Molecular cloning and expression of a cDNA encoding the secretin receptor

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Secretin is a 27 amino acid peptide which stimulates the secretion of bicarbonate, enzymes and potassium ion from the pancreas. A complementary DNA encoding the rat secretin receptor was isolated from a CDM8 expression library of NG108-15 cell line. The secretin receptor expressed in COS cells could specifically bind the iodinated secretin with high and low affinities. Co-expression of the secretin receptor with the α -subunit of rat Gs protein increased the concentration of the high affinity receptor in the membrane fraction of the transfected COS cells. Secretin could stimulate accumulation of cAMP in COS cells expressing the cloned secretin receptor. The nucleotide sequence analysis of the cDNA has revealed that the secretin receptor consists of 449 amino acids with a calculated M_r of 48 696. The secretin receptor contains seven putative transmembrane segments, and belongs to a family of the G protein-coupled receptor. However, the amino acid sequence of the secretin receptor has no significant similarity with that of other G protein-coupled receptors. A 2.5 kb mRNA coding for the secretin receptor could be detected in NG108-15 cells, and rat heart, stomach and pancreatic tissue.

Key words: cAMP/expression cloning/G protein/receptor/secretin

Introduction

Secretin was discovered in 1902 by Bayliss and Starling in the duodenal mucosa as a hormone which stimulates the secretion of bicarbonate, enzymes and potassium ions from the pancreas (Bayliss and Starling, 1902). Vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), glucagon, growth hormone releasing factor (GRF) and gastric inhibitory peptide (GIP) are related to secretin, and constitute a family of peptide hormones (Rosselin, 1986; Gozes and Brenneman, 1989). They have various pharmacological effects on a variety of tissues including pancreas, liver, heart, intestine and kidney (Robberecht *et al.*, 1990). In addition, since these hormones can be found in the central and peripheral nervous system, it is likely that

they act not only as hormones, but also as neurotransmitters or neuromodulators (Gozes and Brenneman, 1989).

The 27 amino acid rat secretin is synthesized as a precursor protein of 134 amino acids in specific endocrine cells, S cells, in the mucosa of the small intestine (Kopin *et al.*, 1990). The transcript for the secretin precursor was also detected in rat hypothalamus, brain stem and cortex, although the expression level was far less than that in small intestine (Kopin *et al.*, 1990). In gut, secretin works as hormone which stimulates secretion of enzymes and electrolytes. In brain and adrenal pheochromocytoma PC-12 cells, secretin regulates tyrosine hydroxylase which is the rate-limiting enzyme in the biosynthesis of catecholamines, suggesting secretin may also work as a neuromodulator (Roskoski *et al.*, 1989).

Diverse functions of peptide hormones of the secretin family are mediated by the interaction with their respective receptors (Rosselin, 1986). Since secretin, VIP and glucagon increase intracellular cAMP, and GTP regulates the interaction of hormones with their receptors, it was postulated that receptors for these hormones are coupled to the cyclase-stimulated G protein (Gs) (Roth *et al.*, 1984; Freneau *et al.*, 1986). However, except for the VIP receptor (Couvineau *et al.*, 1990), the purification and biochemical characterization of receptors for these peptide receptors were hampered by the low abundance of the receptors. In the case of secretin receptor, high and low affinity-secretin binding sites were found in the membrane fraction from rat pancreatic acini (Bissonnette *et al.*, 1984). Chemical cross-linking analyses of the receptor with [¹²⁵I]secretin indicated that the secretin receptor in rat gastric glands (Bewab *et al.*, 1988) and pancreatic acini (Gossen *et al.*, 1989) have M_r values of 62 000 and 51 000, respectively.

In this report, we have isolated a complementary DNA encoding rat secretin receptor from NG108-15 cells. Secretin bound specifically to COS cells transfected with the cloned cDNA and stimulated the intracellular accumulation of cAMP. The nucleotide sequence analysis of the cDNA has revealed that the secretin receptor is a new type of the G protein-coupled receptor with seven transmembrane segments.

Results

Expression cloning of the secretin receptor

To identify the cDNA clone encoding secretin receptor, we utilized a direct expression cloning strategy, which has been used to isolate various cytokine receptor cDNAs (Munro and Maniatis, 1989; Fukunaga *et al.*, 1990). In this method, a cDNA library constructed with a mammalian expression vector was expressed in COS cells, and the transfected cells were subsequently assayed for the ability to bind radioactive ligands. Secretin stimulates adenylate cyclase through the interaction of its receptor with Gs protein (Roth *et al.*, 1984;

Freneau *et al.*, 1986). In β -adrenergic systems, association of the receptor with Gs results in an increase in the affinity of the receptor for ligands (Cerione *et al.*, 1984). We screened the secretin receptor cDNA by its direct expression in a COS cell line (COSGs1) which overexpresses the α -subunit of the Gs-protein (Gs α), in the hope that the receptor would bind [125 I]secretin with a higher affinity than in normal COS cells.

A cDNA library was constructed with the CDM8 vector (Seed, 1987) using poly(A) RNA from NG108-15 cells that express a relatively large number of the secretin receptor (Roth *et al.*, 1984). Plasmid DNA from pools of 750–1000 individual transformants was transfected into COSGs1 cells. Expression of the secretin receptor was screened by incubating cells with [125 I]secretin, followed by exposure to an IP plate (Fuji BAS 2000 system) and image analysis. This method reduced the exposure time about 50 times compared with the normal autoradiography using an X-ray film. After screening 400 pools (~350 000 clones) in this manner, we identified a pool which consistently gave a positive signal. About 3000 independent clones from the positive pool were then subjected to sib selection to isolate the pQ17 clone.

Binding characteristics of the cloned receptor

To confirm that the cloned cDNA encoded the secretin receptor, the pQ17 cDNA was expressed in COS cells with or without rat Gs α cDNA (Itoh *et al.*, 1986). [125 I]secretin bound to the membranes prepared from COS cells co-transfected with pQ17 and a mammalian expression vector pEF-BOS (Mizushima and Nagata, 1990) in a saturating manner, whereas no specific binding of secretin was observed with the membranes from untransfected COS cells. A Scatchard analysis of the binding data revealed the presence of two classes of binding sites with apparent dissociation constants (K_d) of 0.57 nM and 20.1 nM (Figure 1a), which were similar to the values obtained using the membranes from NG108-15 cells (0.44 nM and 14.5 nM) (Figure 1b). The concentration of high and low affinity binding sites in membranes from COS cells was 0.022 pmol, and 1.22 pmol/mg protein, respectively. When COS cells were co-transfected with pQ17 and pEF-BOS-Gs, the concentration of high affinity binding sites for secretin in the membranes increased ~10-fold (0.21 pmol/mg protein), while low affinity binding sites remained at approximately the same level (Figure 1a). These results indicate that the secretin receptor associates with the Gs α protein to form the high affinity binding site.

The binding specificity of the cloned secretin receptor was then examined. Figure 2a shows the ability of various unlabeled peptides to compete with the binding of [125 I]secretin to the transfected COS cell membrane. The results indicated that secretin is the most potent agent in displacing the binding of [125 I]secretin. The C-terminal peptide of secretin (secretin₅₋₂₇) and VIP were ~1000 times less potent than secretin in inhibiting the binding of [125 I]secretin to the membrane. Glucagon had a very weak ability to compete with the binding of secretin to the secretin receptor expressed in COS cells. The apparent half-maximal concentrations for the inhibition (IC_{50}) of secretin, secretin₅₋₂₇ and VIP were 4.5 nM, 9.5 μ M and 4.5 μ M, respectively. These values agreed well with the results obtained with membranes from NG108-15 cells (Figure 2b) and guinea pig pancreatic acini (Jensen *et al.*, 1983).

Fig. 1. Scatchard analysis of [125 I]secretin binding to membranes from COS cells transfected with the secretin receptor cDNA and NG108-15 cells. Membrane fractions from COS7 cells or NG108-15 cells were incubated with various concentrations of [125 I]secretin with or without an excess of unlabeled secretin as described in Materials and methods. The binding data were analyzed using the MacLigand Program (Munson and Rodbard, 1980). (a) Scatchard plot of [125 I]secretin binding data with membranes from COS cells. COS cells were cotransfected with pQ17 and pEF-BOS ($\circ$) or with pQ17 and pEF-BOS-Gs ($\square$). (b) Scatchard plot of [125 I]secretin binding data with membranes from NG108-15 cells.

Intracellular accumulation of cAMP mediated by the cloned secretin receptor

To examine whether the cloned secretin receptor expressed in COS cells could transduce the signal, stimulation of the adenylate cyclase activity by secretin was studied (Figure 3). Secretin stimulated the accumulation of cAMP in COSGs1 cells transfected with pQ17, whereas COSGs1 cells did not respond to secretin in order to accumulate intracellular cAMP. The response was dose-dependent, and the half-maximal response was obtained with 1 nM secretin, which was very close to the value obtained with NG108-15 cells (data not shown). VIP functioned as an agonist to stimulate adenylate cyclase activity in the pQ17-transfected COSGs1 cells, but ~60-fold molar excess of VIP than secretin was needed to show the half-maximal response (Figure 3). These results agree with the previous report for the native secretin receptor using rat gastric glands (Gespach

Fig. 2. Displacement of $[^{125}\text{I}]$ secretin binding by secretin, secretin₅₋₂₇, VIP and glucagon. The binding of $[^{125}\text{I}]$ secretin to membranes was determined in the presence of indicated concentrations of secretin ($\circ$), secretin₅₋₂₇ ($\bullet$), VIP ($\square$) and glucagon ($\triangle$). (a) Displacement of $[^{125}\text{I}]$ secretin binding to membranes from COS7 cells cotransfected with pQ17 and pEF-BOS-Gs. (b) Displacement of $[^{125}\text{I}]$ secretin binding to membranes from NG108-15 cells.

et al., 1986), and confirm that the pQ17 cDNA codes for a functional secretin receptor.

Primary structure of secretin receptor

Figure 4a shows the complete nucleotide sequence (1796 nucleotides long) and the deduced amino acid sequence (449 amino acids) for the secretin receptor. There are two potential AUG initiation codons at amino acid positions -22 and -18 (nucleotide positions 213-215 and 225-227, respectively) in the same reading frame. Although the neighboring sequence of the second AUG agrees better with the consensus sequence (CCA/GCCAUGG) proposed by Kozak (1987), the first AUG from the 5' terminus was tentatively assigned as the initiation codon for the secretin receptor. A hydropathy plot of the amino acid sequence of the secretin receptor (Figure 4b) indicated the presence of a signal sequence at the N-terminal and predicted seven membrane-spanning segments each consisting of 20-24 amino acids (Figure 4a). By comparing the 5' portion of the sequence with the typical signal peptide cleavage site (von

Fig. 3. Stimulation of cAMP production in COSGsl cells expressing the rat secretin receptor by secretin, VIP and glucagon. COSGsl cells transfected with pQ17 were incubated for 45 min with various concentrations of secretin ($\circ$), VIP ($\square$) or glucagon ($\triangle$). As a control, COSGsl cells transfected with the CDM8 vector were treated with 10 μM secretin ($\bullet$), VIP ($\blacksquare$) or glucagon ($\blacktriangle$). The intracellular amount of cAMP was measured as described in Materials and methods and is expressed per 1×10^5 cells.

Heijne, 1986), the 23rd amino acid (Ala) from the initiation codon was tentatively assigned as the N-terminal amino acid of the mature protein. Thus, the mature secretin receptor may consist of 427 amino acids with a calculated M_r of 48 696, which is 2-13 kd smaller than the value estimated for the secretin receptor in rat gastric glands or pancreatic acini by cross-linking studies (Bewab *et al.*, 1988; Gossen *et al.*, 1989). The difference is probably due to the glycosylation at some of the 5 potential N-glycosylation sites (Asn-X-Thr/Ser) found in the putative extracellular domain of the secretin receptor (Figure 4a).

The NG108-15 cell line used to prepare the cDNA library is a hybrid cell line between mouse N18TG neuroblastoma and rat C6Bu-1 glioma cell lines (Klee *et al.*, 1974). To determine the origin of the secretin receptor cDNA (pQ17), a set of oligonucleotides (nucleotides from positions 1476-1495 for the forward primer and 1705-1724 for the reverse primer) was synthesized, and genomic DNAs from rat and mouse were analyzed by polymerase chain reaction (PCR). Amplification of rat and mouse DNAs gave a single discrete band of about 250 nucleotides (data not shown), and the partial nucleotide sequences of these DNA fragments were determined after subcloning into pUC119. The DNA fragment amplified from the rat genomic DNA had a completely identical nucleotide sequence to that of pQ17 cDNA while the nucleotide sequence of the DNA fragment from mouse genomic DNA had a 17% mismatch with that of pQ17. These results indicate that the secretin receptor mRNA corresponding to the pQ17 cDNA was derived from the gene on rat genome present in the NG108-15 hybrid cell line.

Tissue distribution of secretin receptor mRNA

Secretin has pharmacological effects not only in the pancreas but also in other tissues including kidney, intestine, heart and brain (Christophe *et al.*, 1984; Roth *et al.*, 1984;

the stomach and pancreas, but was not found in the lung, liver, kidney and brain. Secretin receptor mRNA was detected in RNA from the hybrid NG108-15 and its parental mouse N18 cells but not from the parental rat C6 glioma cells, which agreed with the results obtained from

Fig. 5. Northern hybridization analysis of the secretin receptor mRNA. Poly(A) RNA (0.5 μ g) from rat tissues and cell lines was electrophoresed on a 1.2% agarose gel containing 6.6% formaldehyde and analyzed by Northern hybridization using the 32 P-labeled 0.7 kb *Nco*I–*Sph*I fragment of pQ17 as a probe.

[125 I]secretin binding (Roth *et al.*, 1984). However, as pointed out above, the secretin receptor cDNA cloned from the hybrid NG108-15 was cDNA for rat mRNA. Therefore, the rat secretin receptor gene from the C6 cell line appears to be transcriptionally activated in the rat/mouse hybrid NG108-15 cell line.

Discussion

Previously, Munro and Maniatis have developed a method to isolate cDNA clones for cell surface receptor by using a radioactive ligand to probe the COS cell monolayer transfected with a expression cDNA library (Munro and Maniatis, 1989). In this report, we have successfully used this method with a slight modification to clone the secretin receptor. Because of the low specific activity of [125 I]secretin and the low affinity of the receptor to [125 I]secretin, the autoradiography of the COS cell monolayer with the ordinary X-ray film was impracticable in exposure times. This problem was overcome by using the IP plate instead of X-ray film. Namely, the 18 h exposure to the IP plate gave an almost identical signal to that obtained with the 40 day exposure to the X-ray film. This modified method may be applied to screen cDNAs for other cytokine and peptide hormone receptors.

We have shown that the secretin receptor can interact with the α -subunit of Gs protein (Figure 1) and activate the adenylate cyclase in COS cells (Figure 3). Accordingly, the secretin receptor contains seven putative transmembrane segments (Figure 4) which are topologically similar to the photoreceptor rhodopsin and other G protein-coupled receptors (O'Dowd *et al.*, 1989). The structure of many G protein-coupled receptors such as adrenergic receptor (O'Dowd *et al.*, 1989), tachykinin receptors (Shigemoto *et al.*, 1990), and endothelin receptors (Arai *et al.*, 1990; Sakurai *et al.*, 1990) have been elucidated, showing that they constitute a large superfamily. Each subfamily consists of several members of receptors with ~50–70% similarity among them, whereas the similarity between the family is

~20–30%. The amino acid sequence of the secretin receptor has little similarity to any members of the G protein-coupled receptors. Furthermore, the amino acid residues conserved in the superfamily such as the Arg residue in the second intracellular domain, and the Asn-Pro residues in the seventh transmembrane segment were not found in the secretin receptor (Figure 4). These results suggest that the secretin receptor is a novel type receptor with seven transmembrane segments. Another notable feature of the secretin receptor is that it contains a signal sequence and a relatively large extracellular region (121 amino acids) preceding the transmembrane segment I. These properties were found in lutropin-choriogonadotropin (McFarland *et al.*, 1989) and endothelin receptors (Arai *et al.*, 1990; Sakurai *et al.*, 1990), but not other members of G protein-coupled receptors. The extracellular domain of the secretin receptor is rich in cysteine residues (7 residues) and may be involved in the binding of secretin (Robberecht *et al.*, 1984).

VIP could weakly compete with [125 I]secretin for binding to the secretin receptor (Figure 2) and stimulated the cAMP production in COS cells expressing the secretin receptor (Figure 3). Previously, Couvineau *et al.* (1990) have shown that secretin weakly competes with VIP for the VIP receptor purified from porcine liver. These results suggest that the secretin and VIP receptors are structurally related. The availability of the secretin receptor cDNA will facilitate the isolation and characterization of cDNAs for the VIP receptor and also the receptors for other secretin-related hormones such as glucagon, GRF and PHI.

Although the previous reports with [125 I]labeled secretin have suggested the existence of the secretin receptor in membranes of rat brain (Fremau *et al.*, 1983), the Northern hybridization analysis did not show the secretin receptor mRNA in brain (Figure 5). As previously suggested (Fremau *et al.*, 1983), the secretin receptor may be localized in the specific regions of brain. The *in situ* hybridization with the labeled DNA or RNA probe for the secretin receptor may reveal the specific localization of the receptor in brain in more detail, and clarify the possibility that secretin works as a neurotransmitter or a neuromodulator.

Materials and methods

Cells and peptides

The cell line NG108-15 (Klee *et al.*, 1974), which was a hybrid cell line of mouse N18TG2 neuroblastoma and rat C6Bu-1 glioma, was obtained from Dr H. Higashida (University of Kanazawa), and was cultured in Dulbecco's modified Eagle's medium (DMEM, Nissui) containing 10% fetal calf serum (FCS, Hyclone), and 0.1 mM hypoxanthine, 0.6 μ M aminopterin and 5 μ M thymidine (HAT). Monkey COSG1 and COS7 cells were maintained in DMEM supplemented with 10% FCS with or without 600 μ g/ml G-418, respectively.

Porcine secretin (Mutt *et al.*, 1970) was synthesized using a peptide synthesizer (Applied Biosystems, model 431A), and purified by HPLC. The authenticity of the synthesized secretin was confirmed by Edman degradation using a gas-phase protein sequenator (Applied Biosystems, model 477A). The synthetic porcine secretin₅₋₂₇ fragment was purchased from Bachem Fine Chemicals, Inc., while VIP and glucagon were from Peptide Institute Inc.

Secretin was labeled at the N-terminal histidine residue by the chloramine T method and purified essentially according to Chang and Chey (1980). The specific radioactivity of [125 I]secretin, determined by self-displacement analysis, was routinely 500–1000 Ci/mmol.

Construction of the CDM8 cDNA library

Total RNA was prepared from NG108-15 cells as described (Chomczynski and Sacchi, 1987), and poly(A) RNA was selected by oligo(dT)–cellulose

column chromatography. Double-stranded cDNA primed with random hexamer oligonucleotides (pdN₆) was synthesized as described previously (Fukunaga *et al.*, 1990) except that AMV reverse transcriptase was replaced by M-MLV RNase H⁻ reverse transcriptase (BRL). After addition of the *Bst*XI adapter, cDNA larger than 1.0 kb was recovered from the agarose gel, ligated with *Bst*XI-digested CDM8 vector (Seed, 1987), and introduced into *E. coli* MC1061/p3 cells by electroporation. A total of 350 000 bacterial colonies were prepared as 400 pools of 750–1000 clones, and plasmid DNAs were prepared from each pool by the alkaline lysis method (Sambrook *et al.*, 1989).

Establishment of COSGs1 cells

Rat cDNA for Gs α (Itoh *et al.*, 1986) was inserted at the *Xba*I site of pDEF-BOS, which was generated by deleting the 0.25 kb of *Hind*III fragment containing the SV40 replication origin from pEF-BOS (Mizushima and Nagata, 1990). The resultant expression plasmid (pEFGs1) was introduced into COS7 cells by the Ca-phosphate coprecipitation method in the presence of 0.1 mM chloroquine (Luthman and Magnusson, 1983) together with pEFneo which carried the neomycin phosphotransferase gene under the human EF-1 α promoter. After selection in medium containing 1 mg/ml G-418 for 14 days, one of transformants (COSGs1) expressed a reasonable amount of Gs α , identified by Western blotting analysis using rabbit anti-rat Gs α antibody (a kind gift from Dr T. Kozasa).

Transfection and screening in COSGs1 cells

1.5 $\times 10^5$ COSGs1 cells per well in 6 well plate were transfected with 1.5 μ g of plasmid DNA by the DEAE–dextran method (Fukunaga *et al.*, 1990). At 60 h after transfection, cells were incubated for 90 min at 37°C with 3.0 $\times 10^5$ c.p.m. of [¹²⁵I]secretin in 1 ml of DMEM containing 10 mg/ml BSA, 2 mg/ml bacitracin and 20 mM HEPES (pH 7.4). Cells were washed four times with phosphate-buffered saline containing 0.5 mM MgCl₂ and 0.7 mM CaCl₂ (PBS⁺), and fixed for 15 min at room temperature with 2% glutaraldehyde in PBS⁺. After washing once with PBS⁺, cells were air-dried, and vertical edges of the plates were removed. The plates were exposed to an IP plate (Fuji Photo Film Co. Ltd) for 18 h, and the bound [¹²⁵I]secretin was visualized by autoradiography using the Fuji BAS 2000 Bio-image analyzer (Fuji Photo Film Co. Ltd).

Binding of [¹²⁵I]secretin to membranes from COS cells and NG108-15 cells

COS7 cells grown in 15 cm plates were transfected by the Ca-phosphate coprecipitation method with 10 μ g each of plasmid pQ17, and the pEF-BOS (Mizushima and Nagata, 1990) or pEF-BOS-Gs which carries rat Gs α cDNA in the pEF-BOS vector. The crude cell membrane fractions were prepared from COS cells or NG108-15 cells as described by Katada *et al.* (1982) except that DTT was omitted from all buffers. 20 μ g of membrane protein was incubated at 37°C for 90 min with [¹²⁵I]secretin in 100 μ l of a solution containing 25 mM HEPES (pH 7.4), 5 mM MgCl₂, 1 mM EGTA, 50 mM NaCl, 10 mg/ml BSA, 2 mg/ml bacitracin, 0.1 mM (p-aminodiphenyl)methanesulfonyl fluoride hydrochloride and 100 μ g/ml leupeptin. The reaction was terminated by centrifugation at 14 000 g for 3 min. The pellet was suspended in 1 ml of ice-cold PBS, and the suspension was filtered through a Whatman GF/C filter which had been presoaked for 24 h in 0.3% polyethyleneimine at room temperature. The radioactivity retained on the filter was counted using a γ -counter. To determine the non-specific binding of [¹²⁵I]secretin to the membrane, a large excess of unlabeled secretin (10 μ M) was included in the assay mixture, and subtracted from the total binding to yield the specific binding.

cAMP assay

The intracellular cAMP levels were assayed essentially as described by Zhou *et al.* (1990). COSGs1 grown in 75 cm³ flask were transfected with 6 μ g of pQ17 or CDM8 by the DEAE dextran method. Cells were split into 6 well microtiter plates 24 h after glycerol shock and grown for 40 h in DMEM supplemented with 10% FCS. Cells were washed twice with incubation buffer (DMEM containing 1 mg/ml BSA and 0.5 mM 1-methyl-3-isobutylxanthine), and incubated at 37°C for 45 min with various concentrations of peptide hormone dissolved in incubation buffer. After aspirating the buffer, the reaction was terminated by adding 1 ml of ethanol, and transferred to 1.5 ml Eppendorf tubes. After centrifugation at 14 000 g for 3 min, the supernatants were dried, and the level of cAMP was quantified using a cAMP assay system from Amersham.

Northern hybridization and nucleotide sequence analysis

Total RNA was prepared from various rat tissues as described previously (Chomczynski and Sacchi, 1987), and poly(A) RNA was selected using

oligotex-dT30 (Takara Shuzo, Japan). Northern hybridization was carried out as described (Sambrook *et al.*, 1989). As a probe, the 0.7 kb *Nco*I–*Sph*I DNA fragment of pQ17 was labeled with ³²P by the random primer labeling method (Sambrook *et al.*, 1989).

DNA sequencing was performed by the dideoxy nucleotide chain termination method using T7-DNA polymerase (Pharmacia) and [α -³⁵S]dATP α S (Amersham).

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References

- Arai, H., Hori, S., Aramori, I., Ohkubo, H. and Nakanishi, S. (1990) *Nature*, **348**, 730–732.
- Bayliss, W. M. and Starling, E. H. (1902) *J. Physiol.*, **28**, 325–353.
- Bewab, W., Gespach, C., Marie, J.-C., Chastre, E. and Rosselin, G. (1988) *Life Sci.*, **42**, 791–798.
- Bissonnette, B. M., Collen, M. J., Adachi, H., Jensen, R. T. and Gardner, J. D. (1984) *Am. J. Physiol.*, **246**, G710–G717.
- Cerione, R. A., Codina, J., Benovic, J. L., Lefkowitz, R. J., Birnbaumer, L. and Caron, M. G. (1984) *Biochemistry*, **23**, 4519–4525.
- Chang, T.-M. and Chey, W. Y. (1980) *Dig. Dis. Sci.*, **25**, 529–552.
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.*, **162**, 156–159.
- Christophe, J., Waelbroeck, M., Chatelain, P. and Robberecht, P. (1984) *Peptides*, **5**, 341–353.
- Couvineau, A., Voisin, T., Guizarro, L. and Laburthe, M. (1990) *J. Biol. Chem.*, **265**, 13386–13390.
- Freneau, R. T. Jr, Jensen, R. T., Charlton, C. G., Miller, R. L., O'Donohue, T. L. and Moody, T. W. (1983) *J. Neurosci.*, **3**, 1620–1625.
- Freneau, R. T. Jr, Korman, L. Y. and Moody, T. W. (1986) *J. Neurochem.*, **46**, 1947–1955.
- Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y. and Nagata, S. (1990) *Cell*, **61**, 341–350.
- Gespach, C., Bataille, D., Vauclin, N., Moroder, L., Wunsch, E. and Rosselin, G. (1986) *Peptides*, **7**, suppl. 1, 155–163.
- Gossen, D., Poloczek, P., Svoboda, M. and Christophe, J. (1989) *FEBS Lett.*, **243**, 205–208.
- Gozes, I. and Brenneman, D. E. (1989) *Mol. Neurobiol.*, **3**, 201–236.
- Itoh, H., Kozasa, T., Nagata, S., Nakamura, S., Katada, T., Ui, M., Iwai, S., Ohtsuka, E., Kawasaki, H., Suzuki, K. and Kaziro, Y. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 3776–3780.
- Jensen, R. T., Charlton, C. G., Adachi, H., Jones, S. W., O'Donohue, T. L. and Gardner, J. D. (1983) *Am. J. Physiol.*, **245**, G186–G195.
- Katada, T., Amano, T. and Ui, M. (1982) *J. Biol. Chem.*, **257**, 3739–3746.
- Klee, W. A. and Nirenberg, M. (1974) *Proc. Natl. Acad. Sci. USA*, **71**, 3474–3477.
- Kopin, A. S., Wheeler, M. B. and Leiter, A. B. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 2299–2303.
- Kozak, M. (1987) *Nucleic Acids Res.*, **15**, 8125–8148.
- Kyte, J. and Doolittle, R. F. (1982) *J. Mol. Biol.*, **157**, 105–132.
- Luthman, H. and Magnusson, G. (1983) *Nucleic Acids Res.*, **11**, 1295–1308.
- McFarland, K. C., Sprengel, R., Phillips, H. S., Kohler, M., Rosenblat, N., Nikolics, K., Segaloff, D. L. and Seeburg, P. H. (1989) *Science*, **245**, 494–499.
- Mizushima, S. and Nagata, S. (1990) *Nucleic Acids Res.*, **18**, 5322.
- Munro, S. and Maniatis, T. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 9248–9252.
- Munson, P. J. and Rodbard, D. (1980) *Anal. Biochem.*, **107**, 220–239.
- Mutt, V., Jorpes, J. E. and Magnusson, S. (1970) *Eur. J. Biochem.*, **15**, 513–519.
- O'Dowd, B. F., Lefkowitz, R. J. and Caron, M. G. (1989) *Annu. Rev. Neurosci.*, **12**, 67–83.
- Robberecht, P., Waelbroeck, M., Camus, J.-C., De Neef, P. and Christophe, J. (1984) *Biochim. Biophys. Acta*, **773**, 271–278.
- Robberecht, P., Cauvin, A., Gourlet, P. and Christophe, J. (1990) *Arch. Int. Pharmacodyn.*, **303**, 51–66.
- Roskoski, Jr, R., White, L., Nowlton, R. and Roskoski, L. M. (1989) *Mol. Pharmacol.*, **36**, 925–931.
- Rosselin, G. (1986) *Peptides*, **7**, Suppl 1, 89–100.

- Roth,B.L., Beinfeld,M.C. and Howlett,A.C. (1984) *J. Neurochem.*, **42**, 1145–1152.
- Sakurai,T., Yanagisawa,M., Takuwa,Y., Miyazaki,H., Kimura,S., Goto,K. and Masaki,T. (1990) *Nature*, **348**, 732–735.
- Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, 2nd edition.
- Seed,B. (1987) *Nature*, **329**, 840–842.
- Shigemoto,R., Yokota,Y., Tsuchida,K. and Nakanishi,S. (1990) *J. Biol. Chem.*, **265**, 623–628.
- von Heijne,G. (1986) *Nucleic Acids. Res.*, **14**, 4683–4690.
- Zhou,Q.-Y., Grandy,D.K., Thambi,L., Kushner,J.A., Van Tol,H.H.M., Cone,R., Pribnow,D., Salon,J., Bunzow,J.R. and Civelli,O. (1990) *Nature*, **347**, 76–80.

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Note added in proof

The nucleotide sequence data reported here will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X59132.