0026-895X/90/060971-07$02.00/0

MOLECULAR PHARMACOLOGY, 37:971-977

Selectivity for Binding of Peptide Analogs to Vascular Receptors for Vasoactive Intestinal Peptide

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Received September 13, 1989; Accepted February 28, 1990

SUMMARY

The structure-activity relationships for vasoactive intestinal peptide (VIP) receptor binding were studied using N-terminally modified VIP analogs, VIP fragments, and VIP receptor antagonists. Tissue sources included bovine coronary artery, rat mesenteric artery, rat pituitary, rat brain synaptosomes, and rat liver. Experimental conditions for receptor binding were maintained as near to identical as possible. The competitive binding curves for VIP analogs were similar in the bovine and rat vascular preparations. However, appreciable differences were observed between the vascular and other preparations. The vascular receptors discriminated between [D-His^1]VIP and [Phe^1]VIP, whereas the receptors in other tissues did not. The greatest selectivity was found for [D-Ala^4]VIP, which was among the lowest affinity analogs tested on the vasculature but among the highest affinity analogs in the other preparations. The rank orders of analog potencies were comparable for the rat brain and pituitary receptors. The rat liver VIP receptor differed from its counterpart in brain and pituitary predominantly by discriminating between [D-Phe^2]VIP and [D-Arg^2]VIP. The two VIP receptor antagonists bound weakly and nonselectively to all receptor preparations. Integrity of the full VIP molecule was necessary for full potency of binding to the vascular receptor. We conclude that the vascular VIP receptor possesses recognition properties that are distinct from those for VIP receptors in liver, pituitary, or brain.

VIP is a 28-residue neuropeptide with a diversity of biological actions and potential target organs (1). The widespread anatomical distribution of VIP in the central and peripheral nervous systems is consistent with its hypothesized role as a chemical messenger subserving numerous regulatory functions. Specific receptors for VIP have been identified on a variety of VIP-responsive tissues and they display characteristics of selective recognition, high affinity, and, in most cases where studied, coupling to adenylate cyclase as an intracellular transduction mechanism (2, 3). The extensive anatomical distribution and spectrum of biological effects of VIP provoke the question of whether heterogeneity or subtypes of VIP receptors may exist, by analogy with other neurotransmitter systems. This hypothesis accrues support from studies of the selectivity of adenylate cyclase activation by VIP, VIP analogs, and homologous peptides (4–7). However, adenylate cyclase is susceptible to activation by receptor systems other than that for VIP, most importantly the secretin receptor (8). Radioligand binding studies utilizing structurally modified VIP analogs provide an alternative approach that directly probes the structure-activity relationships required for VIP receptor occupancy (5–7, 9). Also in support of the concept of VIP receptor heterogeneity, affinity labeling experiments have identified VIP binding sites of varying molecular sizes in different tissues and cells, most extensively studied in the rat (reviewed in Refs. 3 and 10).

The present study examined the recognition properties of the VIP receptor in bovine and rat arteries, rat anterior pituitary gland, rat brain synaptosomes, and rat liver. Competitive receptor binding was studied using VIP analogs with modifications to the biologically critical amino terminus, carboxyl terminal VIP fragments (11), VIP receptor antagonists (12, 13), and apamin, a peptide derived from bee venom (14). The structure-activity relationships for the VIP receptors in blood vessels, rat pituitary, and rat brain had not previously been examined with these particular analogs. The major finding of the study was an appreciable difference in the recognition properties of the vascular VIP receptor in both bovine and rat species from those of the VIP receptor in rat pituitary, brain, or liver. The rat liver receptor differed to a lesser extent from the others, particularly in terms of the relative potency for binding of [D-Phe^2]VIP.

This study was supported by the Medical Research Council of Canada, Alberta Heart and Stroke Foundation, the Alberta Heritage Foundation for Medical Research (AHFMR), and the National Institutes of Health (Grant DK-30167 to D.H.C). O.P.R. is a scholar of the AHFMR. I.W. is a clinical fellow of the AHFMR.

ABBREVIATIONS: VIP, vasoactive intestinal peptide; GRF, growth hormone-releasing factor; PHI, peptide histidine isoleucine; BSA, bovine serum albumin; HPLC, high pressure liquid chromatography.

Experimental Procedures

Materials. Synthetic VIP (identical in structure to native rat and bovine VIP), [4Cl-D-Phe^6,Leu^{17}]VIP, and [*N*-Ac-Tyr^1,D-Phe^2]GRF(1–29)-NH_2 were purchased from Peninsula Laboratories (San Carlos, CA). VIP analogs and fragments were prepared by solid phase synthesis and purified by ion exchange chromatography and partition chromatography as previously described (11, 15). Bacitracin, BSA (fraction V), phenylmethylsulfonyl fluoride, Polybrene, sucrose, and Trizma base were obtained from Sigma Chemical Co. (St. Louis, MO). Apamin was purchased from Serva (Heidelberg, FRG). Na^{125}I (100 mCi/ml) was from Amersham (Arlington Heights, IL).

Membrane preparation. The superior mesenteric artery, anterior pituitary gland, brain, and liver were dissected from male Sprague Dawley rats (250–350 g; Charles River Canada Inc., St-Constant, Quebec). Coronary arteries (the circumflex branch and the left descending coronary artery) were dissected from bovine hearts that were obtained from a local abattoir and transported to the laboratory on ice. All preparative steps involving the different tissues were performed either on crushed ice or at 4°. Arterial membranes were prepared as previously described (16).

Liver membranes were prepared following the method of Neville (17). Briefly, rat liver was minced with scissors and homogenized in a Dounce manual tissue grinder in 20 volumes (based on tissue wet weight) of buffer (20 mM Tris·HCl, pH 7.4, containing 2 mM $MgCl_2$, 2 mM EDTA, and 0.5 mM phenylmethylsulfonyl fluoride). After centrifugation at 270 × *g* for 15 min, the pellet was washed twice with the same buffer and suspended in 20 mM Tris·HCl (pH 7.4) containing 0.25 M sucrose. The suspension was centrifuged at 100,000 × *g* for 1.5 hr in a discontinuous sucrose density gradient consisting of 0.8, 1.0, 1.2, and 1.4 M sucrose in 20 mM Tris·HCl buffer (pH 7.4), using a SW 28 rotor in a Beckman ultracentrifuge. The plasma membrane fraction at the interface of 1.2 and 1.4 M sucrose was collected, washed with 20 mM Tris·HCl (pH 7.4) containing 0.25 M sucrose, and stored in the same buffer at −70°.

The procedure for the preparation of synaptosomal membranes was based on the method of Gray and Whittaker (18). Gray matter of cerebral cortex was homogenized in 20 volumes of 25 mM Tris·HCl (pH 7.4) containing 0.32 M sucrose, in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 1,000 × *g* for 10 min and the supernatant obtained was centrifuged at 12,000 × *g* for 20 min to sediment the crude mitochondrial fraction. The latter was suspended in 12.5 mM Tris·HCl buffer (pH 7.4) and fractionated at 100,000 × *g* for 2 hr in a Beckman SW 28 rotor, using a discontinuous sucrose density gradient consisting of 0.8, 1.0, and 1.2 M sucrose in 20 mM Tris·HCl (pH 7.4). The fraction that was enriched in synaptosomal membranes, at the interface between 1.0 and 1.2 M sucrose, was collected, washed with 25 mM Tris·HCl buffer (pH 7.4) containing 2 mM $MgCl_2$, 0.25 g/liter bacitracin, and stored in the same buffer at −70°.

Anterior pituitary membranes were prepared as previously described (19). In summary, rat anterior pituitary glands were cut into 1–2-mm fragments and homogenized in 150 volumes of buffer (25 mM Tris·HCl, pH 7.4, containing 2 mM EDTA and 2 mM $MgCl_2$), using a Dounce homogenizer. The homogenate was centrifuged at 700 × *g* for 10 min and, after filtration through two layers of gauze, the supernatant was centrifuged at 12,000 × *g* for 30 min. The resulting pellet was washed twice with 25 mM Tris·HCl buffer (pH 7.4) containing 0.25 M sucrose and was stored in the same buffer at −70°.

VIP binding assay. The radioligand used in the VIP binding assay was monoiodinated homogeneous [^{125}I-Tyr^{10}]VIP, prepared by radioiodination of synthetic VIP using the lactoperoxidase/glucose oxidase method as previously described (20). The standard assay measured the binding of [^{125}I-Tyr^{10}]VIP (55–65 pM) to membrane preparations in a total volume of 0.2 ml containing 25 mM Tris·HCl (pH 7.4), 2 mM $MgCl_2$, 0.25 g/liter bacitracin, and 1% BSA (fraction V). The assay was conducted at 21° for 30 min for arterial, liver, and synaptosomal preparations and at 31° for 1 hr for the pituitary preparation. The amount of membrane protein present in the assay was coronary and mesenteric artery, 8–11 μg; liver, 3–4 μg; synaptosomes, 14–16 μg; and anterior pituitary, 20 μg. Nonspecific binding was measured in parallel in the presence of 1 μM synthetic VIP. Free and bound radioligand were separated by filtration under reduced pressure through glass fiber filters (GF/B) that had been presoaked in 0.3% aqueous Polybrene for at least 1 hr (21). Radioactivity was quantitated using a Beckman model 5500 γ counter with 74% counting efficiency. In competitive binding studies, nonradioactive VIP or other peptides were added to the binding assay at specified concentrations. Results were expressed as a percentage of radiolabeled VIP specifically bound in the absence of nonradioactive peptides. Protein in tissue preparations was measured by the method of Lowry *et al.* (22), using BSA as a standard.

The radioligand binding properties of the bovine coronary and rat mesenteric arteries (16) and the rat pituitary (19) have been reported previously by our laboratory. The binding properties of VIP receptors in membranes from rat brain (23) and rat liver (24, 25) have been published by other investigators. The binding properties obtained by our laboratory for the five tissues studied are shown in Table 1. Competitive binding data obtained using unlabeled VIP were analyzed according to the method of Scatchard (26) and generated an upwardly concave curve for each tissue. Analysis of these curves with the least squares nonlinear regression program LIGAND (Elsevier-BIOSOFT, version 3.0, 1986) (27) resulted in the best fit with a two-site binding model in the case of each tissue, with equilibrium dissociation constants (K_D) shown in Table 1.

Stability of [^{125}I-Tyr^{10}]VIP and VIP analogs. Stability of [^{125}I-Tyr^{10}]VIP during a receptor binding experiment was assessed by HPLC analysis of the radioligand recovered after the incubation period. Our previous studies of the vascular and pituitary tissues had demonstrated less than 13% degradation of [^{125}I-Tyr^{10}]VIP during the binding assay (16, 19). Incubation with rat brain synaptosomes and rat liver membranes resulted in degradation of 5% of [^{125}I-Tyr^{10}]VIP. To assess metabolism of the analogs, radioiodinated derivatives of four selective analogs were prepared by a procedure similar to that used for VIP, followed by similar purification by HPLC (20). The radioiodinated derivatives predicted to be iodinated on Tyr^{10} by examination of the HPLC profile (20) were incubated with each of the five tissues under receptor binding conditions. The percentage of metabolism was determined by HPLC analysis similarly as for [^{125}I-Tyr^{10}]VIP. The ^{125}I-labeled VIP analogs were only slightly metabolized by each of the five tissues, ranging as follows: ^{125}I-labeled [D-His^1]VIP, 3–11%; ^{125}I-labeled [Phe^1]VIP, 2–6%; ^{125}I-labeled [D-Ser^2]VIP, 3–11%; and ^{125}I-labeled [D-Ala^4]VIP, 3–8%.

Results

Receptor-peptide affinities were estimated by competitive binding of peptides with [^{125}I-Tyr^{10}]VIP to the five tissue preparations. Concentration-response curves are portrayed in Figs. 1 to 5 and the IC_{50} values and relative potencies of peptides are

TABLE 1

Radioligand binding parameters for different tissues

Percentage of specific binding was calculated as specifically bound cpm divided by total bound cpm times 100. K_{D1} and K_{D2} are the equilibrium dissociation constants for two binding sites determined by Scatchard analysis, as described in Experimental Procedures, given as mean ± standard error. K_D values for bovine coronary arteries, rat mesenteric arteries, and rat pituitary have been published previously (16, 19). Five experiments were performed with rat brain and four with rat liver.

	Specific binding	K_{D1}	K_{D2}
	%	nM	nM
Bovine coronary artery	80–90	0.12 ± 0.03	21 ± 6
Rat mesenteric artery	78–88	0.22 ± 0.02	14 ± 8
Rat pituitary	60–70	0.19 ± 0.03	26 ± 16
Rat brain	65–75	0.87 ± 0.18	90 ± 27
Rat liver	90–93	0.23 ± 0.06	51 ± 21

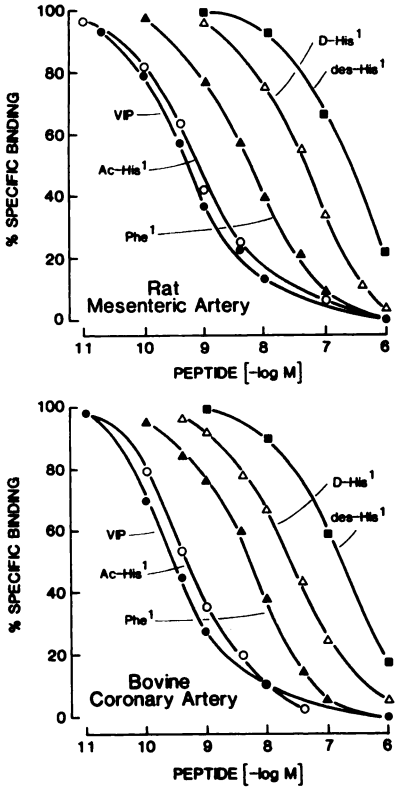

Fig. 1. Competition of binding of [^{125}I-Tyr^{10}]VIP to preparations of rat mesenteric arteries and bovine coronary arteries by VIP analogs modified at amino acid residue 1. Experimental conditions are described in Experimental Procedures. Arterial preparations were incubated with the indicated concentrations of VIP (●), [$N\alpha$-Ac-His^1]VIP (○), [Phe^1]VIP (▲), [D-His^1]VIP (△), and des-His^1-VIP (■). Percentage of specific binding indicates the percentage of [^{125}I-Tyr^{10}]VIP specifically bound to the arterial preparation in the presence of nonradioactive peptide, compared with that bound in the absence of nonradioactive peptide. Each point is the mean of at least three experiments performed in duplicate. Standard error bars are omitted for clarity. The standard error was below 15% of mean values where percentage of specific binding was greater than 20%.

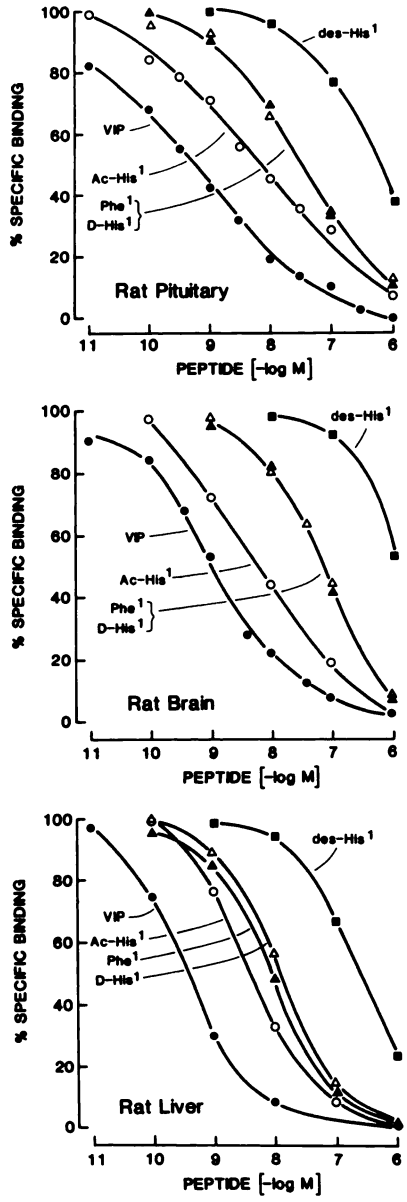

Fig. 2. Competition for binding of [^{125}I-Tyr^{10}]VIP to preparations of rat anterior pituitary, rat brain synaptosomes, and rat liver by VIP analogs modified at amino acid residue 1. Data presentation is similar to that in Fig. 1, for VIP (●), [$N\alpha$-Ac-His^1]VIP (○), [Phe^1]VIP (▲), [D-His^1]VIP (△), and des-His^1-VIP (■). Each point is the mean of at least three experiments performed in duplicate. Standard error bars are omitted for clarity. The standard error was below 15% of mean values where percentage of specific binding was greater than 20%

in Table 2. In general, the competitive binding curves using rat and bovine arteries were more similar between the two species than between arteries and any of the other tissues, including those from the rat. The rank order of potencies of VIP peptides containing modifications at amino acid residue 1 for the rat and bovine vascular VIP receptors was VIP > [$N\alpha$-Ac-His^1]VIP > [Phe^1]VIP > [D-His^1]VIP > des-His^1-VIP. In rat pituitary, brain, and liver preparations, the rank order was only slightly different, VIP > [$N\alpha$-Ac-His^1]VIP > [Phe^1]VIP = [D-His^1]VIP > des-His^1-VIP. Certain differences in the relative potencies of selected analogs were notable. [$N\alpha$-Ac-His^1]VIP was more potent in arterial than in other tissues. [Phe^1]VIP showed more than 4-fold greater potency compared with [D-His^1]VIP in the arterial preparations, whereas the two analogs had similar potency in the nonvascular tissues. The VIP analogs modified in positions 2 to 4 were more tissue selective in receptor binding. The rank order for the vascular tissues was VIP > [D-Ser^2]VIP = [D-Asp^3]VIP > [D-Arg^2]VIP = [D-Phe^2]VIP ≧ [D-Ala^4]VIP. In the case of the rat pituitary and brain, the order was VIP > [D-Ala^4]VIP = [D-Asp^3]VIP > [D-Ser^2]VIP > [D-Arg^2]VIP > [D-Phe^2]VIP. In rat liver, the order was VIP > [D-Ala^4]VIP > [D-Asp^3]VIP > [D-Ser^2]VIP > [D-Phe^2]VIP > [D-Arg^2]VIP. Relative potency differences were greatest between [D-Ser^2]VIP and [D-Ala^4]VIP. In arterial preparations [D-Ser^2]VIP was 12 to 14 times more potent than [D-Ala^4]VIP, whereas in pituitary, brain, and liver tissue [D-Ala^4]VIP was 1.8 to 7 times more potent than [D-Ser^2]VIP. Comparison of the relative potencies between [D-Phe^2]VIP and [D-Arg^2]VIP showed little difference among arteries, pituitary, and brain. However, in the liver preparation [D-Phe^2]VIP was 7-fold more potent than [D-Arg^2]VIP.

The following peptides did not demonstrate tissue selectivity in competitive binding to the five tissue preparations: [D-Asp^3]VIP, des-His^1-VIP, [4Cl-D-Phe^6,Leu^{17}]VIP, and [N-Ac-Tyr^1,D-Phe^2]GRF(1–29)-NH$_2$. Deletion of the His^1 residue from VIP resulted in a reduction of binding potency to less than 1/400th that of intact VIP in all tissues studied (Figs. 1 and 2). The

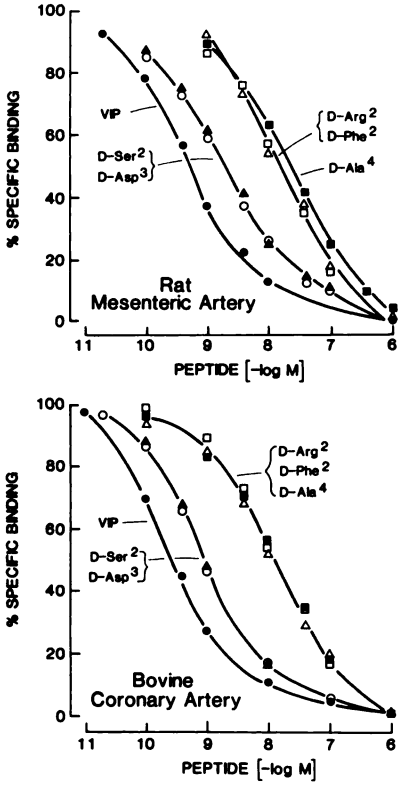

Fig. 3. Competition for binding of [^{125}I-Tyr^{10}]VIP to preparations of rat mesenteric arteries and bovine coronary arteries by VIP analogs modified at amino acid residues 2 to 4. Data presentation is similar to that in Fig. 1, for VIP (●), [D-Ser^2]VIP (○), [D-Asp^3]VIP (▲), [D-Arg^2]VIP (△), [D-Ala^4]VIP (■), and [D-Phe^2]VIP (□). Each point is the mean of at least three experiments performed in duplicate. Standard error bars are omitted for clarity. The standard error was below 15% of mean values where percentage specific binding was greater than 20%.

following fragments from the carboxyl half of VIP, tested at 1 μM concentration in two experiments, did not compete for binding of [^{125}I-Tyr^{10}]VIP to rat mesenteric artery receptors: VIP(14–28), VIP(15–28), VIP(20–28), and VIP(21–28). Because of their lack of binding to the vascular receptor and their weak or absent effect in other published experimental preparations (11, 28, 29), they were not investigated further in this study. Both VIP receptor antagonists competed weakly for binding of [^{125}I-Tyr^{10}]VIP but were not tissue selective in the preparations tested (Fig. 5). Apamin competed either weakly or not at all for VIP binding to the five tissue preparations. The maximal effect of 1 μM apamin was a reduction of specific binding of [^{125}I-Tyr^{10}]VIP to rat pituitary receptors of only 14%.

Discussion

The principal conclusion of this study is that the different concentration-response relationships for receptor binding of N-terminally modified VIP analogs support a hypothesis of heterogeneity of VIP receptors among different tissues in the same species. Comparison of the vascular VIP receptors in two species, rat and bovine, revealed considerable similarity in the structure-activity relationships for analogs modified at amino acid positions 1 and 2 to 4. However, comparison of the vascular with pituitary, brain, and liver receptors showed that the vascular receptor discriminated between [D-His^1]VIP and [Phe^1]

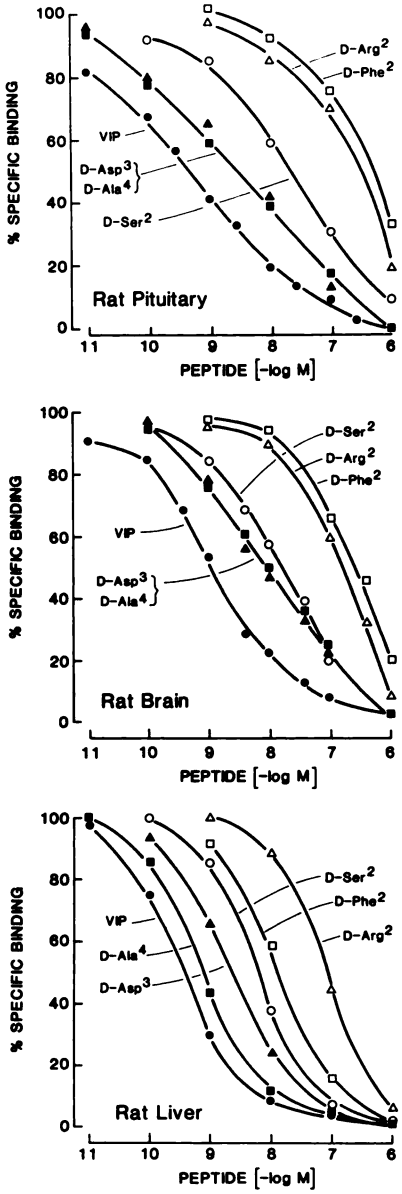

Fig. 4. Competition for binding of [^{125}I-Tyr^{10}]VIP to preparations of rat anterior pituitary, rat brain synaptosomes, and rat liver by VIP analogs modified at amino acid residues 2 to 4. Data presentation is similar to that in Fig. 1, for VIP (●), [D-Ser^2]VIP (○), [D-Asp^3]VIP (▲), [D-Arg^2]VIP (△), [D-Ala^4]VIP (■), and [D-Phe^2]VIP (□). Each point is the mean of at least three experiments performed in duplicate. Standard error bars are omitted for clarity. The standard error was below 15% of mean values where percentage of specific binding was greater than 20%

VIP, whereas the receptors in the other tissues did not. The selectivity of the vascular receptor for position 2–4-modified VIP analogs was also apparent with respect to binding of [D-Ala^4]VIP. This analog was among those with lowest affinity for the vascular receptor but among those with the highest affinity in the other tissues. In addition, the vascular receptor did not distinguish between [D-Asp^3]VIP and [D-Ser^2]VIP, whereas the receptors in the other three tissues did. The structure-activity relationships for the liver receptor were distinguishable from those of the pituitary and brain receptors. The liver receptor differentiated between [D-Phe^2]VIP and [D-Arg^2]VIP, in terms of [D-Phe^2]VIP being more potent than [D-Arg^2]VIP in liver but slightly less potent in brain and pituitary. The liver receptor

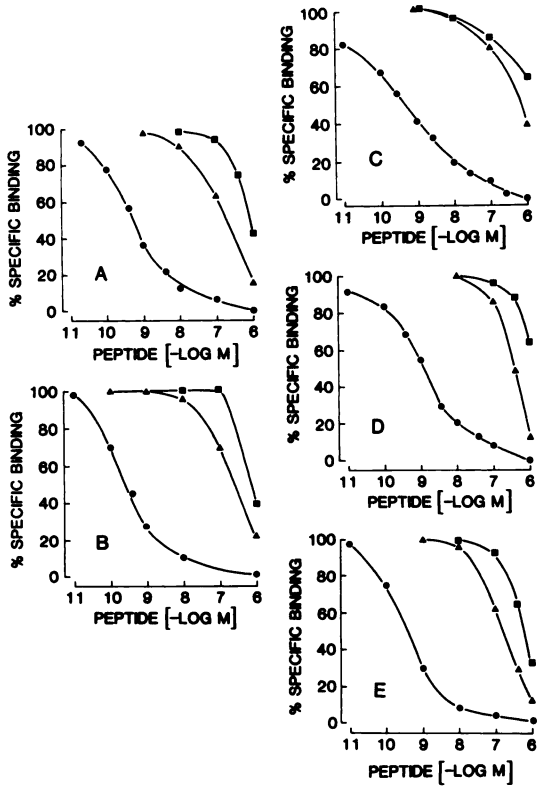

Fig. 5. Competition for binding of [^{125}I-Tyr^{10}]VIP to preparations of rat mesenteric artery (A), bovine coronary artery (B), rat anterior pituitary (C), rat brain synaptosomes (D), and rat liver (E). Data presentation is similar to that in Fig. 1, for VIP (●), [4Cl-D-Phe^6-Leu^{17}]VIP (▲), and [*N*-Ac-Tyr^1,D-Phe^2]GRF(1–29)-NH_2 (■). Each point is the mean of at least three experiments in the case of rat mesenteric artery, brain,and pituitary preparations and two experiments in the case of bovine coronary artery and rat liver preparations, all performed in duplicate. The standard error was below 15% of mean values where percentage of specific binding was greater than 20%.

also distinguished between [D-Ala^4]VIP and [D-Asp^3]VIP, in contrast to the pituitary and brain, which did not. The competitive binding curves for rat pituitary and brain were comparable for all analogs tested. Selective metabolism of the analogs cannot account for the difference in binding observed between tissues, because degradation of radiolabeled VIP and four selected analogs was minimal when measured directly.

The pharmacological properties of the vascular VIP receptor have been described using bovine, porcine, and rat arteries and cultured smooth muscle cells from rat aorta (16, 30–33). Characteristics that are similar to those of VIP receptors in many other tissues include the existence of both high and low affinity binding sites in the same tissue and coupling to adenylate cyclase (16, 30–33). Also typically for VIP receptors, the high affinity binding of VIP to vasculature is decreased by guanosine triphosphate analogs or dithiothreitol reduction of disulfide bonds (34). The VIP receptor in rat mesenteric artery and bovine cerebral and coronary arteries recognizes several native homologs of VIP, such as the PHI and GRF peptides (16, 32). The rank orders of potency for the binding of these peptides to rat and bovine vascular receptors are similar, with the exception of a small between-species difference in terms of the GRF peptides being slightly more potent at rat than at bovine receptors (16). Rat GRF is more potent than either bovine or human GRF at the vascular VIP receptor, probably because of the presence of an N-terminal histidine residue in rat GRF, compared with a tyrosine residue in bovine and human GRF.

Integrity of the N-terminal region of VIP is critical for VIP binding to its receptor, as observed in this and other studies (5–7, 9). The potency of His^1-modified analogs in the present study generally corresponded inversely to the extent of perturbation, specifically, acetylation of His^1, substitution with a bulky hydrophobic phenylalanine residue, perturbation of the conformation at His^1 by substitution of D-His^1, and finally deletion of His^1. Substitution of the naturally occurring residues at positions 2 and 3 with the D-isomers, to generate [D-Ser^2]VIP and [D-Asp^3]VIP, had less effect on potency than did the same modifications in [D-His^1]VIP and [D-Ala^4]VIP. Substitution of Ser^2 with a bulkier Phe residue or a positively charged Arg residue resulted in analogs with low affinity. Interestingly, [D-Ala^4]VIP had much lower potency at vascular than at other receptors. VIP has been proposed to have a β turn as part of the conformation of its N-terminal tetrapeptide (35). The vascular VIP receptor may be more sensitive to the perturbation of this structure in [D-Ala^4]VIP than are the receptors in the other tissues. The amino terminal fragment VIP(1–12) has no

TABLE 2

Comparisons of competitive binding of VIP and GRF analogs to VIP receptors on rat and bovine tissues

The experimental conditions are described in Experimental Procedures and the legends to Figs. 1 to 5. The IC_{50} is the concentration of peptide that reduced maximal specific binding of [^{125}I-Tyr^{10}]VIP by 50%. The IC_{50} values were derived from the competitive binding curves plotted using the mean data points in Figs. 1 to 5. The number of experiments and the standard error values are given in the figure legends. Potency is the ratio of IC_{50} for an analog to the IC_{50} of VIP multiplied by 100.

Peptides	Bovine coronary artery		Rat mesenteric artery		Rat pituitary		Rat brain		Rat liver	
	IC_{50}	Potency	IC_{50}	Potency	IC_{50}	Potency	IC_{50}	Potency	IC_{50}	Potency
	nM		*nM*		*nM*		*nM*		*nM*	
VIP	0.25	100	0.55	100	0.56	100	1.2	100	0.37	100
[*N*-α-Ac-His^1]VIP	0.47	53	0.78	71	6.6	8.5	6.1	20	3.8	9.7
[Phe^1]VIP	5.5	4.5	5.8	9.5	31	1.8	72	1.6	8.9	4.2
[D-His^1]VIP	26	0.96	48	1.1	31	1.8	72	1.6	12	3.0
des-His^1-VIP	160	0.16	250	0.22	480	0.12	>1000	<0.12	280	0.13
[D-Ser^2]VIP	0.91	27	1.8	31	20	2.8	18	6.7	6.3	5.9
[D-Phe^2]VIP	13	1.9	16	3.4	480	0.12	280	0.42	12	3.0
[D-Arg^2]VIP	13	1.9	16	3.4	350	0.16	180	0.67	85	0.43
[D-Asp^3]VIP	0.91	27	1.8	31	3.7	15	10	12	2.4	15
[D-Ala^4]VIP	13	1.9	22	2.5	3.7	15	10	12	0.83	45
[4-Cl-D-Phe^6, Leu^{17}]VIP	213	0.12	141	0.39	480	0.12	390	0.26	162	0.22
[*N*-Ac-Tyr^1,D-Phe^2]GRF (1–29)-NH_2	740	0.03	890	0.06	>1000	<0.06	>1000	<0.12	790	0.05

vasorelaxant activity (30). Secretin, which has considerable amino terminal homology with VIP, notably does not bind to the vascular VIP receptor (16, 32) or activate vascular adenylate cyclase (31). Carboxyl terminal fragments of VIP shorter than des-His^1-VIP bind to vascular receptors either very weakly, in the case of VIP(10–28) tested previously (16), or not at all at 1 μM concentrations, in the case of VIP(14–28), VIP(15–28), VIP(20–28), and VIP(21–28) in the present study. In agreement, VIP(10–28) did not activate vascular adenylate cyclase (30) or relax cerebral arteries (31) and the other VIP fragments were inactive in bioassays of rat blood pressure and rabbit coronary artery perfusion pressure (11). The effect of modifications to the interior of the VIP molecule on vascular activity has been tested using an *in vitro* arterial relaxation bioassay (30). The derivatives [I-Tyr^{10}]VIP , [I-Tyr^{22}]VIP, [O-Met^{17}]VIP, and [O_2-Met^{17}]VIP retained full vasorelaxant activity, whereas [I-Tyr^{10},O-Met^{17}]VIP, and [I-Tyr^{22},O-Met^{17}]VIP had 77% and 42% the effect of VIP (20, 30). Previous detailed structure-activity studies using rat brain synaptosomes and VIP fragments have emphasized the importance of the integrity of the entire VIP molecule for receptor binding (9).

Experimental conditions for radioligand binding to the different tissues were maintained as close to identical as possible in our study. In the case of the pituitary receptor, it was necessary to deviate slightly from standard conditions by using 31° instead of 21° and 60-min instead of 30-min incubation in order to achieve satisfactory specific binding. However, the results obtained comparing rat pituitary and brain receptor binding were similar and, moreover, agreed with previous reports of activation of adenylate cyclase in rat pituitary and brain using the same analogs as used herein (4, 5). Likewise, our experiments on the structure-activity relationships of the rat liver VIP receptor yielded results comparable to studies by Robberecht *et al.* (4, 5) utilizing assays of adenylate cyclase activation and radioligand binding. Notably, the experimental methods for radioligand binding to the rat liver receptor in the present study and that of Robberecht *et al.* (5) differed in radioligand preparation and details of the binding assay. The concordance of results in spite of differences in experimental conditions argues that the small differences in methodology in the present study should not influence our findings comparing the pituitary and other VIP receptors.

The existence of a specific VIP class of receptor distinct from receptors recognizing other related peptides, such as secretin, is generally accepted (2, 3). Heterogeneity of VIP receptor binding is frequently demonstrable within a particular tissue membrane preparation, represented by two binding sites of high and low affinity (Table 1) (16, 19, 23). The question of heterogeneity of VIP receptors between different tissues has proven more difficult to resolve. One line of evidence for VIP receptor heterogeneity between tissues has been comparison of the potency and efficacy for adenylate cyclase activation of naturally occurring homologous peptides and structurally modified VIP analogs, using a variety of rat tissues (4–7). Direct determinations of receptor-analog affinities by radioligand methods have shown similar rank orders for N-terminally modified VIP analogs in rat liver and pancreatic membranes (5, 6). The rank order of potencies of N-terminally modified analogs for binding to human lung membranes resembles the present findings more closely for rat liver, brain, and pituitary than for the vasculature (7). Between-species heterogeneity of VIP receptors has been demonstrated in analog binding and adenylate cyclase studies using rat and human intestinal membranes (28, 36, 37). These and the present study utilizing VIP analogs to probe structure-activity relationships cannot distinguish between the two possibilities that, first, the VIP receptors are different molecular entities, perhaps including different degrees of glycosylation (10), or, second, the membrane environment in which a common receptor resides exerts an influence on its recognition properties. Affinity labeling experiments have identified heterogeneity in the molecular size of VIP receptors found in rat liver, lung, brain, intestine, pancreas, and clonal pituitary tumor cells (3, 10). However, further molecular characterization of VIP receptors is needed to elucidate the question of VIP receptor subclasses.

The vasculature represents a promising model for the study of VIP receptor heterogeneity between tissues. In addition to its selective recognition properties, the vasculature has the advantage of not containing cross-reactive receptors for other homologous peptides such as secretin, glucagon, or PHI (16, 32, 38). Unlike VIP receptors in other tissues, the vascular VIP receptor does not recognize secretin (16, 30–32), which is an additional observation in favor of the hypothesis of VIP receptor heterogeneity. Also, in other VIP-responsive tissues the presence of receptors for homologous peptides such as secretin presents the challenges of overlapping functions and properties. The selectivity of the vascular VIP receptor, compared with its counterpart in other tissues, also has potential relevance for selective pharmacological actions of VIP analogs on the vasculature.

Acknowledgments

We thank Ms. Lisa Potapoff for preparing the manuscript.

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