Purification and Characterization of the Receptor for Pituitary Adenylate Cyclase-activating Polypeptide*

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The abbreviations used are: PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide; biotin-HPDP, N-[6-(biotinamido)hexyl]-3'-N-ethyl-2'-pyridyldithio)propionamide; BSOCOES, bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone; CHAPS, 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; PAGE, polyacrylamide gel electrophoresis; G-protein, guanine nucleotide-binding regulatory protein; GTPγS, guanosine 5'-O-(thio)triphosphate; HPLC, high performance liquid chromatography.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38-amino acid peptide (PACAP38) or a truncated peptide with the same 27 amino-terminal residues (PACAP27). The PACAP receptor was solubilized from bovine brain membranes with digitonin and purified 30-fold by the combination of DEAE-Toyopearl and hydroxylapatite chromatographic analyses. The partially purified PACAP receptors were mixed with biotinylated PACAP27 to form receptor-ligand complexes and then adsorbed onto avidin-agarose. The adsorbed PACAP receptors were eluted with an acidic buffer containing 1.0 M NaCl (pH 4.0). The eluted receptors were purified further by hydroxyapatite and gel filtration chromatography. A single protein band with a Mr = 55,000-60,000 was found in the final preparation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining. Affinity labeling of the purified receptors with [125I]PACAP27 labeled the Mr = 55,000-60,000 protein specifically. The dissociation constant and the specific activity of the purified receptors were 25.8 pm and 17.2 nmol of ligand binding per mg of protein, respectively. Inhibitory constants determined by competitive binding experiments were 30.0 pm for PACAP27, 4.6 pm for PACAP38, and 37.3 nm for vasoactive intestinal peptide. Therefore, the purified PACAP receptor retained high affinity and ligand specificity. The sequence of the amino-terminal 29 residues was derived from the purified receptor.

(2). The structure of PACAP, which is highly conserved between rats, sheep, and humans (3, 4), has 68% similarity to vasoactive intestinal peptide (VIP) in the amino-terminal 28 residues.

PACAP evoked the secretion of pituitary hormones from pituitary cells (1, 5) and interleukin-6 from follicular stellate cells (6). However, PACAP is not only a hypothalamic hormone; it seems to have a much wider spectrum of physiological functions. PACAP stimulates adenylate cyclase, mobilizes intracellular Ca2+, and causes the secretion of catecholamines from adrenal chromaffin cells (7) and insulin from the pancreas (8).

Binding sites for PACAP occur in several organs such as the brain (9-12), lung (9), and pancreas (12). Shivers et al. (13) classified PACAP binding sites into two types: one type is a PACAP-specific binding site with comparable affinity for PACAP38 and PACAP27 (type I), and the other is a binding site shared by PACAP and VIP (type II). They found that the type I binding site predominates in the hypothalamus, anterior pituitary, and adrenal gland, while the type II binding site predominates in the lung and liver. The type I binding site was also found in several cell lines such as primary glial cells (14), pancreatic AR4-2J cells (15), adrenal PC12h cells (16), and NB-OK cells (17). These cells increase their intracellular cAMP concentration in response to PACAP27/38, indicating that their type I binding sites are functionally coupled to adenylate cyclase. Deutsch and Sun (18) found that PACAP38 potently stimulated phospholipase C activity in PC12 cells but PACAP27 was much less potent. The existence of a PACAP38-specific binding site in the brain was suggested from binding experiments with [125I]PACAP38 (19). The type II binding site appears to correspond to the VIP receptor as demonstrated by expression of the cloned VIP receptor (20).

We have studied the properties of the type I binding site in the brain. An in vitro autoradiographic study demonstrated that the type I binding site is abundantly distributed throughout the brain (21). Affinity-labeling experiments identified a Mr = 60,000 protein (including the cross-linked [125I]PACAP27) as the brain PACAP receptor (11, 22). The Mr = 60,000 protein was shown to have the properties of the type I binding site and to be coupled to G-proteins (11). The type II binding site was not labeled under the same conditions, confirming the predominance of the type I binding site in brain membranes (11). In this report, we describe the purification of the brain type I PACAP receptor in a fully active form using a unique biotinylated ligand. Furthermore, the partial amino acid sequence was derived from the purified receptor, which enabled us to proceed to molecular cloning of the cDNA for the PACAP receptor.

EXPERIMENTAL PROCEDURES

Materials—Biotin-HPDP, BSOCOES, avidin-agarose, and Coomassie protein assay reagent were purchased from Pierce Chemical Co.
The solubilized membrane protein (usually 3,000 mg of protein/4,500 ml) was mixed with the same volume of Buffer A containing varying concentrations of digitonin. The mixture was stirred gently at 4 °C overnight, and the washed fractions were combined (usually amounts up to near 150 ml) and incubated with 1.5 × 10^-5 M biotinylated PACAP27 at 4 °C overnight to form receptor-ligand complexes. The preformed receptor-ligand complexes were adsorbed to avidin-agarose (130–150 ml) by gentle stirring at 4 °C for 4 days. The avidin-agarose was packed into a column (Bio-Rad, 5 × 15 cm) and washed with 10 bed volumes of Buffer B containing 1.0 M NaCl and with 5 bed volumes of Buffer B containing 1.0 M NaCl. Then, the PACAP receptors were eluted with a 20 ml magnesium acetate buffer containing 1.0 M NaCl, 0.1% digitonin, 10% glycerol, and the protease inhibitors (pH 4.0). The acidic fractions (10 ml) were immediately neutralized with 2.5 ml of 3 M Tris-HCl (pH 7.4).

**Small Hydroxylapatite and Superose 6 Chromatography**—The peak fractions of the activity from the avidin-agarose column were loaded onto a small hydroxylapatite column (1.6 ml in a Poly-Prep Column, Bio-Rad) at a flow rate of 0.25 ml/min. Elution was done with Buffer C and Buffer D at a flow rate of 0.25 ml/min as follows. Buffer D was increased from 0 to 50% for 60 min, held at 50% for 10 min, and then jumped to 100% and held there for 80 min. Fractions were collected for each 1 ml.

The peak fractions of the activity were concentrated to 0.1 ml by Centricon 10 (Amicon) and gel-filtered on a Superose 6 column (1 × 30 cm) that was equilibrated with Buffer C containing 0.2 mM NaCl at a flow rate of 0.5 ml/min. Fractions were collected for each 0.5 ml.

**Receptor Binding Experiments**—Binding experiments were performed as described by Masuda et al. (24) with minor modifications.

Ligand binding activity in chromatographic fractions was assayed as follows. Fractions were diluted with assay Buffer D (the same as Buffer C except that the digitonin concentration was decreased to 0.05% and 0.1% bovine serum albumin was included) as indicated in the figure legends. An aliquot (90 μl) of the diluted samples was mixed with 10 μl of 1 nM[^125]I-PACAP27 dissolved in assay Buffer C (the digitonin in assay Buffer D was replaced with CHAPS) for 75 min at 25 °C. The mixtures were diluted with 1.5 ml of assay Buffer C and passed through GPF filters (Whatman) which were pretreated with polyethyleneimine. The filters were subjected to γ-ray counting after washing with 1.5 ml of assay Buffer C. Total binding of[^125]I-PACAP27 including nonspecific binding is shown in the figures.

Saturation binding experiments were carried out as described above except with varying concentrations of[^125]I-PACAP27. Nonspecific binding was determined in the presence of 1 μM PACAP27. Specific binding was obtained by subtracting nonspecific binding from total binding. Binding data were analyzed following a Scatchard plot.

Competitive binding experiments were done with varying concentrations of unlabeled PACAP27, PACAP28, or VIP. The concentration of a competing peptide causing 50% inhibition of the specific binding (IC50) was derived by fitting the data to a pseudo-Hill equation:

\[
\log(\%SPB)/(100 - \%SPB) = nR(\log C - \log IC50) \quad (Eq. 1)
\]

where %SPB is the percent of specific binding to maximum specific binding, nR is a Hill constant, and C is the concentration of the competitor. The inhibition constant (IC50) of a competing peptide was derived from the equation of Chang et al. (25):

\[
K_C = IC50/(1 + KL) \quad (Eq. 2)
\]

where L is the concentration of[^125]I-PACAP27, and KL is the dissociation constant of[^125]I-PACAP27 determined by saturation binding experiments. Saturation binding experiments were carried out as described above except with varying concentrations of[^125]I-PACAP27. Nonspecific binding was determined in the presence of 1 μM PACAP27. Specific binding was obtained by subtracting nonspecific binding from total binding. Binding data were analyzed following a Scatchard plot.

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Following quenching of unreacted BSOOCES by the addition of 1 mM Tris (pH 7.5), the reaction mixtures were concentrated 10-fold by ultracentrifugation in Centricon 10 and then lyophilized. The lyophilized materials were dissolved in an SDS-PAGE sample buffer (125 mM Tris, 6% SDS, 50 mM dithiothreitol, and 5 mM EDTA; pH 7.6) and subjected to electrophoresis on an 8% SDS-polyacrylamide gel (26). The gel was silver-stained and dried to a transparent thin film. The dried gel was exposed to an XAR film (Eastman) with an intensifying screen for 3 days at -70 °C. For the determination of nonspecifically labeled proteins, the experiment was done in the presence of 1 μM PACAP27.

**RESULTS**

**Solubilization of the PACAP Receptor**—The brain PACAP receptor was solubilized with varying concentrations of digitonin at 1 mg of protein/ml. As shown in Fig. 1, the solubilized protein and the 125I-PACAP27 binding activity increased and reached a plateau as the concentration of digitonin was increased. Digitonin at 0.5% (digitonin to protein ratio of 5:1) was sufficient for the maximum solubilization of the activity. The presence of higher concentrations of digitonin up to 1.25% did not cause a drastic decrease in the binding of 125I-PACAP27 to the solubilized receptor (Fig. 2). These results indicate that digitonin is convenient for solubilization of the PACAP receptor.

For preparation of the solubilized PACAP receptor on a large scale, solubilization was performed with 1.5% digitonin at protein concentration of 3 mg/ml (digitonin to protein ratio of 5:1). The dissociation constant \( K_d \) and the number of binding sites per mg of protein \( (B_{max}) \) were determined by saturation binding experiments followed by Scatchard plot analysis. As shown in Fig. 3, the plotted points fitted a single straight line, indicating that a single class of binding sites was included in the solubilized proteins. The \( K_d \) and the \( B_{max} \) were 21.8 ± 1.86 pm (mean ± S.E.M.) and 3.2 ± 0.06 pmol/mg of protein, respectively (Table 1). The dissociation constant of the membrane PACAP receptors determined under the same conditions was 55.5 ± 5.1 pm. This result indicates that the solubilized receptors retained a high affinity for PACAP, which was slightly higher than the affinity of the membrane bound receptors. Treatment of the solubilized PACAP receptors with GTP or GTP\(\gamma\)S in a magnesium buffer (assay buffer D/Mg) did not reduce 125I-PACAP27 binding significantly (data not shown), although the same treatment of the membrane receptors reduced the 125I-PACAP27 binding as shown by a previous investigation (11). This implies that the solubilized receptors were no longer coupled to G-proteins. About 60% of the total binding sites found in the membranes were recovered in the solubilized material (Table 1).

**Partial Purification of the PACAP Receptor**—The solubilized proteins were subjected to DEAE-Toyopearl chromatography in the presence of 0.1% digitonin. About half the amount of loaded
Purification of the PACAP Receptor

### TABLE I

<table>
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<tr>
<th>Step</th>
<th>Total activity</th>
<th>Total protein</th>
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<td>0.0505</td>
<td>17,200</td>
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</table>

* Determined by saturation binding experiments followed by Scatchard plot analysis.
† Prepared from 10 brains.
‡ Determined by the method of Schaffner and Weissmann (28).
§ ND, not determined.

The data are from a single preparation which is representative of four similar experiments.

Proteins but no $^{125}$I-PACAP27 binding activity flowed through the DEAE column. The peak fractions of the $^{125}$I-PACAP27 binding activity which were eluted behind the peak fractions of protein (Fig. 4) were pooled and analyzed by saturation binding experiments. As shown in Table I, this step achieved a 4-fold purification with a recovery that was reproducibly near 100% (Table I).

The pooled fractions were further chromatographed on a hydroxylapatite column. Higher concentrations (0.3 M) of potassium phosphate, which usually elutes most of the adsorbed proteins, could not elute the $^{125}$I-PACAP27 binding activity. A concentration of potassium phosphate as high as 0.6 M was required for elution of the $^{125}$I-PACAP27 binding activity (Fig. 5).

The combination of the two conventional chromatographic analyses finally achieved a 30-fold partial purification (Table I). The $K_d$ of the partially purified PACAP receptors was 18.1 ± 0.4 pm, which was in the same range as the initial solubilized receptors. An avidin-binding protein with a $M_r$ = 57,000 that was abundant in the brain membrane extract was removed by this partial purification. Direct application of the crude extract to avidin-agarose results in co-elution of the $M_r$ 57,000 protein and the PACAP receptor. Therefore, this partial purification was indispensable.

**Application of Avidin-Biotin Interactions to the Purification of the PACAP Receptor**—A biotinylated derivative of PACAP was synthesized from PACAP27-Cys-NH$_2$. Reaction with an excess of biotin-HDPD (10:1 molar ratio) converted PACAP27-Cys-NH$_2$ into PACAP27-Cys-(S-(CH$_2$)$_5$-CO-NH-(CH$_2$)$_3$-NH-biotin)-NH$_2$ (simply designated as PACAP27-Cys(biotin)-NH$_2$ hereafter) stoichiometrically (Fig. 6). Peak 3 was rechromatographed to yield a pure preparation of PACAP27-Cys(biotin)-NH$_2$. The biotinylated peptide was found to retain high affinity.
to the solubilized PACAP receptor by competitive binding experiments (Fig. 7). The inhibitory constant ($K_{i}$), which was calculated from the equation of Chang et al. (25), was $37.0 \pm 4$ pm for PACAP27 and $35.8 \pm 0.6$ pm for PACAP27-Cys(biotin)-NH$_2$.

The partially purified receptor preparation was treated with avidin-agarose in the absence of the biotinylated ligand to remove residual avidin-binding proteins, and then incubated with $1.5 \times 10^{-6}$ M PACAP27-Cys(biotin)-NH$_2$ at 4°C overnight. The concentration of the biotinylated PACAP27 required for saturating the partially purified receptors was determined from the result of displacement experiments (Fig. 8). The displacement curve indicated that $1-2 \times 10^{-6}$ M biotinylated PACAP27 was required, which was 30 times of the receptor concentration determined by saturation binding experiments. After washing the avidin-agarose with a low salt and a high salt buffer, the PACAP receptors were eluted with a high salt-acidic buffer (pH 4.0) as shown in Fig. 9. Saturation binding followed by Scatchard plot analysis indicated that the pooled fractions of the acidic eluate retained high affinity for 125I-PACAP27 ($K_{d} = 54.4 \pm 3$ pm).

Final Purification of the PACAP Receptor—The eluate from the avidin-agarose column was applied to a small hydroxyapatite column for further purification and concentration. A diffused band with a $M_r = 55,000-60,000$ was observed in the peak fractions of the 125I-PACAP27 binding activity (Figs. 10 and 11). Contaminating proteins were washed out in the earlier fractions. The peak fractions were concentrated by ultrafiltration and then gel filtered on a Superose 6 FPLC column for the final purification. The single broad band with a $M_r = 55,000-60,000$ was enriched in the purified PACAP receptor preparation (Fig. 11).

Characterization of the Purified PACAP Receptor—The purified PACAP receptors were characterized by receptor binding and affinity-labeling experiments. As shown in Fig. 3, Scatchard plot analysis of saturation binding experiments demonstrated that the purified preparation included a single class of high affinity binding sites. The dissociation constant was $0.7$ pm. This value was comparable to that of the solubilized receptors. No GTP sensitivity was detected in the purified receptors. The $B_{max}$ of the purified receptors was $17,200 \pm 690$ pmol/mg of protein. Assuming that each receptor with a $M_r = 57,000$ has a single binding site, the theoretical concentration of the biotinylated PACAP27 required for saturating the partially purified receptors was determined (0.6 pm for PACAP27-Cys(biotin)-NH$_2$, indicating that the PACAP receptor molecule itself. The molecular weight of the purified receptor is in a good agreement with the molecular weight predicted from the results of previous affinity-labeling experiments (11).

![Figure 7](image.png)  
**Fig. 7.** Competitive binding of 125I-PACAP27 to crude solubilized PACAP receptors. The crude solubilized receptors (50 pm) were incubated with 130 pm 125I-PACAP27 and increasing concentrations of PACAP27 or PACAP27-Cys(biotin)-NH$_2$ at 25 °C for 75 min. Non-specific binding was determined in the presence of 1 pm PACAP27. Percent of specific binding to the maximum specific binding was plotted versus the concentration of PACAP27 (○) or PACAP27-Cys(biotin)-NH$_2$ (○).

![Figure 8](image.png)  
**Fig. 8.** Competitive binding to partially purified PACAP receptors. The concentrated PACAP receptors (52 pm) after the partial purification was incubated with 100 pm 125I-PACAP27 and increasing concentrations of PACAP27 or PACAP27-Cys(biotin)-NH$_2$. Percent of total binding to the maximum total binding was plotted versus the concentration of PACAP27 (○) or PACAP27-Cys(biotin)-NH$_2$ (○).

![Figure 9](image.png)  
**Fig. 9.** Avidin-agarose chromatography in the presence of biotinylated PACAP. Partially purified PACAP receptors were incubated with 1.5 pm PACAP27-Cys(biotin)-NH$_2$ and then adsorbed to avidin-agarose. The adsorbed proteins were eluted with Buffer B, Buffer B containing 1.0 M NaCl, and finally with a 20 m magnesium acetate buffer containing 1.0 M NaCl (pH 4.0). Total binding to 300-fold diluted fractions was determined.

$B_{max}$ for a homogeneous receptor is 17,500 pmol/mg. Therefore, the receptors were purified to very near homogeneity. Competitive binding experiments demonstrated that the purified receptors preserved the ligand binding specificity (Fig. 12) that is characteristic of the type I PACAP receptor (10–12, 19). The $K_i$ for PACAP27, PACAP38, or VIP was 30.0 ± 4.6 pm, 4.6 ± 0.89 pm, or 37.3 ± 4.8 nm, respectively. The Hill coefficient was $1.0 \pm 0.07$ for PACAP27, $1.1 \pm 0.1$ for PACAP38, and $0.95 \pm 0.05$ for VIP.

The purified PACAP receptors were incubated with 125I-PACAP27, cross-linked with BSOCOES, subjected to SDS-PAGE, and then visualized by both silver staining and autoradiography. As shown in Fig. 13, a single labeled band was observed at the same position as the $M_r = 55,000-60,000$ silver-stained band. No labeling was detected in the presence of 1 pm unlabeled PACAP27, indicating that the $M_r = 55,000-60,000$ band was specifically affinity-labeled with 125I-PACAP27. From these results, we concluded that the purified protein with a $M_r = 55,000-60,000$ is the PACAP receptor molecule itself.
FIG. 10. Hydroxyapatite rechromatography. The pooled fractions from avidin-biotin affinity chromatography were applied to a small hydroxyapatite column and eluted with increasing concentrations of potassium phosphate (---). Total $^{125}$I-PACAP27 binding to 1,000-fold diluted fractions was determined.

FIG. 11. SDS-PAGE analysis of the purified PACAP receptor. A, the fractions from the second hydroxyapatite chromatography (see Fig. 10) were analyzed by SDS-PAGE and visualized by silver staining. B, the pooled fractions from the second hydroxyapatite chromatography were concentrated and analyzed by SDS-PAGE (lane 2). The concentrated sample was further purified by gel filtration on a Superose 6 column. The peak fractions of ligand binding activity were concentrated and analyzed by SDS-PAGE (lane 3). Molecular weight markers are shown in lane 1.

Amino Terminal Sequence of the PACAP Receptor—Amino-terminal sequence analysis of the $M_r$ 55,000–60,000 protein was carried out with an automatic protein sequencer. The sequence of the amino-terminal 29 residues was: MHSDXIFK-KEQAMXLEXIQRVNDLMGLND (X means an undetermined residue). Both Xs were later found to be C during the cDNA cloning of the receptor.2

DISCUSSION

During the last several years, cDNA expression cloning of G-protein-coupled receptors has been highly successful in elucidating receptor structures and functions. Biochemical studies, however, have not advanced as rapidly because most of the biochemical approaches encountered obstacles during the purification of the receptors. The difficulty of receptor purification was caused by several problems. The first problem confronted is solubilization of receptors. The choice of a detergent is crucial for success in the purification of membrane proteins. CHAPS is one commonly used, versatile, detergent. Indeed, there is a growing list of papers that describe the successful solubilization of G-protein-coupled receptors with CHAPS, including our previous report of the solubilization of the PACAP receptor from bovine brain membranes (24). The previous study was done under the conditions of low CHAPS concentration versus high protein concentration (CHAPS to protein ratio of 5:7). In this study, we increased the detergent concentration to yield much more solubilized receptor. However, increasing concentrations of CHAPS destroyed the ligand-binding activity of the PACAP receptor (data not shown). Similar results have been reported during the solubilization of other receptors such as the gastrin-releasing peptide receptor (29), neurotensin receptor (30), or gonadotropin-releasing hormone receptor (31). It was reported for the former two receptors that the addition of cholesterol hemisuccinate ester was necessary to obtain soluble receptors in an active form. These results indicate that CHAPS is not suitable for some G-protein-coupled receptors.

Digitonin was excellent for the solubilization of the PACAP receptor. Excess digitonin did not denature PACAP receptors and did not abolish the $^{125}$I-PACAP27 binding to solubilized receptors. The dissociation constant of the digitonin-solubilized
receptors was rather lower than that of the membrane PACAP receptors. On the other hand, the dissociation constant of the CHAPS-solubilized receptors was two times higher than that of the membrane receptors (24). The use of digitonin was also advantageous for the subsequent steps of the purification. The DEAE ion-exchange chromatography in the presence of digitonin provided a high yield of the 125I-PACAP27 binding activity, whereas the chromatography in the presence of CHAPS destroyed the activity of the PACAP receptors completely (data not shown). Similar results were also obtained from gel filtration experiments. The denaturation during chromatography could be due to the deprivation of intrinsic phospholipids and/or the increasing of CHAPS to protein ratio with advancing separation. The addition of cholesteryl hemisuccinate ester to CHAPS (weight ratio = 1:10) protected the PACAP receptor a little.

Design of ligand-affinity chromatography is another important aspect of purification experiments. Initially, we prepared an affinity column of simple design, which immobilized PACAP covalently through its amino residues to activated carboxyl groups of aminoethyl-Sepharose (Thiopropyl-Sepharose 6B, Pharmacia). The solution of immobilized ligands was well adsorbed to the immobilized ligands. However, its use was not practical because once activity was absorbed, very little was released in an active form and many nonspecifically bound proteins were also found in the eluate.

Recently, the successful application of avidin-biotin interactions to the purification of receptors such as the gonadotropin-releasing hormone receptor (31), the endothelin receptor (33), and the somatostatin receptor (34) has been reported. This application requires the development of a biotinylated ligand that retains high affinity for both the receptor and avidin. We derived a biotinylated PACAP, PACAP27-Cys(-biotin)-NHS, from PACAP27-Cys-NH2. The affinity of the biotinylated ligand for the PACAP receptor was comparable to PACAP27. Furthermore, the potential stereic interference between the PACAP receptor and avidin should be minimal because the presumed receptor binding site and the biotinyl group are located on the opposite sides of the ligand and the ligand has a long spacer arm between the Cys residue and the biotinyl group. Indeed, preformed complexes of the partially purified receptors and the biotinylated ligands were adsorbed to avidinagarose. The adsorbed receptors were recovered by simple elution with an acidic salt-buffer. The recovery of an active PACAP receptor, which was barely attained by affinity resins with directly immobilized ligands, is the great advantage of this method. Moreover, the present method reduced nonspecifically bound proteins.

The ligand binding affinity of most of G-protein-coupled receptors is known to decrease in the presence of GTP or GTPyS. The membrane-bound PACAP receptors had a similar property (11, 22). These guanine nucleotides promote the dissociation of G-protein trimers into α and βγ subunits, which no longer associate with the receptors individually. Therefore, an interaction with G-proteins is required for expression of high affinity ligand binding. On the contrary, the purified PACAP receptor itself had a high affinity for the ligand. This result, however, is not surprising, since it is consistent with the property of the crude solubilized receptors. The crude solubilized receptors possessed high affinity for the ligand, although it seemed to be uncoupled from G-proteins as indicated by the loss of sensitivity to guanine nucleotides. A plausible explanation for the result is that the solubilization stabilized the conformation of the PACAP receptor in a high affinity form independent of coupling to G-proteins. A similar explanation was offered by Feldman et al. (29) for their purified gastrin-releasing hormone receptor. For further substantiation of the proposed conformational difference between the membrane receptors and the digitonin-solubilized receptors, reconstitution of the purified receptors into phospholipids bilayers should be undertaken. It is interesting to know the effects of defined reconstituting phospholipids or cholesterol to the ligand-binding properties. Furthermore, reconstitution with purified G-proteins will help us to understand the regulatory mechanism of the ligand-affinity interaction by G-proteins.

We have purified the brain type I PACAP receptor to near homogeneity. The purified receptors retained both high affinity and ligand specificity. The purified receptor gave a single broad band with Mr = 55,000-60,000. The amino-terminal portion of the PACAP receptor was sequenced. A synthetic DNA probe was designed from this sequence and the cDNA for the bovine PACAP receptor was cloned.2 On the other hand, the cDNA for the rat PACAP receptor was cloned recently by cross-hybridization with the rat VIP receptor cDNA (35).

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REFERENCES

Purification of the PACAP Receptor