum of drug-naive rats (125% of basal). The dopamine D_1 receptor antagonist, SCH 23390 (2 μM), almost completely blocked the effect of 10 μM SKF 81297 on cyclic AMP production.

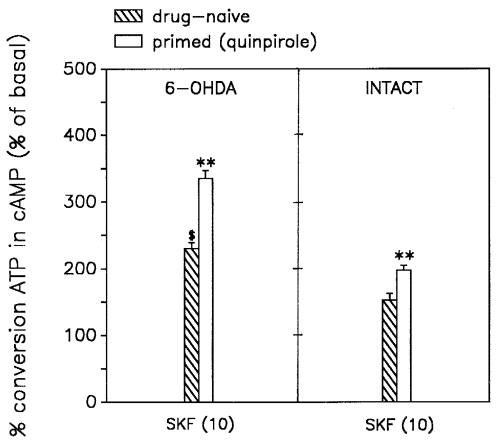

Fig. 2. Effect of priming on cyclic AMP formation in 6-hydroxydopamine-lesioned or intact striatum. Rats were primed with quinpirole (0.2 mg/kg s.c.). Cyclic AMP formation was expressed as % conversion of ATP to cAMP (% of basal values). Cyclic AMP formation was measured in the absence of drugs (basal value = 100%), and in presence of 10 μM SKF 81297. Data are mean ± S.E.M. values from 9 observations obtained in 3 independent experiments. ** $P < 0.001$ primed 6-hydroxydopamine-lesioned or intact striatum vs. drug-naive 6-hydroxydopamine-lesioned or intact striatum; $ $P < 0.001$ drug-naive 6-hydroxydopamine-lesioned striatum vs. drug-naive intact striatum.

3.2. Quinpirole priming

Administration of the dopamine D_2 receptor agonist, quinpirole (0.2 mg/kg s.c.), induced contralateral turning behavior in all rats. Since dopamine and SKF 81297 showed similar effects on cyclic AMP production after L-DOPA priming, we used only SKF 81297 for the measurement of dopamine D_1 receptor-mediated cyclic AMP production after quinpirole priming.

As shown in Fig. 2, SKF 81297 induced a significant stimulation (153% of basal) of cyclic AMP production in the intact striatum of drug-naive rats. Exposure of the lesioned striatum of drug-naive rats to SKF 81297 resulted in a cyclic AMP production amounting to 230% of the basal value. Priming led to a further potentiation of the SKF 81297-induced cyclic AMP production in the lesioned striatum to 335% of basal production. There was a smaller, but still statistically significant, potentiation of SKF 81297-induced cyclic AMP production in the intact striatum of primed rats (198% of basal) as compared to the intact striatum of drug-naive rats (153% of basal).

4. Discussion

As expected, lesioning of the nigrostriatal system with 6-hydroxydopamine increased cyclic AMP production in striatal tissue (as compared to non-lesioned tissue) on in vitro dopamine D_1 receptor stimulation, either with dopamine or SKF 81297 in drug-naive rats. Both compounds were used in a supramaximal concentration to induce maximal cyclic AMP production in striatal tissue. Blockade of the effects of SKF 81297 by SCH 23390 indicated that cyclic AMP production was the consequence of dopamine D_1 receptor stimulation. It has been demonstrated (Krueger et al., 1976) that lesioning of the nigrostriatal dopaminergic cells with 6-hydroxydopamine increases the effectiveness of dopamine D_1 receptor-mediated cyclic AMP production in the striatum. This has been explained by a 'receptor supersensitivity' that develops as a compensatory mechanism for the lack of dopaminergic input.

The new and intriguing finding in the present study was that priming of 6-hydroxydopamine lesioned rats both with L-DOPA and with the dopamine D_2 receptor agonist, quinpirole, led to further potentiation of the dopamine D_1 receptor-mediated cyclic AMP production. Unknowingly, in studies investigating dopamine receptor supersensitivity, many investigators have administered the dopamine D_1/D_2 receptor agonist, apomorphine, to 6-hydroxydopamine-lesioned rats to verify, by measuring turning behavior, whether the unilateral lesion was effective. Thus, it is most likely that in those studies, the effect of apomorphine priming also contributed to the reported increases in cyclic AMP production.

The potentiation of the dopamine D_1 receptor-mediated cyclic AMP production in primed rats as compared to that in drug-naive rats was consistent with behavioral data indicating that priming is a prerequisite to allow dopamine D_1 receptor agonists to induce turning behavior (Morelli et al., 1993a). One possible explanation for the effect might thus be a more effective signal transduction via the dopamine D_1 receptor. L-DOPA might directly sensitize dopamine D_1 receptors, which is reflected in a further increase in dopamine D_1 receptor-stimulated cyclic AMP production in striatal slices of 6-hydroxydopamine-lesioned rats. However, this explanation is unlikely to apply. Firstly, in vitro studies have demonstrated that a consequence of 'preactivation' of dopamine D_1 receptors is desensitization rather than sensitization, which translates as a decrease in cyclic AMP production (Balmforth et al., 1990). Secondly, the present results indicated that priming of the dopamine D_2 receptor with quinpirole also leads to an increase in dopamine D_1 receptor-mediated stimulation of cyclic AMP production.

It is tempting to assume that the basal ganglia-thalamocortical circuits are involved in the priming effects. In these circuits, dopamine D_1 receptor as well as dopamine D_2 receptors play a crucial role in exerting the effects of dopaminergic compounds on motor behavior. In addition, other neuronal elements of these circuits, like γ-aminobutyric acid (GABA) and glutamate, also exert their influence on various components of motor behavior. The finding that blockade of the *N*-methyl-D-aspartate (NMDA) receptor by MK 801 prevents priming (Morelli and Di Chiara, 1990), supports the idea that priming with L-DOPA or quinpirole does not involve exclusively a subpopulation of striatal cells expressing dopamine D_1 receptors. It is more likely that activation or blockade of various elements participating in the basal ganglia circuits leads to long-term changes in the 'tuning' of these circuits. The demonstration of changes in striatal cyclic AMP production that follow dopamine D_1 receptor stimulation may show only one aspect of the question.

Although not the primary aim of this study, it was shown that the 'intact' striatum of the unilaterally 6-hydroxydopamine-lesioned rats also shows, after priming, albeit to a lesser extent, potentiation of dopamine D_1 receptor-mediated cyclic AMP production. This might have been a result of a slight reduction in dopamine levels as a consequence of the contralateral 6-hydroxydopamine lesion. Alternatively, it might have been the consequence of priming as such. We suggest that the priming model will (a) ultimately lead to a better understanding of the mechanisms underlying motor fluctuations following long-term treatment with dopaminergic drugs, and (b) reveal possibilities for better pharmacotherapeutic interventions in patients with Parkinson's disease.

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