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Adenosine A_{2A} receptor antagonism potentiates L-DOPA-induced turning behaviour and c-*fos* expression in 6-hydroxydopamine-lesioned rats

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Abstract

In order to investigate the role of adenosine A_{2A} receptor blockade on dopamine-mediated motor responses, contralateral turning behaviour and expression of the early-gene c-*fos* was evaluated in rats with a unilateral 6-hydroxydopamine lesion of the dopaminergic nigrostriatal pathway. SCH 58261, (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) a potent and selective antagonist of adenosine A_{2A} receptors (5 mg/kg i.p.), induced a 70-fold increase in the contralateral turning behaviour induced by a low dose (2 mg/kg i.p.) of the dopamine precursor L-DOPA (L-3,4-dihydroxyphenylalanine). Expression of c-*fos* as measured by Fos-like immunoreactivity after SCH 58261 plus L-DOPA was also potentiated as compared with L-DOPA alone, both in striatum and globus pallidus of the 6-hydroxydopamine-lesioned side of the brain. SCH 58261 induced a less marked potentiation (7-fold) of turning behaviour induced by dopamine D_2 receptor stimulation with quinpirole, while Fos-like immunoreactivity in the striatum and globus pallidus was not affected. Previous studies have shown that SCH 58261 strongly potentiated dopamine D_1 receptor-mediated responses. The results of the present study therefore indicate that the positive interaction between SCH 58261 and L-DOPA, in 6-hydroxydopamine-lesioned rats, is mainly due to an interaction with dopamine D_1 receptors. The data also suggest that adenosine A_{2A} receptor antagonists might be useful for potentiating the effects of L-DOPA in Parkinson's disease.

Keywords: L-DOPA (3,4-dihydroxyphenyl-L-alanine); Striatum; Early gene; Parkinson's disease; Dopamine D_1 receptor; Dopamine D_2 receptor

1. Introduction

Contralateral turning behaviour in rats with unilateral 6-hydroxydopamine lesions of the dopaminergic nigrostriatal pathway is a currently utilized animal model of Parkinson's disease (Ungerstedt, 1971). In this model caffeine and theophylline, two antagonists of adenosine receptors, induce contralateral turning behaviour when administered alone and potentiate the turning behaviour induced by direct dopamine agonists (Fuxe and Ungerstedt, 1974; Fredholm et al., 1983; Herrera-Marschitz et al., 1988; Casas et al., 1989; Jiang et al., 1993; Garrett and Holtzman, 1995).

These results have led to the suggestion that adenosine antagonists might be beneficial to the treatment of Parkinson's disease. Studies in humans, however, are contradictory since some reports showed that caffeine produced no changes in the therapeutic response to antiparkinsonian drugs such as L-DOPA (L-3,4-dihydroxyphenylalanine) or bromocriptine (Shoulson and Chase, 1975; Kartzinel et al., 1976), while others have reported an improvement of tremor but only after prolonged treatment (Mally and Stone, 1995). This indicates that further studies are needed to clarify whether blockade of adenosine receptors might potentiate the efficacy of the dopamine agonists used in the treatment of Parkinson's disease. The lack or poor effects of caffeine and theophylline in Parkinson's disease could be related to the low affinity and poor selectivity of these compounds in binding the adenosine receptor types mostly involved in the modulation of dopamine-mediated motor behaviours.

Four types of adenosine receptors have been identified, namely A_1, A_{2A}, A_{2B} and A_3 (for review, see Fredholm et al., 1994). While adenosine A_1 and A_{2A} receptors have

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been pharmacologically characterized, both adenosine A_{2B} and A_3 receptors await further studies. Behavioural studies using selective adenosine agonists for adenosine A_1 and A_{2A} receptors have shown that the motor behaviours induced by dopamine receptor stimulation are mostly modulated by adenosine A_{2A} receptors (Ferré et al., 1992; Vellucci et al., 1993; Morelli et al., 1994; Popoli et al., 1994; Pinna et al., 1996).

Recently, selective and potent antagonists for adenosine A_1 and A_{2A} receptors have been synthesized. The availability of these new compounds have opened the possibility of studying whether selective adenosine A_1 or A_{2A} receptors antagonists could influence in a more significant way than caffeine the contralateral turning behaviour induced by dopamine agonists in 6-hydroxydopamine-lesioned rats.

A first study of our group has shown that SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) a new potent and selective antagonist of the adenosine A_{2A} receptors (Zocchi et al., 1996), markedly increased turning behaviour and striatal c-*fos* expression induced by stimulation of dopamine D_1 receptors by SKF 38393 ([±]-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diolhydrochloride), while blockade of adenosine A_1 receptors had little influence on dopamine D_1 receptor-mediated turning behaviour and none on the expression of the early gene c-*fos* (Pinna et al., 1996).

On the basis of that study, we have explored whether blockade of adenosine A_{2A} receptors by SCH 58261 potentiated the turning behaviour induced by stimulation of dopamine D_1/D_2 receptors with L-DOPA and influenced the expression of the early gene c-*fos* in unilaterally 6-hydroxydopamine-lesioned rats. The immediate-early gene c-*fos* is transiently induced by stimuli or drugs which increase second messenger levels into the neurons. For these reasons the c-*fos*-encoded protein Fos has been used as an index of post-synaptic activity (Sagar et al., 1988).

In order to verify whether the influence of SCH 58261 on L-DOPA was related to an action on dopamine D_1 or D_2 receptors, we have also evaluated whether SCH 58261 potentiated turning behaviour and c-*fos* expression induced by stimulation of dopamine D_2 receptors with quinpirole.

2. Materials and methods

2.1. 6-Hydroxydopamine lesion

In order to lesion the dopaminergic nigrostriatal pathway, male Sprague-Dawley rats (275–300 g) were anesthetized with chloral hydrate (400 mg/kg i.p.) and injected in the left medial forebrain bundle at coordinates A 2.2, L 1.5, V 7.9, according to the atlas of Pellegrino et al. (1979), with 6-hydroxydopamine-HCl (8 μg in 4 μl of saline containing 0.05% ascorbic acid). All rats were pretreated with 10 mg/kg i.p. of desipramine in order to prevent damage to noradrenergic neurons.

2.2. Evaluation of turning behaviour

Two weeks after the lesion, rats were screened on the basis of their contralateral rotation in response to benserazide (30 mg/kg i.p.) + L-DOPA (50 mg/kg i.p.) and then treated, 3 days later, with the various drugs. Any rat not showing at least 300 contralateral rotations during the 3 h testing period was eliminated from the study. For behavioural observation rats were placed in hemispherical bowls and turning behaviour was quantified with automated rotameters by counting, every 10 min, the number of turns (360°) made in 3 min.

2.3. Fos immunohistochemistry

Following behavioural observation the different groups of unilaterally 6-hydroxydopamine-lesioned rats were anesthetized with chloral hydrate 120 min after L-DOPA or quinpirole administration and then perfused transcardially with saline followed by 4% paraformaldehyde dissolved in 0.1 M sodium phosphate buffer, pH 7.4. Their brains, post-fixed in the same solution, were cut coronally on a vibratome (40 μm) 2 days later. Sections were incubated for 48 h with a sheep polyclonal Fos antibody selected from a conserved region of mouse and human c-*fos* (OA-11-824, Cambridge Research Biochemical), at a dilution of 1:1000. The reaction was visualized using biotinylated secondary antisera and by standard avidin-biotin horseradish peroxidase technique. Control sections were incubated in the presence of the Fos peptide.

Fos-like immunoreactivity was quantified with an image analyzer (IBAS, Zeiss, Germany) by counting the number of Fos-like positive nuclei. We considered as Fos-positive only those neurons showing grey levels ranging between 0 and 110/120 (total range was from 0 to 255).

2.4. Drugs

6-Hydroxydopamine-HCl, L-DOPA, and desipramine were purchased from Sigma (Milan, Italy), quinpirole from Research Biochemicals International (Natick, MA, USA). SCH 58261 was kindly donated by Schering-Plough (Milan, Italy), benserazide by Hoffmann-La Roche (Basel, Switzerland).

SCH 58261 was suspended in 0.5% methylcellulose (MTC) and injected in a volume of 1 ml i.p./100 g body weight, 40 min before L-DOPA or quinpirole. The other drugs were dissolved in saline and injected in a volume of 0.3 ml i.p. or 0.1 ml s.c. per 100 g body weight.

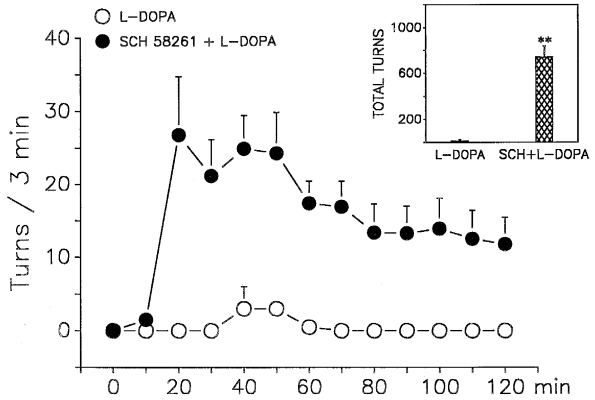

Fig. 1. Contralateral turning behaviour in 6-hydroxydopamine-lesioned rats after vehicle (MTC) + L-DOPA (2 mg/kg i.p.) ($n = 5$) or SCH 58261 (5 mg/kg i.p.) + L-DOPA (2 mg/kg i.p.) ($n = 7$). Ordinate represents the number of contralateral rotations, abscissa indicates the time after L-DOPA administration. The inset shows the number of total contralateral rotations made in 2 h. ** $P < 0.005$.

2.5. Statistics

Mean and S.E.M. of the number of rotations or Fos-like positive nuclei were calculated. Significance between groups was evaluated by analysis of variance followed by a Newman-Keuls post-hoc test.

3. Results

3.1. Effect of SCH 58261 on L-DOPA-induced turning behaviour and Fos-like immunoreactivity

SCH 58261 (5 mg/kg i.p.) markedly increased the number of contralateral rotations induced by a threshold dose of L-DOPA (2 mg/kg i.p.) (Fig. 1). The increase in the number of contralateral rotations was less marked when a higher dose L-DOPA (3 mg/kg i.p.) was administered in combination with SCH 58261 (total turns: L-DOPA = 349 ± 53, SCH 58261 + L-DOPA = 758 ± 127, $P < 0.05$). As shown previously (Pinna et al., 1996), SCH 58261 does not induce any turning behaviour or Fos-like immunoreactivity when administered alone at either 5 or 10 mg/kg i.p.

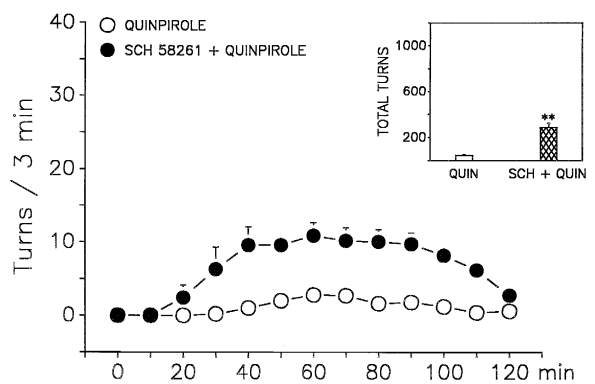

Fig. 2. Contralateral turning behaviour in 6-hydroxydopamine-lesioned rats after vehicle (MTC) + quinpirole (0.03 mg/kg s.c.) ($n = 5$) or SCH 58261 (5 mg/kg i.p.) + quinpirole (0.03 mg/kg s.c.) ($n = 7$). Ordinate represents the number of contralateral rotations, abscissa indicates the time after quinpirole administration. The inset shows the number of total contralateral rotations made in 2 h. ** $P < 0.005$.

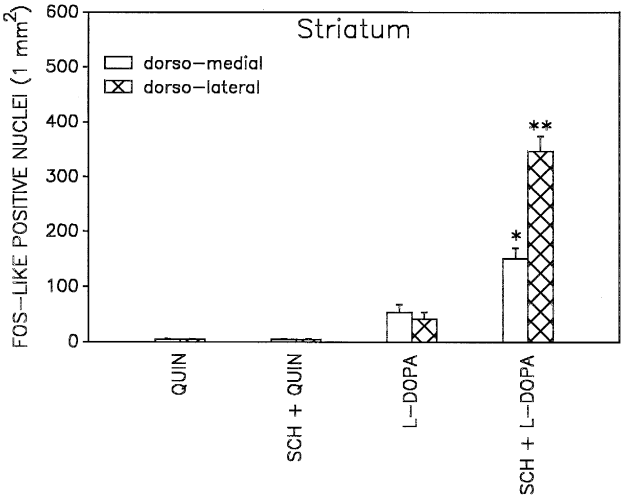

Fig. 3. Number of Fos-like positive nuclei in the 6-hydroxydopamine-lesioned striatum after administration of vehicle (MTC) + quinpirole (0.03 mg/kg s.c.) ($n = 5$) or L-DOPA (2 mg/kg i.p.) ($n = 5$); and SCH 58261 (5 mg/kg i.p.) + quinpirole (0.03 mg/kg s.c.) ($n = 7$) or L-DOPA (2 mg/kg i.p.) ($n = 7$). The rats used in this experiment were the same used in the experiments reported in Figs. 1 and 2. * $P < 0.05$, ** $P < 0.005$.

c-*fos* expression after L-DOPA (2 mg/kg i.p.), as measured by Fos-like immunoreactivity, was potentiated by blockade of adenosine A_{2A} receptors with SCH 58261 in the dorso-medial and dorso-lateral striatum and in the globus pallidus of the 6-hydroxydopamine-lesioned side of the brain (Figs. 3 and 4).

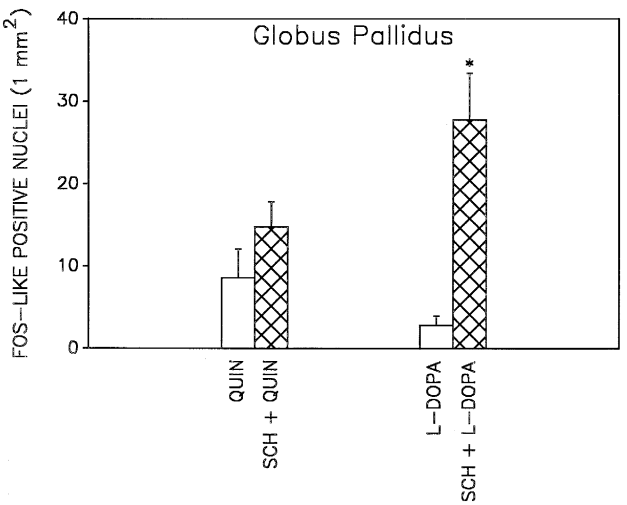

Fig. 4. Number of Fos-like positive nuclei in the 6-hydroxydopamine-lesioned globus pallidus after administration of vehicle (MTC) + quinpirole (0.03 mg/kg s.c.) ($n = 5$) or L-DOPA (2 mg/kg i.p.) ($n = 5$); and SCH 58261 (5 mg/kg i.p.) + quinpirole (0.03 mg/kg s.c.) ($n = 7$) or L-DOPA (2 mg/kg i.p.) ($n = 7$). The rats used in this experiment were the same used in the experiments reported in Figs. 1 and 2. * $P < 0.05$.

3.2. Effect of SCH 58261 on quinpirole-induced turning behaviour and Fos-like immunoreactivity

Stimulation of dopamine D_2 receptors by a low dose of quinpirole (0.03 mg/kg s.c.) induced turning behaviour and expression of Fos-like immunoreactivity in the globus pallidus but not in the striatum of the 6-hydroxydopamine-lesioned side of the brain (Figs. 2–4). Administration of SCH 58261 potentiated the contralateral turning behaviour induced by quinpirole (0.03 mg/kg s.c.); the increase in the number of rotations was, however, less marked than that observed with L-DOPA (Fig. 2). The number of Fos-like positive nuclei induced by combined administration of SCH 58261 + quinpirole in the globus pallidus was not significantly increased as compared to quinpirole alone, although there was a tendency to an increase (Fig. 4).

4. Discussion

In rats with unilateral lesions of the dopaminergic nigrostriatal pathway, the selective antagonist of adenosine A_{2A} receptors SCH 58261 markedly potentiated contralateral turning behaviour and Fos-like immunoreactivity induced by L-DOPA. SCH 58261 increased in a lesser extent turning behaviour induced by stimulation of dopamine D_2 receptors with quinpirole, while dopamine D_2 receptor-stimulated Fos-like immunoreactivity in the globus pallidus was not influenced by adenosine A_{2A} receptor blockade.

These results together with previous studies of our group showing that SCH 58261 induced a marked potentiation of dopamine D_1-mediated turning behaviour and Fos-like immunoreactivity (Pinna et al., 1996), suggest that in 6-hydroxydopamine-lesioned rats, endogenous adenosine release (Pazzagli et al., 1994) through adenosine A_{2A} receptors modulates the functions mediated by dopamine receptors mostly by an indirect interaction with dopamine D_1 receptors.

The present study shows a correlation between turning behaviour and Fos-like immunoreactivity both in the striatum and globus pallidus after L-DOPA plus SCH 58261. The increase in quinpirole-induced turning behaviour by SCH 58261 was instead not reflected in an increase in Fos-like immunoreactivity in the globus pallidus, although a tendency to an increase was observed. Since both the striatum and globus pallidus play an important role in the mediation of turning behaviour, the lack of correlation between turning behaviour and Fos-like immunoreactivity in the globus pallidus is most probably due to the inability of c-*fos* to reflect minor changes in motor behaviour.

Dopamine D_1 and D_2 receptors are segregated on different striatal efferent neurons, the striatonigral and striatopallidal pathway respectively (Gerfen et al., 1990; Le Moine et al., 1991). Adenosine A_{2A} receptors which are selectively located on striatopallidal neurons (Fink et al., 1992; Schiffmann and Vanderhaeghen, 1993) negatively influence the responses mediated by dopamine D_2 receptors (Ferré and Fuxe, 1992). Blockade of adenosine A_{2A} receptors by SCH 58261 might therefore play a direct positive role on quinpirole-mediated turning behaviour, while it would indirectly influence dopamine D_1-mediated responses through an integration, in extrastriatal areas, of the responses mediated by the striatopallidal and striatonigral pathways (Pinna et al., 1996).

Adenosine A_{2A} receptors are located in areas richly innervated by dopamine such as the striatum and olfactory tubercle (Jarvis and Williams, 1989) and play an important role in the mediation of motor behaviour (Ongini and Fredholm, 1996). This discrete localization might be an important feature in order to specifically influence only those areas involved in the control of motor behaviour. Blockade of adenosine receptors with compounds having high affinity and selectivity for adenosine A_{2A} receptors might be therefore a useful approach for potentiating the efficacy of L-DOPA in parkinsonian patients and for reducing the appearance of side effects.

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