The Second Intracellular Loop of the \( \alpha_2 \)-Adrenergic Receptors Determines Subtype-specific Coupling to cAMP Production*

(Received for publication, November 18, 1996)

Johnny Näsman‡§, Christian C. Jansson¶, and Karl E. O. Åkerman‡

From the ‡Department of Physiology and Medical Biophysics, Uppsala University, BMC, Box 572, S-75124 Uppsala, Sweden and the ¶Department of Pharmacology and Clinical Pharmacology, University of Turku, FIN-20520 Turku, Finland

The \( \alpha_2 \)-adrenergic receptors (\( \alpha_2 \)-ARs), which primarily couple to inhibition of cAMP production, have been reported to have a stimulating effect on adenyl cyclase activity in certain cases. When expressed in Spodoptera frugiperda Sf9 cells the \( \alpha_{2A} \) subtype showed only inhibition of forskolin-stimulated cAMP production when activated by norepinephrine (NE), whereas the \( \alpha_{2B} \) subtype displayed a biphasic dose-response curve with inhibition at low concentrations of NE and a potentiation at higher concentrations. To further investigate the subtype-specific coupling, we expressed a set of chimeric \( \alpha_{2A}/\alpha_{2B} \)-ARs at similar expression levels in Sf9 cells to determine the structural domain responsible for the difference between the two subtypes. When the third intracellular loops were interchanged between \( \alpha_{2A} \) and \( \alpha_{2B} \) subtypes, the coupling specificity remained unchanged, indicating that this loop does not confer selectivity toward a stimulating response. A biphasic dose-response curve, typical for the \( \alpha_{2B} \) subtype, could be seen when the second intracellular loop of the \( \alpha_{2B} \) subtype was inserted into the \( \alpha_{2A} \) subtype, suggesting that this loop is important for determining the subtype-specific coupling of \( \alpha_2 \)-ARs to cAMP production. Site-directed mutagenesis of non-conserved amino acids in this second intracellular loop of the \( \alpha_{2A} \) subtype indicated that several residues are involved in the coupling specificity.

The \( \alpha_2 \)-adrenergic receptors (\( \alpha_2 \)-ARs)\(^1\) are members of a large family of heptahelical receptors mediating the extracellular stimuli to the interior of the cell through G proteins. Three different subtypes of \( \alpha_2 \)-ARs, \( \alpha_{2A} \), \( \alpha_{2B} \), and \( \alpha_{2C} \), can be distinguished based on the affinity for selective ligands (1), and this subdivision has been confirmed with the molecular cloning in part by the payment of page charges. This article must therefore be in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\* This study was funded by the Technology Centre of Finland (TEKES), The Orion Corporation, The Waldemar von Frenckell Foundation, The Jussi Foundation, The Ehrnrooth Foundation and The Borg Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Dept. of Physiology and Medical Biophysics, Uppsala University, BMC, Box 572, S-75123 Uppsala, Sweden. Tel.: 46-18-174195 (direct); Fax: 46-18-174938.

| Abbreviations used are: | \( \alpha_2 \)-AR, \( \alpha_2 \)-adrenergic receptor; G protein, guanine nucleotide-binding protein; NE, norepinephrine; p.i., postinfection; PTX, pertussis toxin; CTX, cholera toxin; IBMX, isobutylmethylxanthine; MES, 4-morpholineethanesulfonic acid; MCR, mouse chimeric receptor; 2-loop, second intracellular loop. |

---

**EXPERIMENTAL PROCEDURES**

**Materials**—\(^{[3]}\)H]Adenine (21 Ci/mmol) and \(^{[3]}\)H]RX821002 (48 Ci/mmol) were from Amersham Corp. (Buckinghamshire, UK). \(^{[3]}\)C]cAMP (309 mCi/mmol) was from DuPont NEN. Cholera toxin, isobutylmethylxanthine (IBMX), (-)-norepinephrine, pertussis toxin, propranolol, and quinacrine were from Sigma. Photolamine and UK14,304 were from Research Biochemicals International (Natick, MA).

**Cell Culture**—Sf9 cells were maintained as suspension culture at 25–27 °C in TNM-FH medium (pH 6.3) supplemented with 10% fetal calf serum (Life Technologies, Inc., Paisley, UK), 100 units/ml penicillin (Nordvace Media, Skärholmen, Sweden), and 100 µg/ml streptomycin (Nordvace Media), and 2.5% amphotericin B (Life Technologies, Inc.). For expression, Sf9 cells were subcultured in monolayer and infected with recombinant baculoviruses at a multiplicity of infection of 2–5 for the indicated times.

**Recombinant Baculoviruses**—The cDNAs for the mouse \( \alpha_{2A} \) and \( \alpha_{2B} \)-ARs and three mouse chimeric receptors, MCR1, -2, and -3, were a gift from Dr. B. Kobilka (Howard Hughes Medical Institute, Stanford University). All clones contained a hemagglutinin tag fused to the amino terminus of the receptor constructs. For generation of recombinant baculoviruses, the genes were subcloned into the baculovirus transfer vector plusGRBac1 (9) under the transcriptional regulation of...
the polyhedrin gene promoter. The transfer vectors were then used for cotransfection with wild-type Autographa Californica nuclear polyhedrosis virus DNA, and recombinant viruses were purified essentially as described by Jansson et al. (9).

To generate chimeric receptors MCR4 and MCR5, the cDNA clones for \( \alpha_2A \) and \( \alpha_2C \) ARs were transferred into the SmaI site of Bluescript (Stratagene, La Jolla, CA) in a KS orientation. A SspI restriction site was introduced into the \( \alpha_2A \) sequence at nucleotide position 351 of the coding sequence (equivalent to the position of a SspI site in the \( \alpha_2B \) cDNA) using standard PCR techniques (24). The sequence from the beginning of the \( \alpha_2A \) gene to the novel SspI site was then inserted into pBluescript-\( \alpha_2B \) cut with the same enzymes to generate Bluescript-MCR4.

MCR5 was constructed by isolating a DraIII-fragment (DraIII cuts the cDNAs of \( \alpha_2A \) and \( \alpha_2B \) at an equivalent position and the plasmid DNA at one position) from pBluescript-\( \alpha_2A \) and ligating it into an isolated DraIII-fragment of Bluescript-MCR4 to generate pBluescript-MCR5. The sequences encoding MCR4 and MCR5 were cut out from the plasmid constructs with EcoRI and XbaI and ligated into pFastBac1 digested with the same enzymes. For production of recombinant baculovirus, a BAC-TO-BAC baculovirus expression system kit (Life Technologies, Inc.) was used. The correctness of all plasmid constructs was checked by restriction enzyme mapping and partial DNA sequencing (25).

Site-directed mutagenesis of the \( \alpha_2A \) sequence was performed using PCR as described (24). Mutated \( \alpha_2A \) sequences were subcloned into Bluescript, and the mutations were verified by sequencing. The mutated sequences were subsequently transferred to pFastBac1 with EcoRI and NotI. Recombinant baculoviruses were generated using the BAC-TO-BAC baculovirus expression system kit.

**Measurement of Cellular cAMP—**Sf9 cells were plated on tissue culture dishes and allowed to attach for 1 h before infection with respective recombinant baculovirus for the indicated times. The cells were incubated with 5 \( \mu \)Ci/ml \( [\text{H}] \)adenine for 2–3 h and thereafter were scraped off, pelleted, and washed in MES-buffered medium (130 mM NaCl, 5.4 mM KCl, 1.2 mM MgCl\(_2\), 4.2 mM NaHCO\(_3\), 7.3 mM NaHPO\(_4\), 20 mM MES, 63 mM sucrose, 10 mM glucose, and 1 mM CaCl\(_2\), pH 6.3). The pellet was resuspended and washed as described in Materials and Methods. The washing procedure was repeated once, and the pelleted cells were resuspended in 1 ml of 0.33 M perchloric acid containing about 2000 cpm \([\text{H}]\text{cAMP}\). Cyclic AMP was isolated by sequential Dowex/alumina ion exchange chromatography (26), and radioactivity was determined in a liquid scintillation counter (Wallac, Turku, Finland). The conversion of \([\text{H}]\text{ATP} \) to \([\text{H}]\text{cAMP}\) was calculated as a percentage of total cellular \([\text{H}]\text{ATP}\) and normalized to the recovery of \([\text{H}]\text{cAMP}\).

**Receptor Binding Assay—**Infected cells from monolayer cultures were harvested in phosphate-buffered saline solution and centrifuged 1500 \( \times \)g for 5 min. The cell pellet was resuspended in cold potassium phosphate buffer (40 mM K\(_2\)HPO\(_4\), 10 mM KH\(_2\)PO\(_4\), pH 7.4) and homogenized with an Ultra-Turrax homogenizer (Janke and Kunkel, Germany). 100–200 \( \mu \)g of protein of the homogenate was incubated with 10 nM \([\text{H}]\text{RX821002} \) in a volume of 0.3 ml potassium phosphate buffer at 25 °C for 30 min. 10 \( \mu \)M phentolamine was used to determine nonspecific binding. The reactions were terminated by filtration through prewetted sheets of filtermat B/HS scintillator (Wallac) using a Harvester 96 (Tomtec Inc., Orange, CO). After the filters had dried, a MeltiLex B/HS scintillator sheet was melted on them, and radioactivity was determined in a Microbeta scintillation counter (Wallac).

**RESULTS**

**Expression of Mouse \( \alpha_2-\text{AR Subtypes}—**Infection of Sf9 cells with baculovirus harboring the genes for the mouse \( \alpha_2A \) or \( \alpha_2B \)-AR resulted in a time-dependent increase in receptor density as determined by specific binding of \([\text{H}]\text{RX821002} \) (data not shown). Cells infected with wild-type virus did not show specific binding of \([\text{H}]\text{RX821002} \). When assayed for functional coupling to regulation of cAMP production, the \( \alpha_2A \) subtype maximally inhibited the forskolin stimulation (Fig. 1A), whereas the \( \alpha_2B \) subtype showed a biphasic response with inhibition at low concentration (1 \( \mu \)M) of norepinephrine (NE) and a potentiating effect at higher concentrations (100 \( \mu \)M) (Fig. 1B). The potentiation appeared to reach a maximum around 38 h postinfection (p.i.) and then to decline with longer infection times. For further characterization, 48 h p.i. was chosen because the magnitudes of inhibition versus potentiation of the forskolin response between the two subtypes were similar at this time point.

In Fig. 2, the dose-response curves for the two receptor subtypes with two different agonists, NE and UK14,304, are shown. UK14,304 was used as a control agonist for inhibition since this compound has been shown to be very weak in potentiating forskolin stimulation with the \( \alpha_2B \) subtype while being full agonist for the inhibition (27). Both NE and UK14,304 inhibited cAMP production in \( \alpha_2A \)-expressing cells to the same extent and with similar potencies. In cells expressing the \( \alpha_2B \) subtype, NE displayed a biphasic response with an inhibition at low concentrations, that reached a maximum at 1 \( \mu \)M, and that potentiated the forskolin stimulation at higher concentrations. UK14,304 elicited mainly inhibition with the \( \alpha_2B \) subtype, confirming that UK14,304 is much less effective in elic-
Role of i2-Loop in α2-AR Function

Interchange of Third Intracellular Loop Does Not Affect Coupling Specificity—To try to delineate the domain in the α2B subtype responsible for a potentiation of cAMP production, we expressed a set of chimeric α2A/α2B-receptors (Fig. 4).

MCR1 and MCR2, α2A and α2B with interchange of the third intracellular loop (i3-loop), respectively, show similar dose-response curves with NE as the parent receptor (Fig. 5). MCR1 showed a reduced maximal inhibition compared with the α2A subtype, but no biphasic response mode could be seen. MCR2 displayed a typical biphasic response but with a reduced potency. Employing UK14,304 as the agonist increased the maximal inhibition with MCR1 compared with the parent α2A receptor (Fig. 6). This construct exhibited a biphasic dose-response curve with NE (Fig. 5), which indicated that the change in coupling specificity could not be attributed to differences in the expression levels of the chimeric receptor.

Site-directed Mutagenesis of the Second Intracellular Loop—There are six amino acid residues that differ between the α2A and α2B subtypes in the second intracellular loop (Fig. 4.) Three of these residues are essentially similar (Ile-135 in α2A versus Val at the corresponding position in α2B, Thr-136 versus Thr-136 in α2A versus Ser in α2B, Ser-137 in α2A versus Thr in α2B). To test the involvement of the i2-loop in the different coupling modes, we expressed a chimeric α2A receptor in which the i2-loop had been exchanged to α2B receptor sequence, MCR5. This construct exhibited a similar biphasic dose-response curve with NE as the α2B subtype 48 h p.i. (Fig. 7). Since this viral construct expressed receptors at somewhat higher density compared with the other constructs, we measured the dose-response relationship at 38 h p.i. when the receptor level was comparable to the parent α2A subtype (see Fig. 4.). At 38 h p.i., this construct stimulated cAMP production very potently, showing almost no inhibition with NE (Fig. 7), which indicated that the change in coupling specificity could not be attributed to differences in the expression levels of the chimeric receptor.
Ser, and Ile-139 versus Leu). The three other non-conserved residues differ in terms of polarity (Ser-134 in \(\alpha_2A\) versus Ala at the corresponding position in \(\alpha_2B\), Gln-137 versus Arg, and Leu-143 versus Ser). Site-directed mutagenesis of each of these three amino acids of the \(\alpha_2A\) subtype to corresponding residues of the \(\alpha_2B\) subtype did not give clear indications which residues might be responsible for the coupling specificity (Fig. 8). These mutants all showed a lower degree of inhibition with NE compared with the \(\alpha_2A\) subtype, and no marked biphasic response could be seen. On the contrary, a double mutant, S137A, L143S, exhibited a biphasic response with NE similar to the \(\alpha_2B\) subtype although with a smaller magnitude of stimulation. All of the mutated constructs were expressed at receptor levels comparable with the \(\alpha_2A\) subtype, and the inhibition with UK14,304 was 40–50% of forskolin stimulation with all four constructs (not shown).

**DISCUSSION**

During the last few years, it has become evident that G protein-coupled receptors can couple to multiple G proteins to elicit different cellular responses (7, 28–30). The \(\alpha_2\)-ARs have been shown to couple to both negative and positive regulation of adenylyl cyclase activity (5, 7, 9, 13–15). If coupling to both pathways occurs simultaneously but with different potencies, one could expect to obtain a biphasic dose-response curve. With the \(\alpha_2\)-ARs, this is often the case; an inhibition of forskolin-stimulated cAMP production is seen with low concentrations of agonist and a stimulation or potentiation of cAMP production with higher concentrations (5, 7, 13, 15). Earlier studies have indicated that the stimulatory effect of \(\alpha_2\)-ARs is cell-type-specific (15) and/or dependent on the expression level of the receptors (7). This response also seems to be subtype-specific, the \(\alpha_2B\) subtype having a more pronounced stimulatory effect than the \(\alpha_2A\) and \(\alpha_2\)-ARs when expressed in the same cells (8, 9, 13, 15, 31). In the present study, we expressed the mouse \(\alpha_2A\), and \(\alpha_2\)-AR subtypes in Sf9 cells at comparable expression levels and obtained a marked difference in the coupling specificity between the subtypes. The \(\alpha_2B\) subtype showed
a biphasic dose-response curve with a potentiation of the forskolin stimulation at high concentration of NE, whereas the α2A subtype displayed a monophasic inhibitory dose-response curve. With prolonged infection times, which parallel an increase in receptor density, the maximal inhibition with α2A was increased. This is in contrast to the finding by Eason et al. (7) that an increase in receptor density promotes the stimulatory pathway with the human α2A subtype (α2A-C10). The reason for this discrepancy is unclear but might involve interspecies variation of the receptor subtypes or differences in the types of G proteins expressed by the two different cell lines used. A reduction in the maximal stimulation with longer infection times for the α2B subtype was also seen, which indicates a more efficient coupling to the inhibitory component at higher expression levels. The imidazoline-like agonist UK14,304 was very weak in eliciting a biphasic response with the α2B subtype. The ability of UK14,304 to promote coupling to a stimulatory pathway with the α2B subtype has been shown to be very weak in Chinese hamster ovary cells (27).

PTX treatment, which is known to reveal a stimulatory pathway to cAMP production with the α2-ARs (7, 19, 27), increased the stimulation over 2-fold with the α2A subtype. This supports the hypothesis that coupling to both pathways occur simultaneously, and thereby, an elimination of either pathway would result in an enhancement of the other. PTX treatment of cells expressing the α2A subtype did not reveal any significant coupling to a stimulatory pathway although the inhibition was abolished. This confirms that the ability of the receptors to potentiate cAMP production is, apart from being related to expression levels, also subtype-specific.

Treatment of the cells with CTX, which would abolish the stimulation if it was Gs-mediated, drastically reduced the inhibition with both subtypes but did not seem to affect the stimulatory component with the α2B subtype. These data are difficult to interpret, however, since CTX stimulated the adenylyl cyclase activity several-fold over the forskolin-stimulated activity, and this activity may be difficult to inhibit (9, 32).

α2-AR-mediated stimulation of adenylyl cyclase activity has been suggested to occur through a rise in intracellular calcium concentration in PC12 cells (15). Since we observed a small but significant elevation of intracellular Ca2+ in SF9 cells expressing the α2B subtype when assayed with fura-2 fluorescence (data not shown), we measured the change in cAMP production in the presence of EGTA. The potentiating response did not differ significantly from the control experiment while EGTA largely prevented the Ca2+ elevation in the fura-2 assay. This suggests that Ca2+ elevation is not the stimulating factor in the α2B-mediated potentiation of cAMP production in these cells although Ca2+ may enhance the stimulatory response.

The third intracellular loop of G protein-coupled receptors has been implicated in G protein selectivity and activation (for review, see Ref. 33). When we expressed chimeric α2A/α2B-ARs with interchange of the i3-loops, the responses with NE were very similar to the parent receptor subtypes. A reduction of the potentiating response of α2B was seen with MCR2, and a reduction of the inhibition of α2A could be seen with MCR1. This may be related to a more efficient coupling to Gi proteins through the i3-loops of the receptors. Another possibility is that the change of the i3-loop might alter the general conformation of the coupling device leading to slightly altered responses.

In an earlier study, where part of the carboxyl-terminal tail of the α2A subtype was introduced into the β2-AR, a small reduction in isoproterenol-stimulated adenylyl cyclase activity was seen, suggesting an involvement of the carboxyl-terminal tail in G protein coupling (21). In this study, the exchange of the carboxyl-terminal tail of α2A subtype for α2B sequence (MCR3) did not alter the coupling mode for the α2A subtype (Fig. 6), indicating that this domain, if involved in coupling to G proteins in the α2-ARs, probably interacts with a Gi protein. The chimeric receptor MCR4 displayed a typical biphasic dose-response curve. This was also expected since this chimera contains α2B sequences in all the proposed G protein-coupling domains. We also constructed a chimeric α2A receptor with α2B sequence from the amino terminus to the distal end of the i2-loop, but this construct was not expressed at such a density that a functional characterization could be accomplished.
could increase coupling to the stimulatory component and thereby mask a coupling to the inhibitory component. Second, a Gβγ-coupling domain in the third intracellular loop of the human α2A subtype has been identified based on studies with chimeric α/5-HT1A receptors (34). Exchange of the i2-loop from the α2B subtype might result in a receptor that couples more tightly to a stimulating G protein.

The i2-loop has been implemented in G protein selectivity in a study using muscarinic m1β-adrenergic receptor chimeras (22). The role of the i2-loop in G protein activation has also been studied using synthetic peptides derived from α2ARs and muscarinic receptors (35, 36). In the study by Okamoto and Nishimoto (35), the peptide derived from the i2-loop of the human α2A subtype (α2-C10) potently stimulated GTPγS binding to Gα proteins. Although this finding is in contrast to our results that the α2A receptor is strictly inhibiting, other regions of the receptor are probably also involved in governing the selectivity.

The i2-loop sequences of the α2A and α2B ARs differ at six positions when predicted from the cDNA sequences. There are three non-conserved amino acid substitutions between the two subtypes that will change the polarity of the i2-loop, Ser-134, Gln-137, and Leu-143, in the α2A subtype. Single point-mutated receptors with each of these non-conserved residues substituted with corresponding α2B residues showed no clear biphasic responses with NE. In contrast, a double mutant with S134A and L143S responded in a similar way to the phasic responses with NE. In contrast, a double mutant with human α2A substituted with corresponding carinic receptors (35, 36). In the study by Okamoto and Nishimoto, the i2-loop tightly couples to a stimulating G protein.

In conclusion, we have presented evidence that the coupling of α2-ARs to cAMP production is subtype-specific and that the second intracellular loop of the α2A subtype determines the different coupling specificity leading to stimulation of cAMP production. Site-directed mutagenesis of non-conserved amino acid residues indicates that the whole structure of the second intracellular loop is important for efficient coupling.

Acknowledgments—We thank Dr. Brian Kobila for providing the cDNA clones, Katariina Pohjanoksa for preparing the cDNA inserts, Kent Rönnholm for technical assistance, and Jyrki Kukkonen for constructive criticism.

REFERENCES
1. Bylund, D. B., Ray-Frenger, C., and Murphy, T. J. (1988) J. Pharmacol. Exp. Ther. 245, 600–607
2. Kobila, B. K., Matsui, H., Kobila, T. S., Yang-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J., and Regan, J. W. (1987) Science 238, 650–656
3. Regan, J. W., Kobila, T. S., Yang-Feng, T. L., Caron, M. G., Lefkowitz, R. J., and Kobila, B. K. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6301–6305
4. Lomasney, J. W., Lorenz, W., Allen, L. F., King, K., Regan, J. W., Yang-Feng, T. L., Caron, M. G., and Lefkowitz, R. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5094–5098
5. Fraser, C. M., Arakawa, S., McCombie, W. R., and Venter, J. C. (1989) J. Biol. Chem. 264, 11754–11761
6. Coteccia, S., Kobila, B. K., Daniel, K. W., Nolan, R. D., Lapetina, E. Y., Caron, M. G., Lefkowitz, R. J., and Regan, J. W. (1990) J. Biol. Chem. 265, 63–69
7. Eason, M. G., Kurose, H., Holt, B. D., Raymond, J. R., and Lefkowitz, B. S. (1992) J. Biol. Chem. 267, 15785–15801
8. Jansson, C. C., Marjamäki, A., Lauomala, K., Savola, J. M., Scheinin, M., and Akerman, K. E. O. (1994) Eur. J. Pharmacol. 266, 105–174
9. Jansson, C. C., Karp, M., Oker-Blom, C., Nissén, J., Savola, J. M., and Akerman, K. E. O. (1995) Eur. J. Pharmacol. 280, 75–83
10. Maenulty, E. E., McClue, S. J., Carr, I. C., Jess, T., Walekam, M. J. O., and Milligan, G. (1992) J. Biol. Chem. 267, 2149–2156
11. Michel, M. C., Brass, L. F., Williams, A., Bokoch, G. M., Lamorte, V. J., and Motulsky, H. J. (1989) J. Biol. Chem. 264, 4869–4891
12. Kagaya, A., Mikumi, M., Yamamoto, H., Muraoka, S., Yamaki, S., and Takahashi, K. (1992) J. Neural. Transm. 88, 25–36
13. Peppier, D. J., and Regan, J. W. (1993) Mol. Pharmacol. 44, 802–809
14. Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992) Nature 356, 159–161
15. Dutz, E. and Lanier, S. M. (1992) J. Biol. Chem. 267, 24045–24052
16. Ullrich, S., and Wellheim, C. B. (1984) J. Biol. Chem. 259, 4111–4115
17. Paris, H., Galitzy, J., and Senard, J. M. (1989) Mol. Pharmacol. 35, 345–354
18. Chabre, O., Conklin, B. R., Brandson, S., Bourne, H. R., and Llimbird, L. E. (1994) J. Biol. Chem. 269, 5730–5734
19. Jones, S. B., Halenda, S. P., and Bylund, D. B. (1991) Mol. Pharmacol. 39, 239–245
20. Kobila, B. K., Kobila, T. S., Daniel, K. K., Regan, J. W., Caron, M. G., and Lefkowitz, R. J. (1988) Science 240, 1310–1316
21. Liggett, S. B., Caron, M. G., Lefkowitz, R. J., and Hnatowich, M. (1991) J. Biol. Chem. 266, 4816–4821
22. Wong, S. K. P., Parker, E. M., and Ross, E. M. (1990) J. Biol. Chem. 265, 6219–6224
23. Schneider, H., Feyen, J. H. M., and Swenew, K. (1994) FEBS Lett. 351, 281–285
24. Hipushi, R. (1989) in PCR Technology (Erlich, H. A., ed) pp. 61–70, Stockton Press, New York
25. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
26. Salomon, Y., Londos, C., and Rodbell, M. (1974) Anal. Biochem. 58, 541–548
27. Eason, M. G., Jacinto, M. T., and Lefkowitz, R. J. (1994) Mol. Pharmacol. 45, 696–702
28. Dell’Anno, M. L., Carrell, R. C., and Peralta, E. G. (1993) J. Biol. Chem. 268, 5676–5685
29. Prather, P. L., Loh, H. H., and Law, P. Y. (1994) Mol. Pharmacol. 45, 997–1003
30. Negishi, M., Irie, A., Sugimoto, Y., Namba, T., and Ichikawa, A. N. (1995) J. Biol. Chem. 270, 16122–16127
31. Oker-Blom, C., Janson, C., Karp, M., Lindqvist, C., Savola, J. M., Vlak, J., and Akerman, K. (1995) Biochem. Biophys. Acta 1176, 269–275
32. Dittman, A. H., Weber, J. P., Hinds, T. R., Choi, E. J., Migeon, J. C., Nathanson, N. M., and Storm, D. R. (1994) Biochemistry 33, 943–951
33. Savarese, T. M., and Fraser, C. M. (1992) Biochem. J. 283, 101–119
34. Eason, M. G., and Lefkowitz, B. S. (1995) J. Biol. Chem. 270, 24753–24760
35. Okamoto, T., and Nishimoto, I. (1992) J. Biol. Chem. 267, 8342–8346
36. McClue, S. J., Baron, B. M., and Harris, B. A. (1994) Eur. J. Pharmacol. 267, 185–193
The Second Intracellular Loop of the \( \alpha_2 \)-Adrenergic Receptors Determines Subtype-specific Coupling to cAMP Production
Johnny Näsman, Christian C. Jansson and Karl E. O. Åkerman

J. Biol. Chem. 1997, 272:9703-9708.
doi: 10.1074/jbc.272.15.9703

Access the most updated version of this article at http://www.jbc.org/content/272/15/9703

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 25 of which can be accessed free at http://www.jbc.org/content/272/15/9703.full.html#ref-list-1