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Sensitization of Endogenous and Recombinant Adenylate Cyclase by Activation of D_2 Dopamine Receptors

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SUMMARY

Prolonged stimulation of G_i-coupled receptors often sensitizes adenylate cyclase to subsequent activation by forskolin or G_s-coupled receptors. To identify mechanisms of heterologous sensitization, we characterized sensitization of cAMP accumulation that was induced by activation of recombinant dopamine D_2 receptors expressed in C_6 glioma and human embryonic kidney (HEK)293 cells. Pretreatment with dopamine or other agonists for 2 hr induced heterologous sensitization that was blocked by the D_2 antagonist spiperone but not by the β-adrenergic receptor antagonist propranolol. Sensitization was evident after 15 min of treatment with dopamine and persisted for at least 2 hr after washout. The EC_{50} value for sensitization by dopamine in HEK-D_{2L} cells was 100 nM, ~80-fold higher than the IC_{50} value for dopamine inhibition of cAMP accumulation. The D_2 receptor agonists quinpirole, 7-hydroxy-dipropylaminotetralin, and pergolide also induced sensitization, whereas the high affinity ergot agonists bromocriptine and lisuride did not. Stimulation of either D_{2L} or D_{2S} receptors sensitized cAMP accumulation to similar extents, but stimulation of D_3 receptors did not. In C_6-D_{2L} cells, sensitization of isoproterenol-stimulated activity was manifested as a >100% increase in maximal response, with no change in potency. In contrast, the potency for forskolin-stimulated activity was increased 4-fold, with no apparent change in maximal response. Overnight treatment with pertussis toxin (25 ng/ml) had little effect on isoproterenol or forskolin activation of adenylate cyclase *per se* but prevented D_2 receptor-mediated sensitization in both C_6-D_{2L} and HEK-D_{2L} cells, indicating an involvement of one or more of the pertussis toxin-sensitive G proteins, G_i/G_o. D_2 receptor stimulation also sensitized type I and type II adenylate cyclases, each expressed in HEK293 cells together with D_{2L} dopamine receptors. Rapid D_2 receptor-mediated heterologous sensitization may be the result of enhanced interaction of G_s with adenylate cyclase and may represent a novel mechanism for modulation of neural activity by D_2 receptors.

The enzyme adenylate cyclase (EC 4.6.1.1) is highly regulated by receptors coupled to both stimulatory (G_s) and inhibitory (G_i) G proteins (1). Receptors that couple to G_s enhance adenylate cyclase activity, thus stimulating the accumulation of cAMP, whereas activation of G_i-coupled receptors decreases the synthesis of cAMP. Considering the multiplicity of receptors present on any given cell type, it is of basic importance to understand how cells integrate multiple stimuli that may differ both temporally and chemically. Multiple studies indicate that long term (hours to days) stimulation of G_i-coupled receptors results in the sensitization of adenylate cyclase to subsequent stimulation (2–5). Sensitization may be one mechanism by which cells adapt to prolonged inhibition of cAMP synthesis. Such an adaptive mechanism may play a role in narcotic addiction and withdrawal, as well as hypertensive crises after clonidine withdrawal (6). Long term treatment with G_i-coupled receptor agonists alters the expression of both G_s-coupled receptors (7) and $G_{\alpha i}$ subunits (8, 9). The nature of these changes depends upon receptor and cell type but often involves changes in steady state levels of mRNA for receptors and G proteins (5, 10).

Short term (≤2 hr) stimulation of G_i-coupled receptors can also result in sensitization of adenylate cyclase activity (11–14). In contrast to long term sensitization, short term heterologous sensitization may be independent of changes in gene expression (5) and most likely involves changes in the phosphorylation and subcellular localization of proteins. Pretreatment of HT 29 cells with α_2-adrenergic receptor agonists that inhibit adenylate cyclase sensitizes both forskolin- and vasoactive intestinal peptide-stimulated cAMP accumulation (13). Sensitization occurs rapidly (within ~5 min) and is

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ABBREVIATIONS: D_{2S} receptor, short (415-amino acid) form of D_2 receptor; D_{2L} receptor, long (444-amino acid) form of D_2 receptor; HEK, human embryonic kidney; 7-OH-DPAT, 7-hydroxy-dipropylaminotetralin HBr; CBS, calf bovine serum; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PKA, protein kinase A; PMA, phorbol-12-myristate-13-acetate; SCH 23390, 7-chloro-8-hydroxy-3-methyl-1-2,3,4,5-tetrahydro-1*H*-3-benzazepine HCl.

antagonized by α_2-adrenergic receptor antagonists. The rapid sensitization is blocked by pertussis toxin but not by several modulators of other second messenger systems. Port *et al.* (14) reported that pretreatment of DDT_1MF-2 cells expressing A1 adenosine receptors with A1 agonists for 60 min enhances isoproterenol-stimulated adenylate cyclase activity and that the enhancement is blocked by activators of PKA. In contrast to the report by Jones and Bylund (13), there was no change in forskolin-stimulated adenylate cyclase activity. These observations highlight the biochemical differences in agonist-induced sensitization in two different cell systems.

Long term (>8-hr) treatment of both D_{2S} and D_{2L} dopamine receptors expressed in cultured cells enhances subsequent basal and forskolin-stimulated adenylate cyclase activity (4, 15–17). We recently reported that short term (2-hr) stimulation of D_2 receptors also results in sensitization of isoproterenol-stimulated cAMP accumulation, whereas stimulation of D_3 receptors does not (18). Long term sensitization can be readily explained by changes in receptor and G protein levels within the cell, but cellular correlates of short term sensitization of adenylate cyclase activity have been elusive. The purpose of the present study was to characterize possible mechanisms for short term D_2 agonist-induced sensitization in cultured cells expressing D_{2S}, D_{2L}, and D_3 dopamine receptors, as well cells expressing D_{2L} dopamine receptors in combination with type I or type II adenylate cyclase. We now report that several D_2 dopamine receptor agonists sensitized adenylate cyclase in C_6-D_{2L}, C_6-D_{2S}, and HEK-D_{2L} cells but not in cells expressing D_3 receptors. This heterologous sensitization was blocked by the D_2 antagonist spiperone and by pertussis toxin (25 ng/ml, overnight). In contrast, other modulators of several second messenger systems were unable to alter D_2 agonist-mediated sensitization. Stimulation of D_{2L} receptors enhanced the activity of both type I and type II adenylate cyclases, suggesting a common mechanism of sensitization via G_s.

Experimental Procedures

Materials. [^{3}H]Spiperone was purchased from Amersham (Arlington Heights, IL), and [^{3}H]cAMP from DuPont-New England Nuclear (Boston, MA). Spiperone, quinpirole, 7-OH-DPAT, bromocriptine, lisuride, and forskolin were purchased from Research Biochemicals International (Natick, MA). Pergolide (Lilly) and rat D_{2S} and D_{2L} cDNAs (Dr. O. Civelli, Oregon Health Sciences University) were generous gifts. Dopamine (3-hydroxytyramine) and most other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Production of cell lines. The production of C_6 cells expressing D_{2S}, D_{2L}, and D_3 receptors has been described previously (18, 19). Transfection of HEK-D_{2L} cells was carried out by electroporation (0.17 kV, 950 μF, 0.4-cm cuvette gap). HEK293 cells (8×10^6) were resuspended in DMEM, supplemented with 10% CBS and 5 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, in a total volume of 400 μl combining pcDNA1-D_{2L} cDNA (15 μg) with pBabe Puro (2 μg), to confer resistance to puromycin (20). HEK293 cells expressing types I and II adenylate cyclase were obtained from Dr. Daniel Storm and Mark Nielsen (University of Washington) and were transfected with pcDNA1-D_{2L} as described above, creating the cell lines ACI/D2L and ACII/D2L, respectively.

Cell culture. C_6-D_{2S}, C_6-D_{2L}, and C_6-D_3 cells were maintained in DMEM supplemented with 3% FBS, 2% CBS, 50 units/ml penicillin/50 μg/ml streptomycin, and 2 μg/ml puromycin. HEK-D_{2L} cells were maintained in DMEM supplemented with 5% FBS, 5% CBS, penicillin/streptomycin, and 2 μg/ml puromycin. ACI/D2L and ACII/D2L cells were maintained in DMEM supplemented with 5% FBS, 5% CBS, penicillin/streptomycin, 2 μg/ml puromycin, and 460 units/ml hygromycin. Cells were grown in a humidified incubator at 37°, in the presence of 10% CO_2.

cAMP accumulation assays. Cells were plated at concentrations between 100,000 and 150,000 cells/well in 48-well tissue culture clusters. Confluent cells were washed with 200 μl of assay buffer (Earle's balanced salt solution containing 0.02% ascorbic acid and 2% CBS) and used for sensitization, desensitization, or inhibition experiments. For sensitization and desensitization experiments, cells were routinely preincubated for 2 hr in the presence of drugs at 37° in a humidified incubator with 10% CO_2, and then washed three times for 3–4 min each with 200 μl of assay buffer. In sensitization experiments, forskolin (10 μM), isoproterenol (1 μM), A23187 (10 μM), or PMA (100 nM) was then added in the presence of 1 μM spiperone to preclude acute effects of D_2 dopamine receptor activation by residual agonist. The inclusion of spiperone in the stimulation assay was required because residual agonist often resulted in biphasic sensitization dose-response curves, in which increases in the concentration of D_2 receptor agonist during the pretreatment resulted in inhibition rather than sensitization of cAMP accumulation (data not shown). Such biphasic curves were rarely observed when stimulated cAMP accumulation was assessed in the presence of spiperone. Biphasic dose-response curves have been observed by several others examining sensitization of adenylate cyclase by G_i-coupled receptors (21–23). The incubation with spiperone and isoproterenol, forskolin, A23187, or PMA was carried out for 15 min at 37°. In desensitization experiments, after dopamine (1 μM) pretreatment and extensive washing, the cells were used for cAMP inhibition experiments as described below, except that there was no 10-min preincubation. For inhibition experiments, cells were preincubated with 200 μl of assay buffer for 10 min and placed on ice. Increasing concentrations of D_2 agonists were added to wells and then 10 μM forskolin (HEK-D_{2L} cells) or 1 μM isoproterenol (C_6-D_{2L} cells) was added. Incubations were carried out for 10 min at 37°. For all experiments, the medium was removed after the appropriate incubation time and the cells were placed on ice and lysed with 3% trichloroacetic acid. The 48-well plates were then centrifuged at $1000 \times g$ for 15 min and stored at 4° for at least 1 hr.

Quantification of cAMP. cAMP was quantified using a competitive binding assay adapted, with minor modifications, from the method of Nordstedt and Fredholm (24). Duplicate samples of the cell lysate (10–20 μl) were added to reaction tubes containing cAMP assay buffer (100 mM Tris·HCl, pH 7.4, 100 mM NaCl, 5 mM EDTA). [^{3}H]cAMP (1 nM final concentration) was added to each tube, followed by cAMP-binding protein (~100 μg in 200 μl of cAMP buffer). The reaction tubes were incubated on ice for 3 hr. The tubes were then harvested by filtration (Whatman GF/C filters) using a 96-well Tomtec cell harvester (Orange, CT). Filters were allowed to dry, and 50 μl of BetaPlate scintillation fluid was added to each sample. Radioactivity on the filters was determined using an LKB/Wallac BetaPlate scintillation counter (Gaithersburg, MD). cAMP concentrations in each sample were estimated in duplicate from a standard curve ranging from 0.1 to 100 pmol of cAMP/assay.

Radioligand binding assays after agonist treatment. Cells in 10-cm-diameter plates or six-well tissue culture clusters were washed with assay buffer (Earle's balanced salt solution containing 0.02% ascorbic acid and 2% CBS). Intact cells were treated with drugs and then washed as described for sensitization/desensitization experiments. To harvest, cells were lysed with ice-cold hypotonic buffer (1 mM Na^+-HEPES, pH 7.4, 2 mM EDTA). After swelling for 10–15 min, the cells were scraped from the plate and centrifuged at $24,000 \times g$ for 20 min. The resulting crude membrane fraction was resuspended in Tris-buffered saline with a Brinkman Polytron homogenizer (Westbury, NY), at setting 6 for 10 sec, and used for radioligand binding assays. The binding of [^{3}H]spiperone was as-

sessed essentially as described (19). Aliquots of the membrane preparation (3–40 μg of protein) were added to duplicate assay tubes containing the following (final concentrations): 50 mM Tris·HCl, pH 7.4, with 155 mM NaCl (Tris-buffered saline), 0.001% bovine serum albumin, radioligand, and appropriate drugs. (+)-Butaclamol (2 μM) was used to define nonspecific binding. Incubations were carried out at 37° for 45 min, in a volume of 1.0 ml, and were terminated by filtration as described above.

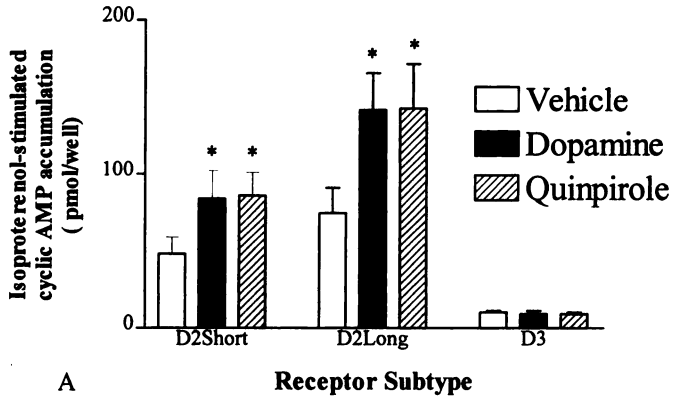
Data analysis. Dose-response curves for cAMP accumulation and saturation analysis of radioligand binding data were analyzed by nonlinear regression using the programs GraphPAD and Prism (GraphPAD, San Diego, CA). The free concentration of radioligand was calculated as the concentration added minus the concentration specifically bound. Statistical comparisons were made using analysis of variance followed by Dunnett's *post hoc t* test comparing vehicle and drug-treated groups, except where indicated in the figure legends.

Results

Desensitization of D_2 receptor-mediated inhibition of cAMP accumulation. Pretreatment of C_6-D_{2L} cells with 1 μM dopamine for 2 hr resulted in a rightward shift (5-fold) in the potency for dopamine inhibition of cAMP accumulation, where the IC_{50} value in control cells was 15 ± 5 nM and that in dopamine-treated cells was 71 ± 24 nM (three experiments). There was no significant change in maximal inhibition of isoproterenol-stimulated cAMP accumulation, expressed as a percentage of total stimulated activity (data not shown)

D_2 agonist-induced sensitization of cAMP accumulation. Acutely, dopamine and other D_2 agonists inhibited cAMP accumulation in C_6 glioma and HEK293 cells expressing D_{2L} and D_{2S} receptors. Pretreatment with the same drugs, however, resulted in a heterologous sensitization of adenylate cyclase activity. Fig. 1 demonstrates that pretreatment with dopamine or the D_2-selective agonist quinpirole enhanced isoproterenol- and forskolin-stimulated cAMP accumulation by approximately 2-fold in C_6-D_{2S} and C_6-D_{2L} cells but not in C_6-D_3 cells. Whereas the absolute levels of drug-stimulated cAMP varied among cell lines, the percentages of sensitization were similar in C_6-D_{2S} and C_6-D_{2L} cells (Fig. 1). The low level of stimulation in the C_6-D_3 cells appears to be the result of clonal variation, because other C_6-D_3 clones had levels of stimulation similar to those of the C_6-D_{2S} and C_6-D_{2L} cells (data not shown). D_2 dopamine receptor-mediated sensitization in C_6-D_{2L} cells occurred rapidly, becoming evident within 15 min for both forskolin- and isoproterenol-stimulated cAMP accumulation (Fig. 2). The sensitized adenylate cyclase response remained stable for at least 2 hr after the termination of 2-hr agonist treatment (data not shown). Similarly, pretreatment of HEK-D_{2L} cells, but not HEK-D_3 cells, with dopamine or quinpirole potentiated forskolin-stimulated cAMP accumulation (Table 1). Whereas agonist treatment of C_6-D_{2L} cells enhanced forskolin-stimulated cAMP accumulation by 2–3-fold, treatment of HEK-D_{2L} cells produced a >7-fold increase of forskolin-stimulated activity. In contrast to the findings with dopamine and quinpirole, we found that pretreatment with another D_2 agonist, bromocriptine, decreased subsequent cAMP accumulation in C_6-D_{2L} and HEK-D_{2L} cells (Table 1; see below).

Effects of antagonists on sensitization of cAMP accumulation. Antagonist (spiperone or butaclamol) pretreatment did not alter the accumulation of cAMP (Table 1; data not shown for butaclamol). Inclusion of spiperone (1 μM) during the agonist pretreatment prevented sensitization of isoproterenol-stimulated cAMP accumulation in C_6-D_{2L} cells and forskolin-stimulated accumulation in C_6-D_{2L} and HEK-D_{2L} cells (Table 1). The D_1 dopamine receptor antagonist SCH 23390 had no effect on D_2 agonist-induced sensitization (data not shown).



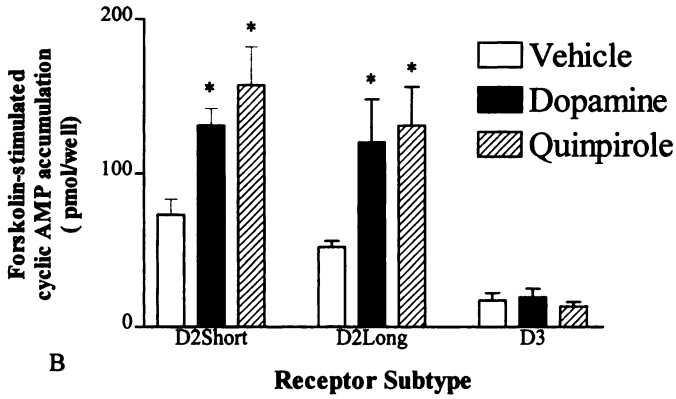


Fig. 1. Heterologous sensitization of cAMP accumulation in C_6 glioma cells. C_6-D_{2S}, C_6-D_{2L}, and C_6-D_3 cells were treated for 2 hr with 1 μM dopamine, 1 μM quinpirole, or drug vehicle and then washed extensively. cAMP accumulation was stimulated with 1 μM isoproterenol (A) or 10 μM forskolin (B) for 15 min. Data shown are the mean ± standard error for three to eight independent determinations, each assayed in duplicate. *, significant difference, compared with vehicle-treated cells (Dunnett's *post hoc* repeated-measures analysis of variance, $p < 0.05$).

Characterization of dose-response curves for isoproterenol- and forskolin-stimulated cAMP accumulation. Pretreatment with dopamine for 2 hr enhanced maximal responsiveness to isoproterenol without changing the EC_{50} value (Fig. 3A, Table 2). In contrast, dopamine pretreatment caused a 4-fold increase in the potency of forskolin; the EC_{50} value for control cells was 60 ± 10 μM and that for dopamine-treated cells was 14 ± 2 μM (four experiments). No change in the maximal stimulation was observed (Fig. 3B). To ensure that the effects of dopamine pretreatment did not involve dopamine stimulation of the β-adrenergic receptors endogenously expressed by C_6 cells, dopamine pretreatment was carried out in the presence of the β-adrenergic receptor antagonist propranolol (1 μM). Pretreatment with propranolol

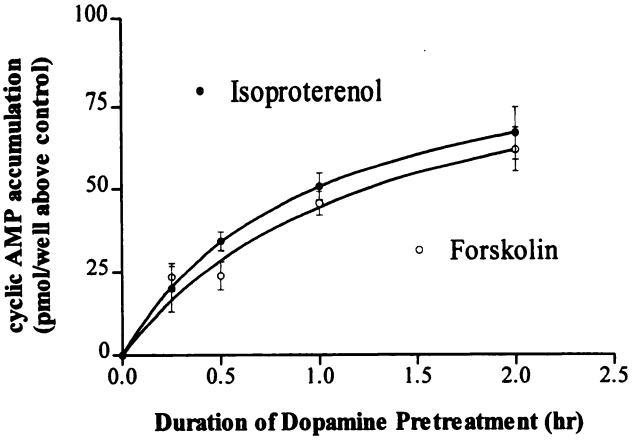


Fig. 2. Time course for dopamine-induced sensitization. C_6-D_{2L} cells were treated for 15 min to 2 hr with 1 μM dopamine and then washed three times with assay buffer. cAMP accumulation was stimulated with 1 μM isoproterenol (●) or 10 μM forskolin (○) for 15 min. Data shown are expressed as cAMP accumulation above control (isoproterenol, 30.3 ± 5.5 pmol/well; forskolin, 40.0 ± 13 pmol/well) in matched wells and are the mean ± standard error for three independent determinations, each assayed in duplicate.

markedly reduced the potency of isoproterenol, presumably because of residual antagonist present during the isoproterenol stimulation of cAMP accumulation; dopamine-induced changes in maximal stimulation, however, were essentially identical to changes in the absence of propranolol (Table 2). The potency of forskolin for stimulation of cAMP accumulation in HEK-D_{2L} cells was reduced, compared with its potency in C_6-D_{2L} cells (Fig. 3, B and C). After pretreatment with dopamine, we obtained dose-response curves for forskolin-stimulated cAMP accumulation in HEK-D_{2L} cells. Dopamine pretreatment appeared to produce a leftward shift in the dose-response curve for forskolin-stimulated cAMP accumulation (Fig. 3C), but the decreased potency of forskolin in HEK-D_{2L} cells precluded the determination of maximal stimulation of cAMP accumulation for the accurate estimation of EC_{50} values.

Effects of other D_2 dopamine receptor agonists. We also examined the ability of other agonists to sensitize adenylate cyclase. Like dopamine and quinpirole, 7-OH-DPAT and pergolide pretreatment enhanced forskolin- and isoproterenol-stimulated cAMP accumulation to similar extents in C_6-D_{2L} and C_6-D_{2S} cells (approximately 100% above vehicle-treated cells; data not shown). In C_6-D_{2S} cells, for example, 7-OH-DPAT (1 μM) and pergolide (1 μM) enhanced cAMP accumulation by 109 ± 17% (four experiments) and 108 ± 24% (four experiments), respectively, above isoproterenol-stimulated cAMP accumulation in vehicle-treated cells.

In contrast, two ergot agonists, bromocriptine and lisuride, dramatically decreased isoproterenol- and forskolin-stimulated cAMP accumulation (Tables 1 and 2, Fig. 3A; data not shown for lisuride) in both C_6-D_{2L} and HEK-D_{2L} cells. The effects of bromocriptine on isoproterenol- and forskolin-stimulated cAMP accumulation were blocked by 1 μM spiperone (Table 1). Analysis of dose-response curves for isoproterenol indicated that pretreatment with bromocriptine (10 nM) induced a >70% reduction in the maximal response of isoproterenol-stimulated cAMP accumulation, with no change in the EC_{50} value for isoproterenol-stimulated cAMP accumulation (Table 2, Fig. 3A). Bromocriptine treatment was also carried out in the presence of propranolol, to preclude any effects mediated by endogenous β-adrenergic receptors, and the results were similar to those in the absence of propranolol, except for a propranolol-induced decrease in the apparent potency of isoproterenol (Table 2B). To evaluate the hypothesis that residual bromocriptine may be responsible for the apparent heterologous desensitization, we conducted a set of experiments in which the cells were washed and then exposed to 1 μM spiperone for 1 hr to prevent agonist rebinding after preincubation with bromocriptine (10 nM). The resulting dose-response curves were similar to those obtained

TABLE 1

Antagonism of D_2 agonist-induced heterologous regulation of cAMP accumulation.

Cells expressing D_{2L} dopamine receptors were incubated with dopamine agonists in the absence or presence of spiperone (1 μM) for 2 hr. The cells were extensively washed, and cAMP accumulation was stimulated by forskolin (10 μM) or isoproterenol (1 μM). Values are the mean ± standard error, with the number of individual determinations shown in parentheses.

Cell-receptor	Agonist	cAMP accumulation	
		No spiperone	+Spiperone (1 μM)
		pmol/well	
Forskolin, 10 μM			
C_6-D_{2L}	Vehicle	58 ± 8 (6)	43 ± 2 (3)
	Dopamine, 1 μM	117 ± 15 (6)[a]	47 ± 5 (3)
	Quinpirole, 1 μM	122 ± 12 (4)[a]	37 ± 5 (3)
	Bromocriptine, 10 nM	18 ± 2 (6)[a]	35 ± 3 (3)
HEK-D_{2L}	Vehicle	15 ± 4 (9)	10.3 ± 3.0 (4)
	Dopamine, 1 μM	118 ± 29 (9)[a]	9.6 ± 2.1 (4)
	Quinpirole, 1 μM	134 ± 32 (6)[a]	10.3 ± 2.1 (4)
	Bromocriptine, 10 nM	1.5 ± 0.2 (9)[b]	11.6 ± 2.4 (4)
HEK-D_3	Vehicle	28.6 ± 2.2 (3)	
	Dopamine, 1 μM	27.4 ± 2.2 (3)	
Isoproterenol, 1 μM			
C_6-D_{2L}	Vehicle	12.7 ± 1.6 (6)	10.8 ± 1.1 (6)
	Dopamine, 1 μM	42.0 ± 3.5 (6)[a]	13.6 ± 2.0 (6)
	Quinpirole, 1 μM	36.8 ± 3.1 (6)[a]	11.0 ± 0.8 (6)
	Bromocriptine, 10 nM	1.0 ± 0.1 (6)[a]	10.6 ± 0.7 (6)

[a] Significant difference, compared with vehicle-treated cells (Dunnett's *post hoc* analysis of variance, $p < 0.05$).
[b] $p > 0.05$ for Dunnett's *post hoc* analysis of variance, $p < 0.05$ for Student's *t* test versus vehicle-treated cells.

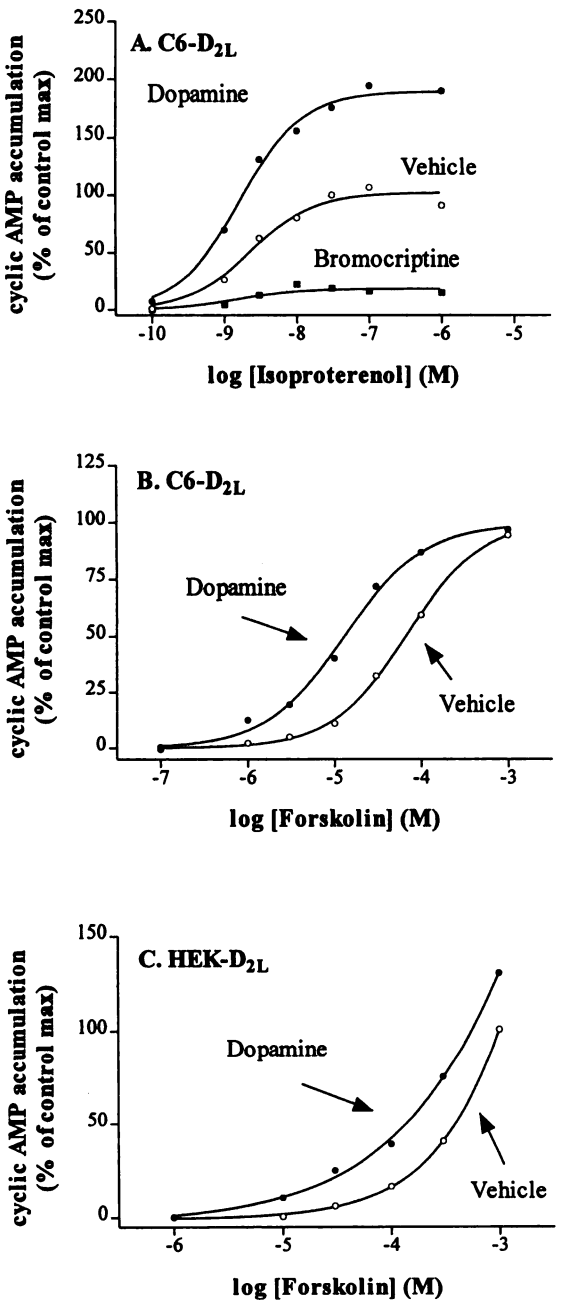


Fig. 3. Dose-response curve for D_2 agonist-mediated regulation of cAMP accumulation. Data are expressed as the percentage of maximal control stimulation, and a representative experiment performed in duplicate is shown for each graph. A, C_6-D_{2L} cells were incubated with vehicle (○), 1 μM dopamine (●), or 10 nM bromocriptine (■) for 2 hr. After washing, cAMP accumulation was stimulated with isoproterenol (0.1 nM to 1 μM) for 15 min (Table 2A). In this representative experiment, the EC_{50} values were as follows: control, 2.3 nM; dopamine-treated, 1.6 nM; bromocriptine-treated, 1.5 nM. The values for maximal stimulation (as a percentage of maximal stimulation in control cells, which was 154 pmol/well) were as follows: dopamine-treated, 186%; bromocriptine-treated, 21%. B, C_6-D_{2L} cells were incubated with vehicle (○) or 1 μM dopamine (●) for 2 hr. cAMP accumulation was stimulated with forskolin (100 nM to 1 mM) for 15 min. In this experiment the EC_{50} value for control cells was 67 μM and for dopamine-treated cells was 13 μM. The maximal stimulated cAMP accumulation for dopamine-treated cells was 99% of control maximal stimulation (390 pmol/well). C, HEK-D_{2L} cells were incubated with vehicle (○) or 1 μM dopamine (●) for 2 hr. cAMP accumulation was stimulated with forskolin (1 μM to 1 mM) for 15 min. The data are expressed as a percentage of maximal stimulation in vehicle-treated cells, which was 423 pmol/well.

in cells that did not undergo the prolonged wash with spiperone (Table 2C). We also measured the density of D_{2L} receptors by saturation analysis of the binding of [^{3}H]spiperone. Bromocriptine pretreatment decreased the density of D_2 dopamine receptors by 57% in a washed membrane preparation from HEK-D_{2L} cells. The B_{max} values were 1460 ± 340 fmol/mg (three experiments) in control cells and 631 ± 120 fmol/mg in cells pretreated with 10 nM bromocriptine. There was no bromocriptine-induced change in the K_d value for binding of [^{3}H]spiperone, indicating that, if residual bromocriptine was present, its binding was not reversible during the 45-min incubation with [^{3}H]spiperone.

Mechanism of sensitization of cAMP accumulation. In preliminary studies, we determined that overnight treatment with 25 ng/ml pertussis toxin was sufficient to abolish D_2 receptor-mediated inhibition of cAMP accumulation in both C_6-D_{2L} and HEK-D_{2L} cells (data not shown) but had only minor effects on isoproterenol- and forskolin-stimulated accumulation of cAMP (Table 3). The same pretreatment with pertussis toxin completely blocked D_2 receptor-mediated sensitization of forskolin- and isoproterenol-stimulated cAMP accumulation (Table 3). Pertussis toxin also abolished the bromocriptine-induced decrease in isoproterenol-stimulated cAMP accumulation (Table 3).

Agonist pretreatment was also conducted in the presence of two activators of PKA, i.e., 8-bromo-cAMP and dibutyryl-cAMP. cAMP accumulation appeared to be enhanced under all conditions in cells treated with cAMP analogs, presumably because of binding of residual analog to the cAMP-binding protein used to quantify cAMP. Nevertheless, D_2 agonist-induced sensitization of isoproterenol-stimulated cAMP accumulation was not altered in D_{2L} or D_{2S} cells (Table 4; data not shown for D_{2S} cells). Similar results were obtained when we assessed the effect of PKA activators on D_2 agonist-induced sensitization of forskolin-stimulated cAMP accumulation (data not shown).

Potency of D_2 agonists for sensitization of cAMP accumulation. We examined the potency of D_2 receptor agonists for the sensitization of adenylate cyclase activity by using HEK-D_{2L} cells because of the greater magnitude of the response, compared with the magnitude of the response in C_6-D_{2L} cells. The potencies of two agonists for sensitization were nearly 2 orders of magnitude lower than their potencies for inhibition of cAMP accumulation (Fig. 4). The EC_{50} value for dopamine for sensitization of forskolin-stimulated cAMP accumulation was 95 ± 14 nM (six experiments), compared with its IC_{50} value for the inhibition of cAMP accumulation, 1.1 ± 0.6 nM (four experiments). Results of experiments with the selective D_2 agonist quinpirole were similar [EC_{50} for sensitization, 103 ± 31 nM (five experiments); IC_{50} for inhibition, 1.4 nM (two experiments)].

Additional potential mechanisms. Because a phosphorylation event may be responsible for sensitization of cAMP accumulation, we examined the ability of H-7, an inhibitor of PKA and protein kinase C, to block sensitization. Because D_2 dopamine receptors couple to multiple signal transduction systems, we also examined the ability of amiloride, a Na^+/H^+ antiporter blocker (25), and nifedipine, a Ca^{2+} channel antagonist, to alter sensitization. None of the compounds altered D_2 agonist-mediated sensitization at concentrations up to 100 μM (data not shown). Disruption of microtubules has been shown to alter the coupling of G_s and adenylate cyclase

TABLE 2

Effects of agonist pretreatment on isoproterenol-stimulated cAMP accumulation.

EC_{50} values and maximal cAMP accumulation were estimated by nonlinear regression analysis of isoproterenol dose-response curves. The maximal cAMP accumulation values are expressed as the percentage of maximal control stimulation for each condition. Values are the mean ± standard error, with the number of individual determinations shown in parentheses. A, C_6-D_{2L} cells were incubated with D_2 agonists for 2 hr in assay buffer and washed three times for 3–4 min each before quantification of isoproterenol-stimulated cAMP accumulation. B, C_6-D_{2L} cells were incubated with D_2 agonists for 2 hr in the presence of 1 μM propranolol, to preclude any effect mediated by endogenous β-adrenergic receptors, and then washed as in A. C, C_6-D_{2L} cells were incubated with D_2 agonists and washed as described for A and then incubated with 1 μM spiperone for 1 hr. The cells were washed and dose-response curves for isoproterenol-stimulated cAMP accumulation were determined.

	Agonist	Antagonist coincubation	Isoproterenol-stimulated cAMP accumulation	
			Isoproterenol EC_{50}	Maximal cAMP accumulation
			nM	*% of control*
A.	Vehicle	None	1.54 ± 0.17 (8)	100
	Dopamine, 1 μM		2.55 ± 0.36 (8)	240 ± 19[a]
	Bromocriptine, 10 nM		1.96 ± 0.49 (5)	19 ± 2[a]
	Quinpirole, 1 μM		2.86 ± 0.46 (3)	204 ± 22[a]
B.	Vehicle	Propranolol, 1 μM	620 ± 230 (3)	100
	Dopamine, 1 μM		1280 ± 220 (3)	234 ± 27[a]
	Bromocriptine, 10 nM		580 ± 96 (3)	22 ± 6[a]
C.	Vehicle	None, followed by 1-hr wash with spiperone, 1 μM	3.48 ± 0.51 (3)	100
	Dopamine, 1 μM		3.73 ± 0.88 (3)	201 ± 20[a]
	Bromocriptine, 10 nM		4.30 ± 1.05 (3)	29 ± 7[a]

[a] Significant difference, compared with vehicle-treated cells (Dunnett's *post hoc* analysis of variance, $p < 0.05$).

TABLE 3

Effect of pertussis toxin on D_2 agonist-induced sensitization

C_6-D_{2L}, C_6-D_{2S}, and HEK-D_{2L} cells were pretreated with pertussis toxin (25 ng/ml) for 18 hr. Cells were then treated with 1 μM dopamine or 1 μM quinpirole for 2 hr. The cells were extensively washed and cAMP accumulation was stimulated by forskolin (10 μM) or isoproterenol (1 μM). Values are the mean ± standard error, with the number of individual determinations shown in parentheses.

Cell-receptor	Treatment (2 hr)	cAMP accumulation	
		No pertussis toxin	+Pertussis toxin
		pmol/well	
Forskolin, 10 μM			
C_6-D_{2L}	Vehicle	68 ± 4 (3)	46 ± 3 (3)
	Dopamine, 1 μM	204 ± 13 (3)[a]	53 ± 8 (3)
	Quinpirole, 1 μM	193 ± 13 (3)[a]	43 ± 6 (3)
C_6-D_{2S}	Vehicle	50 ± 4 (3)	43 ± 4 (3)
	Dopamine, 1 μM	99 ± 14 (3)[a]	44 ± 7 (3)
	Quinpirole, 1 μM	111 ± 2 (3)[a]	42 ± 2 (3)
HEK-D_{2L}	Vehicle	21 ± 6 (4)	11 ± 4 (4)
	Dopamine, 1 μM	105 ± 19 (4)[a]	9 ± 3 (4)
	Quinpirole, 1 μM	95 ± 9 (4)[a]	15 ± 7 (4)
Isoproterenol, 1 μM			
C_6-D_{2L}	Vehicle	111 ± 9 (10)	132 ± 10 (10)
	Dopamine, 1 μM	167 ± 21 (10)[a]	138 ± 13 (10)
	Quinpirole, 1 μM	184 ± 10 (10)[a]	132 ± 10 (10)
	Bromocriptine, 10 nM	34 ± 3 (7)[a]	134 ± 12 (7)
C_6-D_{2S}	Vehicle	62 ± 3 (3)	76 ± 7 (3)
	Dopamine, 1 μM	93 ± 3 (3)[a]	75 ± 5 (3)
	Quinpirole, 1 μM	97 ± 11 (3)[a]	88 ± 9 (3)

[a] Significant difference, compared with vehicle-treated cells (Dunnett's *post hoc* analysis of variance, $p < 0.05$).

(26); however, colchicine (100 μM) did not alter D_2 agonist-induced sensitization (data not shown).

Sensitization of the activity of type I and type II adenylate cyclases. We also examined sensitization in cell lines expressing the D_{2L} dopamine receptor and type I (Ca^{2+}-stimulated) adenylate cyclase (ACI/D2L cells) or the D_{2L} receptor and type II (phorbol ester-stimulated) adenylate cyclase (ACII/D2L cells). Acute studies indicated that in ACI/D2L cells D_2 receptor agonists inhibited the cAMP accumulation induced by the calcium ionophore A23187. In ACII/D2L cells dopamine potentiated isoproterenol-stimulated (100 nM) cAMP accumulation, consistent with the acute activation of type II adenylate cyclase via release of βγ subunits (data not shown). No activation by A23187 was observed in HEK cells expressing type II adenylate cyclase, and no activation by PMA was observed in cells expressing type I adenylate cyclase (data not shown). After those studies, drug-stimulated cAMP accumulation was examined after pretreatment with dopamine agonists for 2 hr. Dopamine pretreatment enhanced type I adenylate cyclase-catalyzed (A23187-stimulated) cAMP accumulation in ACI/D2L cells (Fig. 5A), whereas bromocriptine pretreatment did not (Fig. 5A). In ACII/D2L cells, both dopamine and bromocriptine pretreatment resulted in sensitization of phorbol ester-stimulated cAMP accumulation (Fig. 5B). Additionally, pretreatment with D_2 agonists under conditions where activation of

TABLE 4

Effects of PKA activators on agonist-induced sensitization of adenylate cyclase activity

C_6-D_{2L} cells were incubated with D_2 agonists, in the absence or presence of PKA activators, for 2 hr. The cells were washed three times for 3–4 min and stimulated with 1 μM isoproterenol for 15 min. Values are the mean ± standard error from four independent experiments conducted with duplicate determinations. The percentage of drug-induced sensitization under each condition is given in parentheses.

	cAMP accumulation		
	None	8-Bromo-cAMP	Dibutyryl-cAMP
		pmol/well	
Basal	1.8 ± 0.5	14 ± 2[a]	3.1 ± 1.1
Isoproterenol-stimulated			
Vehicle	23 ± 2	49 ± 10[b]	39 ± 4
Dopamine, pretreatment, 1 μM	73 ± 19[b] (317%)	108 ± 23[b] (220%)	90 ± 23[b] (230%)
Quinpirole, pretreatment, 1 μM	70 ± 12[b] (304%)	105 ± 13[b] (214%)	93 ± 15[b] (238%)

[a] Significant difference, compared with basal levels in cells not treated with a cAMP analog (Dunnett's *post hoc* repeated-measures analysis of variance, $p < 0.05$.
[b] Significant difference, compared with vehicle-treated cells (Dunnett's *post hoc* repeated-measures analysis of variance, $p < 0.05$).

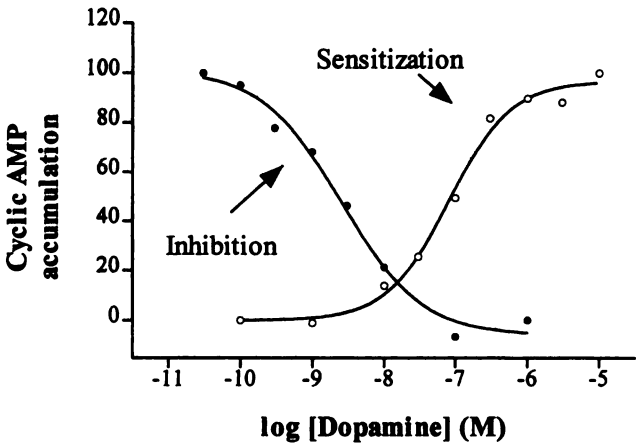


Fig. 4. Potency of dopamine for inhibition and sensitization of forskolin-stimulated cAMP accumulation. Values are expressed as a percentage of forskolin-stimulated activity in the absence of dopamine (inhibition) or after 2-hr pretreatment with 10 μM dopamine (sensitization). For inhibition (●), dose-response curves for dopamine inhibition of cAMP accumulation were determined in HEK-D_{2L} cells stimulated with 10 μM forskolin. For sensitization (○), HEK-D_{2L} cells were treated with increasing concentrations of dopamine for 2 hr and washed, and forskolin-stimulated cAMP accumulation was determined. Data shown are representative experiments completed in triplicate for inhibition or in duplicate for sensitization. In the experiment shown, the IC_{50} value for the inhibition of cAMP accumulation was 2.5 nM and forskolin stimulation in the absence of dopamine was 16 pmol/well. The EC_{50} value for sensitization of forskolin-stimulated cAMP accumulation by dopamine was 85 nM; maximal stimulation of cAMP accumulation was 152 pmol/well.

D_2 receptors stimulated cAMP accumulation (ACII/D2L cells pretreated in the presence of isoproterenol) resulted in sensitization of subsequent phorbol ester-stimulated cAMP accumulation (Fig. 5C).

Discussion

Prolonged treatment (hours) with high concentrations of D_2 dopamine receptor agonists increases the density of D_2 dopamine receptors in several cell lines expressing D_2 dopamine receptors (15–17, 19, 27, 28) and results in the sensitization of subsequent basal and forskolin-stimulated adenylate cyclase activity (4, 15–17). Many studies have also examined desensitization of D_2 dopamine receptors, yielding conflicting observations regarding changes in the potency and efficacy of dopamine-induced inhibition of cAMP accumulation (4, 15–17, 19). Thus, we examined desensitization of D_2 dopamine receptors under conditions that result in heterologous sensitization of cAMP accumulation. We found that dopamine pretreatment for 2 hr produced a 5-fold decrease in the potency of dopamine for inhibition of cAMP accumulation, with no change in efficacy. Although several studies report that desensitization and sensitization are separate events (3, 4, 6), sensitization could influence the results of desensitization studies, because agonist pretreatment that increases the potency of a stimulatory agonist would result in an apparently decreased potency of the inhibitory agonist (29). In the present study sensitization and desensitization both occurred rapidly (within 2 hr). Because there was no change in the potency of isoproterenol (see below), the resulting decrease in the potency of dopamine appears to be distinct from sensitization.

Short term treatment with a number of D_2 dopamine receptor agonists greatly elevated subsequent stimulation of adenylate cyclase activity. This heterologous sensitization was characterized by an increase in both forskolin- and isoproterenol-stimulated cAMP accumulation. The two forms of the D_2 dopamine receptor, D_{2S} and D_{2L}, were able to sensitize both forskolin- and isoproterenol-stimulated cAMP accumulation to similar extents, whereas occupation of D_3 receptors did not, in agreement with previous work from this laboratory (18). When assessed using a single concentration of forskolin (10 μM), the degree of sensitization appeared to vary among cell types. In C_6-D_{2L} cells, maximal sensitization was generally 200–300%, compared with vehicle-treated cells. In HEK-D_{2L} cells, however, the sensitization of forskolin-stimulated cAMP accumulation was always greater than in C_6-D_{2L} cells when expressed as a percentage of control stimulation, ranging from 500 to 800%. This observation may be explained by differences in the potency of forskolin for stimulating cAMP accumulation in HEK-D_{2L} cells, compared with C_6-D_{2L} cells; in control HEK-D_{2L} cells 10 μM forskolin produces little stimulation over basal activity, whereas the same concentration substantially elevates cAMP levels in control C_6-D_{2L} cells. Agonist-induced sensitization in these cells was found to require activation of D_2 receptors, because it was completely blocked by the D_2 antagonist spiperone and not by antagonists of β-adrenergic or D_1 receptors.

The sensitization described in this report differs from the results reported by Bates *et al.* (4), who used Ltk^- cells expressing the D_{2S} dopamine receptor. We found that dopamine receptor-mediated sensitization of adenylate cyclase occurred rapidly and could be observed after only 15 min of

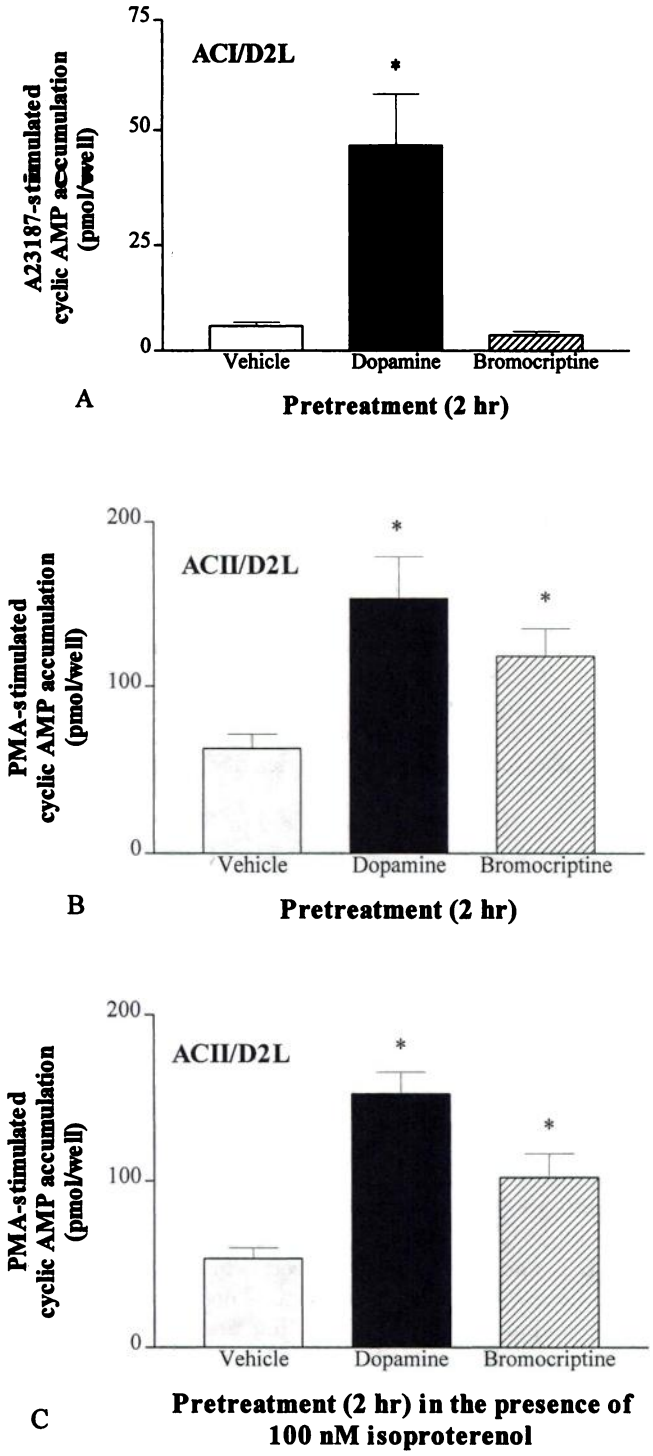


Fig. 5. Heterologous sensitization of cAMP accumulation in HEK293 cells expressing D_{2L} receptors and type I adenylate cyclase or D_{2L} receptors and type II adenylate cyclase. Data shown are the mean ± standard error for four to six independent determinations, each assayed in duplicate. A, ACI/D2L cells were treated for 2 hr with 1 μM dopamine, 10 nM bromocriptine, or drug vehicle and then washed extensively. cAMP accumulation was stimulated with 10 μM A23187 for 15 min. B, ACII2/D2L cells were treated for 2 hr with 1 μM dopamine, 10 nM bromocriptine, or drug vehicle and then washed extensively. cAMP accumulation was stimulated with 100 nM PMA for 15 min. C, ACII/D2L cells were treated for 2 hr with drugs or vehicle in the presence of 100 nM isoproterenol, and cAMP accumulation was stimulated with 100 nM PMA for 15 min. *, significant difference, compared with vehicle-treated cells (Dunnett's *post hoc* repeated-measures analysis of variance, $p < 0.05$).

pretreatment, whereas Bates *et al.* found no sensitization after 1 hr of dopamine pretreatment. Two other reports failed to detect D_2 receptor-mediated rapid sensitization of cAMP accumulation in HEK cells. Boundy *et al.* (28) reported that pretreatment of HEK-D_{2L} cells with quinpirole (5 μM, for 1.5 hr) did not result in sensitization of cAMP accumulation. One explanation for our ability to detect rapid sensitization could be our inclusion of spiperone in assessments of adenylate cyclase stimulation. We have found that sensitization is more variable in the absence of the D_2 antagonist, particularly after pretreatment with higher concentrations of agonist, presumably because of acute inhibition resulting from rebinding of residual agonist to D_2 receptors. Thomas and Hoffman (30) reported that quinpirole pretreatment (0.5 μM for 30 min) did not sensitize endogenous adenylate cyclase activity in HEK cells expressing the D_2 receptor. This could result from the use of cells transiently transfected with the receptor, so that the percentage of cells expressing the receptor and the receptor densities on those cells are unknown. Other studies of D_2 receptor-mediated sensitization did not describe results of drug treatments for periods shorter than 18 hr (15–17).

D_2 agonist-induced sensitization of isoproterenol-stimulated cAMP accumulation differed from sensitization of forskolin-stimulated cAMP accumulation. Pretreatment with D_2 agonists doubled the maximal response to isoproterenol, with no change in the EC_{50} value. In contrast, sensitization of forskolin-stimulated activity was manifested as increased potency with no change in the maximal stimulation. Similarly, Jones and Bylund (13) found that α_2-adrenergic receptor-mediated sensitization (short term) increased the maximal response to vasoactive intestinal peptide but increased the potency of forskolin for stimulation of cAMP accumulation. Studies of long term sensitization have reported similar results (3, 7). The different effects of sensitization on the dose-response curves for these activators of adenylate cyclase may reflect differences in their mechanisms of action. Isoproterenol activates G_s via stimulation of β-adrenergic receptors, whereas forskolin directly activates adenylate cyclase and also influences the interaction of G_s and adenylate cyclase (31, 32). The increase in the potency of forskolin may be related to its ability to synergistically enhance the activation of adenylate cyclase by G_s (31, 33), and this suggests that sensitization is the result of activation of G_s or enhanced coupling of G_s and adenylate cyclase. This hypothesis is consistent with the observation that activators of G_s enhance the potency of forskolin (34).

Treatment with pertussis toxin (25 ng/ml, overnight) had little effect on isoproterenol- or forskolin-stimulated cAMP accumulation but abolished D_2 receptor agonist-induced sensitization of cAMP accumulation, demonstrating that D_2 agonist-induced sensitization is mediated by a pertussis toxin-sensitive G protein. This is consistent with reports describing short term sensitization of adenylate cyclase by other G_i-coupled receptors (13, 35). Bates *et al.* (4) reported that pertussis toxin blocks long term sensitization by D_2 dopamine receptor agonists in Ltk-D_{2S} cells but that pertussis toxin treatment alone produces a >2-fold sensitization of adenylate cyclase. Such an effect of pertussis toxin alone makes interpretation of sensitization data difficult but is consistent with the findings of Katada *et al.* (36) that pertussis toxin dose-dependently enhances isoproterenol-stimu-

lated adenylate cyclase activity. In the present study we were able to identify a concentration of pertussis toxin (25 ng/ml) that prevented D_2 receptor coupling to G_i/G_o (as assessed by inhibition of cAMP accumulation) and prevented sensitization but had little effect on either forskolin- or isoproterenol-stimulated adenylate cyclase activity.

Previous studies have demonstrated that short term sensitization by adenosine receptors can be inhibited by activators of PKA (14), consistent with the hypothesis that G_i-coupled receptor-induced sensitization results from prolonged inhibition of PKA activity. In contrast, there is one report that activators of PKA enhance long term D_2 agonist-mediated sensitization (4). In the present study, we found that activators of PKA had no effect on D_2 receptor-mediated sensitization of adenylate cyclase activity. Additional evidence against a role of PKA comes from a comparison of potencies for sensitization and inhibition of cAMP accumulation by dopamine. The potency for agonist-induced sensitization was lower (dopamine, $EC_{50} \sim 100$ nM) than the potency for inhibition of cAMP formation ($IC_{50} \sim 1$ nM). Interestingly, the potency for sensitization was much greater than that required for D_2 agonist-mediated up-regulation of D_2 receptors in HEK-D_{2L} cells (27) or in C_6-D_{2L} cells (19).

Our studies also demonstrate that D_2 receptor-mediated heterologous sensitization does not discriminate between several isoforms of adenylate cyclase. HEK293 cells endogenously express type III adenylate cyclase (37), and C_6 glioma cells predominantly express type VI adenylate cyclase (38). Although these forms are regulated by calcium in opposing fashions, the activity of either form of adenylate cyclase is enhanced by prior activation of D_2 receptors. More direct evidence for sensitization of multiple forms of adenylate cyclase was obtained in cells expressing type I or type II adenylate cyclase in combination with the D_{2L} dopamine receptor. Type I adenylate cyclase is stimulated by Ca^{2+}/calmodulin and inhibited by G protein $\beta\gamma$ subunits (39–41). The activity of type II adenylate cyclase is enhanced by the phorbol ester PMA and by $\beta\gamma$ subunits in combination with activated G_s (40–42). Pretreatment of either ACI/D2L or ACII/D2L cells with dopamine enhanced subsequent stimulation of cAMP accumulation, suggesting a mechanism through which sensitization occurs. Type I and type II adenylate cyclases differ in their responses to many stimuli but are both activated by G_s. Furthermore, type I adenylate cyclase is synergistically activated by G_s and Ca^{2+} (43) and type II adenylate cyclase is synergistically activated by G_s and PMA (42). These observations suggest that stimulation of D_2 receptors persistently activates or enhances the coupling of G_s to adenylate cyclase and that enhanced responses of the adenylate cyclases to forskolin, Ca^{2+}, or PMA reflect synergistic interactions of these activators with G_s.

The present results with recombinant adenylate cyclases differ in several respects from the results of Thomas and Hoffman (30). In the latter study, for example, stimulation of m_2 muscarinic receptors did not sensitize type I and type II adenylate cyclases expressed in HEK cells. Furthermore, D_2 receptor stimulation did not sensitize endogenous adenylate cyclase in HEK cells. These discrepancies could be the result of several differences in experimental design. For example, in the work of Thomas and Hoffman, D_2 and lutropin receptors and the adenylate cyclases were all transiently expressed. Furthermore, the response of each type of adenylate cyclase varied markedly according to the agent used to stimulate cAMP accumulation, but specific activators of type I and type II adenylate cyclases (e.g., A23187 and PMA) were not used. Alternatively, the mechanisms for heterologous sensitization by m_2 muscarinic receptors and D_2 receptors could differ. This is supported by the rapidity of the time course of sensitization by m_2 receptors (30), compared with D_2 receptors (present results).

One unexpected finding was that two D_2 receptor ergot agonists, bromocriptine and lisuride, decreased subsequent isoproterenol- and forskolin-stimulated adenylate cyclase activity. The apparent heterologous desensitization by bromocriptine was prevented by pretreatment with pertussis toxin and was mediated by activation of D_2 receptors, because it was blocked by the D_2 antagonist spiperone but not by the β-adrenergic antagonist propranolol or the D_1 antagonist SCH 23390. The bromocriptine-induced decrease in isoproterenol- and forskolin-stimulated adenylate cyclase activity persisted even after a 1-hr wash in the presence of spiperone. Bromocriptine also had unusual effects on the apparent density of D_{2L} receptors in HEK293 cells. Treatment with 10 nM bromocriptine for 2 hr decreased receptor number (B_{max}) with no change in the K_d value for [^{3}H]spiperone in saturation radioligand binding experiments. In contrast, most D_2 agonists do not alter receptor number after a 2-hr incubation and increase the density after longer incubations (15, 16, 19).

Bromocriptine and lisuride appear to dissociate very slowly from D_2 receptors, so that the apparent down-regulation of D_2 receptors and heterologous desensitization of adenylate cyclase may be acute effects of persistently bound agonist. This hypothesis is supported by the observation that bromocriptine and lisuride share extremely high affinity for D_2 dopamine receptors (apparent affinity of <1 nM).[1] Sensitization studies in cells coexpressing type II adenylate cyclase with the D_{2L} receptor also lend support to this hypothesis. In ACII/D2L cells, acute treatment with agonists (including bromocriptine) potentiated isoproterenol-stimulated cAMP accumulation in ACII/D2L cells, and a 2-hr pretreatment with bromocriptine resulted in an apparent sensitization of PMA-stimulated cAMP accumulation. Thus, it appears that, in cells where D_2 receptors inhibit cAMP accumulation (C_6-D_{2L}, HEK-D_{2L}, and ACI/D2L cells), pretreatment with bromocriptine produces apparent heterologous desensitization resulting from quasi-irreversible binding and subsequent acute inhibition of drug-stimulated cAMP accumulation. On the other hand, this loss of responsiveness is not observed for cells in which D_2 agonists potentiate G_s-stimulated cAMP accumulation (ACII/D2L cells), because persistently bound bromocriptine would tend to potentiate rather than inhibit adenylate cyclase activity.

Identification of the potential second messenger involved in D_2 dopamine receptor-mediated sensitization would have been greatly aided in a cell-free system (cell membranes). However, when cell membranes were prepared after agonist treatment, there was no evidence of sensitization in either C_6-D_{2L} or HEK-D_{2L} cells. Although others have reported similar findings (11, 13, 35), this result is in contrast to recent reports by Port *et al.* (14) and Johnson *et al.* (44). The reasons for discrepant results are unclear and may reflect

[1] K. A. Neve and V. J. Watts, unpublished observations.

methodological differences such as the degree of sensitization, the precise nature of the membrane preparation, or the buffer composition for adenylate cyclase assays. On the other hand, it may be that in some systems the cell membrane must remain intact and that sensitization requires the integrity of cytoskeletal elements that can influence receptors, G proteins, and their effectors (45–47). Although the exact mechanism responsible for sensitization remains to be elucidated, studies have provided insight into the site of action for sensitization of adenylate cyclase. Ammer and Schulz (48) have provided evidence of enhanced receptor-G_s protein coupling associated with heterologous sensitization. Jones and Bylund (35) found a sensitization-induced increase in the number of [^{3}H]forskolin-labeled sites, which would likely increase G_s-adenylate cyclase coupling efficiency. More recently, Chen and Rasenick (49) have shown that increased G_s-adenylate cyclase coupling is responsible for antidepressant-mediated sensitization of adenylate cyclase. The present study showing sensitization of both receptor- and forskolin-stimulated cAMP accumulation favors the hypothesis that G_s-adenylate cyclase coupling is enhanced under sensitizing conditions.

In summary, we have demonstrated that short term treatment with D_2 receptor agonists results in sensitization of forskolin- and isoproterenol-stimulated cAMP accumulation. Taken together, our results suggest the hypothesis that short term stimulation of D_2 receptors acts via an interaction with a pertussis toxin-sensitive G protein to induce a persistent increase in the activity of G_s or to enhance its interaction with the adenylate cyclases. The nature of the modification of G_s has not yet been determined, but the prevention of sensitization of type VI adenylate cyclase by sequestration of $\beta\gamma$ subunits (30) suggests that $\beta\gamma$ could be involved in the transmission of the signal from the pertussis toxin-sensitive G protein to G_s.

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