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Functional selectivity of dopamine D_1 receptor agonists in regulating the fate of internalized receptors

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Abstract

Recently, we demonstrated that D_1 agonists can cause functionally selective effects when the endpoints of receptor internalization and adenylate cyclase activation are compared. The present study was designed to probe the phenomenon of functional selectivity at the D_1 receptor further by testing the hypothesis that structurally dissimilar agonists with efficacies at these endpoints that equal or exceed those of dopamine would differ in ability to influence receptor fate after internalization, a functional endpoint largely unexplored for the D_1 receptor. We selected two novel agonists of therapeutic interest that meet these criteria (the isochroman A-77636, and the isoquinoline dinapsoline), and compared the fates of the D_1 receptor after internalization in response to these two compounds with that of dopamine. We found that dopamine caused the receptor to be rapidly recycled to the cell surface within 1 h of removal. Conversely, A-77636 caused the receptor to be retained intracellularly up to 48 h after agonist removal. Most surprisingly, the D_1 receptor recovered to the cell surface 48 h after removal of dinapsoline. Taken together, these data indicate that these agonists target the D_1 receptor to different intracellular trafficking pathways, demonstrating that the phenomenon of functional selectivity at the D_1 receptor is operative for cellular events that are temporally downstream of immediate receptor activation. We hypothesize that these differential effects result from interactions of the synthetic ligands with aspects of the D_1 receptor that are distal from the ligand binding domain.

Keywords: D_1 dopamine receptors; Receptor internalization; Receptor recycling; Adenylate cyclase; Functional selectivity; A-77636; Dinapsoline; Dopamine

1. Introduction

The dopamine receptors are a superfamily of heptahelical G protein-coupled receptors (GPCRs) that have historically been partitioned into "D_1-like" and "D_2-like" subfamilies (Kebabian and Calne, 1979; Garau et al., 1978). The dopamine D_1 receptor is a member of the "D_1-like" subfamily, and couples to adenylate cyclase through stimulatory G proteins G_s and G_{olf} (Herve et al., 1993). The early steps in the regulation of the D_1 receptor following the binding of dopamine have been addressed in model cell lines. After the binding of dopamine to the D_1 receptor, receptor phosphorylation is complete within minutes (Gardner et al., 2001). This can be mediated by

Abbreviations: cAMP, cyclic AMP (adenosine 3′,5′-cyclic monophosphate); A-77636, (1*R*,3*S*)-3-(1′adamantyl)-1-aminomethyl-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyran); DA, dopamine; DNS, dinapsoline (8,9-dihydroxy-2,3,7,11*b*-tetrahydro-1*H*-naph[1,2,3-*de*]isoquinoline); GRK, G protein-coupled receptor kinase; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid; RIA, radioimmunoassay; SCH23390, 7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; TMx, transmembrane spanning section X.

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GRKs (Gardner et al., 2001; Tiberi et al., 1996) and/or protein kinase A (PKA) (Mason et al., 2002). Both types of kinases may facilitate D_1 desensitization, and the contribution of each to the overall extent of receptor phosphorylation and desensitization is probably highly dependent on the cell line being studied. Receptor phosphorylation allows arrestin to bind to the third intracellular loop of the receptor (Kim et al., 2004) leading to D_1 receptor internalization. Arrestin is not trafficked into the cell with the receptor, thus the D_1 receptor is considered a "Class A" GPCR (Oakley et al., 2000). Following dopamine-induced internalization, the D_1 receptor is rapidly recycled back to the cell surface (Vickery and von Zastrow, 1999; Vargas and von Zastrow, 2004). Recent studies indicate that a signal sequence within the proximal C-terminal region of the receptor mediates this process (Vargas and von Zastrow, 2004).

The effects of D_1 agonists other than dopamine itself on regulatory events downstream of receptor activation are not well characterized. Besides heuristic interest in these questions, several of the D_1 agonists that have been tested as anti-parkinson agents in human and non-human primates caused a very rapid tolerance evidenced as an almost complete loss of response within a day or so (Asin and Wirtshafter, 1993; Kebabian et al., 1992; Lin et al., 1996; DeNinno et al., 1991a; Johnson et al., 1992). Thus, such molecular events may be important in understanding the cellular mechanisms that contribute to the development of this therapeutic tolerance. Previously, we have observed that desensitization of adenylate cyclase responsivity and receptor down-regulation are highly dependent upon the agonist used, but largely independent of adenylate cyclase activity and agonist affinity in a stably transfected C6 glioma cell line (Lewis et al., 1998). Recently, we explored the relationship between agonist structure, receptor affinity, and efficacy of receptor internalization and adenylate cyclase activation in greater depth by constructing an HEK cell line stably transfected with a hemaglutinin-tagged human D_1 receptor and comparing these endpoints in 13 agonists from three different structural families. We found that D_1 agonists exhibit functional selectivity at these early endpoints following receptor activation that are apparently independent of agonist structure or binding affinity (Ryman-Rasmussen et al., 2005).

These results suggested the major hypothesis tested herein, that D_1 agonists are functionally selective in regulating receptor function at the endpoint of intracellular trafficking of the D_1 receptor, an endpoint that temporally lies downstream of adenylate cyclase activation and internalization, events more immediate of receptor activation. We selected two agonists of therapeutic interest, A-77636 and dinapsoline (DNS), for comparison with dopamine at this endpoint. Both of these synthetic ligands have efficacies of internalization and adenylate cyclase activation comparable to that of dopamine in the HA-hD_1 HEK cell line (Ryman-Rasmussen et al., 2005). The isochroman A-77636 elicits profound and rapid *in vivo* tolerance occurring within approximately 24 h, preventing its use in Parkinson's disease therapy (Lin et al., 1996). Conversely, DNS does not cause such tolerance in a rat model of Parkinson's disease (Gulwadi et al., 2001). The mechanisms of tolerance are unknown, but presumably result from cellular adaptations that lie temporally downstream of receptor internalization and adenylate cyclase activation. The current data demonstrate that although these agonists cause functional changes identical to dopamine immediately following receptor binding, with time they modify D_1 receptor trafficking, and thus show a novel pattern of functional selectivity.

2. Methods

2.1. Materials

Dopamine and A-77636 [(−)-(1*R*,3*S*)-3-adamantyl-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyran hydrochloride)] were purchased from RBI/Sigma-Aldrich (St. Louis, MO). Dinapsoline (8,9-dihydroxy-2,3,7, 11*b*-tetrahydro-1*H*-naph[1,2,3-de]isoquinoline) and [^{3}H]SCH23390 were synthesized according to published procedures (Ghosh et al., 1996; Wyrick et al., 1986). All other reagents and materials were from Sigma Chemical Company (St. Louis, MO), unless otherwise stated.

2.2. HA-hD_1 HEK model cell line

The HA-hD_1 HEK cell line was constructed as described previously (Ryman-Rasmussen et al., 2005). Cells were maintained in DMEM-H, 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.6 mg/mL geneticin at 37 °C and 5% CO_2. Assay plates were coated with 4 μg/mL human fibronectin (Enzyme Research Laboratories Inc., South Bend, IN) at 37 °C for 1 h or overnight, followed by addition of polylysine to 0.2 mg/mL for an additional 30 min to prevent cell loss. Coating medium was aspirated and cells were immediately plated at a density of 500–1000 cells per mm^2.

2.3. Radioreceptor assays

HA-hD_1 receptor expression level and affinity for dopamine, A-77636, and DNS were determined by saturation binding and competition assays. Membrane homogenates from HA-hD_1 HEK cells were prepared as previously described (Lewis et al., 1998). Receptor density and affinity of SCH23390 were measured by incubation of membrane homogenates in 0–2 nM [^{3}H]SCH23390 in 50 mM HEPES, 4 mM $MgCl_2$, 0.01% ascorbic acid, pH 7.4 for 15 min at 37 °C. Yohimbine and propranolol (50 nM) were included to block endogenous adrenergic receptors. Non-specific binding was determined by parallel incubations with 1 μM SCH23390. Radioreceptor assays were done using either a Molecular Dynamics/Skatron harvester and LKB 1209 RackBeta counter, or a Packard 96 Filtermate Harvester and TopCount Counter, using appropriate glass fiber filters and scintillation fluid. The total protein concentration of membrane preparations used in the saturation binding assays was determined by use of the BCA™ assay according to the manufacturer's instructions (Pierce, Rockford, IL). Affinity of dopamine, A-77636, and DNS for the HA-hD_1 receptor was determined by competition (10^{-10} to 10^{-4} M) versus 0.3 nM [^{3}H]SCH23390 in the presence of 50 nM propranolol and yohimbine.

2.4. Adenylate cyclase functional potency and efficacy

Adenylate cyclase activation by dopamine, A-77636, and DNS was measured in whole HA-hD_1 HEK cells as previously described (Ryman-Rasmussen et al., 2005). Triplicate wells in 24-well plates were untreated or incubated with increasing concentrations of dopamine, A-77636, or DNS in the presence of 500 μM IBMX at 37 °C for 15 min. Duplicate wells at the highest concentration of agonist included the D_1 receptor-selective antagonist, 50 μM SCH23390, as a negative control. All wells contained 50 nM (*S*)-propranolol and yohimbine to antagonize endogenous β-adrenergic receptors. The reaction was quenched with 0.1 N HCl, and cAMP was quantified by a modified radioimmunoassay based on a previously published method (Harper and Brooker, 1975).