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Short sequence-paper

cDNA cloning of a mouse pituitary adenylate cyclase-activating polypeptide receptor [1]

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Abstract

A cDNA clone for mouse pituitary adenylate cyclase-activating polypeptide (PACAP) receptor (PACAP-R) was obtained from the brain using reverse transcription-polymerase chain reaction (RT-PCR). The recombinant PACAP receptor expressed in COS cells bound PACAP with about 1000-times higher affinity than vasoactive intestinal polypeptide (VIP), and PACAP stimulated adenylate cyclase through the cloned PACAP receptor. The mouse PACAP receptor consists of 496 amino acids, contains seven transmembrane segments and has 98.4%, 93.0%, and 92.5% identity with the rat, bovine, and human PACAP-R, respectively.

Keywords: Pituitary adenylate cyclase-activating polypeptide receptor; PACAP receptor; cDNA cloning; Vasoactive intestinal polypeptide; (Mouse)

PACAP is a member of the vasoactive intestinal polypeptide (VIP)/secretin/glucagon family [1]. PACAP has a variety of biological actions: it stimulates neurite-outgrowth of sympathetic neuroblast and PC-12 cells [2,3], increases the release of acetylcholine from the hippocampus [4], catecholamines from adrenal chromaffin cells [5], insulin from the pancreas [6], melatonin from pinealocytes [7]. These diverse biological actions of PACAP are mediated by two types of high-affinity PACAP-R [1–3]: both PACAP and VIP bind to the type II receptor with similar affinity, while PACAP shows 100–1000-times higher affinity than VIP to the type I receptor. The rat VIP1 receptor cloned by Ishihara et al. showed similar affinity to both PACAP and VIP, indicating that it was the type II receptor [8]. Recently, several groups, including ours, have cloned the rat type I PACAP-preferring receptor (PACAP-R) [9–13]. Another subtype of the type II receptors, VIP2 receptor, has also been cloned [14,15].

More recently, we have isolated the mouse PACAP-R gene and determined its structural organization [16]. In this study, we obtained a cDNA clone encoding the mouse PACAP-R using RT-PCR. Here we present the nucleotide sequence of the mouse PACAP-R and the properties of the receptor expressed in COS cells transfected with the cDNA.

PACAP (Human PACAP-38) and VIP were purchased from the Peptide Institute (Osaka, Japan). [^{125}I]PACAP ([^{125}I]PACAP-27, 2200 Ci/mmol) was obtained from NEN. Total cellular RNA was prepared from the brain of 8-week-old male DDY mouse, and poly(A)-containing RNA was enriched and transcribed into random-primed, single-stranded cDNA as described [9]. A set of oligonucleotides of 20-mer containing sequences for the exons 1 and 16 of the mouse PACAP-R gene [16] (exon 1: 5′-ATTCGCGGGTTGCGCGTCCT-3′ for the forward primer and exon 16: 5′-TCAGGTGGCCAAGTTGTCGG-3′ for the reverse primer) was synthesized. The PCR was performed using Taq DNA polymerase and Taq Extender PCR Additive (Stratagene) according to the following schedule: 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 3 min. The PCR products were subcloned into pBluescript and sequenced. Clone 17 was selected and further subcloned into the eukaryotic expression vector, pEF-BOS [17] to generate pCAPM17. Transfection of the cDNA to COS7 cells, preparation of membrane fractions, binding

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[1] The nucleotide sequence reported in this paper has been submitted to the EMBL/GenBank/DDBJ Data Libraries under the accession number D82935.

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study of [^{125}I]PACAP, and cAMP assay were performed as described previously [9].

The nucleotide sequence and the predicted amino acid sequence of pCAPM17 were shown in Fig. 1A. The nucleotide sequence of pCAPM17 matched the genomic sequence corresponding to exons of the mouse PACAP-R

A

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                                                  1
                                                  ATTCGCGGGTTGCGCGTCCTCCTGCGCCTTCAGCCCCTGCGCCCAGCAGTCCCGACCAGCCC
CGAGGCCATCAGCGCTGCATGCACAGTGGAGGGTGGTGACTGACTCCCCAAGACTGGGAGCAACAGCCAGAGACAGTGGCTGGGAAGCACC

154                                                         200
ATG GCC AGA ACC CTG CAG CTC TCC CTG ACT GCT CTC CTC CTG CTG CCT ATG GCT ATT GCT ATG CAC TCT
Met Ala Arg Thr Leu Gln Leu Ser Leu Thr Ala Leu Leu Leu Leu Pro Met Ala Ile Ala Met His Ser
-20                                                                          -1   1

GAC TGC ATC TTC AAG AAG GAG CAA GCC ATG TGC CTG GAG AGG ATC CAG AGG GCC AAC GAC CTG ATG GGC
Asp Cys Ile Phe Lys Lys Glu Gln Ala Met Cys Leu Glu Arg Ile Gln Arg Ala Asn Asp Leu Met Gly

CTA AAT GAG TCT TCC CCA GGT TGC CCT GGC ATG TGG GAC AAT ATC ACA TGT TGG AAG CCT GCT CAA ATA
Leu Asn Glu Ser Ser Pro Gly Cys Pro Gly Met Trp Asp Asn Ile Thr Cys Trp Lys Pro Ala Gln Ile
     ★                                              ★
                                                    400
GGT GAG ATG GTC CTT GTG AGC TGC CCT GAG GTC TTC CGG ATC TTC AAC CCG GAC CAA GTC TGG ATG ACA
Gly Glu Met Val Leu Val Ser Cys Pro Glu Val Phe Arg Ile Phe Asn Pro Asp Gln Val Trp Met Thr
50

GAA ACC ATA GGG GAT TCT GGC TTT GCT GAT AGT AAT TCC TTG GAG ATC ACA GAC ATG GGG GTC GTG GGC
Glu Thr Ile Gly Asp Ser Gly Phe Ala Asp Ser Asn Ser Leu Glu Ile Thr Asp Met Gly Val Val Gly

CGG AAC TGC ACT GAG GAT GGC TGG TCG GAG CCC TTC CCC CAT TAC TTC GAT GCT TGT GGG TTT GAT GAC
Arg Asn Cys Thr Glu Asp Gly Trp Ser Glu Pro Phe Pro His Tyr Phe Asp Ala Cys Gly Phe Asp Asp
     ★              100
                                            600
TAT GAG CCC GAG TCT GGG GAT CAG GAT TAT TAC TAC CTG TCG GTG AAG GCC CTC TAC ACA GTC GGC TAC
Tyr Glu Pro Glu Ser Gly Asp Gln Asp Tyr Tyr Tyr Leu Ser Val Lys Ala Leu Tyr Thr Val Gly Tyr
                                                                I

AGC ACC TCC CTC GTC ACC CTC ACC ACT GCC ATG GTC ATC TTG TGC CGC TTC CGG AAG CTG CAC TGT ACC
Ser Thr Ser Leu Val Thr Leu Thr Thr Ala Met Val Ile Leu Cys Arg Phe Arg Lys Leu His Cys Thr
                                150

CGT AAC TTC ATC CAC ATG AAC CTG TTT GTA TCC TTC ATG CTG AGA GCT ATC TCT GTC TTC ATC AAA GAC
Arg Asn Phe Ile His Met Asn Leu Phe Val Ser Phe Met Leu Arg Ala Ile Ser Val Phe Ile Lys Asp
    II
                                    800
TGG ATC TTG TAT GCC GAG CAG GAC AGC AGT CAT TGC TTC GTT TCC ACC GTG GAA TGC AAA GCT GTC ATG
Trp Ile Leu Tyr Ala Glu Gln Asp Ser Ser His Cys Phe Val Ser Thr Val Glu Cys Lys Ala Val Met
                                                200                             III

GTT TTC TTT CAC TAC TGC GTG GTG TCC AAC TAC TTC TGG CTG TTC ATT GAA GGC CTA TAC CTC TTT ACA
Val Phe Phe His Tyr Cys Val Val Ser Asn Tyr Phe Trp Leu Phe Ile Glu Gly Leu Tyr Leu Phe Thr

CTG CTG GTG GAG ACC TTC TTC CCT GAG AGG AGA TAT TTC TAC TGG TAT ACC ATC ATT GGC TGG GGG ACA
Leu Leu Val Glu Thr Phe Phe Pro Glu Arg Arg Tyr Phe Tyr Trp Tyr Thr Ile Ile Gly Trp Gly Thr
                                                                250 IV
                            1000
CCT ACT GTG TGT GTA ACT GTG TGG GCT GTG CTG AGG CTC TAC TTT GAT GAT GCG GGA TGC TGG GAT ATG
Pro Thr Val Cys Val Thr Val Trp Ala Val Leu Arg Leu Tyr Phe Asp Asp Ala Gly Cys Trp Asp Met

AAT GAC AGC ACA GCT CTG TGG TGG GTG ATC AAA GGC CCT GTA GTT GGC TCT ATA ATG GTT AAC TTT GTG
Asn Asp Ser Thr Ala Leu Trp Trp Val Ile Lys Gly Pro Val Val Gly Ser Ile Met Val Asn Phe Val
★                   V                                                           300

CTT TTC ATC GGC ATC ATC ATC ATC CTT GTG CAG AAG CTG CAG TCC CCA GAC ATG GGA GGC AAT GAG TCG
Leu Phe Ile Gly Ile Ile Ile Ile Leu Val Gln Lys Leu Gln Ser Pro Asp Met Gly Gly Asn Glu Ser

                1200
AGC ATC TAC TTC AGC TGC GTG CAG AAA TGC TAC TGC AAG CCA CAG CGG GCT CAG CAG CAC TCT TGC AAG
Ser Ile Tyr Phe Ser Cys Val Gln Lys Cys Tyr Cys Lys Pro Gln Arg Ala Gln Gln His Ser Cys Lys

ATG TCA GAA CTA TCC ACC ATT ACT CTA CGG CTG GCC CGC TCC ACC CTG CTG CTC ATC CCA CTC TTT GGA
Met Ser Glu Leu Ser Thr Ile Thr Leu Arg Leu Ala Arg Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly
    350                                             VI

ATC CAC TAC ACA GTA TTT GCC TTC TCT CCA GAG AAC GTC AGC AAG AGG GAA AGA CTT GTG TTT GAG CTT
Ile His Tyr Thr Val Phe Ala Phe Ser Pro Glu Asn Val Ser Lys Arg Glu Arg Leu Val Phe Glu Leu
                                             ★                                       VII
    1400
GGG CTG GGC TCC TTC CAG GGC TTT GTG GTG GCT GTA CTC TAC TGC TTC CTG AAT GGG GAG GTA CAG GCA
Gly Leu Gly Ser Phe Gln Gly Phe Val Val Ala Val Leu Tyr Cys Phe Leu Asn Gly Glu Val Gln Ala
                    400

GAG ATT AAG AGG AAA TGG AGG AGC TGG AAG GTG AAC CGT TAC TTC ACT ATG GAC TTC AAG CAC CGG CAT
Glu Ile Lys Arg Lys Trp Arg Ser Trp Lys Val Asn Arg Tyr Phe Thr Met Asp Phe Lys His Arg His

                                                                                        1600
CCA TCC CTG GCC AGC AGT GGA GTG AAC GGG GGC ACC CAG CTG TCC ATC CTG AGC AAG AGC AGC TCC CAG
Pro Ser Leu Ala Ser Ser Gly Val Asn Gly Gly Thr Gln Leu Ser Ile Leu Ser Lys Ser Ser Ser Gln
                                    450
                                                    1644
CTC CGC ATG TCC AGC CTC CCG GCC GAC AAC TTG GCC ACC TGA
Leu Arg Met Ser Ser Leu Pro Ala Asp Asn Leu Ala Thr End
                                                476
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gene (data not shown). The neighboring sequence of the potential AUG initiation codon agrees well with the consensus sequence proposed by Kozak [18]. pCAPM17 cDNA contains an open reading frame coding for a protein of 496 amino acids. The first N-terminal 20 amino acids seem to serve as a signal sequence [19]. The mature mouse PACAP-R protein, thus, consists of 476 amino acids with a calculated molecular mass of 54523. The hydropathy analysis of the PACAP-R indicated that it contains 7 transmembrane regions (shown by I–VII in Fig. 1A). In the predicted extracellular regions, there are 5 potential *N*-glycosylation sites (Asn-X-Thr/Ser) (Fig. 1A). It has been reported that the rat PACAP-R consists from several isoforms with one or two insertions at the third intracellular loop [13]. The two alternative exons (Hip and Hop) corresponding to these insertion sequences have been found in the mouse PACAP-R gene [16]. The receptor that was encoded by pCAPM17 had the 84 bp insertion (nucleotides from 1200 to 1283 in Fig. 1A) which were derived from the exon Hop. The expression of three other forms of the splicing variants of the mouse PACAP-R was confirmed by RT-PCR and nucleotide sequencing analysis (Fig. 1B).

As shown in Fig. 2, the deduced amino acid sequence of the mouse PACAP-R showed high overall sequence homology to the rat [9–13], bovine [20], and human PACAP-R [21] (98.4%, 93.0%, and 92.5%, respectively) (the human PACAP-R was compared with the mouse PACAP-R lacking a 28 amino acid portion derived from the exon Hop). Five potential *N*-glycosylation sites were conserved among the four receptors, except for that of the second extracellular loop of the bovine receptor. The extracellular region of members of this receptor family contains highly conserved cysteine and tryptophan residues [9]. These were also conserved in the mouse PACAP-R (Fig. 2).

Transfection of the cDNA into COS7 cells conferred on them high affinity binding of [^{125}I]PACAP (Fig. 3A). The membranes from mock-transfected cells did not show any significant binding of [^{125}I]PACAP (data not shown). Competitive binding experiments revealed that PACAP potently displaced [^{125}I]PACAP binding (IC_{50} = 0.2 nM) while VIP was about 1000-times less potent than PACAP (IC_{50} > 1 μM). To examine whether the cloned receptor transduces the signals, the accumulation of cAMP in the COS7 cells expressing the PACAP-R was quantified. As shown in Fig. 3B, PACAP markedly stimulated the accumulation of cAMP in the cells (EC_{50} = 0.3 nM). In accordance with the weak binding activity of VIP to the PACAP-R, it stimulated cAMP accumulation at concentrations higher than 10 nM. These results indicate that the cloned PACAP-R cDNA encodes the PACAP-R (type I receptor).

In conclusion, we have cloned and expressed a mouse PACAP-R cDNA. The cDNA clone isolated in this study will be useful for the study such as in situ hybridization and ribonuclease protection assay in mice.

We gratefully acknowledge Dr. Shigekazu Nagata for

B

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PACAP-R     AGCATCTACTT----------------------------------------------------------------------------------
            SerIleTyrLe
PACAP-R     AGCATCTACTTAACAAATTTAAGACTGAGAGTCCCCAAGAAAGCCCGAGAGGACCCCCTGCCTGTGCCCTCAGACCAGCATTCACCCCCTTTCCT
(Hip)       SerIleTyrLeuThrAsnLeuArgLeuArgValProLysLysAlaArgGluAspProLeuProValProSerAspGlnHisSerProProPheLe
PACAP-R     AGCATCTACTT----------------------------------------------------------------------------------
(Hop)       SerIleTyrPh
PACAP-R     AGCATCTACTTAACAAATTTAAGACTGAGAGTCCCCAAGAAAGCCCGAGAGGACCCCCTGCCTGTGCCCTCAGACCAGCATTCACCCCCTTTCCT
(Hip-Hop)   SerIleTyrLeuThrAsnLeuArgLeuArgValProLysLysAlaArgGluAspProLeuProValProSerAspGlnHisSerProProPheLe


            ----------------------------------------------------------------------------ACGGCTGGCC
                                                                                        uArgLeuAla
            ----------------------------------------------------------------------------ACGGCTGGCC
                                                                                        uArgLeuAla
            CAGCTGCGTGCAGAAATGCTACTGCAAGCCACAGCGGGCTCAGCAGCACTCTTGCAAGATGTCAGAACTATCCACCATTACTCTACGGCTGGCC
            eSerCysValGlnLysCysTyrCysLysProGlnArgAlaGlnGlnHisSerCysLysMetSerGluLeuSerThrIleThrLeuArgLeuAla
            CAGCTGCGTGCAGAAATGCTACTGCAAGCCACAGCGGGCTCAGCAGCACTCTTGCAAGATGTCAGAACTATCCACCATTACTCTACGGCTGGCC
            uSerCysValGlnLysCysTyrCysLysProGlnArgAlaGlnGlnHisSerCysLysMetSerGluLeuSerThrIleThrLeuArgLeuAla
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Fig. 1. Sequence of the mouse pituitary adenylate cyclase-activating polypeptide (PACAP) receptor (PACAP-R) cDNA and polypeptide. (A) The nucleotide and predicted amino acid sequences of the mouse PACAP-R cDNA clone pCAPM17. Numbers above and below each lane refer to the nucleotide and amino acid positions, respectively. Numbering of the amino acids begins at Met-1 of the postulated mature receptor, with negative numbers for the signal peptide. The seven putative transmembrane segments are underlined, and the potential *N*-glycosylation sites in the extracellular region are marked by stars. The 84 bp sequence derived from the alternative exon Hop is indicated by undulating lines. (B) Sequences of three other splice variants of PACAP-R. The 84 bp sequence derived from the exon Hop can be deleted. It can also be replaced by, or included together with the 84 bp insertion derived from the exon Hip.

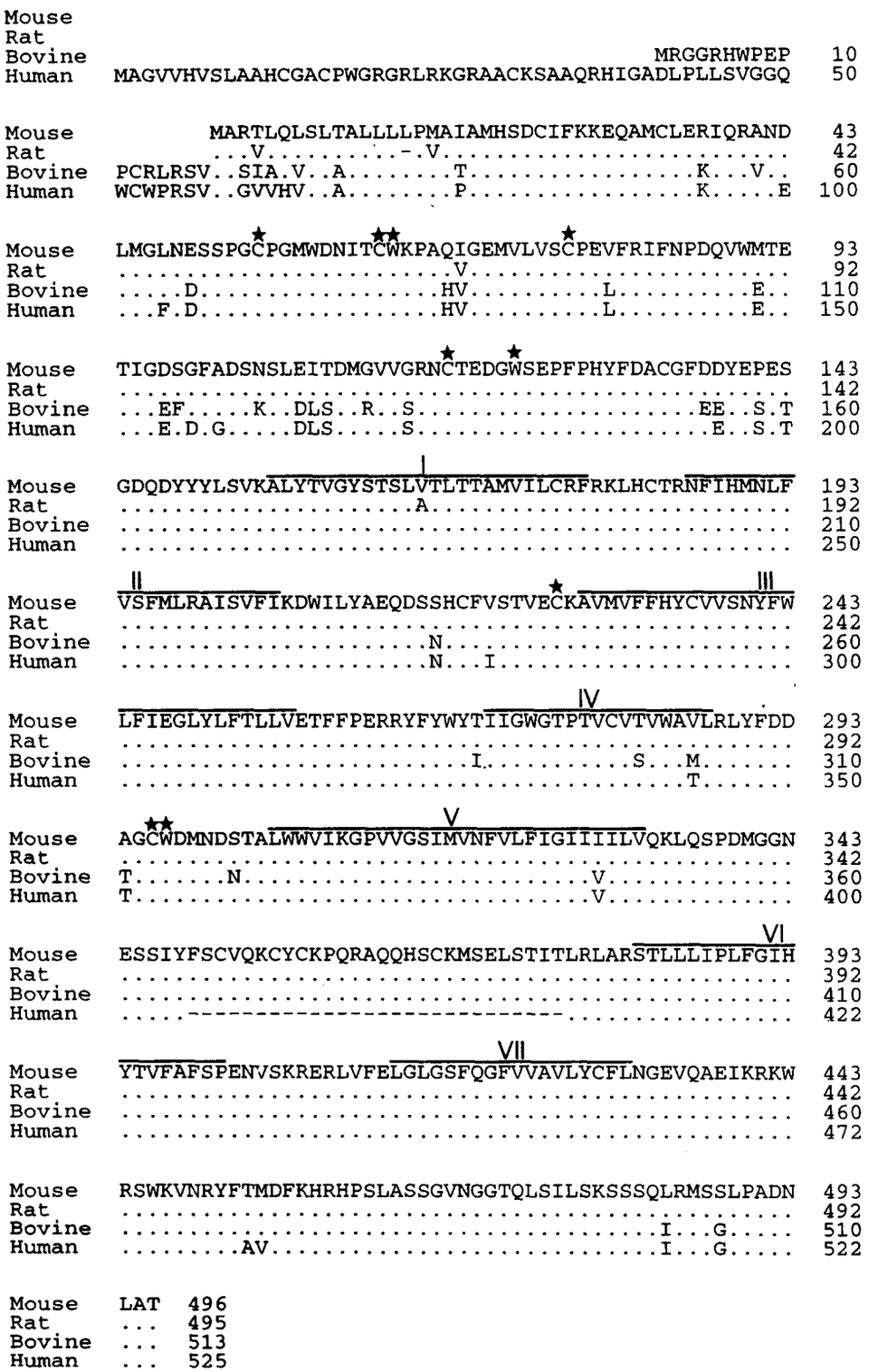

Fig. 2. Amino acid sequence comparison of the PACAP-R of various species. The deduced amino acid sequences of the mouse, rat [9–13], bovine [20], and human PACAP-R [21] are aligned. Dots and hyphens indicate identical and deleted amino acids, respectively, compared with those of the mouse. The amino acids are numbered from the N-terminus of the precursor protein of each receptor. The seven putative transmembrane segments are indicated by bars. In the extracellular region, the cysteine and tryptophan residues conserved among members of this receptor family [9] are marked by stars.

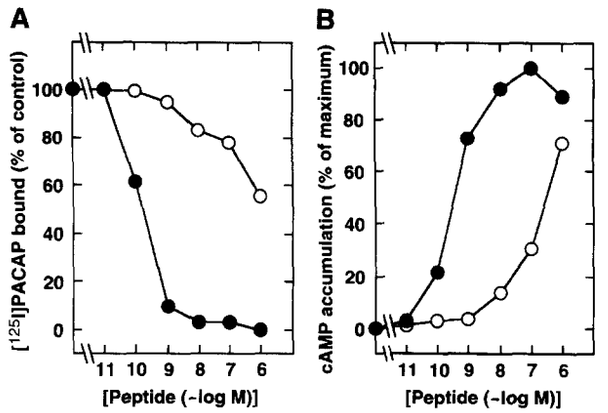

Fig. 3. Pharmacological characteristics of the mouse PACAP-R. (A) Binding specificity of the mouse PACAP-R. Membrane fractions from COS7 cells transfected with the PACAP-R expression plasmid were incubated with [^{125}I]PACAP in the presence of various concentrations of unlabeled PACAP (●) and VIP (○). The binding activity obtained in the absence of competitors was taken as 100%. The binding assays were performed in triplicate, and the average values are plotted. The values obtained in triplicate assays agreed within 10% error. (B) Accumulation of intracellular cAMP in COS7 cells expressing the mouse PACAP-R. COS7 cells transfected with the mouse PACAP-R expression plasmid were incubated with various concentrations of PACAP (●) and VIP (○), and the cAMP accumulated in the cells was quantified. The assays were performed in duplicate, and the average values are plotted. The values in duplicate agreed within 5% error.

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