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The role of the phosphatidyinositol-linked D_1 dopamine receptor in the pharmacology of SKF83959☆

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Abstract

SKF83959, previously described as an antagonist of the D_1 dopamine receptor, has been shown to be a potent anti-parkinsonian agent. However, its mechanism of action is unknown. The present communication was designed to study the mechanism by which SKF83959 exerts its pharmacological effects. SKF83959 induced contralateral rotations in the unilateral 6-OHDA-lesioned rat model of Parkinson's disease (PD). The rotations were completely blocked by the D_1 dopamine receptor antagonist, SCH23390. The response was not affected by the serotonin receptor antagonist, mesulergine and was transiently attenuated by α_1 adrenergic or D_2 dopamine receptor antagonists, prazosin or spiperone, respectively. Injection of 0.5 and 1 mg/kg SKF83959 elicited significant elevations in IP_3 accumulation in lesioned as compared to intact striata. This effect was blocked by SCH23390 at a dose that completely obviated the rotational response to SKF83959, suggesting that activation of the PI-linked D_1 dopamine receptor and the PLC/IP_3 pathway may be the underlying mechanism for the rotational activity induced by SKF83959. The present data provide the first evidence that the PI-linked D_1 dopamine receptor plays a role in regulating motor activity in striatum and that modulation of the D_1 dopamine receptor/PLC/IP_3 pathway may be a novel target in the discovery of drugs for the treatment of Parkinson's disease.

Keywords: Phosphatidylinositol; Dopamine receptor; Parkinson's disease (PD); 6-hydroxydopamine (6-OHDA); Inositol 1,4,5-triphosphate (IP_3)

1. Introduction

Recent progress in the identification of a novel dopamine receptor-coupled second messenger system has been achieved. In addition to the $Gs/_{olf}$-coupled classical D_1 dopamine receptor that increases the formation of cAMP, a D_1-like dopamine receptor that couples to Gq protein and stimulates phospholipase Cβ (PLCβ) has been described (Felder et al., 1989; Undie and Friedman, 1990; Undie et al., 1994; Frail et al., 1993; Yu et al., 1996; Pacheco and Jope, 1997). Activation of PLC stimulates hydrolysis of membrane phosphatidyinositol (PI) and generates diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). While DAG activates protein kinase C (PKC), IP_3 stimulates the release of calcium via the IP_3 receptor channel that is located on intracellular membranes. The PI-linked D_1 dopamine receptor is distinct from the classical D_{1A} receptor in its neurochemistry as demonstrated by the facts that PI hydrolysis is not stimulated in cells that express only the classical D_{1A} receptor (Frail et al., 1993; Jin et al., 2003), and stimulation of the PLC/PI pathway by D_1 agonists is not disrupted in brains of transgenic mice in which the classical D_{1A} receptor has been knocked-out (Friedman et al., 1997). However, the functional role of the PI-linked D_1-like dopamine receptor in brain is completely unknown.

Pharmacological evidence also supports the existence of a PI-linked dopamine receptor. SKF83959, a benzazepine compound, exhibits high affinity for the D_1 dopamine receptor and inhibits dopamine-stimulated cAMP production (Andringa et al., 1999a; Jin et al., 2003). This compound was demonstrated to have excellent anti-parkinsonian actions in an experimental primate model of the disease (Andringa et al., 1999b). The mechanism for this

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action is, however, unclear although it is known that the cAMP pathway is not involved (Deveney and Waddington, 1995; Andringa et al., 1999b; Jin et al., 2003). The recent finding that SKF83959 is a selective agonist for the PI-linked D_1 dopamine receptor (Panchalingam and Undie, 2001; Jin et al., 2003), suggests that the PLC/PI cascade may play a role in the anti-parkinsonian action exerted by SKF83959. In the present communication we report on the action of SKF83959 in the unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model which has been widely used in testing for potential drugs for the treatment of Parkinson's disease. The results demonstrate that stimulation of the PI-linked D_1 dopamine receptor by SKF 83959 increases brain IP_3 concentration in striata ipsilateral to the lesion. Moreover, D_1 receptor inhibition attenuates elevation of IP_3 accumulation and SKF83959-induced contralateral rotations in 6-OHDA-lesioned rats. The results provide the first evidence that stimulation of the PI-linked D_1 dopamine receptor and its associated PLC/IP_3 pathway is involved in mediating rotational behavior in the unilateral lesioned rat and therefore suggests that this receptor may play a role in the anti-parkinsonian action of SKF 83959.

2. Materials and methods

2.1. Materials

SKF 83959 was kindly provided by the NIMH synthesis program (Menlo Park, CA 94025). Apomorphine, pargyline HCl, and prazosin HCl were purchased from Sigma (St. Louis, MO). R(+)-SCH-23390 HCl, and mesulergine HCl (*N*′-[(8α)-1,6-dimethylergolin-8-yl] *N,N*-dimethylsulfamide HCl), were purchased from RBI (Natick, MA). Spiperone was obtained from ICN Biochemicals (Cleveland, OH). The IP_3 assay kit was purchased from Amersham (Piscataway, NJ). Other reagents were purchased from standard laboratory suppliers.

2.2. Animal surgery and behavioral assessment

The procedures used were previously described (Cai et al., 2002; Zhen et al., 2002). Male Sprague–Dawley rats weighing 175 to 200 g were purchased from Taconic (Germantown, NY). Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and received a single stereotactic injection of 8 μg 6-OHDA in 4 μl artificial cerebrospinal fluid and 0.05% ascorbic acid into the mid-forebrain bundle using the following coordinates: AP −2.5 mm, Lat +2.0 mm and Dev −8.5 mm using bregma as the starting point. To limit damage to adrenergic neurons, desipramine–HCl (25 mg/kg) was administrated i.p. 30 min prior to 6-OHDA. The success of the lesion was assessed by the number of contralateral rotations in response to an injection of apomorphine (0.2 mg/kg, s.c.) 3 weeks after surgery. Lesioned rats were placed in 50 cm bowls and acclimated to the environment for 30 min prior to apomorphine injection. Animals demonstrating fewer than 50 rotations per 5 min were excluded from the experiments. Dopamine levels were measured by high performance liquid chromatography. Ipsilateral striatal dopamine levels were found to be less than 10% of the control side in all selected rats, indicating severe loss of striatal dopaminergic nerve terminals. In some experiments, commercially available 6-OHDA-lesioned rats (Taconic) that were testing with apomorphine were also used. Rats were killed by decapitation at designated times, striata were collected from both lesioned and control sides and the samples were rapidly frozen in liquid nitrogen and stored at −80 °C prior to use. The experimental protocols were approved by the Institutional Animal Care and Use Committees and have met the guidelines of the responsible governmental agency.

2.3. Brain IP_3 accumulation in situ in response to in vivo SKF83959 challenge

Rats were injected intraperitoneally with 0.8 mg/kg SKF 83959 or vehicle and decapitated 8, 15 or 25 min thereafter. Brains were removed, and frontal cortices and hippocampi were dissected. Tissues were homogenized in 1.5 ml of 1 M trichloroacetic acid (TCA) and placed on ice for 15 min before centrifugation at 13,000×*g* for 10 min. The pellets were washed three times with distilled water and digested in 1 M NaOH and protein content was determined by the Bradford method (BioRad). One milliliter of trichloro trifluoro ethane–trioctylamine (3:1) was added to 500 μl of TCA tissue extract in a polypropylene eppendorf tube, mixed vigorously for 15 s and centrifuged for 1 min at 10,000×*g*. Aliquots of the upper phase were taken for IP_3 determination according to instructions provided by the manufacturer of the kit. IP_3 concentrations are expressed as pmol/mg protein.

2.4. Statistical analysis

Data are presented as mean±S.E. ANOVA followed by Newman–Keuls test was used to test for significance unless otherwise stated in the figure legends. Significance was considered at $p<0.05$.

3. Results

3.1. Characterization of SKF83959-induced rotation in 6-OHDA-lesioned rats

SKF83959 and apomorphine were examined with regard to their ability to induce rotations in the unilateral 6-OHDA-lesioned rat. As shown in Fig. 1, at the dose of 0.8 mg/kg, SKF83959 elicited a robust rotational response in the 6-OHDA rat. This response was greater than that elicited by a comparable dose of the dopamine receptor agonist, apo-

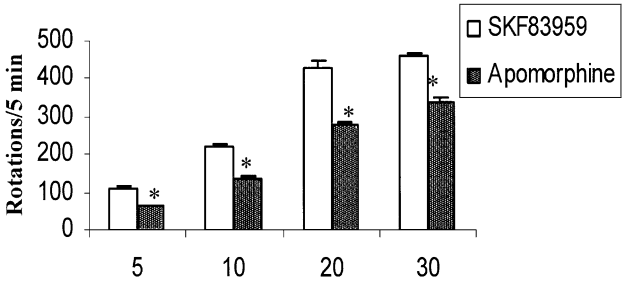

Fig. 1. Comparison of SKF83959- and apomorphine-induced contralateral rotations in the 6-OHDA-lesioned rat model of Parkinson's disease. Rats were injected with 0.8 mg/kg SKF83959, intraperitoneally, or 0.8 mg/kg apomorphine hydrochloride, subcutaneously and rotational behavioral was recorded at the indicated times after drug administration. Data are expressed as mean±S.E. (turns) obtained in 5 min from at least 6 animals in each group. *p<0.01, compared to respective apomorphine-treated group at each time point.

morphine. The difference was obvious throughout the testing period (5–30 min). Since it was previously shown that SKF83959 exhibits weak to mild affinities for the adrenergic, serotonergic and D_2 dopamine receptors, we tested whether these receptors contribute to the effect of SKF83959 on rotation. As shown in Fig. 2A, the 5-$HT_{2A/2C}$ receptor antagonist, mesulergine did not significantly affect SKF83959-induced rotations in lesioned rats. However, the D_2 receptor antagonist, spiperone, and the α_1 adrenergic receptor antagonist, prazosin significantly attenuated SKF83959-induced rotations (Fig. 2B), suggesting that these receptors contribute to the effect of the drug. However, the actions of these receptor antagonists appear to be transient in nature, since the SKF83959-induced rotations recovered 30 min after pretreatment with these antagonists. The D_1 receptor antagonist, SCH23390, at the doses of 0.2 or 0.5 mg/kg, completely blocked SKF83959-induced rotations (Fig. 2C). The effects of SCH23390 on catalepsy might confound the results. However, at the dose range tested SCH23390-induced catalepsy lasts approximately 60 min (Undie and Friedman, 1988), while the effect on rotation was sustained for at least the 2-h observation period (data not shown), suggesting that SKF83959-mediated rotational behavior is primarily mediated via a D_1-like dopamine receptor.

3.2. Enhanced IP_3 accumulation in lesioned striata is associated with SKF83959-stimulated rotation

Since previous work indicated that SKF83959 selectively stimulates the PI-linked D_1 dopamine receptor (Panchalingam and Undie, 2001; Jin et al., 2003), we attempted to test if the PI-linked D_1 receptor mediates rotational behavior in the unilateral lesioned rat model. We first assessed the effect of SKF83959 on phosphoinositol metabolism in striata of 6-OHDA lesioned rats. Administration of 0.5 or 1.0 mg/kg SKF83959, doses which elicited contralateral rotations, resulted in enhanced stimulation of IP_3 accumulation in lesioned striata assessed 8 min after drug administration

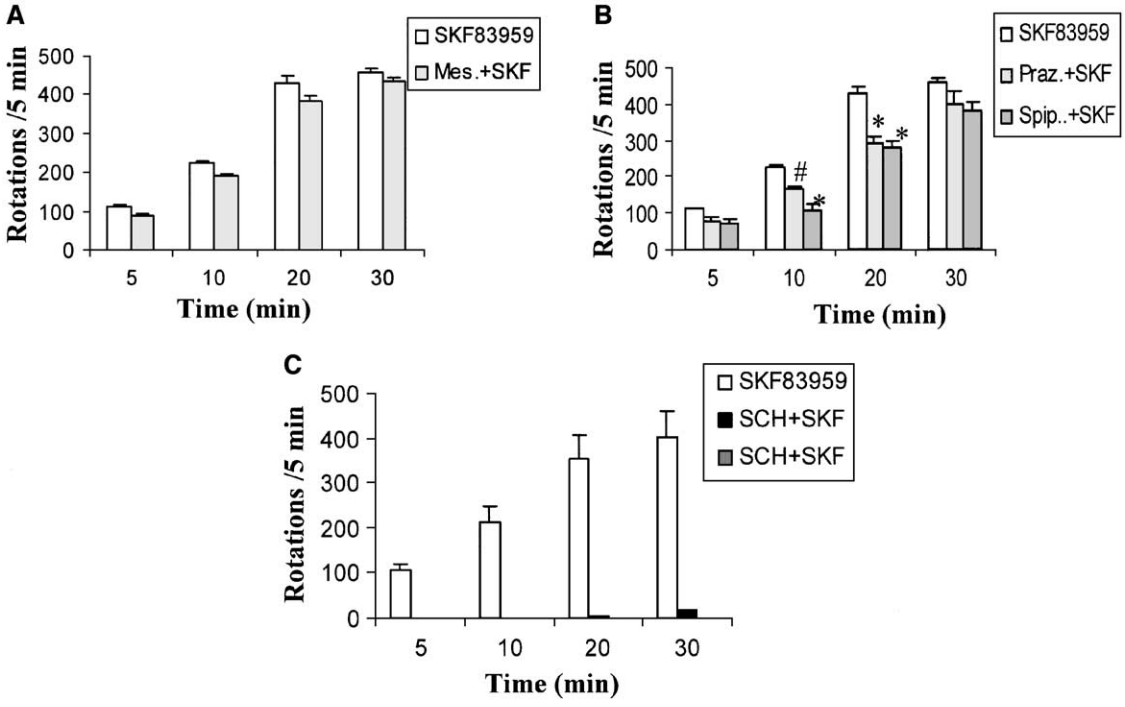

Fig. 2. Effects of various receptor antagonists on SKF83959-induced rotations in 6-OHDA-lesioned rats. A. Rats were administered 0.1 mg/kg mesulergine or vehicle subcutaneously, 15 min before the injection of 0.5 mg/kg SKF83959. B. Rats received 0.1 mg/kg prazosin or 2 mg spiperone, 10 min before 0.5 mg/kg SKF83959. C. Rats were pretreated with 0.2 (SCH1) or 0.5 (SCH2) mg/kg SCH23390 and injected 20 min later with SKF83959. Rotations were recorded at the indicated times after the injection of the agonist. Data are the summary of at least 5 animals in each group and presented as mean±S.E. #p<0.05; *p<0.01; compared to respective SKF83959-treated group. Mes.: mesulergine; SKF: SKF83959; Praz.: prazosin; Spip.: spiperone; SCH.: SCH23390.

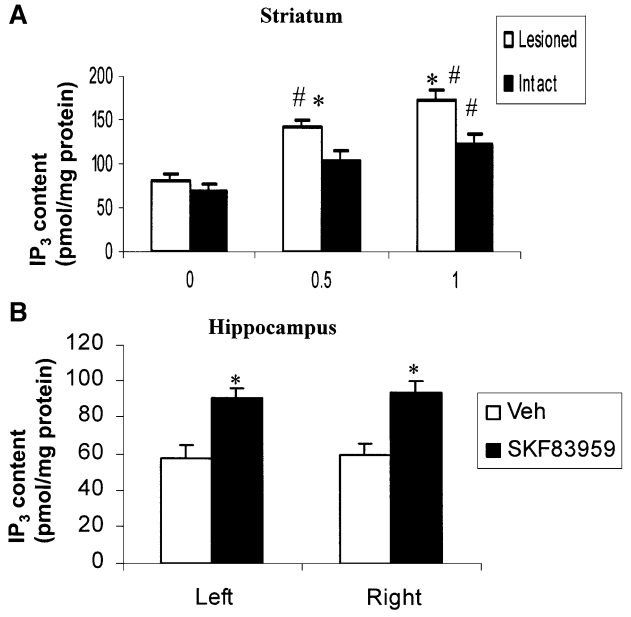

Fig. 3. Effect of SKF83959 on brain IP_3 production in 6-OHDA-lesioned rats. 6-OHDA-lesioned rats were injected with 0.5 or 1 mg/kg of SKF83959 and rats were decapitated 8 min later. Left and right striata were dissected and assayed for IP_3 as described in Methods (A). The left and right hippocampi from control or 1 mg/kg SKF83959-treated rats were also collected for IP_3 assessment (B). Data obtained from at least 5 animals in each group are presented as mean±S.E. A: *p<0.01 lesioned vs. respective intact striata; #p<0.05; ##p<0.01 vehicle vs. different concentration of SKF83959 administration. B: *p<0.01 Vehicle vs. SKF 83959 for each side of hippocampus. Veh: vehicle.

(Fig. 3A). At these doses, SKF83959 induced increases in IP_3 content that were greater than 100% in lesioned (left) as compared to intact (right) striata. In contrast, SKF83959 induced similar elevations in IP_3 in the left and right hippocampi (Fig. 3B). Moreover, pretreatment of rats with 0.5 mg/kg SCH23390, which blocks SKF83959-induced rotations (Fig. 2C), also prevented the elevation in striatal IP_3 content ipsilateral to the lesion (Fig. 4). The results suggest, therefore, that activation of the PLC pathway via the PI-linked D_1 dopamine receptor is involved in mediating SKF83959-induced contralateral rotations in the unilateral 6-OHDA-treated rat.

4. Discussion

The present study confirms that SKF83959 elicits contralateral rotations in the unilateral 6-OHDA-lesioned rat model for Parkinson's disease. Stimulation of rotation by this benzazepine appears to be primarily mediated via activation of D_1 dopamine receptors since the D_1 dopamine receptor antagonist, SCH23390 (but not the D_2 antagonist, spiperone) completely blocked this action. Moreover, we detected a significant increase in IP_3 content in 6-OHDA-lesioned as compared to intact striata in response to a challenge dose of SKF83959, indicating that stimulation of PLC may underlie the rotationals induced by SKF83959. This is further supported by the fact that blockade of SKF83959-induced striatal PI hydrolysis by SCH23390 also resulted in the complete inhibition of rotations elicited by this dopamine agonist. The data clearly implicate the PI-linked D_1 receptor and the PLC/PI pathway as the mechanism by which SKF83959 stimulates rotations in the unilateral lesioned rat.

The 6-OHDA lesioned rat is a widely accepted animal model in screening for agents with potential therapeutic efficacy in Parkinson's diseases (Schwarting and Huston, 1996; Betarbe et al., 2002). SKF83959 was shown in both primate and rat models of Parkinson's disease, to be effective in reducing parkinsonian-like symptoms (Gnanalingham et al., 1995; Andringa et al., 1999b). However, the mechanism for this action is unclear. Previous pharmacological studies demonstrated that SKF83959 does not stimulate cAMP production in cultured cells or in brain tissue although the drug exhibits high affinity for the D_1 dopamine receptor (Jin et al., 2003), suggesting that the classical D_{1A} dopamine receptor, whose actions are modulated via the cAMP pathway, is not involved in mediating SKF83959-induced rotational behavior in the unilateral 6-OHDA-treated rat. The present results, therefore, suggest a role for striatal PI-linked D_1 dopamine receptor in mediating the pharmacotherapeutic action of anti-parkinsonian agents. The results also suggest new strategies for the discovery of novel treatments for Parkinson's disease.

The functional role of the brain PI-linked D_1 dopamine receptor system is unknown. A major obstacle in defining the functional role for this receptor system was the lack of selective tools for probing the effects of this receptor. The recent identification of SKF83959 as a selective agonist for this dopamine receptor provided us with a useful tool that allowed us to begin and characterize the pharmacological/physiological properties of this brain receptor (Panchalingam and Undie, 2001; Jin et al., 2003). The present communication provides evidence that activation of striatal PI-linked D_1 dopamine receptor by SKF83959 mediates contralateral rotations in the 6-OHDA-lesioned rat. To our knowledge, this is the first report defining the involvement

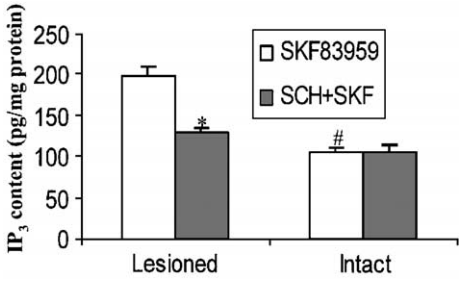

Fig. 4. Effect of SCH23390 on SKF83959-mediated striatal IP3 accumulation. 6-OHDA-lesioned rats were pretreated intraperitoneally with 0.2 mg/kg SCH23390 and injected after 20 min with SKF83959. Rats were killed 8 min later, striata were collected and IP_3 content was assessed in the lesioned or intact striata. Data obtained from at least 5 animals in each group are presented as mean±S.E. *p<0.01: SKF83959 vs. SCH23390+SKF83959; #p<0.01, intact vs. lesioned striata. SCH: SCH23390; SKF: SKF83959.

of this PI-linked D_1 dopamine receptor and its associated signaling pathway in locomotion. Administration of SKF83959 to normal rats was shown to induce other D_1 dopamine receptor-mediated behavioral responses such as orofacial movements, grooming and vacuous chewing (Deveney and Waddington, 1995). The potential contribution of the PI-linked D_1 dopamine receptor in relation to these behavioral responses remains to be studied.

In addition to the present results, we found that stimulation of the PI-linked D_1 dopamine receptor results in inhibition of striatal and substantia nigra GSK3β activity (unpublished data). It is well known that inhibition of neuronal GSK3β activity is an important anti-apoptatic mechanism in preventing various neuronal insults (Kaytor and Orr, 2002; Nagatsu, 2002). It is conceivable that inhibition of GSK3β activity by modulating the PI-linked D_1 dopamine receptor may also have neuroprotective effects. The potential neuroprotective effect of SKF83959 is under investigation in our laboratory.

The conclusions derived from the present communication should, however, be interpreted with caution since the results also indicate that SKF83959-induced rotation was partially and transiently antagonized by spiperone and prazosin, suggesting that the D_2 dopamine and α_1 adrenergic receptors may also contribute to the actions of this drug.

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