Dopamine responsiveness is regulated by targeted sorting of D2 receptors


*Ernest Gallo Clinic and Research Center and †Department of Neurology, University of California, San Francisco, CA 94608; and §Department of Physiological Sciences, Faculty of Medicine, Lund University, S-221 00 Lund, Sweden

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Aberrant dopaminergic signaling is a critical determinant in multiple psychiatric disorders, and in many disease states, dopamine receptor number is altered. Here we identify a molecular mechanism that selectively targets D2 receptors for degradation after their activation by dopamine. The degradative fate of D2 receptors is determined by an interaction with G protein coupled receptor-associated sorting protein (GASP). As a consequence of this GASP interaction, D2 responses in rat brain fail to resensitize after agonist treatment. Disruption of the D2-GASP interaction facilitates recovery of D2 responses, suggesting that modulation of the D2-GASP interaction is important for the functional down-regulation of D2 receptors.

Slice Preparation and Electrophysiology. Horizontal slices (230 µm) containing the VTA were prepared as described (9). Artificial cerebrospinal fluid (ACSF) contained 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH2PO4, 1.2 mM MgCl2, 2.4 mM CaCl2, 18 mM NaHCO3, 11 mM glucose (pH 7.2–7.4 and milliosmolarity 301–305) and was bubbled with 4°C carbogen. After cutting, slices recovered for at least 45 min at 32°C in carbogen-bubbled ACSF. During patch-clamp experiments, slices were submerged and continuously perfused (using a peristaltic pump, ~2 ml/min) with ACSF warmed to 31–32°C, and supplemented with 6-cyano-7-nitroquinoloxaline-2,3-dione (CNQX) (10 µM), picrotoxin, and sodium metabisulfite (50 µM), as described (10). Cells were visualized with an upright microscope using infrared differential interference contrast illumination. Whole-cell voltage clamp recordings were made with 2.5–3.5 MΩ electrodes using a Multiclamp700A amplifier (Axon Instruments) in current clamp mode. The potassium methanesulfonate-based internal solution consisted of 0.95% (vol/vol) KOH, 0.38% (vol/vol) methanesulfonic acid, 20 mM Hesper, 0.2 mM EGTA, 2.8 mM NaCl, 2.5 mg/ml MgATP, 0.25 mg/ml GTP (pH 7.2–7.4, 275–285 milliosmolarity). Data were acquired by passing 2-KHz DC current through an amplifier. Cells were set to −60 mV 10–15 min before each quinpirole application and the membrane potential determined every 2 s (single traces) or 30 s (grouped data).

Cell Culture and Immunocytochemistry. Human embryonic kidney (HEK) 293 cells (American Type Culture Collection) were grown in DMEM (Gibco BRL) supplemented with 10% fetal bovine serum (HyClone). N-terminal FLAG- and/or HA-tagged D1R or D2R constructs (11) were stably expressed in HEK293 cells. GFP-CGASP (3) constructs were either stably or transiently expressed. For generation of clonal stable cell lines, single colonies were chosen and propagated in the presence of selection-containing media. The antibody-feeding immunocytochemistry and recycling

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2Materials and Methods

Animals. Male Sprague–Dawley rats, 22–28 days of age were used. Animal care was in accordance with Ernest Gallo Clinic and Research Center Animal Care and Use Committee.

Drugs and Reagents. Quinpirole, SCH23390, dopamine, raclopride, baclofen, haloperidol, and FLAG antibodies were purchased from Sigma. Hemagglutinin (HA) antibodies were purchased from Co-
experiments were essentially as described (12), except dopamine (10 μM, 60 min), haloperidol (20 μM), or SCH23390 (20 μM) were used. Briefly, cells stably expressing FLAG-tagged D1R or D2R were grown on coverslips to 50% confluency. Live cells were fed M1 antibody (Sigma) directed against the FLAG tag (1:1,000, 30 min). Cells were then treated with agonist (10 μM dopamine, 60 min) or left untreated. Untreated cells were then fixed with 4% formaldehyde in PBS. Residual surface receptors (those not internalized by agonist) in cells treated with agonist were stripped of antibody by washing in PBS without calcium (the M1 interaction is calcium sensitive). Cells were then either fixed as above or first treated with antagonist (20 μM haloperidol for D2R or 20 μM SCH23390 for D1R) for 30 min to assess recycling and then fixed as above. After fixation, cells were permeabilized in blotto with 0.1% Triton X-100 and stained with fluorescently conjugated secondary antibody (1:500, Molecular Probes).

**Biotin Protection Degradation Assay (BPA).** HEK293 cells stably expressing N-terminal FLAG- or HA-tagged D1R or D2R either alone or together with GFP-cGASP were grown to 80% confluency in 10-cm plates and subjected to the BPA protocol as described in ref. 12 except the D1R and D2R agonist dopamine (10 μM), the D2R antagonist haloperidol (20 μM), or the D1R antagonist SCH23390 (20 μM) were used. Briefly, cells were treated with 3 μg/ml disulfide-cleavable biotin (Pierce) for 30 min at 4°C. Cells were then washed in PBS and placed in prewarmed media for 15 min before treatment with ligand (or no treatment) for the specified period. Concurrent with ligand treatment, 100% and strip plates remained at 4°C. After ligand treatment, plates were washed in PBS, and remaining cell surface biotinylated receptors were stripped in 50 mM glutathione/0.3 M NaCl/75 mM NaOH/1% FBS at 4°C for 30 min. Cells were then washed with Tris buffer, then lysed in 0.1% Triton X-100/150 mM NaCl/25 mM KCl/10 mM Tris-HCl, pH 7.4, with protease inhibitors (Sigma). Cleared lysates were immunoprecipitated with anti-FLAG or HA antibodies, treated with PNGase (New England Biolabs) for 2 h and resolved by SDS/PAGE and visualized with streptavidin overlay (Vectorstain ABC immunoperoxidase reagent, Vector Laboratories).

For quantification, at least three blots for each condition were quantified by using Scion IMAGE software package. Agonist treatment for 60 min was designated 100% for each agonist/condition tested.

**Coimmunoprecipitation from HEK293 Cells.** HEK293 cells stably expressing D1R, D2R, or no heterologous receptor were grown to confluency and washed two times with PBS, and lysates were prepared as described (3) in 0.1% Triton X-100/150 mM NaCl/25 mM KCl/10 mM Tris-HCl, pH 7.4, with protease inhibitors (Sigma). Cleared lysate was incubated with M2 anti-FLAG affinity resin (Sigma) for 1 h at 4°C, washed extensively, and deglycosylated with PNGase (New England Biolabs) for 2 h. Precipitates were resolved on a 4–20% gradient Tris-HCl precast gel (Bio-Rad) and transferred to nitrocellulose, and the blots were cut below the 75-kDa marker band to separately immunoblot for either receptor (lower blot) or GASP (upper blot). GASP blots were incubated for 2 h with rabbit anti-GASP (1:1,000) and for 1 h with HRP-conjugated anti-rabbit antibody (NEB) (1:4,000, 1 h at room temperature), then visualized with ECL plus (Amersham Pharmacia). Receptor blots were incubated for 2 h with biotinylated M2 (1:250) (Sigma), then visualized with streptavidin overlay (Vectorstain ABC reagents, Vector Laboratories) and ECL plus.

**GST Competition.** The last 14 amino acids of the D2R were fused to the C terminus of GST, and the fusion protein was generated as described (3). Affinity between in vitro-translated GASP protein and GST-D2R was tested in the presence or absence of rabbit anti-GASP antibodies (250 ng/ml) or control rabbit IgG (250 ng/ml) antibodies.

**GST Pull-Down from Rat Brain.** Rat brain synaptosomal membranes were prepared from adult rats as described (13), and lysates were prepared in 0.1% Triton X-100/150 mM NaCl/25 mM KCl/10 mM Tris-HCl, pH 7.4, with protease inhibitors (Sigma) and Complete (Roche Diagnostics). Cleared lysate was incubated with empty glutathione resin (B, beads), resin bound to empty GST (G), resin bound to a GST-D1R fusion protein (D1), or resin bound to a

**Fig. 1.** D2Rs degrade and D1s recycle. (A) Immunocytochemical analysis of receptor trafficking. (A Upper) HEK cells stably expressing D2Rs were fed antibody to the extracellular FLAG-tag to label surface receptors and treated with dopamine or left untreated (NT, Left). Treatment with dopamine for 60 min (DA60, Center) promotes endocytosis of D2Rs. After agonist treatment, any remaining surface receptors were stripped of antibody and antagonist haloperidol (H) was added to monitor recycling and prevent further endocytosis. Internalized D2Rs fail to return to the surface in the presence of antagonist (Right). (A Lower) HEK293 cells stably expressing FLAG-tagged D1R were treated as above, except the antagonist SCH23390 (SCH) was used. (B) Antagonist prevents dopamine-induced endocytosis. HEK cells stably expressing D2Rs (Upper) or D1Rs (Lower) were fed antibody to the extracellular FLAG-tag to label surface receptors. Cells were then treated simultaneously for 60 min with both dopamine and either haloperidol for the D2Rs (H, Upper), or SCH23390 (SCH, Lower). (C) Stability of endocytosed receptor was assessed by using the BPA on the cell lines used for A and in cells stably expressing both receptors. Cells stably expressing D2R (Top and Upper Middle) or D1R (Lower Middle and Bottom) were biotinylated and treated with the agonists dopamine (for both D1R and D2R) or quinpirole (Q, for D2R) for the times indicated. Remaining surface receptors were then stripped of biotin by using a membrane impermeant reducing agent and the “protected” internalized receptor pool was immunoprecipitated with either anti-FLAG or -HA antibodies and visualized (Lower Middle and Bottom).
GST-D2R fusion protein (D2) as described above, for 2 h at 4°C, then washed extensively. Equivalent protein levels of GST, GST-D1R, and GST-D2R were determined before the GST pull-down experiment and confirmed by Coomassie stain of the gel. Precipitates were resolved on a 4–20% gradient Tris/HCl gel (Bio-Rad), transferred to nitrocellulose, and immunoblotted for GASP by using rabbit anti-GASP (1:1,000) antibody, and incubated for 1 h with HRP-conjugated anti-rabbit antibody (NEB) (1:4,000), then visualized with ECL plus (Amersham Pharmacia).

**VTA Slice Preparation and Immunohistochemistry.** Adult rats were deeply anesthetized with halothane and fixed with 4% paraformaldehyde (Sigma) by using a standard perfusion procedure (14). Double immunofluorescence immunohistochemistry of coronal sections (35 μM) containing the VTA were performed by using rabbit anti-GASP (1:1,000) or rabbit anti-D2R (1:500 Chemicon) and sheep anti-tyrosine hydroxylase (TH), a marker for dopamine production (1:500, Chemicon). Secondary fluorescent antibodies were donkey anti-rabbit Cy-3 and donkey anti-sheep FITC (1:250). Mounted sections were examined by using LSM 510 laser confocal microscope (Zeiss), or Nikon Eclipse E600 microscope equipped with Spot-2 color CCD camera (Technical Instruments, San Francisco, CA). Preincubation of the anti-GASP antibody with the protein used for the immunization, resulted in a loss of immunostaining.

**Coimmunoprecipitation from Rat Brain.** Rat brain synaptosomes were prepared from adult rats as described (13) and lysates prepared in 0.1% Triton X-100/150 mM NaCl/25 mM KCl/10 mM Tris-HCl pH 7.4, with protease inhibitors (Sigma) and Complete (Roche Diagnostics). Cleared lysate was incubated with either anti-mouse dopamine D2 receptor D2R antibody-coated (2.5, 5, and 10 μg/ml, Santa Cruz Biotechnology no. SC5303) protein G-agarose beads (Invitrogen) or 1 mg/ml BSA-coated (Sigma) protein G-agarose beads for 2 h at 4°C, washed extensively with 1% Triton X-100/150 mM NaCl/25
mM KCl/10 mM Tris-HCl, pH 7.4, with protease inhibitors (Sigma and Roche Diagnostics), and then deglycosylated with PNGaseF (New England Biolabs) for 2 h. Sample buffer was added, and the samples were boiled for 5 min at 95°C. Precipitates were resolved on a 4–20% gradient Tris-HCl precast gel (Bio-Rad), transferred to nitrocellulose, and the blots cut below the 75-kDa marker band to separately immuno blot for either D2 receptor (lower blot, monoclonal anti-D2R, Santa Cruz Biotechnology, 1:250 overnight at 4°C) or GASP (upper blot, 1:1000, overnight at 4°C). Immunoreactive bands were detected by using either HRP-conjugated anti-rabbit (GASP) or HRP-conjugated anti-mouse antibody (D2R) (NEB) (1:4,000), then visualized with ECL plus (Amersham Pharmacia).

Supporting Information. For further details, see Supporting Text and Figs. 5 and 6, which are published as supporting information on the PNAS web site.

Results
To directly examine the postendocytic fate of the dopamine D2 and D1 receptors, cell lines were generated that stably expressed epitope-tagged versions of the D2R or the D1R. Immunocytochemistry (Fig. 1A) and BPA (Fig. 1C) experiments (3, 12) demonstrated that both D2Rs and D1Rs were internalized after activation with dopamine (Fig. 1A and C, DA60). D2Rs were also internalized after activation by the small molecule agonist quinpirole (Q) (Fig. 1C Upper, Q60). The D2R antagonist haloperidol (H) inhibited both quinpirole- and dopamine-induced internalization of D2R, whereas the D1R antagonist SCH23390 (SCH) blocked dopamine-induced D1R internalization (Fig. 1B, compare with Fig. 1A Center). D1Rs were efficiently recycled to the plasma membrane after their endocytosis (Fig. 1A Lower Right, DA60 + SCH) and showed no apparent degradation, even after 3 h of agonist exposure (Fig. 1C Upper Middle and Fig. 5A; compare DA60 and DA180 lanes). In contrast, the internalized D2Rs did not return to the cell surface (Fig. 1A Upper Right, DA60 + H30) even in the

Fig. 3. D2Rs bind to GASP in the rat brain. (A) Rat brain. GASP (Upper) coimmunoprecipitates with the D2R (Lower) from rat brain. Increasing concentrations of D2R antibody (αD2R) immunoprecipitate increasing amounts of both D2R and GASP. L, rat brain lysate. B (beads), protein G agarose alone added. The arrow shows the position of the D2R signal. Upper band is IgG heavy chain. (B) GASP from rat brain interacts with the cytoplasmic tail of the D2R but not the D1R expressed as GST fusion proteins. (Upper) Protein eluted from GST resin that was immunoblotted for GASP. (Lower) The Coomassie stain of the input protein. L, brain lysate; B (beads), empty GST resin; G, empty GST protein; D1, GST-D1R c-tail fusion protein; D2, GST-D2R c-tail fusion protein. (C–H) GASP is localized in dopaminergic neurons of the VTA. (C) Coronal slices were stained by using affinity-purified anti-GASP antibody. (D) The GASP signal in C was blocked by preincubation of the antibody with purified GASP protein (block). (Scale bar, 50 μm.) (E and G) GASP (G) colocalizes with TH (E). GASP is distributed in both cell bodies and dendrites (white arrow). (F and H) D2Rs (H) colocalize with TH (F). D2Rs, like GASP, are also distributed on dendrites (white arrow). (Scale bar, 20 μm.) (I) Recovery of D2R responsiveness in the VTA. Slices were pretreated for 25 min with quinpirole (Q), then washed and allowed to recover for 35 min (washout). Quinpirole was then reapplied to monitor recovery of D2R function. D2R function did not recover. The GABAB agonist baclofen (Bacl) still elicited a response.
presence of antagonist, and the internalized receptors were substantially degraded after 3 h of exposure to dopamine or quinpirole (Figs. 1C, 1C–Upper and 5A). D2Rs and D1Rs retained their individual postendocytic fates even when coexposed in the same cells (Fig. 1C Lower Middle and Bottom). This finding suggests that the D2Rs but not D1Rs are targeted for degradation after receptor activation and internalization.

Several mechanisms have been described that contribute to down-regulation of membrane proteins (15, 16). Recently, GASP has been shown to be responsible for the postendocytic sorting of the δ-opioid receptor to the lysosome where it is degraded (3). Hence, we examined whether GASP might be responsible for lysosomal targeting and degradation of the D2Rs. We found that the dopamine D2Rs associate with GASP, whereas D1Rs have a substantially lower affinity for GASP as determined by commounprecipitation experiments in HEK293 cells (Fig. 2A, compare D1R and D2R lanes), suggesting that GASP could be responsible for D2R degradation. Consistent with this hypothesis, transient overexpression of the dominant negative form of GASP, cGASP, which we have previously shown competes with endogenous GASP for receptor binding (3), facilitated recycling of the D2R [Fig. 2C, compare cells with cGASP overexpression (gray stars) and without cGASP overexpression (white arrows)]. To quantify this effect, cells were generated that stably expressed D2R and stably overexpressed cGASP at levels >80-fold over endogenous GASP (Fig. 2B). Overexpression of cGASP delayed degradation of internalized D2Rs [Fig. 2E, compare quinpirole 90 min (Q90) and dopamine 120 min (DA120) in the two different cell lines]. Likewise, GFP-cGASP facilitated D2R recycling of the D2Rs as assessed by fluorescent microscopy (Fig. 2D) and BPA [Figs. 2E and 5B, compare dopamine 90 min plus haloperidol 30 min (DA90/H30) to dopamine 120 min (DA120) in the D2R-GFP-cGASP cell line]. Together, these data suggest that GASP mediates the postendocytic degradation of the D2R in HEK293 cells, and that disruption of the GASP-D2R interaction facilitates recycling of the D2R.

We then determined whether D2Rs interact with GASP in the rat brain. We found that GASP coimmunoprecipitated with the D2Rs from rat brain (Fig. 3A). In addition, GASP in a rat brain lysate also selectively interacted with the cytoplasmic tail of the D2R, but not the D1R expressed as a GST fusion protein (Fig. 3B), suggesting that the GASP-D2R interaction is mediated, at least in part, by the cytoplasmic tail of the D2R.

We next used patch-clamp electrophysiology to examine whether GASP modulated D2R responsiveness in the rat VTA. We found that dopamine D2Rs associate with GASP, whereas D1Rs have a substantially lower affinity for GASP as determined by commounprecipitation experiments in HEK293 cells (Fig. 2A, compare D1R and D2R lanes), suggesting that GASP could be responsible for D2R degradation. Consistent with this hypothesis, transient overexpression of the dominant negative form of GASP, cGASP, which we have previously shown competes with endogenous GASP for receptor binding (3), facilitated recycling of the D2R [Fig. 2C, compare cells with cGASP overexpression (gray stars) and without cGASP overexpression (white arrows)]. To quantify this effect, cells were generated that stably expressed D2R and stably overexpressed cGASP at levels >80-fold over endogenous GASP (Fig. 2B). Overexpression of cGASP delayed degradation of internalized D2Rs [Fig. 2E, compare quinpirole 90 min (Q90) and dopamine 120 min (DA120) in the two different cell lines]. Likewise, GFP-cGASP facilitated D2R recycling of the D2Rs as assessed by fluorescent microscopy (Fig. 2D) and BPA [Figs. 2E and 5B, compare dopamine 90 min plus haloperidol 30 min (DA90/H30) to dopamine 120 min (DA120) in the D2R-GFP-cGASP cell line]. Together, these data suggest that GASP mediates the postendocytic degradation of the D2R in HEK293 cells, and that disruption of the GASP-D2R interaction facilitates recycling of the D2R.

We next used patch-clamp electrophysiology to examine whether GASP modulated D2R responsiveness in rat brain. Dopaminergic neurons of the ventral tegmental area (VTA) were chosen for these studies. D2Rs are expressed on these dopamine projection neurons, where they function as autoreceptors to regulate dopamine release and neuronal firing (7, 8, 17). Importantly, during hyperdopaminergic conditions, D2R function in the VTA is reduced (7, 8), suggesting that there is a mechanism in this region that decreases D2R function. We hypothesized that reduced D2R function in this region during hyperdopaminergic conditions reflects the inability of D2Rs to recycle and resensitize to the presence of the agonist as a consequence of their interaction with GASP. Indeed, we found that GASP is expressed in dopamine cells of the VTA (Figs. 3C–H and 5C), suggesting that it could contribute to D2R sorting in these cells.

To examine whether GASP affected D2R function in the VTA, we performed whole-cell patch-clamp experiments in VTA dopaminergic neurons (see Materials and Methods). We then examined whether D2R responsiveness could resensitize after a prolonged exposure to agonist. Dopaminergic neurons were identified by the presence of an Ih current and large size of the soma (18). Cells were then exposed to the D2R agonist quinpirole (3 μM, Fig. 3I) or dopamine (75 μM, data not shown) for 25 min. Neurons showed a significant hyperpolarization during the first application of agonist attributable to Gβγ-mediated activation of G protein-gated inwardly rectifying K+ (GIRK) channels (19). After 35 min of washing in agonist-free artificial cerebrospinal fluid, neurons were rechallenged with 3 μM quinpirole to determine the degree of recovery of D2R function. D2Rs in slices pretreated with either quinpirole (Fig. 3J) or dopamine (data not shown) failed to recover, as evidenced by the lack of responsiveness to the second application of quinpirole (Fig. 3J). Treatment with 1 μM of the G1-coupled GABA B receptor agonist baclofen demonstrated that receptor-mediated modulation of GIRK channels was still present in these neurons, and that desensitization of D2Rs had not produced heterologous desensitization of GABA B receptors. These results suggest that D2Rs do not resensitize after exposure to agonist even after a prolonged recovery period. This finding is in contrast to other GPCRs, such as the μ opioid receptor, which has been shown to resensitize to agonist within 15 min (20), consistent with its ability to recycle after endocytosis (12).

We next determined whether disruption of the GASP-D2R interaction could affect D2R resensitization. The anti-GASP antibody efficiently disrupted binding of GASP to the D2Rs in vitro (Fig. 4A) (see Materials and Methods), and the anti-GASP antibody could be delivered into the VTA dopaminergic cell bodies (Fig. 4B) via the patch pipette. Thus, we were able to measure the effects of GASP on D2R resensitization by using this antibody to disrupt the
GASP–D2R interaction in neurons of the VTA. Neurons were filled with either control (n = 4) or anti-GASP (n = 6) antibody (Fig. 4C). Neurons filled with anti-GASP antibody showed a significant hyperpolarization upon application of quinpirole indistinguishable from that observed with control IgG (Fig. 4C, filled red circles and green triangles, respectively), suggesting that the anti-GASP antibody did not affect acute D2R function. In addition, the acute desensitization profiles were indistinguishable whether GASP antibody or IgG was added, suggesting disrupting the GASP–D2R interaction did not disrupt acute D2R desensitization. Importantly, in contrast to IgG-filled neurons, anti-GASP antibody-filled neurons showed significant hyperpolarization upon quinpirole rechallenge (Fig. 4C, filled red circles). The second response to quinpirole in the anti-GASP antibody-filled neurons was blocked by the D2R antagonist raclopride (Fig. 4C, open red circles, n = 4), demonstrating that the second quinpirole effect was mediated by the D2R. Baclofen responses were unaffected by treatment with dopamine or raclopride. Specifically, acute GABA<sub>B</sub> responses in naïve cells averaged 12.68 ± 1.2 mV (n = 5), whereas GABA<sub>B</sub> responses after dopamine alone (14.6 ± 1.47 mV, n = 4) or dopamine and raclopride (12.76 ± 3 mV, n = 4) were not significantly different from naïve cell responses. Thus, disruption of the GASP-D2R interaction in VTA neurons could facilitate recovery of functional D2 receptor responses.

Discussion

In summary, we have shown that D2Rs, in contrast to D1Rs, interact with the sorting protein GASP. As a consequence, D2Rs are targeted for degradation, unlike D1Rs that are recycled to the plasma membrane. In addition, disruption of the D2R–GASP interaction in the VTA dopaminergic neurons allows functional recovery of D2R receptor responses after agonist rechallenge. Dominant negative cGASP does not prevent internalization of the D2R (Fig. 2 C–E), nor does disruption of the GASP–D2R interaction alter the rapid desensitization of D2R in the VTA (Fig. 4C). However, disrupting the D2R–GASP interaction with the GASP antibody promotes resensitization of the D2R in the VTA (Fig. 4C), and overexpression of the dominant negative cGASP facilitates postendocytic recycling of the D2R in HEK293 cells (Fig. 2 C–E). Taken together, these data suggest that GASP functions postendocytically to sort the D2R to the degradative pathway.

These results have significant implications toward our understanding of dopaminergic signaling. In vivo, both the D1 and D2 dopamine receptors respond to the same agonist ligand, dopamine. However, they are coupled to G proteins with profoundly different signaling outcomes, with D1 receptors being coupled to the stimulatory G protein G<sub>olf</sub> and the D2 receptor being coupled to G<sub>i/o</sub>. As we have shown, D2Rs and D1Rs have different postendocytic fates. It follows, that although both the D2R and the D1R would internalize in response to dopamine, only the D2Rs would be targeted for degradation. This selective sorting would thereby leave the D1R-G<sub>i</sub> signaling unopposed during the next dopamine exposure. Consequently, a cell, synapse, or circuit that expressed both receptor subtypes would have an altered signaling profile depending on the extent of the previous dopamine exposure. Specifically, the D1 receptor Gs response would be predominating in situations of increased dopamine tone.

D2Rs are down-regulated in several disease states, including drug addiction. One might predict that one way to prevent D2R down-regulation could be through the use of D2R antagonists, which may block endocytosis of the D2R and thereby prevent their subsequent postendocytic degradation. Specifically, antagonists at the D2R inhibit endocytosis of the D2R when administered at high doses in our in vitro models. However, because 20,000 nM haloperidol and 5,000 nM raclopride used in the present in vitro experiments exceeds the therapeutic molarities of 1–4 nM haloperidol and 2–10 nM raclopride in the spinal fluid or water phase of the plasma in animals or treated patients, it will be important to determine whether the lower concentrations used clinically are effective in preventing endocytosis and postendocytic degradation of the D2R. Nevertheless, even if these antagonist drugs could prevent the down-regulation of D2Rs, their effectiveness could still be limited because D2Rs would not respond normally to their endogenous ligand, dopamine, in the presence of the antagonist drug. Thus, preventing down-regulation of D2Rs without blocking their function, perhaps by inhibiting their interaction with GASP, might provide an alternative means to stabilize D2R signaling.

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