Molecular Cloning and Functional Analysis of a Novel P₂
Nucleotide Receptor*

(Received for publication, June 12, 1995, and in revised form, August 17, 1995)

Kyungho Changtš, Kazuo Hanaoka§, Mamoru Kumada¶, and Yoh Takuwaši

From the Departments of Cardiovascular Biology, §Anesthesiology, and Physiology, ¶Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

The cDNA encoding a novel P₂ receptor was isolated from rat aortic smooth muscle cell library and functionally characterized. The cloned P₂ receptor exhibits structural features characteristic of the G-protein-coupled receptor family and shows 44 and 38% amino acid identity with previously cloned rat P₂u and chicken P₂y receptors, respectively. The cloned P₂ receptor is functionally coupled to phospholipase C but not to adenylate cyclase in C6 rat glioma cells transfected with the cloned P₂ expression vector. The rank order of agonist potency as judged by intracellular Ca²⁺ mobilization responses is UTP > ADP = 2-methylthioATP > ADPβS > ATP = ATPγS, which is not compatible with any of the previously characterized P₂ receptor subtypes. The non-selective P₂ antagonists, suramin and reactive blue-2, inhibit nucleotide-induced phospholipase C activation in cells expressing the cloned P₂ receptor. The cloned P₂ receptor mRNA is abundantly expressed in various rat tissues including lung, stomach, intestine, spleen, mesentery, heart, and, most prominently, aorta. The results indicate that the novel metabotropic P₂ receptor has pharmacological characteristics distinct from any of P₂ receptor subtypes thus far identified and suggest the existence of a novel regulatory system by extracellular nucleotides of potential significance.

P₂ nucleotide receptors mediate a wide variety of physiological responses to extracellular nucleotides, including vascular smooth muscle contraction and relaxation, neurotransmission, and endocrine and exocrine secretion (1, 2). In the vascular system, nucleotides activate P₂ receptors on vascular smooth muscle cells to cause contraction (3). On the other hand, when nucleotides activate P₂ receptors on vascular endothelial cells, it stimulates release of the vasorelaxants, prostacyclin and NO, to cause vasorelaxation (4–6). Nucleotide-induced vasoconstriction and relaxation were initially suggested to be mediated via two distinct subtypes of P₂ receptors, P₂u and P₂y purinoceptors, respectively (7). However, recent studies on the agonist specificity and potency rank order have provided evidence for the existence of more than a single class of P₂ receptors in both vascular smooth muscle and endothelium. For example, the pyrimidine UTP as well as the P₂u selective agonist α,β-methylene ATP evokes vasoconstriction in various vascular beds, leading to the suggestion that the third P₂ receptor that can interact with UTP, P₂u, mediates vasoconstriction, because UTP does not serve as a ligand for P₂u or P₂y receptors (8–12). Furthermore, activation of P₂ receptors with UTP and α,β-methylene ATP in smooth muscle cells was demonstrated to lead to activation of different downstream effector molecules (i.e. phospholipase C (2) and a cation channel intrinsic to P₂ receptors (13), respectively). Several studies also demonstrated that depending on vascular beds and animal species, P₂u receptors mediate nucleotide-induced endothelium-dependent relaxation (11, 14, 15). However, because of unavailability of a radioactive P₂ receptor ligand and an agonist and antagonist selective for each of the P₂ receptor subtypes, definitive identification of P₂ receptor subtypes that are expressed in vascular smooth muscle and endothelium has been hampered.

Very recently, the cDNAs encoding for mouse (16), human (17), and rat (18) P₂u receptors and chicken brain P₂y receptor (19) have been isolated. It is, however, unknown whether these P₂ receptor subtypes are expressed in the vascular tissues or in fact mediate vascular responses to nucleotides. It was reported that the cloned chicken brain P₂y receptor, when expressed in Xenopus oocytes, displays a significantly different agonist potency rank order from that for endothelium-dependent relaxation (19). Hence, it is still possible that another P₂ receptor subtype may be responsible for nucleotide-induced vascular responses. In the present report we demonstrate the isolation of a cDNA encoding a novel P₂ receptor subtype from rat aortic smooth muscle cell cDNA library, which is coupled to the Ca²⁺ phospholipase C messenger system and shows a distinct agonist specificity from any other known P₂ receptor. This receptor is predominantly expressed in rat aorta and several other tissues.

MATERIALS AND METHODS

Cdl Culture—Primary cultures of rat aortic smooth muscle (RASM) cells were obtained by the explant method (20). C6–15 rat glioma cells were donated by Dr. Hama (Department of Pharmacology, University of Tsukuba). Both cells were grown in Dulbecco’s modified Eagle’s minimal essential medium containing 10% (v/v) fetal bovine serum (Commonwealth Serum Laboratory, Australia), 100 μg/ml streptomycin, and 100 units/ml penicillin in G under the atmosphere of 95% air plus 5% CO₂. RNA Preparation and cDNA Library Construction—Total RNA was isolated from rat tissues and RASM cells for Northern analysis by the acid guanidium isothiocyanate/phenol/chloroform extraction method as described by Chomczynski and Sacchi (21). For cDNA synthesis, total RNA was isolated from rat kidney by the LiCl-urea method (22) and from RASM cells by the guanidium isothiocyanate/CsCl₂ procedure (23). Poly(A)⁺ RNA was purified by passing twice through an oligo(dT)-cellulose (Type III, Collaborative Research) column. Double-stranded
Cloning and Amino Acid Sequence of a Novel G Protein-coupled Receptor—We screened a cDNA library of rat kidney with a synthetic oligonucleotide encoding a conserved region of the third transmembrane domain between mouse P2y1 and chicken P2y2 receptors, and isolated a single hybridization-positive clone with an insert of 3.8 kbp. The partial sequence of this clone revealed a 984-bp open reading frame encoding a novel 328-amino acid protein. The predicted amino acid sequence was 44 and 38% (in overall) identical to that of rat P2y1 receptor (18) and chicken P2y2 receptor (19), respectively, which is in the expected range for receptors within the same family. Because Northern analysis using the coding region of this clone as a probe revealed a major band of approximately 2.2 kb in mRNA from various rat tissues and a very faint band of 3.8 kb, which is observed only in mRNA from aorta (see Fig. 3), we screened a cDNA library of RASM cells with the coding region of the 3.8-kbp clone as a probe and isolated a clone with a 2.0-kbp insert. The 2.0-kbp clone revealed the 984-bp open reading frame identical to the 3.8-kbp clone and a shorter 3' untranslated region than the 3.8-kbp clone.

Shown in Fig. 1 are nucleotide and deduced amino acid sequences of this clone. An in-frame initiating codon (nucleotides 440–442 in Fig. 1) is in the context of the Kozak translation initiation consensus sequence (30) and is preceded by an in-frame stop codon. The predicted molecular mass of 36.7 kDa of this protein is substantially low among G protein-coupled receptors and close to that of A1 adenosine receptor (31) and several odorant receptors (32) so far reported to have the smallest molecular weight. Hydropathy analysis (33) of the clone reveals seven stretches of hydrophobic amino acids, predicted to represent membrane-spanning domains characteristic of the G protein-coupled receptors. The amino-terminal region preceding the putative first transmembrane domain contains a single potential asparagine-linked glycosylation site, and the third intracellular loop and cytoplasmic tail have two recognition sites (Ser-235 and Thr-320) for phosphorylation by protein kinase C (34, 35). This protein also possesses a number of residues conserved in most of the G protein-coupled receptors such as Asp in the second transmembrane domain, Leu in the second and the seventh transmembrane domains, Arg-Tyr immediately behind the third transmembrane domain, and Pro in the fifth, the sixth, and the seventh transmembrane domains (36, 37). A Cys in the carboxyl-terminal region conserved in many of the G protein-coupled receptors, which may be a membrane-anchoring palmitoylation site, and an Asp in the third transmembrane domain conserved in the G protein-coupled receptors for charged amines are absent in this protein (37). All these characteristics suggest that this protein represents a G protein-coupled receptor.

Shown in Fig. 2 is alignment of the amino acid sequences of this protein and two previously cloned P2y receptors, rat P2y1 and chicken P2y2. 25% of the residues are conserved among these three proteins. The observed similarities are mostly confined to the region between the first and the seventh transmembrane domains, whereas the amino acid sequences and the length of the amino and carboxyl termini are variant. The next most similar proteins found from a data base search are rat G10D orphan receptor (29% identity in a 294-amino acid overlap), rat thrombin receptor (28% identity in a 294-amino acid overlap), and rat type 1b angiotensin II receptor (27% identity in a 300-amino acid overlap).

Tissue Distribution of the mRNA of the Cloned Receptor—In order to assess the tissue expression of the cloned receptor gene and the size of the transcript, poly(A)^+ RNA isolated from various rat tissues was subjected to Northern blot analysis.
P2U receptor, which is expressed predominantly in the skeletal muscle, kidney, liver, and heart, and of the P2Y1 receptor. Because previous pharmacological studies demonstrated in vascular tissues the expression of P2 receptors that respond to UTP (8–12), we compared the expression of P2U receptor and the cloned new P2 receptor in the large capacitance vessel aorta, the mesentery, which includes lots of resistance vessels of smaller calibers, and cultured RASM cells. The 2.8-kb P2U transcript is abundantly expressed in the aorta like the case of the cloned P2 receptor (Fig. 4). The P2U transcript is also readily detected in the mesentery and RASM cells. The signal intensities of both P2U and cloned new P2 receptors transcripts in each tissue and cell appear to be roughly similar (compare Figs. 3 and 4). However, strict quantitative comparison of expression levels of P2U and cloned P2 receptor transcripts in Figs. 3 and 4 may not be possible, although the lengths and specific radioactivities of employed cDNA probes and exposure times of membranes to films in the Northern analyses were similar.

Functional Properties of the Cloned Receptor—Functional characterization of P2 receptors has not been easy, because many of the cells often used for transfection assay, such as COS7, CHO, and Ltk cells, express endogenous P2 receptors, and specific radioactive ligands for P2 receptors have not been available. For transfection we employed a subline of C6 rat glioma cells (C6–15) that we found not to express endogenous P2 receptors at a significant level as evaluated with the measurement of intracellular Ca\textsuperscript{2+} mobilization response to nucleotides. As shown in Fig. 5A (left), in the presence of 1.25 mM extracellular Ca\textsuperscript{2+} the addition of ADP (300 μM) induces a rapid rise in [Ca\textsuperscript{2+}], which peaks within 20 s and then gradually declines to the second plateau in transfected C6–15 cells.

Addition of Co\textsuperscript{2+}, a Ca\textsuperscript{2+} channel blocker, causes complete inhibition of the plateau phase of the [Ca\textsuperscript{2+}], response. In contrast, in the absence of extracellular Ca\textsuperscript{2+}, ADP still elicits an initial transient increase in the [Ca\textsuperscript{2+}], although the amplitude of the peak [Ca\textsuperscript{2+}], is smaller than in the presence of extracellular Ca\textsuperscript{2+} but not the second plateau of the [Ca\textsuperscript{2+}], response (Fig. 5A, right). The profile of the [Ca\textsuperscript{2+}], response is similar between ADP and other effective nucleotides, although the amplitude of the [Ca\textsuperscript{2+}], response is varied (see below). These results indicate that the cloned receptor functions as a P2 receptor for nucleotides, which is coupled to mobilization of Ca\textsuperscript{2+} from both intra- and extracellular pools.

To characterize the agonist specificity of the cloned P2 receptor, C6–15 cells expressing the cloned P2 receptor were stimulated with various concentrations of nucleotides, and the [Ca\textsuperscript{2+}], response was monitored. As shown in Fig. 5B, UTP is the most potent with the EC\textsubscript{50} value of approximately 1 μM, which is similar to the reported EC\textsubscript{50} values for UTP in mouse endothelial cells. The EC\textsubscript{50} value of ADP is less than two orders less potent than UTP. Thus, the cloned new P2 receptor does not exactly fit with any of previously cloned P2 receptors.
without effect on cells transfected with vector alone. The results indicate that the cloned P2 receptor is coupled to phospholipase C. To examine whether the receptor is coupled to phospholipase C via a pertussis toxin-sensitive G protein, we studied the effect of pertussis toxin pretreatment on ADP-induced inositol phosphate production. Pretreatment of pertussis toxin (10 ng/ml) for 24 h does not affect ADP (100 μM)-induced inositol phosphate production (Table I). Thus, the cloned P2 receptor appears to be coupled to phospholipase C via a pertussis toxin-insensitive G protein.

To examine potential coupling of the cloned P2 receptor to adenylate cyclase, the effects of nucleotides on cellular cyclic AMP contents were examined in C6–15 cells transfected with vector alone or the cloned P2-expression vector. Forskolin increases cyclic AMP contents 3.5–4.2-fold in cells transfected with vector alone or the cloned P2-expression vector. Either ADP, ATP, or UTP at 100 μM does not change cyclic AMP contents in forskolin-stimulated cells transfected with either construct. The results indicate that the cloned P2 receptor is not coupled positively or negatively to adenylate cyclase in C6–15 cells.

The results of the nucleotide selectivity of the cloned P2 receptor described above in the present study indicate that the pharmacological feature of the cloned P2 receptor is distinct from that of either mouse and human P2U receptor or chicken P2Y receptor. We further tried to characterize the pharmacological property of the cloned P2 receptor by examining the susceptibility of the cloned P2 receptor to known P2 receptor antagonists (38, 39). As shown in Fig. 7, suramin (100 μM), an antagonist for P2X, P2Y, and P2U receptors, slightly (20%) but significantly inhibited ADP-induced inositol phosphate production. Reactive blue-2 (100 μM), an antagonist for P2X and P2Y receptors, more strongly (77%) inhibited ADP-induced inositol phosphate production. Thus, the cloned P2 receptor is sensitive to known P2 receptor antagonists.
DISCUSSION

Based on their pharmacological properties and signaling mechanisms, mammalian P2 receptors have been classified into five subtypes, P2X, P2Y, P2Z, P2T, and P2U (1, 2). Among them, P2T subtype is expressed in very limited cell types including platelets (1, 2). P2X and P2Z have the properties of ligand-gated ion channels. P2X receptor cDNAs have recently been cloned (40, 41) and found to have only two transmembrane domains and a pore-forming motif. On the other hand, P2Y and P2U are a metabotropic type of P2 receptors and widely expressed in various tissues. Recent molecular cloning of mouse, human, and rat P2U and P2Y receptors revealed that they belong to G protein-coupled receptors and are linked to intracellular Ca\textsuperscript{2+} mobilization (16–19). In the present study we demonstrate the isolation of a cDNA clone encoding a novel member of P2 receptors. Evaluation of functional properties of the new receptor has revealed that it is not categorized into any of the classical P2 receptor subtypes mentioned above.

Sequence analysis of the cloned P2 receptor demonstrates that the receptor possesses structural properties characteristic of the G protein-coupled receptor superfamily and has a con-

TABLE I

| Pretreatment | Stimulation | Total inositol phosphates | % of basal |
|--------------|-------------|--------------------------|-----------|
| None         | none        | 100.0 ± 2.0              |           |
| Pertussis toxin | none        | 214.8 ± 5.5             |           |

TABLE II

| Transfection | Stimulation | Cyclic AMP content | pmol/ well |
|--------------|-------------|-------------------|-----------|
| Vector alone | none        | 1.88 ± 0.13       |           |
| forskolin    | none        | 7.65 ± 1.06       |           |
| forskolin + ADP | none      | 7.55 ± 0.88       |           |
| forskolin + ATP | none      | 8.10 ± 0.45       |           |
| forskolin + UTP | none      | 8.28 ± 0.31       |           |

| P2 receptor | none          | 1.68 ± 0.16     |           |
|            | forskolin    | 5.90 ± 0.46     |           |
|            | forskolin + ADP | none      | 5.27 ± 0.24     |           |
|            | forskolin + ATP | none      | 5.43 ± 0.76     |           |
|            | forskolin + UTP | none      | 5.72 ± 0.16     |           |
considerable homology to P2U and P2Y receptors (44 and 38% amino acid identity, respectively) (Fig. 2). Functional analysis of the cloned P2 receptor shows that this receptor is coupled to phospholipase C and Ca\textsuperscript{2+} mobilization (Fig. 5A), like P2U and P2Y receptors. However, the agonist selectivity of the cloned P2 receptor clearly differs from that of P2U and P2Y receptors; the rank order of agonist potency for the cloned P2 receptor is UTP > 2-methylthioATP = ADP > ATP; UTP > ATP >> 2-methylthioATP = ADP, and for the chicken P2 receptor 2-methylthioATP > ATP > ADP >> UTP. Thus, the cloned P2 receptor resembles the P2U receptor in that it can respond to the pyrimidine UTP with a relatively high sensitivity (Fig. 5B). However, the cloned P2 receptor also share some properties with classical P2Y receptors in that the cloned P2 and classical P2Y receptors respond to ADP and 2-methylthioATP with a higher sensitivity than the P2U receptor and that responses mediated by both cloned P2 and classical P2Y receptors are inhibited by the receptor antagonist, reactive blue-2 (37) (Fig. 7). All these results indicate that the cloned P2 receptor is a novel metabotropic P2 receptor, which, together with the recently cloned P2U and P2Y receptors, constitutes a distinct family of G protein-coupled P2 receptors.

Barnard et al. (42) and Fredholm et al. (31) in their recent reviews have recommended reclassifying cloned P2 receptors into the two major families, the G protein-coupled P2Y receptors and the intrinsic channel type receptor P2X. They proposed terming newly discovered G protein-coupled P2 receptors as P2Y\textsubscript{1}, P2Y\textsubscript{2}, P2X\textsubscript{1}, etc., by consecutively numbering them. They designated the chicken brain P2Y and its rat equivalent as P2Y\textsubscript{2}u mouse and human P2U as P2Y\textsubscript{2}u, and the third G protein-coupled P2 receptor with a strong preference for ADP that they have recently cloned but not published yet as P2Y\textsubscript{3} (42). Our P2 receptor clearly belongs to the G protein-coupled P2 receptor family and should in future be named in a systematic way as proposed (31, 42).

Detection of a strong signal of our cloned P2 receptor transcript in rat aorta (Fig. 3) implicates its physiological role in vascular function. Because the cloned P2 receptor transcript is detected in cultured rat aortic smooth muscle cells, the cloned P2 receptor is likely expressed in the smooth muscle layer of the vascular wall, where it may be involved in the regulation of vascular tone. The cloned P2 receptor transcript is also detected in the mesentery, indicating the expression of the cloned P2 receptor in both large capacitance vessels and smaller resistance vessels (Fig. 3). Because activation of the cloned P2 receptor stimulates phospholipase C to induce an increase in the [Ca\textsuperscript{2+}] (Figs. 5 and 6), the cloned P2 receptor on vascular smooth muscle cells most likely mediates vascular smooth muscle contractile response. In addition, it is an interesting possibility that the cloned P2 receptor on vascular smooth muscle cells may also be involved in promoting vascular smooth muscle cell growth under physiological or pathological conditions, because it was shown that nucleotides induce synthesis of DNA synthesis in cultured rat vascular smooth muscle cells (43, 44). The moderate levels of the cloned P2 receptor transcript are detected in smooth muscle organs, including stomach and intestine, suggesting that the cloned P2 receptor is involved in the functional regulation of nonvascular smooth muscle as well.

A number of recent reports demonstrated that the pyrimidine UTP causes potent vasoconstriction in a variety of vascular beds (8–11). The present study may suggest that the cloned P2 receptor mediates UTP-induced vasoconstriction. We found that the P2\textsubscript{2u} transcript is also detected in rat aorta, mesentery, and cultured RASM cells (Fig. 4). Therefore, both our novel P2 and P2\textsubscript{2u} receptors may be involved in the reported UTP-induced vascular contraction. A previous study (45) demonstrated that the rank order of potency for nucleotide-induced Ca\textsuperscript{2+} mobilization in RASM cells is more consistent with that of P2\textsubscript{2u} receptors (16, 17). This may suggest that P2\textsubscript{2u} rather than our cloned P2 receptor functionally predominates in RASM cells. However, it is possible that yet another P2Y subtype different from our cloned P2 or P2U (P2Y\textsubscript{2u}) are also involved in nucleotide-induced vasoconstriction, because some studies suggested the involvement of a P2Y subtype. Further study on the physiological properties of each P2Y receptor subtype will undoubtedly greatly help to promote understanding the physiology of P2 receptors.

Acknowledgments—We thank Dr. K. Hamada for help in sequencing DNA. We also thank F. Iwase, R. Nakanishi, and N. Miyamoto for excellent technical and secretarial assistance.

REFERENCES

1. Burnstock, G. (1990) in Ann. N. Y. Acad. Sci. 603, 1–18
2. Dubyak, G. R., and El-Moatassim, C. (1993) Am. J. Physiol. 557–566
3. Burnstock, G., and Kennedy, C. (1986) Circ. Res. 58, 319–330
4. Martin, W., Cusack, N. J., Carleton, J. S., and Gordon, J. L. (1985) Eur. J. Pharmacol. 108, 295–299
5. Boenenaoms, J. M., and Pearson, J. D. (1990) Trends Pharmacol. Sci. 11, 34–37
6. Motte, S., Pirrotton, S., and Boenenaoms, J. M. (1993) Circ. Res. 72, 504–510
7. Burnstock, G., and Kennedy, C. (1985) Gen. Pharmacol. 16, 433–440
8. Sefert, R., and Schlit, G. (1989) Trends Pharmacol. Sci. 10, 365–369
9. Saita, B., Milan, D., Allain, H., Rault, B., and Driessche, J. V. D. (1990) Blood Vessels 27, 352–364
10. von Kugelgen, I., and Starke, K. (1990) Naunyn-Schmiedeberg’s Arch. Pharmacol. 341, 538–546
11. Ralevic, V., and Burnstock, G. (1991) Circ. Res. 69, 1583–1590
12. Kitaizima, S., Ozaki, H., and Karaki, H. (1994) Eur. J. Pharmacol. 526, 263–267
13. Benham, C. D., and Tsien, R. W. (1987) Nature 328, 275–278
14. O’Connor, S. E., Dainty, I. A., and Leff, P. (1991) Trends Pharmacol. Sci. 12, 137–141
15. Wilkinson, G. F., Mckechney, K., Dainty, I. A., and Boarder, M. R. (1994) J. Pharmacol. Exp. Ther. 268, 881–887
16. Lustig, K. D., Shiu, A. K., Brake, A. J., and Julius, D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5113–5117
17. Parr, C. E., Sullivan, D. M., Paradiso, A. M., Lazarowski, E. R., Burch, L. H., Olsen, J. C., Erb, L. W., Weisman, G. A., Boucher, R. C., and Turner, J. T. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3275–3279
A Novel G Protein-coupled P₂ Receptor in the Vascular System

18. Rice, W. R., Burton, F. M., and Fiedeldey, D. T. (1995) Am. J. Respir. Cell Mol. Biol. 12, 27–32
19. Webb, T. E., Simon, J., Krishek, B. J., Bateson, A. N., Smart, T. G., King B. F., Burnstock, G., and Barnard, E. A. (1993) FEBS Lett. 324, 219–225
20. Chamly-Campbell, J., Campbell, G. R., and Ross, R. (1979) Physiol. Rev. 59, 1–61
21. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
22. Clemens, M. J. (1984) in Transcription and Translation: A Practical Approach (Hames, B. D., and Higgins, S. J., eds) pp. 211–230, IRL Press, Oxford
23. Sambook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Takebe, Y., Seki, M., Fujisawa, J., Pamala, H., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1989) Molec. Cell. Biol. 8, 466–475
25. Takuwa, Y., Kasuya, Y., Takuwa, N., Kudo, M., Yanagisawa, M., Goto, K., Masaki, T., and Yamashita, K. (1990) J. Clin. Invest. 85, 653–658
26. Libert, F., Pharmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M. J., Dumont, J. E., and Vassart, G. (1989) Science 244, 569–572
27. Isebe, K., Nakai, T., and Takuwa, Y. (1993) Endocrinology 132, 1757–1765
28. Takuwa, N., Takuwa, Y., Yanagisawa, M., Yamashita, K., and Masaki, T. (1989) J. Biol. Chem. 264, 7856–7861
29. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) J. Biol. Chem. 260, 3440–3450
30. Kozak, M. (1983) Nucleic Acids Res. 12, 865–872
31. Fredholm, B. B., Abbracchio, M. P., Burnstock, G., Daly, J. W., Harden, K., Jacobson, K. A., Leff, P., and Williams, M. (1994) Pharmacol. Rev. 46, 143–156
32. Ressler, K. J., Sullivan, S. L., and Buck, L. B. (1993) Cell 73, 597–609
33. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
34. Woodget, J. R., Gould, K. L., and Hunter, T. (1986) Eur. J. Biochem. 161, 177–184
35. Kennelly, P. J., and Krebs, E. G. (1991) J. Biol. Chem. 266, 15555–15558
36. Dohlman, H. G., Thorner, J., Caron, M. G., and Lefkowitz, R. J. (1991) Annu. Rev. Biochem. 60, 653–688
37. Strosberg, A. D. (1991) Eur. J. Biochem. 196, 1–10
38. Fedan, J. S., and Lamport, S. J. (1990) in Ann. N. Y. Acad. Sci. 603, 182–197
39. Hourani, S. M. O., Hall, D. A., and Nieman, C. J. (1992) Br. J. Pharmacol. 105, 453–457
40. Valera, S., Hussy, N., Evans, R. J., Adami, N., North, R. A., Surprenal, A., and Buell, G. (1994) Nature 371, 516–519
41. Brake, A. J., Wagenbach, M. J., and Julius, D. (1994) Nature 371, 519–523
42. Barnard, E. A., Burnstock, G., and Webb, T. E. (1994) Trends Pharmacol. Sci. 15, 67–70
43. Malam-Souley, R., Campaen, M., Gadeau, A. P., and Desgranges, C. (1993) Am. J. Physiol. 265, C783–C788
44. Erlinge, D., Yoo, H., Edvinsson, L., Reis, D. J., and Wahlestedt, C. (1993) Am. J. Physiol. 265, H1089–H1097
45. Tawada, Y., Furukawa K., and Shigekawa, M. (1987) J. Biochem. 102, 1499–1509