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AUTOANTIBODY TO VASOACTIVE INTESTINAL PEPTIDE IN HUMAN CIRCULATION

S. Paul, P. Heinz-Erian and S.I. Said[1]

Department of Medicine, University of Oklahoma Health Sciences Center
and Veterans Administration Medical Center, Oklahoma City, OK 73190

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SUMMARY: In a radioassay for Vasoactive Intestinal Peptide (VIP)-binding, eight out of 33 plasma samples from healthy human subjects exhibited specific binding ranging from 2.6% to 46.7% of total [^{125}I]VIP. This binding was competitively displaced by unlabeled VIP. The structurally homologous peptides, Peptide Histidine Isoleucine (PHI) and secretin, were, respectively, 72-fold and 413-fold less potent than VIP in displacing bound [^{125}I]VIP, whereas the unrelated peptides, neurotensin, eledoisin, bombesin and met-enkephalin, were without effect on the binding. The antibody nature of the VIP-binding factor was suggested by its precipitation with ammonium sulfate, attenuation after absorption with Staphylococcus aureus preparations, precipitation with antisera against human IgG and IgM, and coelution with standard IgG and IgM on anion-exchange and high-performance gel-filtration columns. Pepsin treatment of purified IgG fraction yielded a VIP-binding species with apparent molecular weight of 108 ± 13 kDa that was precipitated by antiserum against the $F(ab)_2$ fragment of the IgG molecule. These results demonstrate the existence in some human plasmas of an autoantibody that binds VIP.

The presence of autoantibodies against peptide hormones or against cell-membrane constituents in human subjects has been documented (1-4). On the other hand, the occurrence of antibodies directed against neurotransmitters has not been demonstrated. We report here evidence for the existence in human plasma of an autoantibody that binds vasoactive intestinal peptide (VIP), a 28-amino acid neuropeptide that is a likely neurotransmitter or neuromodulator in the central and peripheral nervous systems (5,6).

MATERIALS AND METHODS

[^{125}I]VIP: Purified porcine VIP was prepared at Karolinska Institutet, Stockholm (Prof. Viktor Mutt) and was iodinated by the chloramine-T method. The [^{125}I]VIP was purified by reverse phase HPLC on C-18 Novapak columns (Waters Associates, Milford, MA) as described previously (7), and had a specific activity of 1.99 ± 0.21Ci/μmole.

[1]To whom correspondence should be addressed.

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Radioimmunoassay (RIA) for anti-VIP antibody: Peripheral venous blood from healthy volunteers (age: 10-51 years) was collected in EDTA and centrifuged at 1000 xg for 15 min at 4°C. Plasma (50 µl) containing aprotinin (Mobay Chem. Corp., New York, NY, 500 u/ml), was incubated with [^{125}I]VIP (12-15 x 10^3 cpm) for 20-24 hr at 4° C in a final volume of 150 µl of diluent [0.01 M sodium phosphate pH 7.2, containing 0.9% NaCl, 0.5% bovine serum albumin (BSA, Sigma, St. Louis, MO) 0.005% bacitracin, 0.005% protamine sulfate (Sigma) and 0.025M EDTA]. Nonspecific binding was determined by incubation in the presence of excess unlabeled VIP (2.5 µM). Bound [^{125}I]VIP was precipitated by: (i) addition of polyethylene glycol (PEG 5.5 kDa, Sigma, final concentration 7.5% w/v in distilled water), incubation for 10 min at 23° C and centrifugation at 2000 xg (20 min, 4°C), or (ii) addition of 200µ l goat antiserum against human IgM (1:2 in diluent) or IgG (undiluted) (Antibodies Inc., Davis, CA), incubation for 30 min at 4°C and centrifugation. The supernates were aspirated and radioactivity in pellets determined in a Beckman gamma-spectrometer (model 5500). The optimal strength of the second-antibody reagents was determined in preliminary antiserum-dilution experiments. Binding specificity was assessed by measuring displacement with PHI, secretin (Peninsula, Belmont, CA), neurotensin, eledoisin (Beckman, Berkely, CA), bombesin and met-enkephalin (Sigma).

Analysis of the VIP-binding factor: The globulin fraction of plasma samples was precipitated with an equal volume of a saturated solution of ammonium sulfate at 4°C and centrifuged at 3000 rpm (4°C) for 30 min. The pellet was redissolved in 0.1 M sodium phosphate buffer, pH 7.0, and dialysed (48 hr, 2 changes at 4°C). Absorption of plasma with an equal volume of Staphylococus aureus (10% w/v in RIA-diluent, glutaraldehyde fixed, Miles Scientific, Naperville, IL) was done for 12 hr at 4°C. The suspension was centrifuged in a Beckman microfuge (12,000 xg, 10 min) and the supernates assayed for VIP binding activity. A DEAE-cellulose anion exchange column (10.5 x 1 cm) (DE-52, Whatman Inc., Clifton, NJ) was used to prepare the IgG fraction from plasma (previously dialyzed against 200 volumes of the column-buffer for 2 hr). A linear gradient from 0.05 M Tris-HCl, pH 8.0 to 0.3M Tris-HCl, pH 4.0, over 2 hr at a flow rate of 100ml/hr was used for elution. High performance gel-filtration of plasma or the IgG fraction, with and without prior treatment for 4 hr with crystalline pepsin (Worthington, Freehold, NJ) (8), was on a Protein-pak 300sw column (Waters Associates) in 0.1 M sodium phosphate, pH 7.0, at a flow rate of 0.5 ml/min. 0.2 ml fractions were collected and assayed for specific VIP-binding using goat anti-human IgM, IgG or $F(ab)_2$ sera (Accurate Chemicals) as the precipitating agent. The molecular weight markers used were dextran blue, ferritin, aldolase, BSA, ribonuclease (Pharmacia Fine Chemicals, Piscataway, NJ), human IgG and human IgM (Capell Laboratories, Malvern, PA).

RESULTS AND DISCUSSION

VIP-binding activity in plasma: Plasma incubated with [^{125}I]VIP was treated with polyethylene glycol (PEG) to precipitate bound VIP. Specific [^{125}I]VIP binding (i.e., binding displaceable by excess unlabeled VIP) increased with increasing concentration of PEG, and reached a plateau at concentrations greater than 7.5%. Specific [^{125}I]VIP binding decreased progressively with increasing dilution of plasma and was competitively inhibited by increasing concentration of unlabeled VIP (Fig 1). In three different plasma samples,

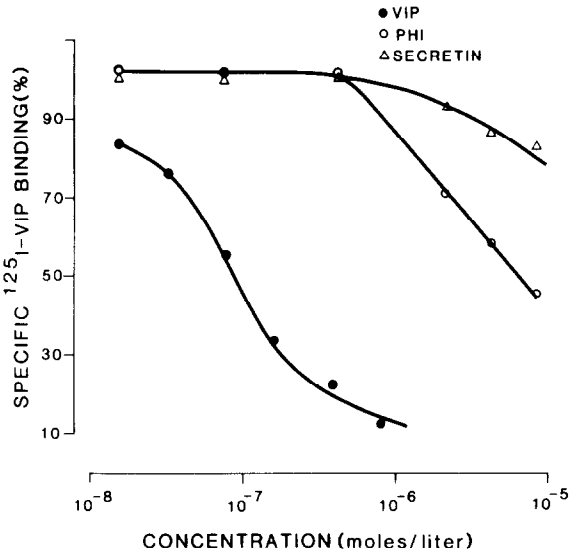

Figure 1. Competitive inhibition of plasma [^{125}I]VIP binding activity by VIP, PHI and secretin. Plasma was incubated with [^{125}I]VIP in the absence and presence of increasing concentrations of VIP, PHI and secretin. Bound VIP was precipitated with polyethylene glycol. Values plotted are mean of 3-replicates and are corrected for nonspecific binding obtained in the presence of 2.5 μM VIP.

the concentration of VIP causing 50% displacement of [^{125}I]VIP ranged from 21 to 815 nM. Analysis of the data according to Scatchard (9) yielded Kd values from 64 to 1500 nM, and VIP-binding capacity values, from 0.19 to 3.1 nmoles/ml plasma. The structurally unrelated peptides eledoisin, met-enkephalin, neurotensin and bombesin, at concentrations up to 5 μ M, did not displace [^{125}I]VIP binding by plasma (N=3). On the other hand, the homologous peptides, PHI and secretin, were, respectively 72-and 413-fold less potent that VIP in displacing the binding of [^{125}I]VIP (Fig. 1).

Eight of thirty-three plasma samples showed [^{125}I]VIP binding that was significantly ($P < 0.01$) greater than nonspecific binding obtained in the presence of 2.5 μ M VIP (range: 2.8% to 46.7% of total [^{125}I]VIP). The eight subjects positive for VIP-binding ranged in age from ten to 51 years; four were male and four female.

Characterization of the VIP-binding activity: Several lines of evidence indicate that the VIP-binding activity in plasma was due to immunoglobulins: (a) The specific-VIP binding activity of three plasma samples was precipitated

with 50% saturated ammonium sulfate. (b) A plasma sample absorbed with Staphylococcus aureus, bacteria that bind IgG by virtue of protein A present on their surface (10), exhibited significantly reduced (by 77%) specific VIP-binding. (c) In the eight plasma samples assayed, seven showed significant specific VIP-binding using anti-human IgG, and all eight using anti-human IgM, to precipitate bound VIP (range: 1.6% to 17.5% of total [^{125}I]VIP). In a control experiment, goat anti-rabbit IgG serum did not precipitate the plasma-VIP binding activity. (d) High performance gel-filtration chromatography of four different plasma samples yielded two discrete peaks of specific VIP-binding activity coeluting with standard IgM and standard IgG. On DEAE-cellulose anion exchange columns, plasma fractions that coeluted with standard IgG exhibited specific VIP-binding activity (Fig. 2; upper panel). The IgG-eluate from the DEAE-cellulose column was subjected to high performance gel-filtration. The specific VIP-binding activity eluted as a single peak along with standard IgG (Fig. 2, lower panel). After digestion for four hours with pepsin, the IgG-fraction retained 96% of its original VIP-binding activity. About 60% of this activity eluted as a lower molecular-weight species (apparent MW 108 kDa $\pm$ 13 kDa) and was precipitated by anti-human-F(ab)$_2$ antiserum.

DISCUSSION

Our studies demonstrate the existence of specific VIP-binding activity in the plasma of a proportion (24%) of healthy human subjects. The immunoglobulin nature of the VIP-binding activity is indicated by its precipitation with ammonium sulfate, partial absorption with Staphylococcus aureus, precipitation with specific anti-human IgG and IgM antisera, coelution with standard human IgG and IgM on anion exchange and gel-filtration columns, and the ability of the F(ab)$_2$ fragment to bind VIP.

The VIP used in these experiments was purified from pig intestine (11), but its amino-acid sequence is identical to that of human VIP (12). The presence of blood-borne IgG and IgM antibodies that bind VIP may, therefore,

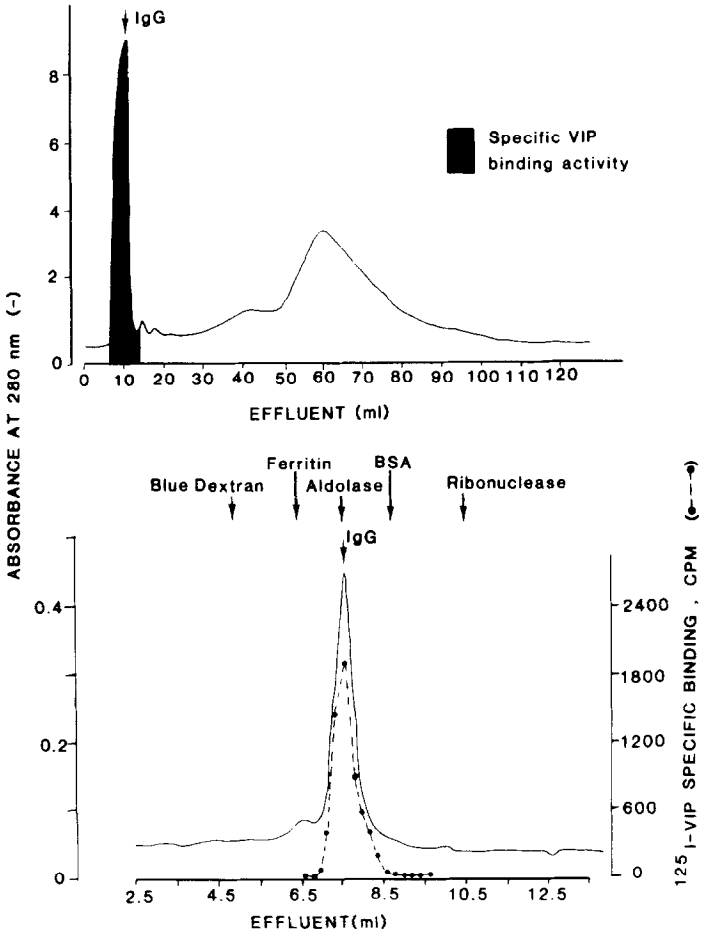

Figure 2. Elution profile of the plasma-VIP binding activity on DE-52 (upper panel) and PP300sw high performance gel-filtration (lower panel) columns. The chromatographic procedures were performed as described in the text. Portions of the eluate fractions were assayed in duplicate for specific [^{125}I]VIP binding using goat anti-human IgG to precipitate bound VIP.

be considered as a manifestation of autoimmunity. Autoantibodies may be generated by exposure to cross-reacting heterologous antigen (13), infectious agents (14) or polyclonal B-lymphocyte activators (15), as well as by disturbances in physiologic immunoregulation by anti-idiotype antibodies (11,15). Which of these may be the original stimulus for formation of the VIP-autoantibody is uncertain. In addition, VIP has been shown to derive from larger precursor molecules (17) that can be expected to exhibit greater immunogenicity than the peptide itself.

A number of endocrine and neurologic diseases (e.g., diabetes, thyroid disorders, myasthenia gravis and multiple sclerosis) are associated with elevated autoantibody levels against hormonal agents or cell-membrane constituents (1,4,19). The presence of antibody reactive with endogeneous antigens is, however, not necessarily of pathologic consequence. Thus, autoantibodies directed against soluble proteins (20) and cellular antigens (21) are found in healthy subjects, and may have a homeostatic function (22). The present study reveals a novel autoimmune specificity, directed against a putative neurotransmitter or neuromodulator peptide. The autoantibody to VIP may: a) neutralize the biologic effects of the peptide in its target tissues, or b) protect it from proteolysis, in a manner analogous to the reduced degradation of insulin due to insulin antibody (18). If the autoantibody is present in sufficient concentrations in the tissue microenvironment, its presence may lead to an alteration in the functional concentration of VIP at its receptors.

The eight subjects we found to have VIP-binding activity are healthy, active individuals without overt manifestation of disease; three are full-time college students, one is a school-going child and four are engaged in non-sedentary vocations. The possibility remains that the demonstration of VIP-binding antibodies may predate the apparent onset of certain diseases, as has been reported for other antibodies (23). The possible relationship of VIP-antibody to disease cannot, therefore, be ascertained from the present results, and will require long-term observation of the subjects with antibody activity. In this regard, we have recently found that plasma mean VIP-binding activity in 37 cystic fibrosis patients was significantly higher (3.1-fold) than in 33 healthy controls (24). Patients with cystic fibrosis often have a polyclonal hypergammaglobulinemia (25). However, the VIP-binding was poorly correlated with both total IgG ($r=0.21$) and IgM ($r=0.01$) serum levels in 21 patients with this disease. The elevated VIP-binding by cystic fibrosis sera cannot therefore be attributed to a non-specific increase in total antibody

levels. The potential physiologic or pathophysiologic significance of autoantibodies to VIP clearly deserves further investigation.

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