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Rotation and immediate-early gene expression in rats treated with the atypical D1 dopamine agonist SKF 83822

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Abstract

Classical agonists of the dopamine D1 receptor activate both adenylyl cyclase and phospholipase C (PLC) signaling pathways. As a result, the extent to which these two pathways are essentially involved in various effects produced by D1 receptor agonists is currently uncertain. In the present report we examined the effects of SKF 83822, a dopamine D1 agonist which has been reported to activate adenylyl cyclase, but not PLC, on behavior and immediate early gene (IEG) expression in rats with unilateral 6-hydroxydopamine lesions. SKF 83822 (25–100 μg/kg) induced dose dependent contralateral rotation in these subjects, and, additionally, stimulated strong expression of the IEG products c-Fos, Fra2, Zif/268 and Arc in the deinnervated striatum. All of these effects could be antagonized by pretreatment with the selective D1 dopamine antagonist SCH 23390 (0.5 mg/kg). Although PLC may be involved in many effects mediated through dopamine D1 receptors, these results suggest that direct activation of PLC is not necessary for the induction of either rotation or IEG expression in dopamine depleted rats.

Keywords: SKF83822; Adenylate cyclase; cAMP; Turning; Circling; 6-OHDA; Fos; Fos related antigen; Activity regulated cytoskeletal protein; Caudate; Fra-2; Zif/268

1. Introduction

The effects produced by stimulation of dopamine D1 receptors have classically been assumed to result primarily from activation of adenylyl cyclase. The ability of D1 agonists to induce a number of behavioral and electrophysiological effects, however, is not correlated with their ability to induce cAMP formation (Arnt et al., 1992; Gnanalingham et al., 1995a,b; Johansen et al., 1991). These findings have suggested that additional signal transduction pathways must be involved in mediating the effects of D1 receptor activation. Although several alternative pathways could be involved (Bergson et al., 2005; Gautam et al., 1998), the greatest amount of interest has centered on the activation of phospholipase C (PLC) and the subsequent hydrolysis of phosphatidylinositol biphosphate. A number of dopamine D1 agonists have been shown to stimulate phosphoinositide (PI) signaling pathways and these effects can be blocked by the dopamine D1 antagonist SCH 23390 (Undie et al., 1994; Undie and Friedman, 1990; Zhen et al., 2005). Furthermore, studies of knockout mice have suggested the existence of D1-like receptor subtypes which are able to interact with PLC but not adenylyl cyclase (Friedman et al., 2005).

Interest in the role of PLC in mediating the effects of dopamine D1 agonists has been stimulated by recent studies of the drug SKF 83959. This agent acts on dopamine D1 receptors to stimulate PI hydrolysis (Arnt et al., 1992; Jin et al., 2003; Panchalingam and Undie, 2001), but has been reported to actually antagonize dopamine mediated stimulation of adenylyl cyclase (Andringa et al., 1999; Arnt et al., 1992; Jin et al., 2003). The selectivity of SKF 83959 could result either from a preferential interaction with a D1-like receptor subtype which is specifically linked to PLC or from an "agonist trafficking" mechanism (Kenakin, 1995). Despite the reported inability of SKF 83959 to increase formation of cAMP, this agent produces a number of effects similar to those induced by classical dopamine D1 receptor agonists. For example, SKF 83959 is able to induce intense grooming behavior (Downes and Waddington, 1993), stimulate orofacial movements (Downes and Waddington, 1993; Tomiyama et al., 2001), reverse parkinsonian symptoms in MPTP treated primates (Gnanalingham et al., 1995b), induce contralateral rotation in rats with unilateral 6-OHDA lesions (Arnt et al., 1992; Gnanalingham et al., 1995a; Wirtshafter and Osborn, 2005) and stimulate striatal

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expression of the immediate-early gene c-*fos* (Wirtshafter and Osborn, 2005). These results suggest that D1 receptors linked to PLC may play a major role in the effects of D1 receptor agonists and raise the possibility that stimulation of adenylyl cyclase may not be involved to as great an extent as has been generally assumed. This possibility is supported by the observation that selective phosphodiesterase inhibitors do not alter the effects of D1 agonists on either rotational behavior or immediate-early gene expression (Thompson et al., 2004), even though they would be expected to potentiate the effects of D1 agonists on levels of cAMP.

Although it has not yet been subjected to intensive study, the drug SKF 83822 is likely to be a useful tool for exploring the involvement of adenylyl cyclase in D1 mediated responses. This benzazepine derivative binds to dopamine D1 receptors with high affinity (Seeman and Niznik, 1988) and stimulates production of cAMP, but, unlike standard D1 agonists, is unable to induce PI hydrolysis (Undie et al., 1994). The behavioral effects of SKF 83822 also appear to differ markedly from those of standard D1 agonists; for example, SKF 83822, unlike other D1 agonists, is not able to induce either intense grooming in rats (O'Sullivan et al., 2004) or dyskenetic movements in sensitized monkeys (Peacock and Gerlach, 2001). These findings suggest that stimulation of adenylyl cyclase is not sufficient to induce these effects, and are consistent with the notion that activation of PLC, rather than adenylyl cyclase, may be responsible for many of the effects of dopamine D1 receptor stimulation. Since SKF 83822 has been studied in a very limited number of situations, however, it is impossible at the present time to evaluate the extent to which its actions differ from those of classical agonists. It would seem of substantial interest to examine the effects of SKF 83822 in other paradigms known to be sensitive to standard D1 agonists. In the current experiments we therefore examined whether SKF 83822 is able to reproduce two of the best established effects of standard D1 receptor agonists, namely the induction of contralateral rotation and the stimulation of expression of immediate-early genes in rats with unilateral dopamine depleting lesions. Most studies of IEG expression have examined only *c-fos*; under certain conditions, however, various IEGs appear to be differentially expressed (Ons et al., 2004; Pollack and Fink, 1995; Simpson and Morris, 1995). We thus examined the effects of SKF 83822 on the expression of four different IEGs, all of which have been shown to be sensitive to the effects of typical dopamine D1 agonists, in order to increase our chances of detecting unusual aspects of the response to SKF 83822.

2. Materials and methods

2.1. Subjects

Subjects were adult, male Sprague–Dawley derived rats obtained from a colony maintained by the Psychology Department of the University of Illinois at Chicago. Rats were housed in individual wire mesh cages throughout the experiment. Experimental procedures were approved by the Animal Care Committee of the University of Illinois at Chicago.

2.2. Surgery

Rats received unilateral injections of 6-hydroxydopamine (6-OHDA, Sigma Chemical Company, St. Louis, MO, 8 μg free base in 4 μl of a 0.1% ascorbic acid vehicle) into the lateral hypothalamus (AP: 5.2, H: 1.2, L: 1.8; Paxinos and Watson, 1997) using standard stereotaxic techniques. Surgery was conducted under sodium pentobarbital anesthesia (40 mg/kg) following pretreatment with the norepinephrine uptake inhibitor desmethylimipramine (25 mg/kg) to reduce damage to noradrenergic neurons. Two weeks later, these subjects were injected with apomorphine (0.3 mg/kg, s.c.) and placed in automated rotometers (San Diego Instruments) for a period of 120 min. All subjects used in the current study showed at least 200 contralateral rotations in this test trial and further studies were conducted about five months following screening with apomorphine.

2.3. Drugs

SKF 83822 (3-allyl-6-chloro-7,8-dihydroxy-1-(3-methylphenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine, molecular weight=424.76) was obtained through the NIMH Chemical Synthesis and Drug Supply Program administered by RTI International (Research Triangle Park, NC). SCH 23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-*H*-3-benzazepine hydrochloride; molecular weight=324.1) was obtained from Research Biochemicals Inc. (Natick, MA).

2.4. Perfusion and immunocytochemistry

Animals were deeply anesthetized with sodium pentobarbital (100 mg/kg) and then rapidly perfused at room temperature with saline followed by 10% formalin using a variable pH protocol (Berod et al., 1981). Brains were then removed from the skulls and post-fixed in the pH 9.5 formalin solution for 1 h at 4 °C. The tissue was then transferred to a solution of phosphate buffered saline (PBS) containing 20% sucrose where it was stored at 4 °C until the next day. Cryostat sections were then cut through the rostral striatum at a thickness of 35 μm and processed using standard immunocytochemical methods as we have previously described in detail (Wirtshafter and Asin, 2001). The primary antibodies were a rabbit anti-c-Fos serum (Oncogene Sciences/Calbiochem, Cambridge, MA, AB5, 25,000×), a rabbit anti-Fra-2 serum (Santa Cruz Biotechnology, Santa Cruz, CA, 1500×), a rabbit anti Zif/268 serum (Santa Cruz, 6000×) and a goat anti-Arc serum (Santa Cruz, 750X). Antigenic sites were visualized using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) employing nickel intensified diaminobenzadine as the chromogen. In control sections in which the primary antibodies was omitted or replaced by nonimmune rabbit or goat serum, no stained nuclei were seen. In order to quantitatively examine the gene expression data, fields measuring 0.6×0.7 mm in the dorsolateral striatum were digitally captured and the number of cells automatically counted using methods we have described in detail elsewhere (Wirtshafter et al., 1995; Wirtshafter and Osborn, 2005).