Identification of pituitary adenylate cyclase-activating polypeptide 1-38-binding factor in human plasma, as ceruloplasmin

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^{125}I-Pituitary adenylate cyclase-activating polypeptide (PACAP) 1-38 is able to bind a factor in human plasma, which can be displaced by unlabelled PACAP 1-38 and PACAP 28-38 but not by the other biologically active form, PACAP 1-27. Likewise, ^{125}I-PACAP 28-38 binds this plasma factor, whereas ^{125}I-PACAP 1-27 does not. Apparent K_d values were measured to be 12.0 ± 1.3 and 3.4 ± 1.5 nM for PACAP 1-38 and PACAP 28-38, respectively, using a competition assay with ^{125}I-PACAP 28-38. Purification of the PACAP 1-38-binding factor from human blood was made by ethanol precipitation of serum followed by Ni^{2+}-chelating and anion-exchange chromatography. A 120-kDa band on SDS/PAGE, as well as some proteolytic products, was blotted on to PVDF membrane and their N-terminal amino acid sequences determined. In combination with a mass-spectrometric fingerprinting of a tryptic digest of the 120-kDa band, this PACAP 1-38-binding factor was identified as ceruloplasmin. Purified commercial ceruloplasmin shows identical mobility on SDS/PAGE to the PACAP 1-38-binding factor and the same binding characteristics to PACAP 1-38, 1-27 and 28-38, using the same amount of ceruloplasmin as was expected to be found in the human plasma. Furthermore, the ability of plasma to bind ^{125}I-PACAP 1-38 or 28-38 disappeared when ceruloplasmin was immunoprecipitated from plasma with rabbit anti-human ceruloplasmin Ig.

Key words: amino acid sequencing, ferroxidase, mass spectrometry, neuropeptide.

INTRODUCTION

Pituitary adenylate cyclase-activating polypeptide (PACAP) belongs to the vasoactive intestinal polypeptide/secretin/glucagon family of peptides and exists in two biologically active α-amidated forms, PACAP 1-38 and the C-terminally truncated form PACAP 1-27, of which PACAP 1-38 is the dominant form in tissue [1]. Both PACAPs, which are derived from the same 176-amino acid precursor, were isolated originally from the hypothalamus [2,3]. The amino acid sequence of PACAP 1-38 is HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRV KNK-NH_2. Besides being present in distinct areas of the central nervous system [4–8], PACAP is also found in neuronal elements of a number of peripheral organs [9–13]. In addition, PACAP is expressed in non-neuronal cells, since PACAP immunoreactivity and PACAP mRNA have been shown in spermatogenic cells of rat testes [14–16] and in steroidogenic cells from rat ovary [17]. Although the functional role of PACAP is not yet fully elucidated, there is evidence that the peptide is a hypothalamic regulatory factor influencing hormonal secretion from the anterior pituitary lobe [18–21]. A neurotrophic role during injury repair [22] as well as a function in early neurogenesis has also been suggested [23]. Furthermore, a hormonal role for the peptide is likely.

During our attempts to elucidate the physiological role of PACAP by measuring the concentration of immunoreactive PACAP 1-38 in plasma, we noticed the presence of binding activity that specifically bound PACAP 1-38 but not PACAP 1-27. In the present study we have isolated and characterized this plasma-binding activity and identified it to be identical with ceruloplasmin.

EXPERIMENTAL

Peptides and proteins

PACAP 1-38 was purchased from Bachem AG (Bubendorf, Switzerland), whereas PACAP 1-27 and 28-38 were purchased from Peninsula Laboratories (Belmont, CA, U.S.A.). Iodination and reverse-phase (RP) C_{18} HPLC purification of ^{125}I-PACAP 28-38 were as described by Hannibal et al. [6], whereas iodination and RP C_{18} HPLC purification of ^{125}I-PACAP 1-38 and ^{125}I-PACAP 1-27 were essentially as described for vasoactive intestinal polypeptide [24]. Human ceruloplasmin was obtained from Sigma (catalogue nos. C4770 and C4519, St. Louis, MO, U.S.A.), whereas rabbit anti-human ceruloplasmin Ig in 0.1 M NaCl/15 mM NaN_3 was obtained from Dako (A0031, Glostrup, Denmark).

Purification of PACAP 1-38-binding factor (PBF) from human blood

Coagulation of human blood was allowed to take place for 3 h at room temperature. Serum was obtained by centrifugation at 1500 ***g*** for 10 min. Ethanol was added to 50 ml of human serum to a final concentration of 33 % at 0 °C, and incubated for

Abbreviations used: PACAP, pituitary adenylate cyclase-activating polypeptide; PBF, PACAP 1-38 binding factor; RP, reverse phase; MALDI-TOF, matrix-assisted laser desorption ionization-time-of-flight.

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15 min. After a 15-min centrifugation at 10000 ***g*** at 4 °C, the pellet was resuspended in 25 ml of 0.1 M sodium phosphate buffer/40 mM NaCl (pH 7.0) and filtered through Whatman paper no. 1. An additional ethanol precipitation was done by the addition of 25 ml of ethanol (0 °C), followed by incubation for 15 min. After a 15-min centrifugation at 10000 ***g*** at 4 °C, the pellet was resuspended with 20 ml of 0.1 M sodium phosphate buffer/40 mM NaCl (pH 7.0) and loaded on to a Ni^{2+}-coated Chelating Sepharose Fast Flow column (1 cm × 26 cm; Pharmacia Biotech, Uppsala, Sweden). The column was pre-equilibrated with buffer A (0.1 M sodium phosphate buffer/0.1 M NH_4Cl, pH 7.0) and elution was done by increasing buffer B (0.1 M sodium acetate buffer/0.1 M NH_4Cl, pH 4.8) by 0.56 %/min after 70 min of isocratic elution with buffer A. The flow rate was 0.5 ml/min. A final concentration of 50 mM sodium phosphate buffer (pH 7.0) was added to the fractions, which were stored at 4 °C and analysed for ^{125}I-PACAP 28-38 binding. The following day, ethanol was added to fractions with ^{125}I-PACAP 28-38-binding capacity to a final concentration of 50 % and incubated for 15 min at 0 °C. After a 15-min centrifugation at 10000 ***g*** at 4 °C, the pellet was resuspended in 15 ml of water and concentrated to 1 ml twice using an Ultrafree-15 unit (Millipore) at 2000 ***g*** at 4 °C. Bis-Tris/HCl was added to a final concentration of 50 mM (pH 6.5), and NaCl was added so that the electrical conductivity was identical to the conductivity of 50 mM Bis-Tris/HCl/150 mM NaCl (pH 6.5) and loaded on to a SOURCE 15Q column (Pharmacia Biotech). Elution was done by increasing the NaCl concentration by 1 mM/min after 70 min of isocratic elution. The flow rate was 0.8 ml/min.

PBF-binding assay of plasma or ceruloplasmin

Plasma (20 μl), 5 mM EDTA or 0.2 mg/ml ceruloplasmin (Sigma catalogue no. C4770) was incubated with 20 μl of 20 mM Hepes/NaOH (pH 7.2)/8.5 nM ^{125}I-PACAP 1-38, 1-27 or 28-38 with or without 10 μM unlabelled PACAP and incubated for 30 min at room temperature. Bound and free ^{125}I-PACAP were separated by agarose electrophoresis.

PBF binding assay of chromatographic fractions

Chromatographic fractions (20 μl) were incubated with 20 μl of 4 g/l human serum albumin/40 mM sodium phosphate buffer (pH 7)/8.5 nM ^{125}I-PACAP 28-38 and incubated for 30 min at room temperature before agarose electrophoresis. ^{125}I-PACAP 28-38 was used as a tracer instead of ^{125}PACAP 1-38 due to the better solubility and stability properties of ^{125}I-PACAP 28-38.

Agarose electrophoresis

Glycerol (5 μl) was added (to obtain higher density) to each sample before it was loaded in to wells of a 0.8 % agarose horizontal gel (20 cm × 14 cm × 1 cm) in a 40 mM Tris/acetate buffer, pH 8.0 (see Figure 3 below) or 20 mM Hepes/NaOH, pH 7.2 (see Figures 1 and 5 below). The electrophoresis was done at 100 V for 2 h. The agarose gels were dried at 60 °C and exposed to a BAS-MP imaging plate (Fuji) for 1 h and analysed by a BAS-1500 scanner and the software program TINA (Fuji).

SDS/PAGE

Protein samples were incubated with 8 % (w/v) SDS/24 % (v/v) glycerol/0.1 M Tris/HCl/0.025 % (w/v) Coomassie G250 (pH 6.8) at 80 °C with or without 40 mM dithiothreitol for 5 min (a final concentration of 0.1 M iodoacetamide was added to the reduced samples) and electrophoresed on 10 % Tricine SDS/polyacrylamide gels according to the methods of Schägger and von Jagow [25]. Silver staining of the gels was performed as described by the manufacturer, Pharmacia Biotech, using the Plusone silver-staining kit.

Western blotting and amino acid sequencing of PACAP 1-38-binding protein

After SDS/PAGE of reduced and carbamidomethylated samples, the proteins were transferred on to PVDF membranes (NOVEX, San Diego, CA, U.S.A.) in 12 mM Tris/96 mM glycine/0.005 % SDS at 0.8 mA/cm² for 1 h using a semi-dry blotting unit (Bio-Rad). The PVDF membranes were stained in 0.1 % Coomassie G250/50 % methanol for 2 min and destained with 50 % methanol. Potential PACAP 1-38-binding proteins were cut out of the membrane and analysed for 10 or 15 residues in an automatic protein sequencer (Procise HT; Perkin-Elmer, Foster City, CA, U.S.A.) with online HPLC detection of the phenylthiohydantoin derivatives. All chemicals and solvents were sequence or HPLC grade (Applied Biosystems).

MS fingerprinting of tryptic digest

Approx. 50 μg of the 120-kDa band was applied to 13 wells and SDS/PAGE (10 % gel) carried out. After Coomassie staining, the 120-kDa bands were cut out of the gel and digested with trypsin as described by Williams et al. [26]. Acetonitrile was added to a final concentration of 5 % and 0.1 % trifluoroacetic acid. The digest was separated at 60 °C by RP C_{18} HPLC on a Vydac column (0.21 cm × 15 cm) using a linear gradient from 5 to 80 % acetonitrile in 0.1 % trifluoroacetic acid at 0.2 ml/min for 60 min. The molecular masses of the HPLC-purified tryptic peptides were determined by matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) MS in a Biflex instrument (Bruker, Bremen, Germany) using α-cyano-4-hydroxycinnamic acid as matrix.

RESULTS

Figure 1 shows ^{125}I-PACAP 1-38 binding to a factor (PBF) in plasma, which can be displaced with 10^{-5} M unlabelled PACAP 1-38 but not by the other biologically active form, PACAP 1-27. The C-terminal extension, PACAP 28-38, displaced ^{125}I-PACAP 1-38 to some extent. ^{125}I-PACAP 1-27 was unable to bind, whereas ^{125}I-PACAP 28-38 also bound to PBF. The three different PACAP forms have theoretical pI values above 10. In

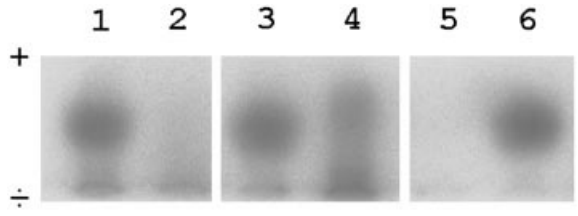

Figure 1 PACAP 1-38, 1-27 and 28-38 binding in plasma

Plasma was incubated with 4 nM ^{125}I-PACAP 1-38 (lanes 1–4) with the following additions: unlabelled 10 μM PACAP 1-38 (lane 2), unlabelled 10 μM PACAP 1-27 (lane 3) and unlabelled 10 μM PACAP 28-38 (lane 4). In lanes 5 and 6, plasma was incubated with 4 nM ^{125}I-PACAP 1-27 and 4 nM ^{125}I-PACAP 28-38, respectively. The samples were applied to a 0.8 % agarose gel in 20 mM Hepes/NaOH (pH 7.4) buffer near the cathode and separated by electrophoresis for 2 h at 100 V. The anode (+, top) and cathode (÷, bottom) are indicated. See the Experimental section for details.

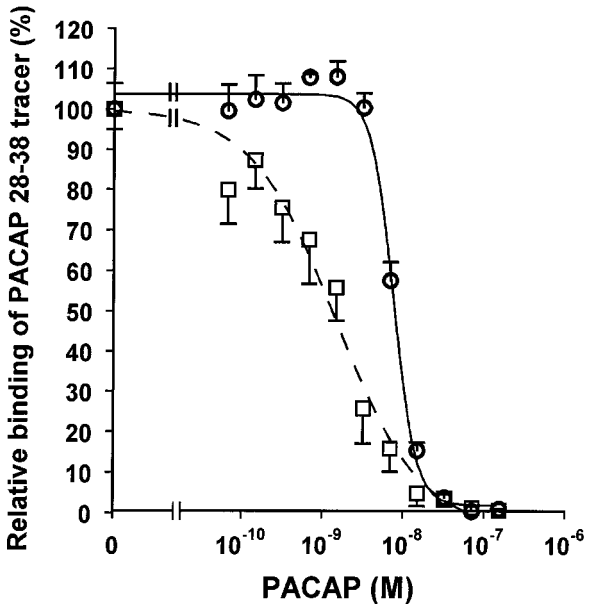

Figure 2 Apparent K_d determination of PACAP 1-38 (○, solid line) or 28-38 (□, dashed line) to PBF in plasma

Competitive-equilibrium experiments were performed using plasma and 5 mM EDTA diluted 6250-fold with 10 mM sodium phosphate buffer (pH 7.2)/3.5 mM KCl/135 mM NaCl/0.1 % (v/v) Tween 20/5 mM EDTA/10 mM dithiothreitol/0.5 nM ^{125}I-PACAP 28-38 and an increasing concentration of unlabelled PACAP 1-38 or 28-38 incubated for 2 h at room temperature. The intensity of the ^{125}I-PACAP 28-38 bound to PBF after agarose electrophoresis was measured and a binding curve produced. The apparent K_d was estimated from a sigmoid dose-response equation, $y = \text{Bottom} + (\text{Top} - \text{Bottom})/\{1 + [(x/K_d)^P]\}$ were x is the concentration, y is the response and P is the slope factor.

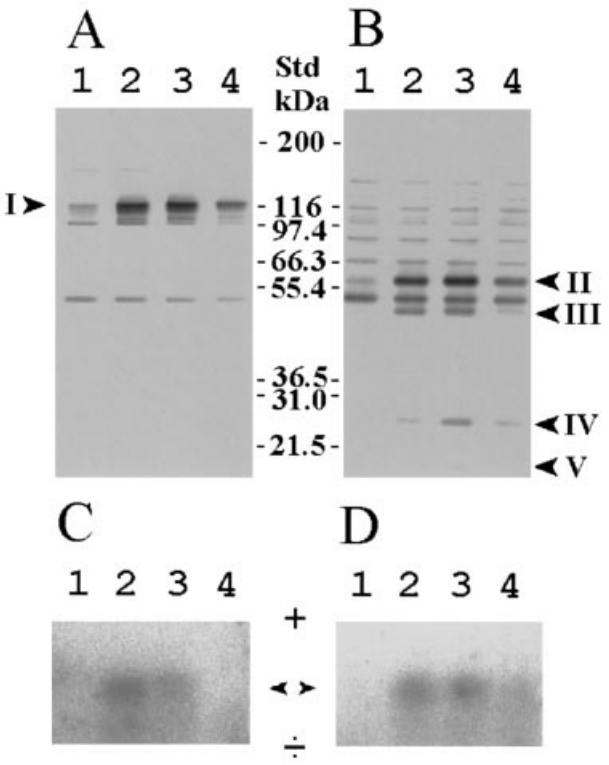

Figure 3 SDS/PAGE and ^{125}I-PACAP 28-38-binding assays of fractions from anion-exchange chromatography

The elution times of the fractions evaluated by SDS/PAGE (**A**) and ^{125}I-PACAP 28-38-binding assays (**C**) were 75–79 min (lanes 1), 79–83 min (lanes 2), 83–87 min (lanes 3) and 87–91 min (lanes 4). Another sample was stored at 4 °C for 2 weeks (in contrast to standard overnight storage at 4 °C) before the final anion-exchange chromatography. Fractions were analysed by SDS/PAGE (**B**) and ^{125}I-PACAP 28-38-binding assays (**D**). The arrows show the bands I–V in SDS/PAGE (**A** and **B**), which have a comparable peak pattern to the ^{125}I-PACAP 28-38-binding assay (**C** and **D**). The molecular masses of marker proteins are indicated (Std kDa).

contrast, the binding complex of ^{125}I-PACAP 1-38 and PBF must have had a pI below 7.2 in order to move towards the anode. The homologous vasoactive intestinal peptide that is 70 % identical to PACAP 1-27 did not bind to PBF (results not shown). Apparent K_d values were determined to be 12.0 ± 1.3 (mean $\pm$ S.E.M.; $n = 5$) and 3.4 ± 1.5 nM ($n = 7$) for PACAP 1-38 and PACAP 28-38, respectively, using a competition assay with ^{125}I-PACAP 28-38, as shown in Figure 2. Triton X-100 was used in the binding assay to enhance solubility of PACAP 1-38, whereas PACAP 28-38 was unaffected by the addition of 0.1 % Triton X-100 in the binding assay (results not shown). PACAP 28-38 showed the expected binding slope with a slope factor of approx. 1, as for a non-cooperative binding. However, PACAP 1-38 showed a steep binding curve (slope factor 3.3), which we expect to be due to remaining non-specific adsorption of PACAP 1-38, which results in lack of displacement of ^{125}I-PACAP 28-38 at low PACAP 1-38 concentrations. Thus the actual K_d for PACAP 1-38 binding could have been lower than 12 nM.

PBF was purified from human blood in order to identify the factor. PBF persists in serum after coagulation of blood and PBF was precipitated from serum by 33 % (v/v) ethanol (0 °C), resuspended in buffer and applied on to a Ni^{2+}-chelating Sepharose column. Fractions able to bind ^{125}I-PACAP 28-38 were pooled and, after a buffer shift, the pool was applied on an anion-exchange column. SDS/PAGE of fractions from the anion-exchange chromatography shows one dominating band (band I, Figure 3A) with a molecular mass of 120 kDa. Another sample, stored at 4 °C for 2 weeks before application to the final anion exchanger, is shown in Figures 3(B) and 3(D). Several smaller bands were seen, whereas the dominating band of 120 kDa had disappeared. The silver-staining intensities at each fraction of band I (Figure 3A) and bands II–V (Figure 3B) were proportional to the abilities of the same fractions to bind ^{125}I-PACAP 28-38 (Figures 3C and 3D). For example, fractions in lane 2 and 3 (Figures 3A and 3C) had the most intense bands, visualized by silver staining, and the greatest ability to bind ^{125}I-PACAP 28-38. The proteins were blotted on to a PVDF membrane and subjected to N-terminal sequence analysis (Table 1). A search in the SwissProt database showed that all bands fitted to sequences in human ceruloplasmin precursor (EC 1.16.3.1, SwissProt accession no. P00450). Bands I and II corresponded to the N-terminus of mature ceruloplasmin (without the signal peptide), whereas the first amino acid in bands III and IV corresponded to amino acid 501 in ceruloplasmin and in band V to amino acid 907. Furthermore, a polyacrylamide gel slice of band I was subjected to trypsin digestion. The digest was loaded on to an RP C_{18} column and selected peaks were analysed by MALDI-TOF MS. The 48 molecular masses obtained were analysed by the peptide-mass fingerprinting tool, MS-Fit (by P. Baker and K. Clauser), from the University of California at San Fransisco Mass Spectrometry Facility (http://prospector.ucsf.edu). Ceruloplasmin was selected as the most likely candidate among all human proteins in the SwissProt database. The MS-Fit program found 34 of the 48 masses within

Table 1 N-terminal sequence analysis of potential PACAP 1-38-binding proteins

The SDS/PAGE samples shown in Figure 3 were blotted on to a PVDF membrane and N-terminal amino acid sequence analysis (10 or 15 cycles) was done for the bands corresponding to bands I–V (see Figure 3).

Band no.	Apparent molecular mass (kDa)	N-terminal sequence	Corresponding first ceruloplasmin residue
I	120	KEKHYYIGII	20 →
II	58	KEKHYYIGIIETTWD	20 →
III	50	SVPPSASHVAPTETF	501 →
IV	27	SVPPSASHVA	501 →
V	20	VFNPRRKLEF	907 →

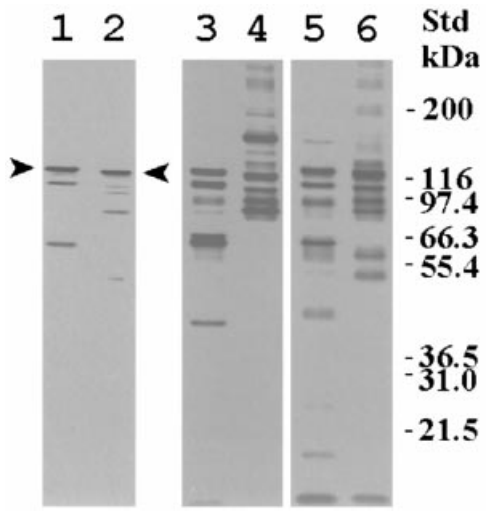

Figure 4 Comparison of the purified PBF (lanes 1 and 2) with two commercial human ceruloplasmin preparations obtained from Sigma, catalogue nos. C4770 (lanes 3 and 4) and C4519 (lanes 5 and 6)

Both reduced (lanes 1, 3 and 5) and unreduced (lane 2, 4 and 6) samples were analysed. Protein (20 ng) were applied to lanes 1 and 2, whereas 100 ng were applied to lanes 3–6. The molecular masses of marker proteins are indicated (Std kDa).

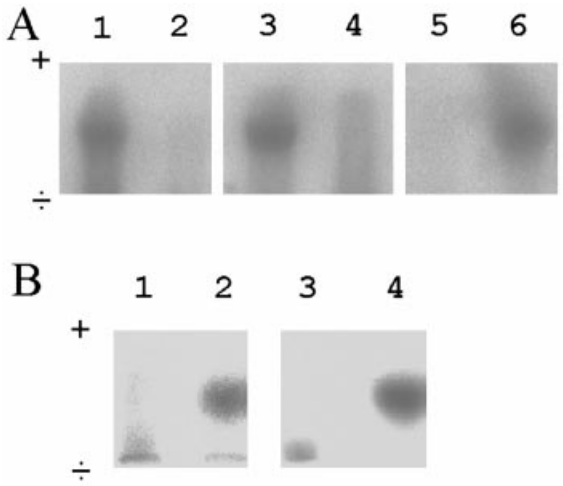

Figure 5 PACAP 1-38, 1-27 and 28-38 binding to human ceruloplasmin

(**A**) The amount applied of human ceruloplasmin, 30 pmol/lane, is comparable with the amount of ceruloplasmin in human plasma used in Figure 1. Incubations were as described in Figure 1. (**B**) Rabbit anti-human ceruloplasmin (30 μl; lanes 1 and 3), or 30 μl of 0.1 M NaCl/15 mM NaN_3 (control; lanes 2 and 4) were added to 40 μl of human plasma and incubated for 30 min at room temperature. After a centrifugation at 10000 ***g*** for 10 min at room temperature, the supernatant was incubated with ^{125}I-PACAP 1-38 (lanes 1 and 2) or ^{125}I-PACAP 28-38 (lanes 3 and 4) and electrophoretically separated on agarose gel (see the Experimental section).

the peptide mass tolerance of 0.1 %. The experimental/theoretical values were: 1205.1/1204.2; 1333.5/1332.4; 1355.6/1356.5; 1361.8/1360.6; 1373.4/1372.6; 1521.7/1520.7; 1599.6/1599.0; 1641.9/1640.9; 1650.0/1648.9; 1905.4/1905.0; 2174.0/2172.4; 2207.1/2205.6; 2223.8/2221.6; 2239.6/2237.6; 2255.7/2253.6; 2296.0/2294.4; 2302.4/2300.5; 2358.6/2356.6; 2366.9/2364.8; 2458.9/2456.8; 2477.2/2475.6; 2486.7/2484.8; 2487.9/2488.8; 2493.1/2491.6; 2509.0/2510.7; 2527.4/2527.8; 2528.4/2528.8; 2535.8/2535.9; 2592.6/2591.7; 2631.1/2633.1; 3326.4/3324.7; 3714.9/3712.0; 4046.9/4049.5; and 4338.0/4339.8 Da. The remaining 14 molecular masses (1251.1, 1296.6, 1551.1, 1582.6, 1583.3, 2166.8, 2260.4, 2297.4, 2312.2, 2461.0, 2501.3, 2567.5, 3465.1 and 3756.5) not found by the MS-Fit program could be post-translationally modified peptides or partially cleaved peptide fragments. Figure 4 shows that the reduced and unreduced sample preparations of a pool of the fractions shown in lanes 1–4, Figure 3(A), have the same mobilities in SDS/PAGE as two different preparations of ceruloplasmin obtained from Sigma. Some impurities/degradation products, however, are seen in these preparations. In Figure 5(A) it is shown that ceruloplasmin (Sigma catalogue no. C4770) has the same binding characteristics to PACAP 1-38, 1-27 and 28-38 as plasma. The same amount of ceruloplasmin was added as was expected to be found in the plasma used in Figure 1. The ability of plasma to bind ^{125}I-PACAP 1-38 or 28-38 disappeared when the plasma was depleted of ceruloplasmin by immunoprecipitation (Figure 5B). Plasma controls incubated under identical conditions without rabbit anti-human ceruloplasmin Ig retained the ability to bind ^{125}I-PACAP 1-38 or 28-38.

DISCUSSION

We found that PACAP 1-38 binds to a factor (PBF) in plasma, whereas the other biologically active form, PACAP 1-27, does not. The truncated form, PACAP 28-38, is also able to bind PBF. Thus the C-terminal part of PACAP 1-38 is responsible for most

or all of the energy of binding to the PBF. Apparent K_d values were determined to be 12.0 ± 1.3 and 3.4 ± 1.5 nM for PACAP 1-38 and 28-38, respectively. PBF was purified by ethanol precipitation of serum followed by Ni^{2+}-chelating and anion-exchange chromatography. The resulting protein had an apparent molecular mass of 120 kDa and it was identified to be ceruloplasmin by N-terminal sequence analysis and MS fingerprinting of a trypsin digest. Native ceruloplasmin is a 132-kDa glycoprotein [27] and the apparent molecular mass of band I is within the variation of molecular-mass determination using SDS/PAGE. The theoretical pI of 5.7, calculated from the amino acid composition of ceruloplasmin + PACAP 1-38, fits with the electrophoretic mobility towards the anode in agarose at pH 7.2. A sample was stored at 4 °C for 2 weeks before the final anion-exchange chromatography and the fractions were analysed by SDS/PAGE and for PBF content as described above. The 120-kDa band had disappeared, but several minor bands on SDS/PAGE appeared. The sum of the apparent molecular masses of bands II, III and V fits with the molecular mass of unprocessed human ceruloplasmin. Proteolytic cleavage of ceruloplasmin during purification has been observed previously with apparent molecular masses of 67, 50 and 19 kDa [27]. Thus human ceruloplasmin is accessible to proteolysis at specific positions without severe instability of the tertiary structure. N-terminal sequence analysis of these bands showed that they were proteolytic products of human ceruloplasmin being cleaved at positions 501 and 907. According to the X-ray structure of human ceruloplasmin, the first cleaved site is positioned in domain 3, whereas the second is positioned between domains 5 and 6 [28]. All three fragments have pI values (5.3–5.6) comparable with full-length ceruloplasmin. Consequently, it cannot be excluded that, for example, domains 1–5 and domain 6 are separated during agarose electrophoresis and that one of these fragments accounts for the binding to ^{125}I-PACAP 28-38, as shown in Figure 3(D). Reduced and unreduced samples of the purified PBF showed the same mobility on SDS/PAGE as commercial preparations of ceruloplasmin. In addition, the commercial ceruloplasmin from Sigma showed the same binding characteristics to PACAP 1-38, 1-27 and 28-38 as seen for plasma, and plasma depleted of human ceruloplasmin by immunoprecipitation was not able to bind ^{125}I-PACAP 1-38 or 28-38. Taken together, our findings provide strong evidence that ceruloplasmin is the PBF in plasma.

Ceruloplasmin is a copper-binding glycoprotein (6 atoms per molecule). Several possible functions for ceruloplasmin have been described: ferroxidase activity, amine oxidase activity, superoxide dismutase activity and copper transport. Human ceruloplasmin is an acute phase protein whose hepatic synthesis and plasma concentration increase in response to a variety of conditions [29]. Most plasma ceruloplasmin derives from hepatic synthesis, but the protein is also produced in other tissues, including brain [30,31], uterus [32], mammary gland [33], lung [34], testis [35] and synovium [36].

The physiological significance of the PBF is at present purely speculative. Potential functions of the binding protein could include blood transport of PACAP, altering of the PACAP clearance rate, inactivation of neuronal overflow to the blood of PACAP released at the synapses, increasing or decreasing biological activity of PACAP and, finally, targeting of PACAP. The high concentration of ceruloplasmin in human plasma (1.7 μM) compared with the reported picomolar concentrations of PACAP 1-38 in the circulation [37] (50–100 pM in hypophyseal portal plasma and 25 pM in peripheral plasma) implies a high binding capacity for PACAP despite the moderate apparent K_d of 12.0 nM for the PACAP 1-38–ceruloplasmin complex. If ceruloplasmin serves as a transport protein for PACAP 1-38 in human blood, the moderate apparent K_d enables PACAP 1-38 to dissociate from the protein and bind to a PACAP receptor.

An interesting finding is the expression of the PACAP-binding protein ceruloplasmin in the central nervous system, where the major form of this molecule is attached to the membranes of astrocytes via a glycosylphosphatidylinositol anchor [38]. It might be speculated that, after neuronal release of PACAP in the brain, subsequent binding to ceruloplasmin could act to limit PACAP to a local action. Alternatively, the complex may function as a signal in its own right if there is a receptor and a signalling pathway associated with the site of the PACAP–ceruloplasmin complex.

In conclusion, a factor in human plasma able to bind PACAP 1-38 has been isolated and identified as ceruloplasmin. The lack of PACAP 1-27 binding to human ceruloplasmin suggests that the 28-38 extension is important for binding.

The skilful technical assistance of Yvonne Søndergaard is gratefully acknowledged. The study was supported by the Danish Biotechnology Center for Cellular Communication.

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Received 22 February 1999/13 April 1999; accepted 5 May 1999