SPECIFICITY OF RECEPTORS TO VASOACTIVE INTESTINAL PEPTIDE IN GUINEA PIG BRAIN

Patrick Robberecht, Wolfgang König⁺, Monique Deschodt-Lanckman,
Philippe De Neef and Jean Christophe

Department of Biochemistry and Nutrition, School of Medicine
Université libre de Bruxelles, Boulevard de Waterloo 115,
B-1000 Brussels, Belgium
and ⁺Hoechst, Aktiengesellschaft, Frankfurt, West-Germany

(Received in final form July 30, 1979)

Summary

The specific binding of VIP to guinea pig brain membranes was tested by 1/ the ability of eight VIP and secretin analogs and fragments to inhibit the binding of ¹²⁵I-VIP and 2/ the capacity of the same peptides to influence basal and VIP-stimulated adenylate cyclase activities. Among all peptides tested, only VIP, secretin, [Val⁵] secretin, and [Gln⁹, Asn¹⁵]secretin (5-27) were able to inhibit ¹²⁵I-VIP binding. The adenylate cyclase activity was stimulated by VIP, secretin and [Val⁵]secretin. [Gln⁹, Asn¹⁵]secretin (5-27) although inactive per se was able to inhibit the VIP-stimulated adenylate cyclase activity competitively.

The presence of specific binding sites for the brain-gut peptide vasoactive intestinal polypeptide (VIP) has been demonstrated in guinea pig brain synaptic membranes (1). VIP activates adenylate cyclase activity in the same preparation (2). Recently, it was also observed that cyclic AMP levels increase in brain slices incubated in the presence of VIP (3). So far, the specificity of VIP receptors coupled to an adenylate cyclase system is broadly established on the following ground : 1) other neuro- or candidate neuro-transmitter peptides do not interfere with ¹²⁵I-VIP binding receptors (1); 2) among parent peptides, natural secretin is the only one capable to recognize VIP receptors (1) but no peptide is able to stimulate adenylate cyclase activity significantly (2).

In order to further characterize VIP brain receptors in guinea pig brain synaptic membranes, we documented the ability of synthetic secretin, of six secretin analogs and fragments, and of one VIP fragment to interfere with VIP binding and VIP activation of adenylate cyclase.

Materials and Methods

The preparation procedure of guinea pig brain synaptic membranes is detailed in previous papers (1,2).

Adenylate cyclase activity was determined according to Salomon et al. (4). The standard adenylate cyclase reaction mixture (final volume 60 µl) contained the following final concentrations : 30 mM Tris-HCl (pH 7.4), 1 mM 2-mercaptoethanol 5 mM MgCl₂, 0.5 mM EGTA, 1 mM cyclic AMP, 1 mM theophylline, 0.5 mM [α -³²P] ATP, 10 mM phospho(enol) pyruvate and 30 µg/ml pyruvate kinase. The reaction was initiated by the addition of membranes (30 to 100 µg protein) and was conducted for 7 min at 37° C. Under all conditions tested, the [³²P]cyclic AMP produced correlated linearly with time and protein concentration.

Abbreviation : VIP, vasoactive intestinal peptide.

0024-3205/79/100879-06\$02.00/0
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^{125}I -labeled VIP was prepared as previously published (1,5). Its binding to brain membranes was conducted at a $3 \cdot 10^{-11}$ M tracer concentration and at 37°C in a final volume of 120 μl consisting of 25 mM Tris-HCl buffer (pH 7.4), 2 mM MgCl_2 , 0.1 mM 2-mercaptoethanol, 500 kallikrein inhibitory units/ml Trasylol, 0.25 mg/ml Bacitracin, ^{125}I -labeled VIP (15 000 - 20 000 counts/min/assay) and 0.5 % bovine serum albumin. The reaction was initiated by the addition of the brain membrane preparation (50-100 μg protein/assay) and was terminated 10 min later by diluting the mixture with 2 ml of ice-cold 25 mM Tris-HCl buffer (pH 7.4), 2 mM MgCl_2 , 0.5 % bovine serum albumin, and immediate filtration through 0.45 μm pore size cellulose acetate Millipore filters. The filters were washed twice with 2 ml of the same ice-cold buffer and counted in a gamma scintillation counter.

Essentially pure natural porcine VIP was prepared by Dr. V. Mutt (GIH Laboratory, Karolinska Institutet, Stockholm, Sweden). The synthetic C-terminal VIP fragment (18-28) and the substituted tricosapeptide secretin fragment [Gln⁹-Asn¹⁵]secretin(5-27) were generous gifts from Dr. M. Bodansky (Case Western Reserve University, Cleveland, Ohio, U.S.A.). Synthetic secretin, [Glu³]secretin, [Asn³]secretin, [Ala⁴]secretin, [D-Ala⁴]secretin, [Val⁵]secretin and the C-terminal secretin fragment(7-27) were prepared as previously described (6,7). Natural porcine glucagon was provided by Novo Industri (Etablissements Couvreur, Brussels, Belgium). The origin of the other chemicals was mentioned in previous papers (1,2).

FIG. 1

Inhibition of ^{125}I -VIP binding to brain synaptic membranes by VIP (o), [Gln⁹,Asn¹⁵]secretin(5-27) (●), [Val⁵]secretin (Δ) and secretin (▲). VIP (18-28), secretin(7-27), [Asn³]secretin, [Glu³]secretin, [Ala⁴]secretin, [Ala⁴]secretin and glucagon were without effect at the concentration tested (□). Membranes were incubated for 10 min at 37°C under the conditions described in Materials and Methods. The specific binding of ^{125}I -VIP was expressed as percentage of radioactivity bound in presence of tracer only. The curves are the means of 4 experiments performed in duplicate.

FIG. 2

Dose-response curves of adenylate cyclase activity in a guinea pig brain synaptic membrane fraction stimulated by VIP and related peptides. Symbols for the different peptides are the same as those presented in Fig. 1. The results were expressed in increases in pmol cyclic AMP formed $\times \text{min}^{-1} \times \text{mg protein}^{-1}$ in excess of the corresponding mean basal value of 175 pmol cyclic AMP. The curves are the means of three experiments performed in duplicate.

Results

1. Ability of VIP and parent peptides to inhibit ^{125}I -VIP binding to guinea pig brain synaptic membranes (Fig. 1).

Only four peptides were able to inhibit the binding of a tracer concentration of ^{125}I -VIP: natural VIP was the most potent followed in decreasing order by $[\text{Gln}^9, \text{Asn}^{15}]$ secretin(5-27), $[\text{Val}^5]$ secretin and secretin. Secretin concentrations higher than 10^{-5} M were required to observe an inhibitory effect and VIP was at least 1000-fold more potent than the three other peptides.

2. Effects of VIP analogs on the activity of guinea pig brain synaptic membrane adenylate cyclase incubated in the absence or in the presence of VIP (Figs. 2 and 3).

Among the nine peptides tested, only natural VIP, $[\text{Val}^5]$ secretin and secretin were able to activate brain membrane adenylate cyclase. VIP was the most potent and the most efficient peptide, half-maximal adenylate cyclase activation occurring at a $2 \cdot 10^{-7}$ M peptide concentration.

A significant adenylate cyclase activation was found at 10^{-5} M $[\text{Val}^5]$ secretin and $4 \cdot 10^{-5}$ M secretin concentrations. The limited availability of these peptides prevented the use of concentrations higher than $4 \cdot 10^{-5}$ M. It was therefore impossible to determine the maximal velocity of adenylate cyclase activity in the presence of these peptides, and the peptide concentration giving half-maximal adenylate cyclase activation. $[\text{Val}^5]$ secretin was significantly more potent than secretin but at least 100-fold less potent than natural VIP.

The inactive peptides were tested in combination with increasing concentrations of VIP. $[\text{Gln}^9, \text{Asn}^{15}]$ secretin(5-27) was the only peptide which

interfered with the VIP stimulation of adenylate cyclase activity. This peptide, when used at a $4 \cdot 10^{-5}$ M concentration, induced a significant shift of the dose-effect curve of VIP activation of adenylate cyclase to the right. Curves in Fig. 3 indicate that [Gln⁹, Asn¹⁵]secretin(5-27) was a competitive inhibitor of VIP-stimulation of adenylate cyclase with an inhibitory constant of $3 \cdot 10^{-5}$ M, according to the calculation of Kauman and Birnbaumer (8).

FIG. 3

Dose-response curves of adenylate cyclase activity in a guinea pig brain synaptic membrane fraction stimulated by VIP in the absence (o) or in the presence (●) of $4 \cdot 10^{-5}$ M [Gln⁹,Asn¹⁵]secretin(5-27). The results were expressed in cyclic AMP formed in excess of the basal unstimulated value. The mean basal activity was 175 pmol cyclic AMP formed $\times$ min⁻¹ $\times$ mg protein⁻¹. The curves are the means of three experiments performed in duplicate.

Discussion

The present results demonstrate that brain VIP receptors were highly specific since they were recognized only by secretin and [Val⁵]secretin, and were competitively blocked by [Gln⁹,Asn¹⁵]secretin(5-27).

a) The occupancy of the VIP brain receptors by secretin at concentrations higher than 10^{-5} M and the weak but significant stimulation of brain adenylate cyclase activity at secretin concentrations higher than 10^{-5} M were at variance with our previously published data (1,2). In these studies, a significant competition between secretin and ¹²⁵I-VIP was already detectable at a 10^{-6} M concentration. A possible explanation is that the batch of natural secretin, previously delivered in ampules with 1 mg cystein by the GIH Unit of the Karolinska Institutet, was less pure than the present synthetic secretin. A highly purified natural secretin batch prepared by Dr. V. Mutt and the synthetic secretin used in this study are found equipotent in inhibiting ¹²⁵I-VIP binding and stimulating adenylate cyclase in rat pancreatic plasma membranes (9).

b) [Val⁵]secretin which is 65 % as active as secretin in stimulating the flow rate of pancreatic juice in dogs (7), was more potent than secretin in stimulating adenylate cyclase activity and inhibiting ¹²⁵I-VIP binding in guinea pig brain membranes. Besides, it proved to be the only secretin analog tested capable to recognize brain VIP receptors. The substitution of the hydrophilic amino acid threonine by the hydrophobic residue valine in the secretin molecule sufficed already to make the secretin molecule more VIP-like (there is a valine residue in the 5-position of VIP). Data on secretin analogs with a substitution in the 5-position are scarce. [Ala⁴,Val⁵]secretin is as active as secretin on pancreatic juice flow rate in perfused rat pancreas and 2.5-fold less potent than secretin on cat pancreatic adenylate cyclase activity (10). In rat pancreatic plasma membranes, [Val⁵]secretin is no more potent than secretin in inhibiting ¹²⁵I-VIP binding, and even less potent than secretin in stimulating adenylate cyclase activity in the same membrane preparation (9). Thus, at variance with the results observed on pancreatic preparations, [Val⁵]secretin appeared more VIP-like when interacting with brain VIP receptors.

[Ala⁴]secretin and its stereoisomer [D-Ala⁴]secretin were inactive although an alanine residue is present in the 4 position of the VIP molecule. Similarly, König et al. (6) reported that [Ala⁴]secretin and [D-Ala⁴]secretin were only 2 % and 8 % as active, respectively, as secretin on dog pancreas. On rat pancreas, [Ala⁴]secretin and [D-Ala⁴]secretin are 20-fold less potent than secretin on adenylate cyclase activation but equipotent to secretin for inhibiting ¹²⁵I-VIP binding (9). There is no explanation available for the relatively poor efficacy of these two peptides. Similar findings were described by Gardner et al. (11) when comparing [Tyr¹⁰]secretin and [Ala⁴,Tyr¹⁰]secretin.

The substitution of the aspartic acid residue in position 3 of secretin by glutamic acid or asparagine made these two peptides unable to occupy VIP₅ receptors in guinea pig brain membranes even when offered at a high $4 \cdot 10^{-5}$ M concentration. The same peptides exert a markedly reduced activity on pancreatic function (6) and pancreatic adenylate cyclase (9) when compared to secretin.

c) The peptide fragment secretin(7-27) and VIP(18-28) did not recognize VIP receptors and consequently could not interfere with VIP-stimulated brain membrane adenylate cyclase activity. Secretin fragments lacking the N-terminal sequence His-Ser-Asp-Gly are usually inactive (8-11) or only extremely weak partial agonists of secretin on other biological preparations (12). They can however recognize secretin receptors and inhibit the biological effect of secretin (13). For instance, secretin(5-27) and secretin(7-27) specifically inhibit the production of cyclic AMP in, respectively, pancreatic acinar cells from guinea pig (13) and rat pancreatic plasma membranes incubated in the presence of secretin (9). Both fragments are by contrast without effect on VIP stimulations.

In [Gln⁹,Asn¹⁵]secretin (5-27), the neutralization of two negative charges present in secretin renders this fragment more VIP-like than secretin-like (14). On brain synaptic membranes, this fragment behaved as a competitive inhibitor of VIP for VIP receptors coupled to an adenylate cyclase. The same substituted secretin fragment inhibits cyclic AMP production in guinea pig pancreatic acinar cells stimulated by both secretin and VIP (15).

Acknowledgments

Aided by Grant 20,403 from the Fonds de la Recherche Scientifique Médicale (Belgium) and Grant RO-1AM-17010 from the National Institutes of Health (U.S.A.).

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