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## Site-directed Mutagenesis of P<sub>2U</sub> Purinoceptors

POSITIVELY CHARGED AMINO ACIDS IN TRANSMEMBRANE HELICES 6 AND 7 AFFECT AGONIST POTENCY AND SPECIFICITY\*

(Received for publication, November 9, 1994, and in revised form, December 23, 1994)

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Two subtypes of G protein-coupled receptors for nucleotides (P<sub>2U</sub> and P<sub>2Y</sub> purinoceptors) contain several conserved positively charged amino acids in the third, sixth, and seventh putative transmembrane helices (TMHs). Since the fully ionized form of nucleotides has been shown to be an activating ligand for both P<sub>2U</sub> and P<sub>2Y</sub> purinoceptors (P<sub>2U</sub>R and P<sub>2Y</sub>R), we postulated that some of these positively charged amino acids are involved in binding of the negatively charged phosphate groups of nucleotides. To investigate the role of the conserved positively charged amino acids in purinoceptor function, a series of mutant P<sub>2U</sub>R cDNAs were constructed so that lysine 107 and arginine 110 in TMH 3, histidine 262 and arginine 265 in TMH 6, and arginine 292 in TMH 7 were changed to the neutral amino acid leucine or isoleucine. The mutated P<sub>2U</sub>R cDNAs were stably expressed in 1321N1 astrocytoma cells and receptor activity was monitored by quantitating changes in the concentration of intracellular Ca<sup>2+</sup> upon stimulation with full (ATP, UTP) or partial (ADP, UDP) P<sub>2U</sub>R agonists. Neutralization of His<sup>262</sup>, Arg<sup>265</sup>, or Arg<sup>292</sup> caused a 100–850-fold decrease in the potency of ATP and UTP relative to the unmutated P<sub>2U</sub>R and rendered ADP and UDP ineffective. In contrast, neutralization of Lys<sup>107</sup> or Arg<sup>110</sup> did not alter the agonist potency or specificity of the P<sub>2U</sub>R. Neutralization of Lys<sup>289</sup> in the P<sub>2U</sub>R, which is expressed as a glutamine residue in the P<sub>2Y</sub> subtype, did not alter receptor activity; however, a conservative change from lysine to arginine at this position altered the rank order of agonist potency so that ADP and UDP were approximately 100-fold more potent than ATP and UTP. A three-dimensional model of the P<sub>2U</sub>R indicates the feasibility of His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup> interactions with the phosphate groups of nucleotides.

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The cDNAs encoding two subtypes of G protein-coupled receptors (GPCRs)<sup>1</sup> for extracellular nucleotides have been isolated (1–4) and, based upon the similarities of the predicted amino acid sequences, these nucleotide receptors apparently form a new subset within the GPCR superfamily (5). These receptors, known as P<sub>2</sub> purinoceptors and recently renamed P<sub>2Y</sub> purinoceptors (6), are linked predominantly via the G<sub>i</sub>/G<sub>o</sub> and G<sub>q</sub>/G<sub>11</sub> families of G proteins to the inhibition of adenylyl cyclase or the mobilization of intracellular calcium (7, 8). Physiological processes involving G protein-coupled P<sub>2</sub> purinoceptors include platelet aggregation and wound healing (9), insulin secretion (10), mitogenesis (11), vasodilation (12, 13), and trans-epithelial ion transport (14, 15), making these receptors an intriguing target for pharmacotherapy. For example, clinical studies in cystic fibrosis patients indicate that local delivery of ATP or UTP to lung epithelia stimulates chloride secretion and promotes mucohydration (16), presumably by activating the P<sub>2U</sub> subtype of purinoceptors. Unfortunately, the lack of stable, selective agonists and antagonists for the various P<sub>2</sub> purinoceptor subtypes has hindered further development of therapeutic applications centered around these receptor molecules. The work described in this paper used site-directed mutagenesis to investigate amino acid residues involved in determining the agonist potency and specificity of the P<sub>2U</sub>R, an important step in the process of rational drug design.

### EXPERIMENTAL PROCEDURES

**Materials**—Pfu DNA polymerase was purchased from Stratagene (La Jolla, CA). G418 (Geneticin) was purchased from Life Technologies, Inc. Human astrocytoma (1321N1) cells were obtained from M. L. Toews (University of Nebraska, Omaha, NE), and a clonal line that was not responsive to extracellular nucleotides was selected. cDNA encoding the murine P<sub>2U</sub>R was provided by K. D. Lustig and D. Julius (University of California, San Francisco).

**Site-directed Mutagenesis of P<sub>2U</sub>R cDNA**—Point mutations in the murine P<sub>2U</sub>R cDNA were made by using enzymatic inverse polymerase chain reaction as described previously (17). The class 2s restriction enzyme used was BsmI (New England Biolabs, Beverly, MA). To reduce the amount of DNA sequencing necessary to confirm the mutation within the cDNA, an approximately 1-kilobase pair BstEII/A/III fragment containing the point mutation was removed and used to replace a similar fragment in the wild type receptor cDNA. All mutations were verified by double-stranded dideoxy sequencing (18).

**Receptor Expression in Mammalian Cells**—To generate stable transfectants expressing the mutant P<sub>2U</sub>Rs, a 2.4-kilobase pair HindIII/NotI fragment encompassing nearly the entire receptor cDNA was excised from each mutant construct in the pBluescript vector and subcloned into the mammalian expression vector pRc/CMV (Invitrogen, San Diego, CA). 1321N1 cells were stably transfected with the wild type or mutant pRc/CMV-P<sub>2U</sub>R plasmids by electroporation as described previously for the transfection of K562 human leukemia cells with pRc/CMV-P<sub>2U</sub>R (7). Transfected 1321N1 cells were selected in growth medium (Dulbecco's modified Eagle's medium, 5% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin) containing 500 µg/ml G418 and maintained in growth medium containing 100 µg/ml G418 at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. Clonal cell lines were isolated and assayed for P<sub>2U</sub>R activity by quantitating changes in the concentration of cytoplasmic free calcium ([Ca<sup>2+</sup>]<sub>i</sub>) upon stimulation with extracellular nucleotides. Preincubation of transfected 1321N1 cells with apyrase (2 units/ml) to hydrolyze endogenously released ATP (3) had no effect on wild type or mutant P<sub>2U</sub>R activity and, therefore, was not used in this study.

<sup>1</sup> The abbreviations used are: GPCR, G protein-coupled receptor; P<sub>2U</sub>R, P<sub>2U</sub> purinoceptor; TMH, transmembrane helix.

	TMH 3		TMH 6		TMH 7	
	107	110	262	265	289	292
RECEPTOR:						
Murine P <sub>2U</sub>	KLVRF <sup>107</sup> LYTNLYCSILFLTCISVHR		KSVRTIALVLAVFALCF <sup>262</sup> LPFHVTRTL		MAYKVTRPLASANSCLDPVLYFL	
Human P <sub>2U</sub>	KLVRF <sup>107</sup> LYTNLYCSILFLTCISVHR		KSVRTIAVLAVFALCF <sup>262</sup> LPFHVTRTL		MAYKVTRPLASANSCLDPVLYFL	
Avian P <sub>2Y</sub>	KLQRFIFHVNLYGSILFLTCISVHR		KSYLVILVLT <sup>262</sup> VFVSVYLPFHVMKTL		TTYQVTRGLASINSCVDPILYFL	
Bovine A <sub>1</sub>	LKVA <sup>107</sup> CPVLILTQSSILALLAIAVDR		KIAKSLALILFLFALSWLFLHILNCI		ILTYIAIFLSHGNSAMNPITYAF	

Fig. 1. Sequence alignment of the murine P<sub>2U</sub>, human P<sub>2U</sub>, chick P<sub>2Y</sub>, and bovine A<sub>1</sub> adenosine receptor. Amino acids that form the third, sixth, and seventh TMHs are shown. Altered amino acids are underlined, and the position within the murine receptor sequence is indicated above each mutagenized residue. Hydrophobicity analysis and two-dimensional modeling of the P<sub>2U</sub>R indicated that the altered amino acids are located near the plasma membrane-extracellular interface.

**Calcium Measurements**—[Ca<sup>2+</sup>]<sub>i</sub> was measured by dual excitation spectrofluorometric analysis of cell suspensions loaded with fura-2 as described previously (19, 20) and assayed in 10 mM Hepes-buffered saline (pH 7.4) containing 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub> unless indicated otherwise. Concentration response data were analyzed with the Prism curve-fitting program (GraphPAD, San Diego, CA).

**Molecular Modeling**—Construction of the P<sub>2U</sub>R three-dimensional model was based on the following five criteria used to model neurotransmitter GPCRs (21). 1) Hydrophobicity analysis and sequence alignment with highly conserved amino acids in other GPCRs were used to select regions of the receptor as probable transmembrane  $\alpha$ -helices. 2) A model for bacteriorhodopsin based on high resolution electron cryomicroscopy was used as a template to position the sixth and seventh anti-parallel  $\alpha$ -helices of the murine P<sub>2U</sub>R. 3) The helices were oriented so that highly conserved residues and charged residues faced the central cleft of the receptor, as is the case for bacteriorhodopsin. 4) The peptide backbone dihedral angles,  $\phi$  and  $\psi$ , were  $-63.0^\circ$  and  $-45.3^\circ$ , respectively, which is consistent for  $\alpha$ -helices in a hydrophobic environment. 5) Energy minimization was performed by using the Kollmann force field analysis in SYBYL (TRIPOS, St. Louis, MO) to optimize the packing of the model.

## RESULTS AND DISCUSSION

The ligand binding sites of most GPCRs, with the possible exception of the glycoprotein and peptide hormone receptors, appear to be located in the transmembrane  $\alpha$ -helical regions (22, 23). With this in mind and since the fully ionized form of nucleotides has been shown to be an agonist for both P<sub>2U</sub> and P<sub>2Y</sub> purinoceptors (19, 24, 25), we postulated that several of the positively charged amino acids in the putative transmembrane helices of G protein-coupled purinoceptors that are conserved between the P<sub>2U</sub> and P<sub>2Y</sub> subtypes may be responsible for binding the negatively charged phosphate groups of the nucleotide ligands.

Sequence alignment of the murine P<sub>2U</sub>R, human P<sub>2U</sub>R, and chick P<sub>2Y</sub>R indicated that five positively charged amino acids (Lys<sup>107</sup>, Arg<sup>110</sup>, His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup>) near the plasma membrane-extracellular interface of TMH 3, TMH 6, and TMH 7 were conserved with only a modest change (from Arg<sup>265</sup> to Lys) occurring in the P<sub>2Y</sub> subtype (Fig. 1). The adenosine A<sub>1</sub> receptor lacks these lysine and arginine residues (Fig. 1), although histidine residues in TMH 6 and TMH 7 of this receptor are involved in ligand binding (26). The conserved positively charged amino acids in TMH 6 and TMH 7 are close (within 1–5 amino acids) to prolines or glycines that have been implicated in forming hinges within transmembrane  $\alpha$ -helices as well as kinks and turns (27, 28). These perturbations of the  $\alpha$ -helix caused by prolines or glycines could serve to create a ligand binding pocket involving juxtaposing residues (29).

To investigate the role of the conserved positively charged amino acids in nucleotide receptor function, we constructed a series of mutant cDNAs that encode P<sub>2U</sub>R proteins with single amino acid substitutions. Concentration response curves generated for the wild type and mutant P<sub>2U</sub>Rs expressed in 1321N1 cells indicate that His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup> are important for establishing the agonist potency and specificity of the P<sub>2U</sub>R. Neutralization of His<sup>262</sup>, Arg<sup>265</sup>, or Arg<sup>292</sup> by substitution with the uncharged amino acid leucine in each case caused a 100–850-fold decrease in the potency of ATP and UTP

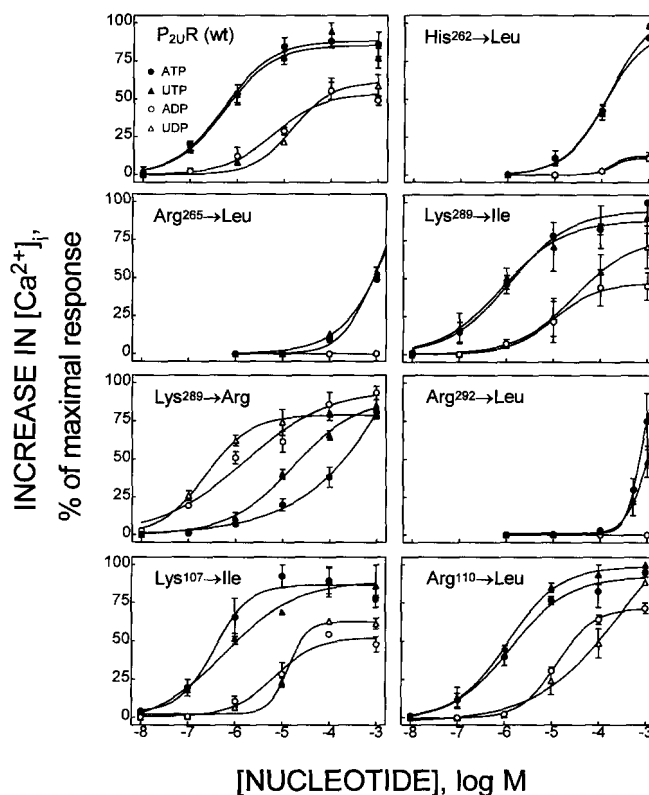


Fig. 2. Agonist stimulation of intracellular calcium mobilization in 1321N1 cells transfected with wild type or mutant P<sub>2U</sub>R constructs. Cells stably transfected with wild type or mutant P<sub>2U</sub>R cDNAs were assayed for P<sub>2U</sub>R activity by quantitating changes in the concentration of cytoplasmic free calcium ([Ca<sup>2+</sup>]<sub>i</sub>) in response to the indicated concentration of ATP, UTP, ADP, or UDP. Data are expressed as a percentage of the maximal increase in [Ca<sup>2+</sup>]<sub>i</sub> elicited by the most effective ligand for each receptor construct and represent the mean  $\pm$  S.E. of three experiments. The maximal increase in [Ca<sup>2+</sup>]<sub>i</sub> obtained for the wild type receptor and each mutant construct ranged from 200–500 nM, except for mutant Lys<sup>289</sup>  $\rightarrow$  Arg, which exhibited a maximal increase of 90 nM. The average resting [Ca<sup>2+</sup>]<sub>i</sub> was 80–150 nM for all constructs.

without decreasing the efficacy of these ligands relative to the wild type receptor (Fig. 2). ADP and UDP, which are partial agonists of the wild type P<sub>2U</sub>R (Fig. 2), were ineffective agonists of the His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup> mutants at concentrations as high as 3 mM (Fig. 2). Neutralization of a nonconserved positively charged residue in TMH 7 (Lys<sup>289</sup>  $\rightarrow$  Ile) as well as the 2 conserved positively charged residues in TMH 3 (Lys<sup>107</sup>  $\rightarrow$  Ile and Arg<sup>110</sup>  $\rightarrow$  Leu) had little effect on the agonist potency or specificity of the P<sub>2U</sub>R (Fig. 2), indicating that these residues are not critical for receptor activation.

Previous studies with the P<sub>2U</sub>R in murine macrophages suggest that this receptor is activated by both ATP<sup>4-</sup> and MgATP<sup>2-</sup> but that ATP<sup>4-</sup> is 10 times more potent than MgATP<sup>2-</sup> (apparent K<sub>0.5</sub> values were 0.65  $\mu$ M and 6.5  $\mu$ M,



TABLE I  
EC<sub>50</sub> values and slope factors of ATP for wild type and mutant P<sub>2U</sub> purinoceptors

The values are derived from concentration response curves as described in Fig. 2 and are generated from cells incubated in the presence (+) or absence (−) of 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub>. EDTA (0.1 μM) was added to the media of cells incubated in the absence of divalent cations. The data represent the mean ± S.E. of three experiments.

Divalent cations	EC <sub>50</sub> values (μM)		Slope factor	
	−	+	−	+
Wild type P <sub>2U</sub> R	0.85 ± 0.13	1.00 ± 0.15	0.72 ± 0.08	0.88 ± 0.12
His <sup>262</sup> → Leu	11.7 ± 1.0	105 ± 20	1.00 ± 0.20	0.95 ± 0.18
Arg <sup>265</sup> → Leu	178 ± 76	457 ± 118	0.55 ± 0.18	1.22 ± 0.30
Arg <sup>292</sup> → Leu	209 ± 25	851 ± 160	1.85 ± 0.28	2.29 ± 0.37

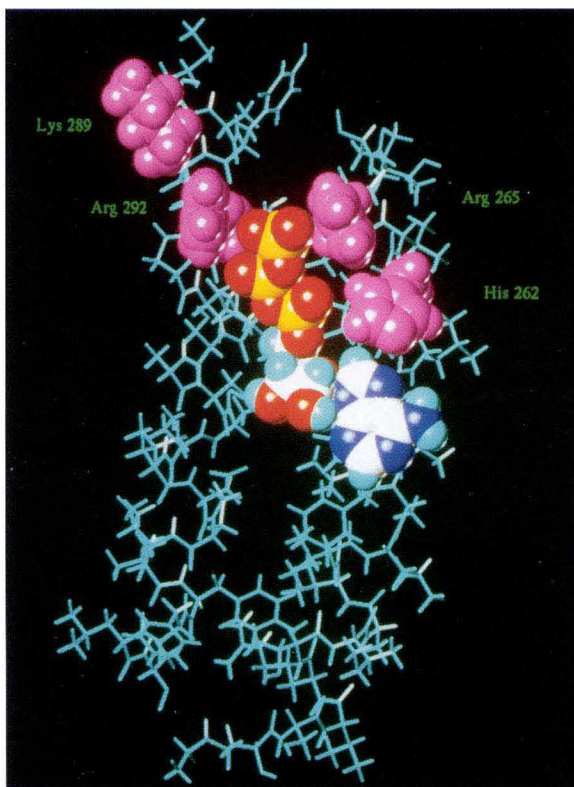


FIG. 3. Molecular model of the P<sub>2U</sub>R sixth and seventh TMHs showing the putative ATP binding site. Shown is a space-filling model of amino acid residues (highlighted in fuchsia) that are thought to interact with the negatively charged phosphate residues of ATP. The viewing direction is in the plane of the plasma membrane and within the central cleft of the receptor. ATP is colored by atom types: white, carbon; red, oxygen; orange, phosphorus; dark blue, nitrogen; light blue, hydrogen.

respectively) (30). Therefore, it was possible that the rightward shift in the concentration response curves observed for the His<sup>262</sup> → Leu, Arg<sup>265</sup> → Leu, and Arg<sup>292</sup> → Leu mutants was caused by a selective decrease in receptor affinity for the fully ionized nucleotide. Activation of these mutant P<sub>2U</sub>Rs in the absence of extracellular divalent cations, however, indicated that ATP<sup>4−</sup> is still the most potent form of agonist and that the potency of ATP<sup>4−</sup>, relative to total ATP, may be enhanced at these mutant receptors (Table I).

Although neutralization of Lys<sup>289</sup> by substitution with isoleucine had little effect on the agonist potency or specificity of the P<sub>2U</sub>R, a conservative substitution of Lys<sup>289</sup> with arginine shifted the agonist potency so that ADP and UDP were more effective agonists than ATP and UTP (Fig. 2). Relative to the wild type P<sub>2U</sub>R, the potencies of ADP and UDP were increased

by 4- and 7-fold, respectively, whereas the potencies of ATP and UTP were decreased by 300- and 26-fold, respectively, for the Lys<sup>289</sup> → Arg mutant. The efficacy of all four ligands, however, was reduced compared to the wild type receptor (Fig. 2), which could be due to a change in the receptor's tertiary structure introduced by the Lys<sup>289</sup> → Arg mutation or a low expression level of this mutant receptor. Nonetheless, the altered rank order of agonist potency of the Lys<sup>289</sup> → Arg mutant cannot be explained by a low level of receptor expression and strongly suggests that this conservative mutation affects ligand binding and not a subsequent step in the signaling pathway.

Interestingly, Lys<sup>289</sup>, which is conserved in the human and murine P<sub>2U</sub>R homologs, is instead the neutral amino acid glutamine in the chick P<sub>2Y</sub>R (Fig. 1). Assuming that the phosphate docking site in these nucleotide receptor subtypes is the same, this could explain why neutralization of Lys<sup>289</sup> in the P<sub>2U</sub>R did not alter receptor activity and suggests that the Lys<sup>289</sup> residue is not directly involved in ligand binding. However, the finding that the Lys<sup>289</sup> → Arg mutation altered the rank order of agonist potency of the P<sub>2U</sub>R could suggest that Lys<sup>289</sup> is positioned close enough to the ligand binding pocket so that substitution of this residue with the slightly larger arginine residue is able to interfere with normal ligand binding.

Molecular modeling of the P<sub>2U</sub>R sixth and seventh transmembrane α-helical regions indicated the feasibility of His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup> interactions with the negatively charged phosphate residues of ATP (Fig. 3). Although ligand binding could not be directly demonstrated in this paper, due to the lack of a reliable assay, the finding that neutralization of His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup> decreased the potency but not the efficacy of ATP and UTP suggests that ligand binding is being affected. Furthermore, the observation that the agonist potency of both ATP and UTP were affected equally by these mutations supports the hypothesis that His<sup>262</sup>, Arg<sup>265</sup>, and Arg<sup>292</sup> interact with the phosphate residues rather than the purine or pyrimidine group of these nucleotide ligands. Unlike the P<sub>2U</sub> purinoceptor subtype, the P<sub>2Y</sub> subtype is not activated by the pyrimidine nucleotides UTP or UDP. Further mutagenesis studies as well as the creation of P<sub>2U</sub>/P<sub>2Y</sub> chimeric receptors should help to delineate which regions of these nucleotide receptors are responsible for selectivity of the nucleoside group.

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#### REFERENCES

- Lustig, K. D., Shiau, A. K., Brake, A. J., and Julius, D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 5113–5117
- Webb, T. A., 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
- Parr, C. E., Sullivan, D. M., Paradiso, A. M., Lazarowski, E. R., Burch, L. H., Olsen, J. C., Erb, L., Weisman, G. A., Boucher, R. C., and Turner, J. T. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 3275–3279
- Filtz, T. M., Li, Q., Boyer, J. L., Nicholas, R. A., and Harden, T. K. (1994) *Mol. Pharmacol.* **46**, 8–14
- Barnard, E. A., Burnstock, G., and Webb, T. A. (1994) *Trends Pharmacol. Sci.* **15**, 67–70
- Fredholm, B. B., Abbracchio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P., and Williams, M. (1994) *Pharmacol. Rev.* **46**, 143–156
- Erb, L., Lustig, K. D., Sullivan, D. M., Turner, J. T., and Weisman, G. A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 10449–10453
- Dubyak, G. R., and El-Moatassim, C. (1993) *Am. J. Physiol.* **265**, C577–C606
- Gachet, C., and Cazenave, J. P. (1991) *Nouv. Rev. Fr. Hematol.* **33**, 347–358
- Loubatieres-Mariani, M. M., Chapal, J., Lignon, F., and Valette, G. (1979) *Eur. J. Pharmacol.* **59**, 277–286
- Boutherin-Ralson, O., Reuse, S., Dumont, J. E., and Boeynaems, J. M. (1990) *Biochem. Biophys. Res. Commun.* **172**, 306–312
- Lustig, K. D., Erb, L., Landis, D. M., Hicks-Taylor, C. S., Zhang, X., Sportiello, M. G., and Weisman, G. A. (1992) *Biochim. Biophys. Acta* **1134**, 61–72
- Boeynaems, J. M., and Pearson, J. D. (1990) *Trends Pharmacol. Sci.* **11**, 34–37
- Yu, H., and Turner, J. T. (1991) *J. Exp. Pharmacol. Ther.* **259**, 1344–1350
- Clarke, L. L., and Boucher, R. C. (1992) *Am. J. Physiol.* **263**, C348–C356
- Knowles, M. R., Clarke, L. L., and Boucher, R. C. (1991) *N. Engl. J. Med.* **325**, 533–538

17. Stemmer, W. P. C., and Morris, S. K. (1992) *BioTechniques* **13**, 215–220
18. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467
19. Lustig, K. D., Sportiello, M. G., Erb, L., and Weisman, G. A. (1992) *Biochem. J.* **284**, 733–739
20. Turner, J. T., James-Kracke, M. R., and Camden, J. M. (1990) *J. Pharmacol. Exp. Ther.* **253**, 1049–1056
21. Hibert, M. F., Trumpp-Kallmeyer, S., Bruinvels, A., and Hoflack, J. (1991) *Mol. Pharmacol.* **40**, 8–15
22. Probst, W. C., Snyder, L. A., Schuster, D. I., Brosius, J., and Sealfon, S. C. (1992) *DNA Cell Biol.* **11**, 1–20
23. Strader, C. D., Fong, T. M., Tota, M. R., Underwood, D., and Dixon, R. A. F. (1994) *Annu. Rev. Biochem.* **63**, 101–132
24. Greenberg, S., Di Virgilio, F., Steinberg, T. H., and Silverstein, S. C. (1988) *J. Biol. Chem.* **263**, 10337–10343
25. Motte, S., Piroton, S., and Boeynaems, J. M. (1993) *Br. J. Pharmacol.* **109**, 967–971
26. Olah, M. E., Ren, H., Ostrowski, J., Jacobson, K. A., and Stiles, G. L. (1992) *J. Biol. Chem.* **267**, 10764–10770
27. Williams, K. A., and Deber, C. M. (1991) *Biochemistry* **30**, 8919–8923
28. MacArthur, M. W., and Thornton, J. M. (1991) *J. Mol. Biol.* **218**, 397–412
29. Ballesteros, J. A., and Weinstein, H. (1992) *Biophys. J.* **62**, 107–109
30. Alonso-Torre, S. R., and Trautmann, A. (1993) *J. Biol. Chem.* **268**, 18640–18647