Differential Regulation of Dopamine D1A Receptor Responsiveness by Various G Protein-coupled Receptor Kinases*

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The role of G protein-coupled receptor kinases (GRKs) in the regulation of dopamine D1A receptor responsiveness is poorly understood. To explore the potential role played by the GRKs in the regulation of the rat dopamine D1A receptor, we performed whole cell phosphorylation experiments and cAMP assays in 293 cells cotransfected with the receptor alone or with various GRKs (GRK2, GRK3, and GRK5). The agonist-dependent phosphorylation of the rat D1A receptor was substantially increased in cells overexpressing GRK2, GRK3, or GRK5. Moreover, we report that cAMP formation upon receptor activation was differentially regulated in cells overexpressing either GRK2, GRK3, and GRK5 under conditions that elicited similar levels of GRK-mediated receptor phosphorylation. Cells expressing the rat D1A receptor with GRK2 and GRK3 displayed a rightward shift of the dopamine dose-response curve with little effect on the maximal activation when compared with cells expressing the receptor alone. In contrast, cells expressing GRK5 displayed a rightward shift in the EC50 value with an additional 40% reduction in the maximal activation when compared with cells expressing the receptor alone. Thus, we show that the dopamine D1A receptor can serve as a substrate for various GRKs and that GRK-phosphorylated D1A receptors display a differential reduction of functional coupling to adenylyl cyclase. These results suggest that the cellular complement of G protein-coupled receptor kinases may determine the properties and extent of agonist-mediated responsiveness and desensitization.

Phosphorylation is an important mechanism involved in the regulation of numerous cellular responses, notably the responsiveness of G protein-coupled receptors (1). This phosphorylation process is believed to be the triggering mechanism that leads to receptor desensitization. The cellular responses elicited upon activation of G protein-coupled receptors are regulated in a dynamic fashion by the action of two classes of serine/threonine kinases. The first class consists of the second messenger-dependent kinases such as protein kinase A and protein kinase C (1). The second class consists of specific kinases that phosphorylate the agonist-occupied or activated form of the G protein-coupled receptors (1–3). These receptor kinases were originally described for rhodopsin (rhodopsin kinase) and the β2-adrenergic receptor (β2-adrenergic receptor kinase) and are referred to as the G protein-coupled receptor kinases or GRKs1 (1–3).

This large family of kinases includes six members (GRK1 to GRK6) whose activities are regulated by phospholipids, post-translational modifications, or G protein βγ subunits (2–6). The GRKs are widely distributed in brain and periphery, suggesting an important role in the regulation of responsiveness of various G protein-coupled receptors (2, 7). Moreover, Arriza et al. (7) have shown that β2-adrenergic receptor kinase 1 (GRK2) and β-adrenergic receptor kinase 2 (GRK3) are found in presynaptic and postsynaptic localizations in various brain regions consistent with a general role for these kinases in the desensitization of neuronal G protein-coupled receptors and their putative role in the regulation of neuronal activity. However, little information exists as to the specificity of the various kinases and as to whether phosphorylation of a given receptor by different kinases results in the same attenuation of the biological signals.

The recent advent of molecular biology techniques has allowed a better understanding of the underlying mechanisms of the dopaminergic neurotransmission. So far, five distinct genes encoding at least six dopamine receptors have been isolated and characterized (8, 9). Dopamine receptors belong to the G protein-coupled receptor superfamily. These dopamine receptors are grouped into D1- and D2-like receptors based upon their similarity at the amino acid level and their ability to couple to the activation (D1A/D1 and D1B/D5) or inhibition (D2Short, D2Long, D3, and D4) of adenylyl cyclase (8, 9). Many of the neurophysiological effects of dopamine in retina and brain are thought to be mediated through the activation of dopamine D1A receptor subtype (10–15). However, the mechanisms involved in the regulation of the D1A receptor responsiveness are poorly understood. Upon exposure to dopamine, D1A receptors have been shown to undergo a desensitization process as evidenced in cellular systems expressing endogenous D1A receptors or heterologous expression systems (16–20). Furthermore, Zhou et al. (21) demonstrated using a protein kinase A inhibitor and a GRK inhibitor (heparin) that D1A receptors, expressed endogenously in SK-N-MC cells, could undergo both protein kinase A- and GRK-mediated desensitization. Although a recent study has shown that D1A receptor overexpressed in Sf9 cells can undergo agonist-dependent desensitization, which was associated with weak receptor phosphorylation (22), a convincing demonstration of a role for direct phosphorylation of the receptor in this process remains to be

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The abbreviations used are: GRK, G protein-coupled receptor kinase; HA, hemagglutinin.
clearly established. Most of the regulation studies performed previously in cellular systems have been limited by the low levels of receptor density (16–20). To avoid this difficulty, we have overexpressed the receptor alone or with various GRKs using a heterologous expression system to investigate the potential role of the GRK pathway in the phosphorylation and desensitization of the dopamine D1A receptor as well as to examine the biochemical and biological specificity of various GRKs. Our results indicate that the agonist-occupied form of the D1A receptor can serve as a substrate for a variety of GRKs. Moreover, we show that receptor phosphorylation by specific members of the GRK family leads to distinct desensitization patterns of dopamine responsiveness.

**EXPERIMENTAL PROCEDURES**

*Expression Constructs—*The dopamine rat D1A (rD1A) receptor was modified using oligo-directed mutagenesis to include the sequence for the hemagglutinin (HA) epitope recognized by a commercially available monoclonal antibody 12CA5 (23). The nine-residue sequence of the epitope (YPYDVPDYA) was inserted after Ala2, and this residue was modified using oligo-directed mutagenesis to include the sequence for the hemagglutinin (HA) epitope recognized by a commercially available monoclonal antibody 12CA5 (23). The nine-residue sequence of the epitope (YPYDVPDYA) was inserted after Ala2, and this residue was modified using oligo-directed mutagenesis to include the sequence for the hemagglutinin (HA) epitope recognized by a commercially available monoclonal antibody 12CA5 (23). 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taining 5% (v/v) fetal bovine serum, gentamicin, and labeled with [3H]adenine (1.5 μCi/ml) for 18–22 h. Determinations of intracellular cAMP were assessed by incubating cells in 10 mM HEPES-buffered minimum essential medium (with no phosphodiesterase inhibitors) in the absence or presence of dopamine at 37°C for various periods of time (26). Assays were terminated by transferring dishes on ice, aspirating the medium, and adding 1 ml of stop solution (2.5% (v/v) perchloric acid, 100 μM cAMP, and ~10,000 cpm of [3H]cAMP. After 20–30 min in the cold, the acid-cell lysates were transferred to tubes containing 0.1 ml of neutralizing solution (4.2 M KOH). The salt precipitates were pelleted by centrifugation, and separation of [3H]cAMP in the cell lysate supernatants was done using a sequential chromatography on Dowex and alumina columns. Data are presented as 1000 times the ratio [3H]cAMP formed over the total uptake measured in the well.

Data Analysis—Radioligand binding and dose-response curves were analyzed using the curve-fitting programs ALLFIT and LIGAND (32, 33). Statistical analysis of data were performed using analysis of variance. Pairwise comparisons were assessed either by the t distribution test or GT2-method (34). Homogeneity of variances was assessed by the Fmax test. The level of significance was established at 5% using one-tailed test. Results are expressed as the mean ± S.E.

Materials—Human embryonic kidney cells (293) were obtained from American Tissue Culture Collection (CRL 1573). Tissue culture reagents were from Life Technologies, Inc. [3H]Adenine, [14C]cAMP, and 32Pi were purchased from DuPont NEN. Dopamine-HCl and flupentixol-HCl were from Research Biochemical International. Protease inhibitors were obtained from Sigma. Nonidet P-40 was purchased from Calbiochem. SANPAH was purchased from Pierce. Biotin-XX succinimidyl ester probe was procured from Molecular Probes. SCH39111 was obtained from Schering-Plough Corp.

RESULTS

Molecular and Biochemical Characterization of the Hemagglutinin Epitope-tagged Dopamine D1A Receptor—Using polymerase chain reaction-based methodology, we have engineered the sequence coding for the hemagglutinin epitope (HA) in the amino terminus of the rat dopamine D1A (rD1A) receptor. To verify that the receptor function was not impaired by the insertion of the epitope, we characterize the binding and coupling properties of the HA-rD1A receptor. Saturation studies revealed that equilibrium dissociation constant (Kd) of the antagonist 125I-SCH39111 for 293 cells expressing either the wild-type or the HA-rD1A receptor was similar (0.51 ± 0.06 μM versus 0.46 ± 0.05 μM, respectively). We subsequently investigated the binding properties of agonists and antagonists. The affinity constants (Kd) of the agonist dopamine and the antagonist were identical at both the wild-type or HA-tagged receptor (data not shown). These results suggest that insertion of HA in the amino terminus does not affect the binding properties of agonists and antagonists displayed at the wild-type rD1A receptor.

The rD1A receptor has been shown to be coupled to the activation of adenyl cyclase when expressed in 293 cells (26). To investigate the coupling properties of the HA-rD1A receptor, dose-response curves to dopamine were performed at different time points (2, 5, and 10 min) using a whole cell cAMP assay in the absence of phosphodiesterase inhibitors. The wild-type or HA-rD1A receptor display similar dose-response curves to dopamine at all time points investigated (Fig. 1). Over the time course studied, at similar receptor expression levels for both forms of the receptor, the maximal activation of adenyl cyclase (Vmax) increased to similar extent, while the basal activity was not statistically different. As depicted in Fig. 1, the effective concentration (EC50) measured for the HA-rD1A receptor was not statistically different from the wild-type receptor (~15 nM).

To determine the apparent molecular weight of the HA-rD1A receptor protein expressed in 293 cells, we performed photoaffinity cross-linking experiments using 125I-SCH39111 (27). As shown in Fig. 2A, the HA-rD1A receptor is expressed at the plasma membrane of 293 cells as a broad protein band of about 80 kDa, an electrophoretic mobility similar to that of the wild-type rD1A receptor. These results suggest that insertion of the epitope in the amino terminus does not interfere with the glycosylation of the D1A receptor. This photoaffinity labeling was specific to the expression of the wild-type or tagged receptor, since no detectable labeling was observed in mock-transfected cells (Fig. 2A). The post-translational modification of the human homologue of the D1A receptor expressed in 293 cells was identical to its rat counterpart (data not shown).

To test the ability of the monoclonal antibody 12CA5 to immunoprecipitate the HA-rD1A receptor, 293 cells expressing the tagged receptor or pCMV/βGal (mock) were conjugated with a succinimidyl ester probe (biotin-XX), solubilized, and subjected to immunoprecipitation as described under “Experimental Procedures.” Dose-response curves were performed with increasing concentrations of dopamine and exposed for 2, 5, and 10 min. Each point represents the mean of two independent experiments. Curves were fitted using the ALLFIT program (32). Determinations of EC50 values at each stimulation times for either the wild type or HA-tagged rD1A receptor were not statistically different (Wild type, EC50 = 15.5 ± 0.9 nM; HA, 15.6 ± 1.5 nM). Maximal activation values for the wild type (, 7.6 ± 0.2; , 9.9 ± 0.2; , 10.4 ± 0.2) and HA-tagged (, 7.6 ± 0.2; , 9.9 ± 0.2; , 11.6 ± 0.2) receptors were increased significantly over the time course studied. Expression levels for the wild type and HA-tagged were 10.6 and 11.1 pmol/mg of membrane protein, respectively.

A

Phosphorylation and Desensitization of Dopamine D1A Receptor

Fig. 1. Time course of dopamine-mediated activation of adenyl cyclase in 293 cells transiently transfected with rat dopamine D1A receptor. A, Wild-type receptor; B, HA-tagged receptor. Whole cell cAMP assays were performed as described under “Experimental Procedures.” Dose-response curves were performed with increasing concentrations of dopamine and exposed for 2, 5, and 10 min. Each point represents the mean of two independent experiments. Curves were fitted using the ALLFIT program (32). Determinations of EC50 values at each stimulation times for either the wild type or HA-tagged rD1A receptor were not statistically different (Wild type, EC50 = 15.5 ± 0.9 nM; HA, 15.6 ± 1.5 nM). Maximal activation values for the wild type (, 7.6 ± 0.2; , 9.9 ± 0.2; , 10.4 ± 0.2) and HA-tagged (, 7.6 ± 0.2; , 9.9 ± 0.2; , 11.6 ± 0.2) receptors were increased significantly over the time course studied. Expression levels for the wild type and HA-tagged were 10.6 and 11.1 pmol/mg of membrane protein, respectively.

To investigate the potential role of GRK-mediated phosphorylation in the regulation of the dopamine D1A recep-
Phosphorylation and Desensitization of Dopamine D1A Receptor

FIG. 2. Photoaffinity cross-linking and biotinylation of the HA-rD1A receptor expressed transiently in 293 cells. A, photoaffinity cross-linking experiments were performed in membranes prepared from 293 cells transfected with β-galactosidase alone (MOCK), HA-tagged rD1A (HA-rD1A) or wild-type receptor (WT-rD1A). Membranes were incubated with 125I-SCH39111 in the absence or presence of 10 μM flupentixol (FLU) as described under “Experimental Procedures.” No detectable binding was measured in mock transfected cells, whereas cells harboring HA-rD1A or wild type receptor expressed 5.6 and 6.5 pmol/mg of membrane protein, respectively. Shown is a representative example of an experiment repeated 3 times. B, cells were transfected with β-galactosidase alone (M) or HA-tagged rD1A receptor (HA), biotinylated, solubilized, and immunoprecipitated using the purified monoclonal antibody 12CA5 as described under “Experimental Procedures.” Immunocomplexes were resolved by SDS-polyacrylamide gel, transferred to nitrocellulose membrane, and blotted with streptavidin-horseradish peroxidase conjugate. The expression level for cells harboring the HA-rD1A receptor was 9.4 pmol/mg of membrane protein.

FIG. 3. Time course of agonist-dependent phosphorylation of HA-rD1A receptor overexpressed alone or with various GRKs in transiently transfected 293 cells. Cells transfected with the HA-rD1A receptor alone (β-galactosidase (βGAL)) or with GRK2, GRK3, or GRK5 were labeled with 32P (0.2 μCi/ml) for 90 min. The cells were then treated with or without 10 μM dopamine for various periods of time. The phosphorylated receptors were solubilized and immunoprecipitated as described under “Experimental Procedures.” The amount of receptor phosphorylation was quantitated by PhosphorImager, and data were expressed as percentage above control basal (as measured in cells transfected with HA-rD1A receptor and βGAL incubated in the absence of agonist). Each curve represents the mean of three to four independent experiments. The receptor expression obtained under the transfection conditions were similar and as follows: β-galactosidase, 11.2; GRK2, 11.6; GRK3, 10.0; and GRK5, 5.2 pmol/mg of membrane protein.

Overexpression of GRKs Diminishes the Dopamine D1A Receptor Activation of Adenyl Cyclase—To assess the functional importance of the GRK-mediated phosphorylation of the D1A receptor, we assessed the accumulation of cAMP in intact cells expressing the receptor alone or with different GRKs (Fig. 5). The basal adenyl cyclase activity of cells harboring the receptor alone or co-transfected with different GRKs was not significantly modified under the same experimental conditions (Fig. 5A). However, a time course of intracellular cAMP accumulation reveals that activation of the D1A receptor by 10 nM dopamine (EC50 dose) was dampened in cells overexpressing different GRKs at each time point studied (Fig. 5B). A time course of the intracellular cAMP formation elicited by stimulation of the HA-rD1A receptor using a maximal concentration of dopamine (10 μM) was not modified in cells overexpressing GRK2 or GRK3 (Fig. 5C). Interestingly, however, cells overexpressing GRK5 display a significant blunting of the adenyl cyclase response under these conditions (Fig. 5C). These results suggest that phosphorylation of the D1A receptor by various GRKs leads to functional uncoupling from the Gs protein as evidenced by a decrease in the rate and extent of adenyl cyclase activation. Moreover, at a high concentration of dopamine, the rate and extent of adenyl cyclase activation appears to be affected only in those cells overexpressing the GRK5.

To further elucidate the differences in the regulation of the dopamine D1A receptor responsiveness by GRK2, GRK3, and GRK5, cells expressing similar levels of the HA-rD1A receptor either alone or with these kinases were stimulated with increasing concentrations of dopamine for 5 min. Under these conditions, cells expressing the receptor alone or with GRK2 displayed a significant blunting of the adenyl cyclase activity. Moreover, a time course of the intracellular cAMP formation elicited by the maximal concentration of dopamine reveals that overexpression of GRK5 leads to functional uncoupling from the Gs protein as evidenced by a decrease in the rate and extent of adenyl cyclase activation.
experimental conditions, dopamine elicited a dose-response curve in cells expressing the receptor alone with an EC\textsubscript{50} of 23 nM with a maximal stimulation of 10–15 fold above basal activity (Fig. 6A). In cells expressing either GRK2 or GRK3, dose-response curves display a statistically significant 3- and 7-fold rightward shift in the EC\textsubscript{50} with values corresponding to 68 nM and 157 nM for GRK2 and GRK3, respectively (Fig. 6A). Expression of GRK2 or GRK3, however, had no significant effect on the fold activation or V\textsubscript{max} (Fig. 6A). In contrast to the GRK2 and GRK3 situation, cells overexpressing the GRK5 displayed the most striking change in the HA-D1A receptor responsiveness depicted by a marked decrease in the maximal stimulation of intracellular cAMP (Fig. 6A). A significant 2-fold rightward shift in the EC\textsubscript{50} for dopamine was also observed (Fig. 6A). Concomitant to these dose-response curves, phosphorylation experiments were performed in the same transfected cells used for the whole cell cAMP assay. Interestingly, stimulation of the HA-rD1A receptor by 10 \mu M dopamine for 5 min leads to a similar amount of the agonist-induced receptor phosphorylation by all the GRK isoforms utilized (Fig. 6B). This suggests that functional differences observed for the D1A receptor responsiveness cannot be explained by differences in the extent of receptor phosphorylation.

**DISCUSSION**

In this report we demonstrate that the agonist-occupied form of the D1A receptor can serve as a substrate for various GRKs. Phosphorylation of the D1A receptor by these GRKs leads to a diminished ability of the receptor to increase intracellular cAMP levels in response to dopamine. For an equivalent extent of phosphorylation of the D1A receptor by various kinases, the attenuation of responsiveness appears to be more pronounced following phosphorylation by GRK5 than GRK2 or GRK3. These results provide evidence that specificity of action can be demonstrated between G protein-coupled receptors and GRKs.

Direct Phosphorylation of Dopamine D1A Receptor by GRKs Regulates Signaling Function—Previous evidence for a regulatory role of phosphorylation in the function of the dopamine D1A receptor has come mostly through indirect means. Zhou et al. (21) used kinase inhibitors to imply a role for protein kinase A- and GRK-mediated phosphorylation of the D1A receptor in the process of agonist-mediated desensitization. In the latter
and other desensitization studies, direct phosphorylation of the D1A receptor could not be demonstrated presumably due to low levels of receptor expression in the various systems used (16–20). To circumvent these difficulties, we have overexpressed an epitope-tagged D1A receptor in 293 cells co-transfected with various GRKs.

In 293 cells, the HA-D1A receptor behaves identically to the wild-type receptor. In agreement with the observation that 293 cells contain low levels of endogenous GRKs (30), only a very weak agonist-mediated desensitization of the signal is observed in cells overexpressing the D1A receptors (data not shown). These findings correlate with the low agonist-mediated increase (~50%) in phosphorylation of the D1A receptor in the absence of exogenous kinases (Fig. 4C). In contrast, overexpres-

**Fig. 5.** Time course of dopamine-mediated adenyl cyclase activation in 293 cells overexpressing the HA-rD1A receptor and various GRKs. A, basal; B, 10 nM dopamine; C, 10 μM dopamine. Whole cell cAMP accumulation was measured following exposure to 0.1 mM ascorbate (basal) or dopamine (DA) for 2, 5, and 10 min. Data are presented as the mean of three independent experiments done in triplicate determinations. Receptor expression for each of the experimental procedures were as follows: β-galactosidase (βGAL), 6.7; GRK2, 5.6; GRK3, 5.2; and GRK5, 5.1 pmol/mg of membrane protein.
fected D1A receptor by the various GRKs supports a role of phosphorylation in regulating the functional state of the D1A receptor.

Distinct Modulation of D1A Receptor Responsiveness Suggests Specificity of GRK Actions—Phosphorylation of the D1A receptor by different GRKs resulted in attenuation of responsiveness with distinguishing characteristics. Indeed, phosphorylation of the D1A receptor by GRK5 afforded a dramatic attenuation of response with both a shift in the EC50 for dopamine and a marked 40% decrease in maximum response. In contrast, GRK2- and GRK3-mediated receptor phosphorylation led to significant rightward shifts of the dose-response curves for dopamine with no change in the maximal response (Fig. 6A). Therefore, GRK5 phosphorylation of the D1A receptor engenders a more profound desensitization than phosphorylation by either GRK2 or GRK3. These differences could not be attributed to differences in the extent of phosphorylation of the D1A receptor as similar levels of phosphorylation were achieved with all three kinases. Taken together, these results suggest that the D1A receptor responsiveness can be regulated differentially according to the GRK subtype expressed in a particular cell. What possible underlying mechanisms could explain such differential modulation of the D1A receptor function by the various GRKs?

First, these results might be explained by the existence of distinct GRK phosphorylation sites located within the cytoplasmic domains of the D1A receptor. Indeed, it is possible that phosphorylation of distinct GRK sites leads to different conformational changes of the phosphorylated D1A receptor, which may potentially display differences in their ability to activate adenyl cyclase. Despite the large amount of evidence for the phosphorylation of G protein-coupled receptors by GRKs, very little is known about the exact nature of the phosphorylation sites for the various characterized GRKs. Distinct specificities have been demonstrated for β-adrenergic receptor kinase 1 (GRK2) and GRK5 using peptide substrates (36, 37). Thus, phosphorylation of distinct sites by various GRKs could result in different extent of attenuation of the response. It is interesting to note that, although GRKs are serine/threonine kinases, of the 22 serine and 14 threonine residues present in the cytoplasmic domains of the D1A receptor, only serine residues appear to be phosphorylated (Fig. 4D). Further studies using purified and GRK-phosphorylated D1A receptor will be required to determine the exact nature of the sites phosphorylated by GRK2, GRK3, and GRK5.

Second, the distinct modulation of D1A receptor responsiveness by the different GRKs might be explained by the potential role played by arrestin-like proteins, which have been demonstrated to be essential for the full extent of receptor desensitization for β-adrenergic receptor kinase 1 (GRK2) (3) and rhodopsin kinase (GRK1) (38). Meanwhile, no such data exist for GRK5-mediated desensitization. Under normal conditions, the levels of arrestin proteins are unlikely to be limiting (39); however, under conditions of overexpression of G protein-coupled receptors in heterologous systems, kinase and arrestin proteins may become limiting (40). Thus, the absence of a diminished Vmax in cells overexpressing GRK2 and GRK3 in our studies might be explained by a limiting level of arrestin proteins. However, this raises the intriguing question about the potential role arrestin proteins play in GRK5-mediated desensitization of the D1A receptor. GRK5 belongs structurally to a different subfamily of kinases than GRK1, GRK2, and GRK3 (2, 41), and therefore receptor desensitization by GRK5 may be potentially elicited independent of the binding of arrestin proteins. In addition, several forms of arrestin proteins have been isolated (3), and it is interesting to speculate that different phosphorylated sites may provide different interaction sites for the various arrestin proteins. Further studies are required to establish the precise role of arrestin proteins in the modulation of D1A receptor function upon its phosphorylation by GRKs. Finally, it is worth mentioning that these effects were observed in whole cell preparations, and therefore we cannot rule out that GRK5 also regulates a downstream effector important for D1A receptor signaling. Studies performed using added GRKs to membranes expressing D1A receptors may help to elucidate potentially these different effects (42).

Regardless of the basis for the observed differences, our data document that under identical conditions, the effect of these various kinases can be significantly different (i.e. selectivity of action exists).

Previously, selectivity, or the lack thereof, in the ability of the different GRKs for phosphorylating different receptors has been documented. Thus, rhodopsin is a better substrate for GRK1 than GRK2 (43, 44); β2-adrenergic and m2-muscarinic receptors are better substrates for β-adrenergic receptor kinase 1 (GRK2) than GRK5 (37, 42), whereas the β2-adrenergic receptor appears to be as effectively phosphorylated by either GRK2, GRK3, or GRK5 (50). The dopamine D1A receptor represents yet a different type of selectivity in that the receptor appears to be covalently modified by the various kinases to a similar extent, but the biological consequence of that phosphorylation (i.e. desensitization) differs.

Physiological Relevance of D1A Receptor Phosphorylation—The demonstration of a role for the GRKs in regulating D1A receptor responsiveness in a heterologous mammalian expression system raises the issue of the physiological relevance of the differential modulation of D1A receptor function by GRK2, GRK3, and GRK5. Cellular co-localization of the D1A receptor (or any other G protein-coupled receptors) with different GRKs is currently unknown. However, in situ hybridization and immunohistochemistry studies have shown that GRK2 and GRK3 are expressed in brain regions that have been shown to contain D1A receptors (7). In addition, Arriza et al. (7) have shown that GRK2 and GRK3 appear to be associated with pre-synaptic and more predominantly postsynaptic localizations in various brain regions, consistent with a putative role of these two GRKs in the desensitization of synaptic G protein-coupled receptors. Moreover, in support of a physiological relevance for D1A receptor regulation by GRK5, the GRK5 mRNA has been found in cortex and in retina that also express the D1A receptor (24, 45). Recently, it has been shown that exposure of D1A receptors to dopaminergic agonists leads to a greater desensitization of the D1A receptor in the retina than in the striatum (46). Thus, it would appear that regulation of D1A receptor function in these two tissues is different, and our results may provide the molecular and biochemical basis for this observation.

Studies using cellular systems have helped to delineate the molecular events involved in G protein-coupled receptor regulation (39). Several transgenic studies have now established the relevance of these mechanisms in various physiological situations (47, 48). Mice overexpressing carboxyl-terminal truncated rhodopsin, which lack the GRK1 phosphorylation sites, display abnormal prolonged flash responses, suggesting that phosphorylation of rhodopsin is essential for turnoff of the light signal in vivo (47). In addition, transgenic mice overexpressing GRK2 specifically in the heart display a reduced cardiac function as measured by a diminution of isoproterenol-stimulated left ventricular contractility, myocardial adenyl cyclase activity, and decreased functional coupling of β-adrenergic receptors (48). Recent studies have also shown that levels and activities of GRKs can be modulated by physiological or pharmacological situations that modulate the levels of hor-
monal or neuronal input (40, 49). Since the D1A receptor mediates several behavioral paradigms and responses to psychostimulants, the regulation of its function by GRK-dependent events is a question of interest that will require further investigation of the underlying mechanisms possibly using genetically altered animals. The present study illustrates the functional importance the multiplicity of GRKs may play in regulating receptor responsiveness in these various physiological situations.

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