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Alternative Splicing of the Dopamine D2 Receptor Directs Specificity of Coupling to G-proteins*

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Two isoforms of the dopamine D2 receptor have been characterized, D2L (long) and D2S (short), generated by alternative splicing from the same gene. They differ by an in-frame insert of 29 amino acids specific to D2L within the putative third intracytoplasmic loop of the receptor. We have previously demonstrated (Montmayeur, J.-P., Guiramand, J., and Borelli, E. (1993) *Mol. Endocrinol.* 7, 161–170) that D2S and D2L, although presenting very similar pharmacological profiles, couple differently to the α -subunit of guanine nucleotide-binding regulatory proteins (G-proteins). In particular, D2L, but not D2S, requires the presence of the α -subunit of the inhibitory G-protein (G α i2) to elicit greater inhibition of adenylyl cyclase activity. The insert present in D2L must therefore confer the specificity of interaction with G α i2. Thus, we introduced substitution mutations within the D2L insert. These mutant receptors were expressed in JEG3 cells, a G α i2-deficient cell line, scoring for those presenting an increased inhibition of adenylyl cyclase by dopamine. Our analysis identified two mutants, S259/262A and D249V, with these properties. These results clearly show that the insert present in D2L plays a critical role in the selectivity for the G-proteins interacting with the receptor.

Dopamine mediates its effect *in vivo* through the activation of five different receptors, which show distinct pharmacological profiles (1). All dopamine receptors belong to the large family of seven-transmembrane domain G-protein¹-coupled receptors

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X55674.

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¹ The abbreviations used are: G-protein, guanine nucleotide-binding regulatory protein; 7TM receptor, seven-transmembrane domain G-protein-coupled receptor; D2L and D2S, two isoforms of the dopamine D2 receptors of 444 (long) and 415 (short) amino acids, respectively; G α i2, α -subunit of the inhibitory G-protein; [³H]SPL, [³H]spiperone;

(7TM receptors). Interestingly, the receptor with a pharmacology corresponding to the D2 subtype is represented by two isoforms generated by alternative splicing of the same gene (2–10). These isoforms, named D2L and D2S, are identical except for an insert of 29 amino acids present in the putative third intracellular loop of D2L. The pharmacological profiles of these isoforms are very similar in transfected cells. Ligand activation of both receptors lowers intracellular cAMP levels (2–10). Thus, it appears that both isoforms have very similar functional properties. However, the D2L insert confers a different specificity for the coupling to G-proteins. Our previous studies have shown that D2L and D2S couple differently to G-proteins (11). Specifically, D2L requires the presence of the α -subunit of the inhibitory G-protein (G α i2) to inhibit adenylyl cyclase more potently (12). Similar results have also been obtained in other systems (13). These observations underline the functional significance of the 29-amino acid insert in the third loop of D2L. In general, the third intracytoplasmic domain of 7TM receptors appears to direct the interaction of the receptor with the appropriate G-proteins. For example, swapping of this region from the α ₁- to the β ₂-adrenergic receptor creates a chimeric receptor with the signal transduction characteristics of an α ₁-adrenergic receptor (14). Similarly, swapping experiments performed between the m1- and m2-muscarinic receptors also demonstrate the importance of this loop in the selective coupling to specific G-protein/effector systems (15).

Analysis of the amino acid composition of the third loop of the known 7TM receptors has shown the presence of highly charged residues in the N- and C-terminal regions of the loop. It is known that an alternation of hydrophobic/hydrophilic amino acids can influence the secondary structure of proteins inducing an amphipathic α -helical structure. Thus, the N- and C-terminal regions of the third loop are postulated to adopt such a structure. Moreover, these highly charged regions of the third loop are strikingly conserved between many different 7TM receptors. Point mutations or deletions affecting these regions disrupt the normal signal transduction by these receptors by altering their binding to G-proteins (16–19).

An interesting feature of the D2 receptor is that the D2L-specific insert is located outside of the regions mentioned above. Furthermore, the insert interrupts a putative α -helical structure present in the D2S third loop and incorporates a novel stretch of alternating hydrophobic/hydrophilic residues.

To establish the importance of this region in determining the D2L coupling characteristics, we generated amino acid substitution mutations in the 29-amino acid insert. We took advantage of the observed D2L requirement for G α i2 in JEG3 cells to score for mutants that display a higher potency of adenylyl

[³H]NPA, (-)-N-propyl[³H]propylnorapomorphine; (-)-IPR, (-)-isoproterenol; DA, dopamine.

cyclase inhibition and, in this respect, that behave similarly to D2S.

Amino acid mutations were generated in the D2L insert in order to identify the residues playing a role in the coupling to G-proteins. Charged amino acids were substituted with valine, a nonpolar amino acid. In addition, proline 264 was substituted with glycine, and serines 259 and 262 with alanines. These mutant receptors were analyzed for their ability to bind D2-specific ligands and to transduce the signal at the cAMP level. We have identified mutants with an associated increase in the inhibition of cAMP levels. This clearly demonstrates that the D2L-specific insert determines the selectivity of coupling of this isoform to G-proteins.

EXPERIMENTAL PROCEDURES

Materials—[³H]Spiperone ([³H]SPI; benzene ring-³H; 920 GBq/mmol) and (-)-N-propyl[³H]propylnorapomorphine ([³H]NPA; 1900 GBq/mmol) were purchased from DuPont NEN. (+)-Butaclamol and (-)-isoproterenol ((-)-IPR) were from Research Biochemicals Inc. (Natick, MA); dopamine (DA) and isobutylmethylxanthine were from Sigma. The cAMP radioimmunoassay kit was obtained from Immunotech.

Construction of Mutant Forms of the D2L Receptor—A 585-base pair *HincII* fragment of the mouse D2L cDNA (10) containing the 87-base pair D2L-specific sequence was subcloned into pBluescript SK (Stratagene, La Jolla, CA) with deleted endogenous *SacI* and *BspHI* sites to generate pD2L-HII. To generate each mutant, pD2L-HII was digested with two restriction enzymes, and the wild-type fragment was substituted with the mutagenized fragment. Pairs of complementary oligonucleotides including the mutations were synthesized corresponding to the sequences between the two restriction sites (see Fig. 1). After annealing, the oligonucleotides were ligated into pD2L-HII, generating the different mutants. The *HincII*-mutated fragments were then sequenced and exchanged with that of wild-type D2L. The D2L mutants were verified by restriction digests and sequencing. The full-length inserts containing the entire D2L coding region were finally subcloned into the pSG5 eucaryotic expression vector (20). To generate mutants K251V and D249V, pD2L-HII was digested with *Bsu36I* and *BspHI* at positions 868 and 896 of the full-length mouse D2L cDNA, respectively (10). For mutants K3R-V, K5R-V, P264G, S259/262A, and D271V, pD2L-HII was digested with *Bsu36I* and *SacI* at positions 868 and 953, respectively. The sequences of the oligonucleotides are presented in Fig. 1.

[³H]SPI and [³H]NPA Binding of Transiently Transfected JEG3 Cells—JEG3 cells were grown in modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum. They were plated at 1×10^5 cells/35-mm well and transfected with 1.6 μ g of total DNA containing 0.4 μ g of specific wild-type or mutated D2 expression vectors using the calcium phosphate coprecipitation technique. Cells were washed 8 h after transfection. [³H]SPI binding experiments were performed 18 h later on whole cells as already described (12).

To perform [³H]NPA binding (21, 22) to transfected JEG3 cell membranes, cells were first harvested in phosphate-buffered saline and pelleted at $500 \times g$. After homogenization in 10 mM Tris-HCl (pH 7.5) and 5 mM EDTA with 10 strokes of a Dounce homogenizer, membranes were isolated by centrifugation at $1000 \times g$ for 10 min. The supernatant was recovered and centrifuged at $45,000 \times g$ for 40 min. The pellet containing the membranes was resuspended in the same buffer and centrifuged again at the same speed. The final pellet was resuspended in 50 mM Tris-HCl (pH 7.7) and stored in aliquots at -80°C . [³H]NPA binding was performed in buffer containing 40 mM Tris-HCl (pH 7.7), 96 mM NaCl, 4 mM KCl, 1.6 mM CaCl₂, 0.8 mM MgCl₂, and 0.1% ascorbic acid. Membranes were incubated in this buffer in the presence of increasing concentrations of [³H]NPA ranging from 0.08 to 5 nM for 30 min at 37°C . Nonspecific binding was determined in the presence of 1 μ M (+)-butaclamol. Incubations were terminated by rapid filtration at $0-4^\circ\text{C}$ through Whatman GF/B filters using a Brandel harvester apparatus, followed by three washes with 2 ml of ice-cold 50 mM Tris-HCl (pH 7.7). Binding data were analyzed with the EBDA-LIGAND program (Elsevier-Biosoft) using the one-site fitting model, allowing the determination of dissociation constant (K_d) and maximal binding capacity (B_{max}) values for each experiment.

Cyclic AMP Measurements—Cells were cultured and transfected as described above, with the exception that 0.4 μ g of pKSVTF, a vector expressing the β_2 -adrenergic receptor, was added to each well. 18 hours after transfection, cells were incubated for 45 min at 37°C with 2 ml of modified Eagle's medium supplemented with 25 mM HEPES (pH 7.5). Then, 100 μ l of a 10 mM solution of isobutylmethylxanthine was added,

and the cells were further incubated for 20 min at 37°C . The cells were then stimulated for 45 min at 37°C with 2 ml of phosphate-buffered saline containing (-)-IPR (10 μ M) and DA at increasing concentrations. To stop the reaction and to extract cAMP, the medium was removed, and 500 μ l of 65% ethanol was added. The extracts were lyophilized, and cAMP was measured by radioimmunoassay using an Immunotech kit as described by the supplier. The maximal inhibition (I_{max}) of the (-)-IPR-evoked cAMP response as well as the concentration of DA required to obtain 50% of the maximal inhibition (IC_{50}) were calculated on each dose-response curve using the EBDA program.

RESULTS

Mutations in the D2L-specific Insert—The 29-amino acid insert characterizing the D2L isoform is located 31 residues C-terminal from the end of the putative fifth domain, as compared with D2S. The computer-predicted secondary structure of this region, using the method of Garnier *et al.* (27), shows alternating α -helices and β -sheets. To establish whether mutations in the D2L-specific insert may alter the coupling characteristics of the receptor, we substituted lysines and arginines for valine. These changes lie within or adjacent to the 29-amino acid insert, creating mutants K251V, K3R-V, and K5R-V (Fig. 1). In the mutant P264G, proline 264 was exchanged with glycine. Serines 259 and 262 were mutated to alanine to create the mutant S259/262A. Finally, aspartates 249 and 271 were replaced by valine in mutants D249V and D271V, respectively.

[³H]SPI Binding Characteristics of Mutant Receptors—Wild-type D2L and D2S receptors and the D2L mutants were transiently transfected in JEG3 cells. [³H]SPI binding on D2L- or D2S-transfected JEG3 cells is saturable, and the Scatchard representation of this binding fits a one-site model (12). Analogous results were obtained with the mutant receptors, indicating that they maintain a conformation able to sustain ligand binding. Thus, we determined the binding characteristics (K_d and B_{max}) of the mutant receptors as compared with the wild-type D2L receptor (Table I).

The K_d of [³H]SPI for the D2L receptor was in agreement with that observed *in vivo* (28, 29). As described previously (12), these binding characteristics are not significantly different from those of the D2S isoform (Table I). Among the different mutants studied, we noticed that mutations affecting the positively charged residues (*i.e.* K251V, K3R-V, and K5R-V) resulted in B_{max} values lower than those for the wild-type D2L receptor (Table I) for equal amounts of transfected vectors. This effect becomes more evident as the number of mutated residues increases (Table I). Indeed, with the K3R-V receptor, in which 4 residues (1 lysine and 3 arginines) were substituted with valine, the B_{max} value is about half that for D2L. For the K5R-V receptor, in which 6 residues (1 lysine and 5 arginines) were mutated, B_{max} values are 40% lower with respect to D2L. In addition, we also observed a slightly higher affinity of [³H]SPI for the K5R-V mutant receptor than for the wild-type D2L receptor (Table I). No significant differences in the [³H]SPI binding to the other mutants have been observed (Table I).

Inhibition of Adenylyl Cyclase by Mutant Receptors—To test whether the G-protein coupling characteristics of the mutant receptors were different from those of D2L, we measured their ability to inhibit the activity of adenylyl cyclase. Transient cotransfection assays were performed in JEG3 cells in the presence of the β_2 -adrenergic receptor and either the wild-type or mutant receptors. This way, upon stimulation by the β_2 -agonist IPR, it is possible to calculate the extent of inhibition of the cAMP level evoked by the wild-type and mutant D2 receptors in the presence of dopamine. The IC_{50} value of DA for D2S receptors is significantly lower than that for the wild-type D2L receptors, indicating a better efficiency of coupling of D2S receptors in these cells (Table I). We have previously shown that this difference is due to the absence of the α -subunit of Gi2 in

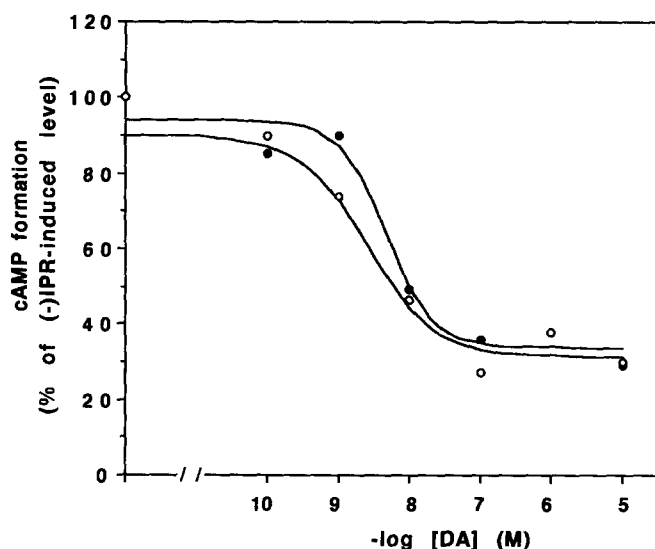


FIG. 2. Dose-response curves for inhibition of cAMP formation by DA in D2L-transfected (●) and S259/262A-transfected (○) JEG3 cells. Cells were cotransfected with 0.4 μ g of plasmid expressing wild-type or mutant D2 receptors and 0.4 μ g of a plasmid expressing the β_2 -adrenergic receptor. 18 hours after transfection, the cells were incubated for 20 min with 500 μ M isobutylmethylxanthine and then stimulated for an additional 45 min with 10 μ M (-)-IPR and increasing concentrations of DA ranging from 0.1 nM to 10 μ M. After extraction, the cAMP was quantified by radioimmunoassay. The results are expressed as percentages of the cAMP concentration. The values of cAMP obtained by stimulation of the transfected cells with (-)-IPR, in the absence of dopamine, were taken as 100%. The fittings of the curves were calculated using the EBDA program. This figure is representative of one experiment performed at least three times, each in duplicate. In this experiment, we obtained the following values: $IC_{50} = 5$ nM and $I_{max} = 64\%$ for D2L and $IC_{50} = 3$ nM and $I_{max} = 65\%$ for S259/262A.

type receptors as this was controlled for by Northern blot analysis of transfected cells (data not shown). We then compensated for the difference in the number of sites by transfecting higher amounts of mutant *versus* D2L expression vector. In this way, the lower potency of these mutants as compared with that of D2L was not observable anymore. This suggests that the basic residues mutated in K3R-V and K5R-V mainly affect the integration of the receptor into the plasma membrane without directly altering the coupling properties of D2L.

Mutations Affecting the Coupling Properties of D2L—In contrast to the results obtained by the substitution of the basic residues in the D2L insert, we found two mutants that displayed a higher potency in the cAMP tests. Indeed, the IC_{50} values for DA of mutants S259/262A and D249V were significantly lower than that of the D2L receptor (Figs. 2 and 3 and Table I). The IC_{50} of mutant S259/262A was almost 3-fold lower than the one obtained for the D2L isoform, which brings the activity of this receptor to the same level as that of D2S. Interestingly, the IC_{50} value for DA of D249V is even lower than that of S259/262A, which is at least 5-fold lower than the IC_{50} of the D2L receptor and is lower to a lesser extent than that of D2S. It thus seems that substitution of serines 259 and 262 and particularly of aspartate 249 creates a D2 receptor similar to or even more potent than the respective wild-type D2 receptor.

Noticeably, all the D2L mutants present an unaltered efficacy (I_{max}) of dopamine inhibition of (-)-IPR-induced cAMP formation, which is comparable to that of the wild-type D2 receptors. This indicates that the D2L insert does not directly participate in the recruitment of the G-proteins to the receptor, in agreement with findings showing that the regions responsible for such function reside elsewhere in the third loop (16–19).

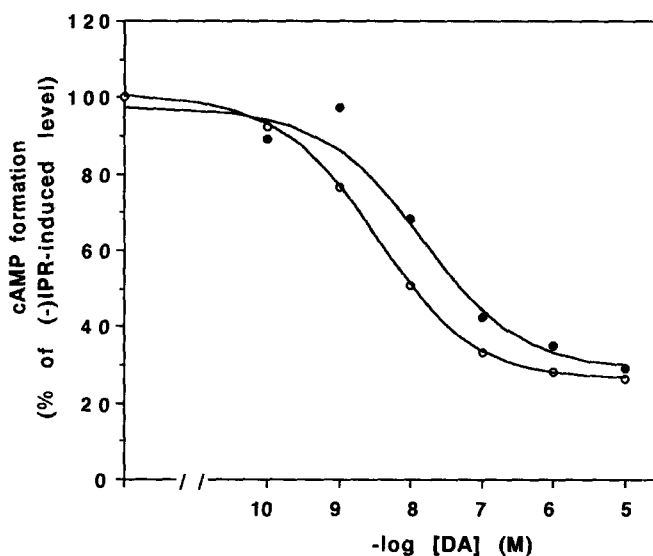


FIG. 3. Dose-response curves for inhibition of cAMP formation by DA in D2L-transfected (●) and D249V-transfected (○) JEG3 cells. The experiments were performed as described for Fig. 2. These data, expressed as percentages of the (-)-IPR-evoked cAMP concentration, are representative of one experiment performed in duplicate and repeated at least three times. Estimated values corresponding to the experiment presented in this figure are as follows: $IC_{50} = 14$ nM and $I_{max} = 71\%$ for D2L and $IC_{50} = 3$ nM and $I_{max} = 74\%$ for D249V.

However, the lower IC_{50} suggests that the insert is implicated in the establishment of the interactions of the D2L receptor with specific G-proteins. Mutants P264G and D271V were not significantly different from the wild-type receptor in the inhibition of cAMP levels (Table I).

[³H]NPA Binding of Mutant D2L Receptors—The increased potency of S259/262A and D249V observed in the cAMP tests led us to investigate whether this effect could have also been at the level of agonist binding to the receptors. Indeed, [³H]NPA binding experiments with membranes of transfected JEG3 cells indicated that these two receptors present higher affinity for the binding of D2-specific agonists. The K_d of [³H]NPA for the D2L receptor was 459 ± 65 ($n = 9$). We then calculated the ratio between the K_d of D2L *versus* the K_d of D249V, S259/262A, and D2S. This way, we obtained the following values: 0.54 ± 0.08 ($n = 3$, $p < 0.05$) for S259/262A, 0.51 ± 0.02 ($n = 3$, $p < 0.05$) for D249V, and finally 0.43 ± 0.08 ($n = 3$, $p < 0.05$) for D2S.

These data show that the increased potencies observed in the cAMP tests for the mutant receptors correlate, as expected, with a gain in the affinity of these receptors for the agonists. They also indicate that these receptors can interact better, with respect to D2L, with the G-proteins available in JEG3 cells, in a manner similar to the D2S isoform.

DISCUSSION

The dopamine D2 receptor represents an interesting paradigm among the characterized 7TM receptors to understand the structure/function relationships of these proteins. Two isoforms of this receptor have been isolated with comparable pharmacological characteristics and anatomical distribution. This is in spite of the structural difference located at the level of the third intracytoplasmic loop. This loop plays a central role in the coupling of this class of receptors to G-proteins (23, 24). In particular, the regions flanking each extremity of the loop, adjacent to transmembrane domains V and VI, are fundamental to the coupling of the receptor with G-proteins (16, 17, 25, 26). The insert present in D2L does not affect these regions, while it adds 29 amino acids at a position 31 residues C-

terminal from transmembrane domain V. Nevertheless, both receptors transduce the signal correctly and are consequently able to interact with G-proteins. Interestingly, in previous reports, we have demonstrated that the D2L isoform requires the presence of G α i2 for maximum inhibition of adenylyl cyclase (12). This indicates that the insert plays a role in specifying the interaction of D2L with G α i2, although even in its absence, this receptor is able to interact less efficiently with other G-protein(s). The results presented in this paper support this hypothesis. Indeed, none of the amino acid substitutions tested creates mutant D2L receptors that are unable to inhibit cAMP levels in transfected cells. In contrast, we show that S259/262A and in particular D249V display a lower IC₅₀ (Figs. 2 and 3 and Table I), demonstrating that these mutants acquire an increased potency with respect to D2L in inhibiting cAMP levels. This is of particular interest given that the wild-type D2L receptor works less efficiently in these cells than in other cell types due to the lack of the α -subunit of Gi2. This indicates that the mutations generate receptors able to interact more efficiently with G-proteins other than G α i2. Indeed, mutants S259/262A and D249V present IC₅₀ values similar to and even higher than that of the D2S isoform, respectively (Table I). We believe that the 29-amino acid insert of D2L generates a structure that confers interaction selectivity for G α i2. Computer analysis of the sequence of the D2L insert, using the method of Garnier *et al.* (27), predicts an α -helical structure. This type of structure has been previously shown to be important for receptor/G-protein interaction (30–33). The substitution of a negatively charged amino acid, such as aspartate 249, with a non-polar amino acid might modify the structure of the neighboring region of the protein, and in this way, the mutant receptor may acquire novel G-protein specificities. This could also apply for mutant S259/262A. Alternatively, serines 259 and 262 might represent phosphorylation target sites. Phosphorylation is believed to be involved in mechanisms of G-protein-coupled receptor desensitization (19, 34); therefore, mutant S259/262A could be more efficient because it is not desensitized as efficiently as the wild-type receptor. However, by sequence comparison, serines 249 and 262 do not constitute consensus substrates for known kinases. While consensus phosphorylation sites for 7TM receptor kinases are not well defined, it seems that acidic residues are required in the proximity of the target Ser or Thr residues (35). This is not the case for either serine 259 or 262. Future experiments will be required to assess whether these serines are phosphorylation sites for known or still uncharacterized kinases. In conclusion, our experiments reinforce the notion of the importance of the D2L-specific insert in determining the functional properties of this isoform with regard to coupling to G-proteins.

Comparison of the dopamine D2 receptor gene in human and mouse demonstrates that its sequence and splicing events have been highly conserved through evolution. This might indicate that the presence of two isoforms and their selective interaction with different G-proteins represent an essential feature of dopamine D2 receptor function *in vivo*. It is tempting to speculate

that *in vivo*, these receptors might serve different functions by activating different G-proteins and possibly different transduction pathways. The simultaneous activation of different G-proteins might be a cellular mechanism to amplify the cellular response to the dopaminergic signal.

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REFERENCES

- Gingrich, J. A., and Caron, M. G. (1993) *Annu. Rev. Neurosci.* **16**, 299–321
- Dal Toso, R., Sommer, B., Ewert, M., Herb, A., Pritchett, D. B., Bach, A., Shivers, B. D., and Seeburg, P. H. (1989) *EMBO J.* **8**, 4025–4034
- Selbie, L. A., Hayes, G., and Shine, J. (1989) *DNA (N. Y.)* **8**, 683–689
- Giros, B., Sokoloff, P., Martres, M.-P., Riou, J.-F., Emorine, L. J., and Schwartz, J.-C. (1989) *Nature* **342**, 923–926
- Monsma, F. J., Jr., McVittie, L. D., Gerfen, C. R., Mahan, L. C., and Sibley, D. R. (1989) *Nature* **342**, 926–929
- Chio, C. L., Hess, G. F., Graham, R. S., and Huff, R. M. (1990) *Nature* **343**, 266–269
- Miller, J. C., Wang, Y., and Filer, D. (1990) *Biochem. Biophys. Res. Commun.* **166**, 109–112
- O'Dowd, B. F., Nguyen, T., Tirpak, A., Jarvie, K. R., Israel, Y., Seeman, P., and Niznik, H. B. (1990) *FEBS Lett.* **262**, 8–12
- Rao, D. D., McKelvy, J., Kebabian, J., and MacKenzie, R. G. (1990) *FEBS Lett.* **263**, 18–22
- Montmayeur, J.-P., Bausero, P., Amlaiky, N., Maroteaux, L., Hen, R., and Borrelli, E. (1991) *FEBS Lett.* **2**, 239–243
- Montmayeur, J.-P., and Borrelli, E. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 3135–3139
- Montmayeur, J.-P., Guiramand, J., and Borrelli, E. (1993) *Mol. Endocrinol.* **7**, 161–170
- Liu, Y. F., Jakobs, K. H., Rasenick, M. M., and Albert, P. R. (1994) *J. Biol. Chem.* **269**, 13880–13886
- Cotecchia, S., Ostrowski, J., Kejelsberg, M. A., Caron, M. G., and Lefkowitz, R. J. (1992) *J. Biol. Chem.* **267**, 1633–1639
- Kubo, T., Bujo, H., Akiba, I., Nakai, J., Mishina, M., and Numa, S. (1988) *FEBS Lett.* **241**, 119–125
- Strader, C. D., Dixon, R. A. F., Cheung, A. H., Condelore, M. R., Blake, A. D., and Sigal, I. S. (1987) *J. Biol. Chem.* **262**, 16439–16443
- O'Dowd, B. F., Hnatowich, M., Regan, J. W., Leader, W. M., Caron, M. G., and Lefkowitz, R. J. (1988) *J. Biol. Chem.* **263**, 15985–15992
- Cotecchia, S., Ekum, S., Caron, M. G., and Lefkowitz, R. J. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 2896–2900
- Savarese, T. M., and Fraser, C. M. (1992) *Biochem. J.* **283**, 1–19
- Green, S., Issemann, I., and Sheer, E. (1988) *Nucleic Acids Res.* **16**, 369–370
- Titeler, M., and Seeman, P. (1979) *Eur. J. Pharmacol.* **56**, 291–292
- Creese, I., Padgett, L., Fazzini, E., and Lopez, F. (1979) *Eur. J. Pharmacol.* **56**, 411–412
- O'Dowd, B. F., Lefkowitz, R. J., and Caron, M. G. (1989) *Annu. Rev. Neurosci.* **12**, 67–83
- Strader, C. D., Sigal, I. S., and Dixon, R. A. F. (1989) *FASEB J.* **3**, 1825–1832
- Wong, S. K.-F., Parker, E. M., and Ross, E. M. (1990) *J. Biol. Chem.* **265**, 6219–6224
- Wess, J., Bonner, T. I., Dorje, F., and Brann, M. R. (1990) *Mol. Pharmacol.* **38**, 517–523
- Garnier, J., Osguthorpe, J., and Robson, B. (1978) *J. Mol. Biol.* **120**, 97–120
- De Lean, A., Kilpatrick, B. F., and Caron, M. G. (1982) *Mol. Pharmacol.* **22**, 290–297
- Seeman, P., Watanabe, M., Grigoriadis, D., Tedesco, J. L., George, S. R., Svensson, U., Lars, J. L. G., and Neumeyer, J. L. (1985) *Mol. Pharmacol.* **28**, 391–399
- Okamoto, T., Katada, T., Murayama, Y., Ui, M., Ogata, E., and Nishimoto, I. (1990) *Cell* **62**, 709–717
- Okamoto, T., and Nishimoto, I. (1992) *J. Biol. Chem.* **267**, 8342–8346
- Sukumar, M., and Higashijima, T. (1992) *J. Biol. Chem.* **267**, 21421–21424
- Wakamatsu, K., Okada, A., Miyazawa, T., Ohya, M., and Higashijima, T. (1992) *Biochemistry* **31**, 5654–5660
- Kobilka, B. (1992) *Annu. Rev. Neurosci.* **15**, 87–114
- Haga, T., Haga, K., and Kameyama, K. (1994) *J. Neurochem.* **63**, 400–412