The D2 dopamine receptor has two isoforms, the short form (D2s receptor) and the long form (D2l receptor), which differ by the presence of a 29-amino acid insert in the third cytoplasmic loop. Both the D2s and D2l receptors have been shown to couple to members of the Ga family of G proteins, but whether each isoform couples to specific Ga protein(s) remains controversial. In previous studies using Ga mutants resistant to modification by pertussis toxin (Ga PT), we demonstrated that the D2s receptor couples selectively to GaPT and that the D2l receptor couples selectively to GaPT (Senogles, S. E. (1994) J. Biol. Chem. 269, 23120–23127). In this study, two point mutations of the D2s receptor were created by random mutagenesis (R233G and A234T). The two mutant D2s receptors demonstrated pharmacological characteristics comparable with those of the wild-type D2s receptor, with similar agonist and antagonist binding affinities. We used human embryonic kidney 293 cells stably transfected with GaPT, GaPT, or GaPT to measure agonist-mediated inhibition of forskolin-stimulated cAMP accumulation before and after pertussis toxin treatment. The two mutant D2s receptors demonstrated a change in Gi coupling specificity compared with the wild-type D2s receptor. Whereas the wild-type D2s receptor coupled predominantly to GaPT, mutant R233G coupled preferentially to GaPT, and mutant A234T coupled preferentially to GaPT. These results suggest that this region of the third cytoplasmic loop is crucial for determining Gi protein coupling specificity.

These isoforms differ only by the presence of an additional 29 amino acids encoded by exon 5 (4) in the third cytoplasmic loop of the D2l receptor.

The D2 dopamine receptor has been shown to couple to the inhibition of adenylyl cyclase in cells of the anterior lobe of the pituitary (5) and the striatum (6). In addition, agonist activation of the D2 dopamine receptor activates K+ channel activity in isolated lactotrophs and transfected GH4 cells (7). Other investigators have shown that dopamine can also inhibit two distinct voltage-gated calcium currents in isolated lactotrophs (8).

The expression of the two D2 dopamine receptor isoforms is coincident in all tissues examined to date, although the ratios differ between tissues (9–11). Localization studies of the two isoforms have demonstrated that the D2l receptor is strongly expressed in the neurons of the striatum and nucleus accumbens and that the D2s receptor is found in the cell bodies and axons of the mesencephalon and hypothalamus (12). These data suggest that the D2s receptor is the presynaptic autoreceptor and that the D2l receptor is predominantly found postsynaptically. Evidence from analysis of genetically engineered mice has confirmed this hypothesis. The evaluation of mice deficient in the D2l receptor has suggested discrete functional roles for the D2s and D2l receptors (13, 14). Overall, it is apparent that the D2 isoforms have unique localization and serve distinct functions.

The G protein coupling specificity of the D2s and D2l receptors remains controversial. A variety of experimental approaches, including the expression of pertussis toxin-insensitive Ga mutants (GaPT) (15–18), antisense ablation of G protein α-subunits (19), overexpression of G protein α-subunits (20), and evaluation of high affinity agonist coupling and stimulation of GTPγS binding in Gaα knockout mice (21), have yielded disparate results. Some investigations have suggested that the insert region of the D2l receptor may be important for coupling to G proteins, as mutations in this region can impact modestly on signaling (20). However, the specific amino acids in the third intracellular loop of the D2s and D2l receptors responsible for dictating the specificity of G protein coupling have not been identified.

We demonstrate in this work that the Gi protein coupling specificity of the D2s receptor is altered by random point mutants in the third cytoplasmic loop. We have examined the Ga coupling of two point mutations of the D2s receptor to identify specific amino acids that influence the receptor/G protein coupling specificity. These results suggest that a key region of receptor interaction with G proteins exists in the third cytoplasmic loop and may play a crucial role in determining the specificity of receptor/G protein interaction.
EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium, fetal calf serum (certified), and G418 sulfate were obtained from Invitrogen. [125]Iodosulpiride, [3H]adenosine, and [14]CICAMP were purchased from PerkinElmer Life Sciences. All of the ligands, including N-propyl-norpomorphine (NPA), and all other chemicals were obtained from Sigma. Pertussis toxin (PTX) was obtained from Calbiochem-Novabiochem. Horseradish peroxidase-conjugated goat anti-rabbit antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Culture—Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5.0% heat-inactivated fetal calf serum and 50 μg/ml gentamycin and grown in a 5% CO2 environment at a constant temperature of 37°C.

PCR Amplification in the Presence of Mn2+—The rat D2s receptor (2) was cloned into the pSelect-1 vector (Promega) using BamHI-PstI sites. This construct was used as the template for the PCR amplification. The PCR amplifications were performed to generate random mutations in the region of the D2s receptor from the beginning of the fifth transmembrane domain to the C terminus using the following primers: primer 1, 5′-GTCACTCTGCTGGTCTATATC-3′; primer 2, 5′-CGTCTTAAGGGAGGT3′; and primer 3, 5′-TGCTAGGGAGGGGT3′. To amplify only the third cysteys paid loop, primers 1 and 2 were used. To amplify the third loop onward to the C-terminal tail of the D2s receptor, primers 1 and 3 were used. PCR was performed under the following reaction conditions: 10 ng of purified plasmid (Wizard preps system, Promega), 0.5 mM dNTPs, 2.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, and 250 μM MnCl2. Amplification was performed with 5 units of Taq polymerase (Promega) under the following reaction conditions: denaturing for 4 min at 94°C, annealing for 2 min at 55°C, and extension for 3 min at 72°C. The PCR amplification products were cloned into the TA vector (Invitrogen) and subjected to DNA sequencing to identify mutations.

Cloning of Mutant D2s Receptors—The D2s receptor was cloned into the BamHI-PstI sites of pc3 (Invitrogen). Interesting point mutants generated by PCR amplification were then cloned into the pCR3-D2s receptor plasmid using appropriate unique restriction sites (MroI, BbsI, and SacI) within the coding region of the receptor. The insert and vectors were ligated and transformed into JM109 bacteria, and the plasmid was isolated using the Wizard miniprep system. The assembly of the plasmid encoding a correct full-length receptor with a point mutation was verified by DNA sequencing.

Generation of Cell Lines Expressing GaPT Mutants—The full-length cDNAs encoding GaPT, GαqPT, and GαsPT (15) were cloned into pREP8 (Invitrogen). The constructs were used to transfect HEK 293–c18 cells (American Type Culture Collection CRL10852) stably expressing the Epstein-Barr virus antigen-1, which allows for episomal replication of the plasmid containing the Epstein-Barr virus origin of replication. The HEK 293 cells were transfected using calcium phosphate as described by Cullen (22); and after 48 h, the cells were put under selection by inclusion of 400 μg/ml hygromycin in the medium. Clonal lines expressing the specific GaPT mutants were generated by passaging and expanding the colonies present after 2 weeks of hygromycin selection.

Transient Expression of Mutant Receptors in GaPT-expressing Cells—The cell lines expressing each of the GaPT mutants were transfected using 20 μg of mutant D2s receptor DNA and Calfclint reagent (Invitrogen) following the manufacturer’s protocol. At the same time, a parallel transfection was performed using the p-SPORT vector (Invitrogen) encoding β-galactosidase to monitor transfection efficiency. Cells were used 48–72 h after transfection.

Agonist and Antagonist Binding As Assayed by [125]Iodosulpiride—Transiently transfected cells were plated and grown to confluence in 75-cm² dishes. The cells were washed with phosphate-buffered saline (PBS), scraped into microcentrifuge tubes, and centrifuged at 13,000 × g for 15 min. The membranes were resuspended in buffer containing 50 mM Tris-HCl (pH 7.4 at 25°C), 120 mM NaCl, 1 mM EDTA, and 10 mM MgCl2. Binding was performed by incubating 20 μg of protein with 100 pm [125]Iodosulpiride and concentrations of displacing ligand ranging from 10−10 to 10−8 M in a total volume of 250 μl. Incubation was carried out for 1 h at ambient temperature with agitation. Non-specific binding was defined by the inclusion of 1 μM (+)-butaclamol in parallel incubations. [125]Iodosulpiride bound to membranes was obtained by filtration through Whatman GF/C membranes and quantified by γ-counting. Displacement curves were modeled with GraphPad Prism software using nonlinear regression analysis.

cAMP Accumulation Assay—Cells were routinely plated at 100,000 cells/well in 24-well cluster plates. On the day prior to assay, the cells were labeled with 1 μCi/well [3H]adenosine (20–40 Ci/mmol). The next day, the medium was aspirated and replaced with Dulbecco’s modified Eagle’s medium containing 500 μM 3-isobutyl-1-methylxanthine and incubated for 15 min at 37°C. Agonists and forskolin were added simultaneously after the 15-min incubation, and the assay was allowed to incubate for an additional 30 min at 37°C. The medium was aspirated, and 1 ml of 10% trichloroacetic acid containing 100 μCAMP and a known quantity of [14]CICAMP was added to each well. The cells were scraped into microcentrifuge tubes and centrifuged at 13,000 × g for 15 min. [3H]AMP in the supernatants was purified by sequential chromatography on Dowex AG-1 X4 and neutral alumina following the method of Salomon et al. (23). The recovery of [3H]AMP was estimated by following the recovery of the [14]CICAMP tracer. The dose-response curves of NPA-mediated inhibition of forskolin-stimulated cAMP accumulation were fit using Prism software. The EC50 values and percent maximal inhibition were obtained for each experiment.

Pertussis Toxin Treatment—HEK 293 cells were seeded at the usual density in 24-well cluster dishes in Dulbecco’s modified Eagle’s medium with serum. PTX treatment was performed overnight for a minimum of 12 h at 37°C using a concentration of PTX (20 ng/ml) shown previously to fully ADP-ribosylate the G, family of G proteins in HEK 293 cells (24).

Western Blotting—The cells were washed with PBS, scraped into microcentrifuge tubes, and centrifuged at 13,000 × g for 15 min. The crude pellet was resuspended in sample buffer containing 5% SDS (a modification of Ref. 25). The samples were subjected to SDS-PAGE, and the gels were transferred for 1 h at 100 V onto nitrocellulose using buffer containing 192 mM glycine, 25 mM Tris, and 20% methanol (by volume). The blots were blocked by a 1-h incubation at ambient temperature with PBS containing 3% nonfat dry milk and 0.05% Tween 20 and then incubated overnight with the primary antibody at 4°C. The next morning, the blots were washed with PBS containing 0.2% Tween 20 for 15 min, followed by extensive washing with PBS. The horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was incubated for 1 h at ambient temperature, followed by extensive washing with PBS. The blots were visualized with chemiluminescence using ECL reagents and Hyperfilm ECL film (both from Amersham Biosciences).

RESULTS

Generation of Point Mutations in the D2s Receptor—Point mutations were generated in the coding region of the D2s receptor by PCR amplification in the presence of Mn2+, followed by DNA sequencing and assembly of the D2s receptor as described under “Experimental Procedures.” In Fig. 1, the positions of D2s receptor mutants A234T and R233G are shown in relation to the position of the 29-amino acid insert of the D2l receptor, which begins after amino acid 241 of the D2s receptor. In addition, the amino acid sequences of the third intracellular loop of both the D2s and D2l receptors are shown for comparison.

Pharmacology of D2s Receptor Mutants R233G and A234T—After transient expression in HEK 293 cells, both mutant D2s receptors and the wild-type D2s receptor were evaluated for ligand binding (Table I). The transfected wild-type D2s receptor displayed both a high (6.5 nm) and a low (4.2 μm) affinity site for the agonist NPA as assessed by displacement of bound [125]Iodosulpiride (n = 4). The values of the high and low affinity sites of NPA binding calculated for the two point mutants are comparable with the values obtained for the wild-type D2s receptor. In addition, the Kd values for the D2 dopamine receptor antagonists (+)-butaclamol, spiperone, and eticlopride were comparable between the wild-type D2s receptor and mutants A234T and R233G (data not shown).

Coupling of the D2s Receptor in HEK 293 Cells—To demonstrate that functional D2s receptor was expressed in transfected HEK 293 cells, transfected cells were evaluated for agonist-mediated inhibition of forskolin-stimulated cAMP accumulation (Fig. 2). The HEK 293 cells transfected with the D2s receptor showed a dose-dependent inhibition of forskolin-stimulated cAMP accumulation in response to NPA (open cir-
Represented by using GraphPAD Prism to a one- or two-site fit. The one-site fit is as described under "Experimental Procedures." The data were modeled using GraphPAD Prism. The EC50 values were obtained for each experiment, and the two-site fit is represented by H for the high affinity site and by L for the low affinity site. The results are expressed as the mean ± S.E. of at least four independent experiments.

### Table I
Characterization of agonist binding of the wild-type D2s receptor and mutants R233G and A234T

| Receptor                | Kd (nM) | H (nM) | L (μM) |
|-------------------------|---------|--------|--------|
| Wild-type D2s receptor  | 47 ± 3  | 6.5 ± 0.7 | 4.2 ± 0.2 |
| Mutant R233G            | 43 ± 2  | 6.1 ± 0.2 | 3.9 ± 0.1 |
| Mutant A234T            | 54 ± 5  | 6.8 ± 0.4 | 4.9 ± 0.4 |

### Table II
Characterization of NPA-mediated inhibition of forskolin-stimulated cAMP accumulation in cells transfected with the wild-type D2s receptor and mutants R233G and A234T

| Receptor                | EC50 (nM) | Maximal inhibition (%) | n  |
|-------------------------|-----------|------------------------|----|
| Wild-type D2s receptor  | 0.3 ± 0.1 | 47 ± 5                 | 34 |
| Mutant R233G            | 0.4 ± 0.2 | 44 ± 3                 | 18 |
| Mutant A234T            | 0.4 ± 0.3 | 51 ± 7                 | 17 |

D2s receptor signaling in HEK 293 cells is sensitive to PTX. HEK 293 cells were transfected with 20 ng of pCR3-D2s receptor plasmid by calcium phosphate precipitation. The cells were split into 24-well cluster dishes and labeled with 1 μCi/well [3H]adenine. Duplicate cluster plates of transfected cells were incubated overnight in the presence (○) or absence (□) of 20 ng/ml PTX. The response of the mock-transfected HEK 293-c18 cells is also shown (△). The assay for NPA-mediated inhibition of forskolin-stimulated cAMP accumulation was performed as described under "Experimental Procedures." The data were normalized to the [3H]cAMP response generated by forskolin alone and are expressed as a percentage. These data represent the mean ± S.E. of four independent experiments.

Expression of GaPT in HEK 293 Cells—Cell lines stably expressing GaPT mutants were generated from HEK 293-c18 cells by transfection with pREP8 vectors carrying the coding region for Gaα1PT, Gaα3PT, or Gaα2PT. To demonstrate that the GaPT proteins were expressed in HEK 293 cells, Western blot analysis was performed. Shown in Fig. 3 are the results from Western blot analysis of an SDS-polyacrylamide gel of samples from HEK 293-c18 cells (control) before and after treatment with PTX and from one Gaα2PT-expressing cell line after PTX treatment. The blot shown in Fig. 3 was visualized with both anti-Gaα2 subtype antibody and anti-Gβ antibody to allow for direct comparison. PTX modification of G protein has been reported to result in a slower migrating protein species upon SDS-PAGE analysis (Van Dop, C., Yamanaka, G., Steinberg, F., Sekura, R. D., Manclark, C. R., Stryer, L., and Bourne, M. R. (1984) J. Biol. Chem. 259, 23–26). In control cells, the Ga subunit migrated with a reduced mobility after PTX modification (compare Control and Control + PTX). In contrast, the...
Fig. 3. Western blot analysis of GoPT-expressing cells. Control HEK 293-c18 cells and cells stably expressing the GoPT mutant were treated overnight with 20 ng/ml PTX, and cell lysates were prepared as described under “Experimental Procedures.” The lysates were subjected to SDS-PAGE following the method of Laemmli (25) and blotted onto nitrocellulose. The blots were visualized by concomitant incubation with anti-Gaα antibody and anti-Gβ antibody. The positions of the two Ga bands and the Gβ band are shown by arrows. The molecular mass markers are indicated in kilodaltons. First lane, control HEK 293 cells (untreated); second lane, control HEK 293 treated overnight with PTX; third lane, HEK 293 cells stably expressing GaPT treated overnight with PTX.

GoPT-expressing cell line showed a doublet corresponding to endogenous Gaα that was modified by PTX and the unmodified GaPT protein. We obtained similar results for GaαPT- and GaPT-expressing cell lines visualized with anti-Gaα subtype antisera (data not shown).

Evaluation of Coupling of the D2s Receptor in Cell Lines Stably Expressing GoPT—We have previously shown that the D2s receptor will preferentially couple to GoPT-expressing cells stably transfected into GH4C1 cells (15). In the present study, we used HEK 293 cells stably transfected with each of the GoPT mutants to demonstrate the G protein coupling of the D2s receptor. The data shown in Fig. 4 (A–C, open symbols) demonstrate that the D2s receptor inhibited forskolin-stimulated cAMP accumulation in all three cell lines in the absence of PTX. Under these experimental conditions, the transfected D2s receptor could couple to both the stably expressed GoPT mutants and endogenous Gα. However, after overnight treatment with PTX to inactivate endogenous Gα proteins, GaαPT and GaαPT did not couple significantly to the D2s receptor, as evidenced by the lack of NPA-mediated inhibition of forskolin-stimulated cAMP accumulation (Fig. 4, A and C, closed symbols). In contrast, GaPT was able to couple efficiently to the D2s receptor in PTX-treated cells (Fig. 4B, closed symbols), with EC50 = 1 nM (n = 13). These results suggest that the D2s receptor signals preferentially through Gaα to inhibit forskolin-stimulated cAMP accumulation in HEK 293 cells.

Evaluation of Coupling of the Mutant D2s Receptors in Cell Lines Stably Expressing GoPT—The D2s receptor mutants (R233G and A234T) were evaluated for Gα coupling in the three cell lines stably expressing a discrete GoPT mutant. Fig. 5 shows the results from experiments in which mutant R233G was transfected into the GoPT mutant-expressing cell lines. The assay for NPA-mediated inhibition of forskolin-stimulated cAMP accumulation was performed under control conditions (Fig. 5, A–C, open symbols) and overnight PTX treatment conditions (closed symbols). Mutant R233G displayed NPA-dependent inhibition of forskolin-stimulated cAMP accumulation in all three cell lines in the absence of PTX. After treatment with PTX, mutant R233G was capable of significantly inhibiting forskolin-stimulated cAMP accumulation only in the GoPT-expressing cell line, with maximal inhibition comparable with that of the wild-type D2s receptor. Mutant R233G inhibited forskolin-stimulated cAMP accumulation, with EC50 = 24 nM (n = 9). Mutant R233G was ineffective in modulating agonist-mediated inhibition of forskolin-stimulated cAMP accumulation in the GaPT-expressing (Fig. 5A) and GoPT-expressing (Fig. 5B) cell lines after overnight exposure to PTX (n = 6).
In contrast, mutant A234T displayed a different pattern of coupling to G proteins. In the absence of PTX treatment, the D2s mutant A234T signaled to the inhibition of forskolin-stimulated cAMP accumulation in all three G\textsubscript{iPT}-expressing cell lines (Fig. 6, A–C, open symbols). Mutant A234T was ineffective in coupling to G\textsubscript{i2PT} and G\textsubscript{i3PT} under conditions of PTX treatment (Fig. 6, B and C, closed symbols). However, mutant A234T coupled to G\textsubscript{i1PT} with an efficiency comparable with that of the D2s receptor control (Fig. 6A, closed symbols). These combined results demonstrate that the D2s receptor point mutations R233G and A234T are capable of coupling to discrete Gi proteins to inhibit forskolin-stimulated cAMP accumulation. Most notably, these mutants demonstrate an altered G protein specificity compared with each other and with the wild-type D2s receptor. The data suggest that the region of the D2s receptor containing these point mutations appears to be crucial for dictating G protein specificity.

**DISCUSSION**

Attempts to identify the particular G protein involved in the D2s receptor-mediated inhibition of adenylyl cyclase has yielded disparate results from a number of different laboratories. Work from our laboratory demonstrated that the G protein coupling specificity of the D2s and D2l receptors is distinct (15). Using G\textsubscript{iPT} mutants, the D2s receptor coupled with high affinity to G\textsubscript{i2}, and the D2l receptor coupled with high affinity to G\textsubscript{i3}, as determined by assessment of NPA-mediated inhibition of forskolin-stimulated cAMP accumulation. Neither receptor could couple significantly to G\textsubscript{i1} using this same assay. In agreement with our previous study (15), Ghahremani et al. (17), using Ltk\textsuperscript{−} cells, showed that the D2s receptor couples to G\textsubscript{i3} to inhibit forskolin-stimulated adenylyl cyclase and ex-
tended the initial observations to show that the D2s receptor couples to G\textsubscript{ai3} to inhibit prostaglandin E\textsubscript{2}-stimulated adenylyl cyclase. In addition, Watts et al. (18) demonstrated that the D2l receptor couples selectively to PTX-insensitive G\textsubscript{ai} mutants in a neuroblastoma cell line (NS20Y). The different results obtained for D2 dopamine receptor/G protein specificity may be due to differences in the cell line used for investigation. One confounding factor of the G\textsubscript{ai}PT mutant approach is the cellular response to expression of the G\textsubscript{ai}PT mutant. We observed that G\textsubscript{i} subunit expression was increased upon expression of G\textsubscript{ai}PT subunits.\textsuperscript{2} It is possible that G\textsubscript{ai}PT expression may result in the formation of different G\textsubscript{ai}PT\gamma heterotrimers depending on the cell line being used for transfection. This phenomenon may underlie some of the disparity found in the literature.

Other experimental approaches have also been used to elucidate the receptor/G protein coupling specificity of the D2s and D2l receptors. Using a baculovirus expression system, Grunewald et al. (27) demonstrated that the D2s receptor prefers G\textsubscript{ai1} over G\textsubscript{ai2}. Liu et al. (19) used an antisense ablation approach to target G proteins and found that partial ablation of G\textsubscript{ai2} affects D2l (but not D2s) receptor signaling. Montmayeur et al. (20) observed that transfection of G\textsubscript{ai2} promotes more potent inhibition of adenylyl cyclase by the D2l receptor in JEG3 cells, a line that has been reported to lack G\textsubscript{ai2} protein. Assessment of D2 dopamine receptor coupling in G\textsubscript{ai} knockout mice demonstrated that the absence of G\textsubscript{ai} abolishes D2 dopamine receptor-stimulated G\textsubscript{i}PT\gamma binding as well as high affinity agonist binding (21).

Deletion and chimera analysis has revealed that N- and C-terminal regions of the third intracellular loop appear to be crucial for G protein interaction of many different G protein-coupled receptors (28–30). Our data suggest that the middle of the third loop is critical for the coupling specificity, and we have identified a region that is exquisitely sensitive to modification. We have shown in this study that two point mutants of the D2s receptor (R233G and A234T) have altered G protein coupling specificity as assessed in the G\textsubscript{ai}PT-specific background. When transfected into HEK 293 cells, the mutants had agonist and antagonist binding properties and signaling properties comparable with those of the wild-type D2s receptor. Only when these point mutants were transfected into a “G-specific” background was the change in specificity of coupling to the receptor revealed.

The expression of G\textsubscript{ai}PT mutants has been used by a number of investigators and is a powerful tool for sorting out G protein coupling specificity (15–18, 31). We have demonstrated here that G\textsubscript{ai}PT mutants have an extremely subtle effect on D2s receptor/G protein coupling specificity. The data presented here show that we have uncovered a region of the third loop that is a determinant of G protein specificity. This region of the third loop has a high degree of a-helical character, as analyzed by several secondary structure prediction algorithms such as Predator (32), and contains a putative hydrophobic and a hydrophilic face (data not shown). The D2s receptor mutant R233G results in replacement of a charged amino acid with a glycin, and the D2s receptor mutant A234T results in replacement of a hydrophobic amino acid with a polar amino acid. Each of these point mutations may impact on the helical character of this region of the receptor and disrupt the receptor/G protein interface. The helical character of the third cytoplasmic loop may be a defining factor in receptor interaction with G proteins. This region is also extremely close to the insert region of the D2 dopamine receptor, which generates the D2l receptor isoform, and may be important for signaling specificity for both isoforms of the D2 dopamine receptor. Future investigations will address these questions.

Acknowledgments—We thank Dr. Mary Dahmer, Ryan Kendall, and Ben Everett for help with manuscript preparation.

REFERENCES

1. Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998) Physiol. Rev. 78, 189–235
2. Bunow, J. R., Van Tol, H. M., Grandy, D. K., Albert, P., Salom, J., Christie, M., Machida, C. A., Neve, K. A., and Civeilli, O. (1988) Nature 336, 785–787
3. Grandy, D. K., Mochizuki, M. A., Makun, H., Stoff, R. E., Alfano, M., Froehlingham, L., Fisher, J. B., Burke–Howie, K. J., Bunow, J. R., Server, A. C., and Civeilli, O. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9762–9766
4. Del Toso, R., Sommer, B., Ewert, M., Herb, A., Pritchett, D. B., Bach, A., Shivers, B. D., and Se ebbe, P. H. (1998) EMBO J. 17, 4025–4034
5. McDonald, W. M., Silbey, D. R., Kilpatrick, B. F., and Caron, M. G. (1982) Mol. Cell. Endocrinol. 36, 201–209
6. Giannattasio, G., DePerracchi, M. E., and Spada, A. (1981) Life Sci. 28, 1605–1611
7. Einhorn, L. C., Gregerson, K. A., and Oxford, G. S. (1991) J. Neurosci. 11, 3727–3737
8. Lledo, P. M., Homburger, V., Roca, J. C., and Vincent, J. D. (1992) Neuron 8, 455–463
9. Mansour, A., Meador-Woodruff, J. J., Bunow, J. R., Civeilli, O., Akl, H., and Watson, S. J. (1990) J. Neurosci. 10, 2587–2600
10. Weiner, D. M., and Brann, M. R. (1989) FEBS Lett. 253, 207–213
11. O'Malley, K. L., Mack, K. J., Gandelman, K. Y., and Todd, R. D. (1990) Biochemistry 29, 1367–1371
12. Khan, Z., Mrzliak, L., Gutierrez, A., De La Calle, A., and Goldman-Rakic, P. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 85, 7731–7736
13. Wang, Y., Xu, R., Sasaoka, T., Tonegawa, S., Kung, M. P., and Sankoorikai, E. B. (2000) J. Neurosci. 20, 8305–8314
14. Usiello, A., Balick, J. H., Ruge–Pont, F., Picetti, R., Dierich, A., Le Meur, M., Piazza, P. V., and Borelli, E. (2000) Nature 408, 199–203
15. Senogles, S. E. (1994) J. Biol. Chem. 269, 21320–21327
16. O'Hara, C. M., Tang, L., Taussig, R. D., Todd, R. D., and O'Malley, K. L. (1996) J. Pharmacol. Exp. Ther. 278, 354–360
17. Ghahremani, M. H., Cheng, P., Lembo, P. M., and Albert, P. R. (1999) J. Biol. Chem. 274, 9238–9245
18. Watts, V. J., Wiens, B. L., Cumby, M. G., Yu, M. N., Neve, R. L., and Neve, K. A. (1996) J. Neurosci. 21, 8092–8098
19. Liu, Y. F., Jakobs, K. H., Raesnick, M. M., and Albert, P. R. (1994) J. Biol. Chem. 269, 13880–13886
20. Montmayeur, J. P., Guiramand, J., and Borelli, E. (1993) Mol. Endocrinol. 7, 161–170
21. Jiang, M., Spicher, K., Boulay, G., Wang, Y., and Birnbaumer, L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3577–3582
22. Cullen, R. B. (1987) Methods Enzymol. 152, 684–704
23. Salomon, Y., Londos, C., and Rodbell, M. A. (1974) Anal. Biochem. 58, 541–548
24. Senogles, S. E. (1995) in Molecular and Cellular Mechanisms of Neostriatal Function (Ariano, M. A., and Surmeier, D., eds) pp. 103–110, R. G. Landes Co., Austin, TX
25. Laemmli, U. K. (1970) Nature 227, 680–695
26. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
27. Grunewald, S., Reilander, H., and Michel, H. (1996) Biochemistry 35, 15162–15173
28. Strader, C. D., Dixon, R. A., Cheung, A. H., Candelore, M. R., Blake, A. D., and Sigal, I. S. (1987) J. Biol. Chem. 262, 16439–16441
29. O'Dowd, B. F., Hnatowich, M., Regan, J. W., Leader, W. M., Caron, M. G., and Leffkowitz, R. J. (1988) J. Biol. Chem. 263, 15895–15902
30. Burnstein, E. S., Spalding, T. A., and Brann, M. (1996) J. Biol. Chem. 271, 2882–2885
31. Taussig, R., Sanchez, S., Rifo, M., Gilman, A. G., and Belardetti, F. (1992) Neuron 8, 789–809
32. Frishman, D., and Argos, P. (1997) Proteins 27, 329–335

\textsuperscript{2} S. E. Senogles, unpublished data.