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CHARACTERIZATION OF VASOACTIVE INTESTINAL PEPTIDE RECEPTORS IN CANINE LIVER MEMBRANES

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Abstract—The binding characteristics of vasoactive intestinal peptide (VIP) in the liver membranes of the dog were examined using radioligand binding assay with ^{125}I-VIP and unlabelled peptides and results were compared with those from the rat. The binding of VIP to canine liver membranes occurred in a reversible, saturable, specific and temperature-dependent manner. Guanine nucleotides dose-dependently inhibited VIP binding. The order of potency in competition experiments with unlabelled peptide was: VIP > pituitary adenylate cyclase activating peptide (PACAP)-27 > PACAP-38 ⩾ peptide histidine isoleucine (PHI) = secretin in the dog, and PACAP-27 > PACAP-38 > VIP > PHI > secretin in the rat. PHI and secretin were about 5000 times less potent than VIP in the dog, but secretin was about 100 times less potent than VIP in the rat. The VIP binding sites in canine liver membranes have recognition sites for VIP which differ from those in rat liver membranes. As most of VIP in the portal vein was removed during its passage through the canine liver, the binding sites of canine liver may play a role in degradation of VIP.

Key words: competitive binding experiments, pituitary adenylate cyclase activating peptide, peptide histidine isoleucine, secretin, guanine nucleotides

Vasoactive intestinal peptide (VIP†) is an octacosapeptide isolated from the porcine upper small intestine, which consists of an amino acid sequence similar to those of secretin and glucagon [1]. It is generally accepted that VIP exerts widespread effects, including stimulation of hormone secretion and smooth muscle relaxation in various tissues via interaction of the peptide with specific receptors. Several peptides structurally related to VIP, such as secretin, peptide histidine isoleucine (PHI), growth hormone releasing factor [2] and helodermin [3], have been shown to bind to VIP receptors. Recently, pituitary adenylate cyclase activating polypeptides (PACAP) with 38 and 27 amino acid residues isolated from ovine hypothalamic tissues [4, 5] were also found to bind to VIP receptors in rat liver membranes [6].

In epithelial membranes of the intestinal tract, the affinity of PHI for human VIP receptors is three orders of magnitude lower than that of VIP but five-fold lower for rat VIP receptors [7]. Moreover, the affinities of secretin and PHI for VIP receptors in pancreatic membranes are much less potent in the guinea-pig than in the rat [8]. Therefore, the relative affinity of VIP analogues for VIP receptors seems to vary depending on the animal species and type of tissues [9]. Binding sites for VIP present in rat liver membrane have been well characterized with competitive binding experiments using the related peptides described above [6, 10–12]. However, the physiological role in receptors of the liver remained obscure. It has been proposed that receptor binding mediates degradation of VIP in pig liver [13].

We therefore compared the binding characteristics of VIP receptors in liver membranes of the dog and rat by competitive radioligand binding experiments and then measured plasma VIP concentrations in the hepatic and portal vein to examine the removal of VIP by the liver.

MATERIALS AND METHODS

Preparation of hepatic membranes. Male Wistar rats (160–180 g) were stunned and killed by bleeding. Mongrel dogs (8–12 kg) were anaesthetized with pentobarbitone (30 mg/kg) and killed by bleeding. The livers isolated from the dogs and rats were homogenized at 4° using an electric blender in 10-fold amounts of 20 mM Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose, 2 mM EDTA and 2 mM $MgCl_2$. The suspension was filtered through two layers of medical gauze. The homogenate was centrifuged for 10 min at 2500 *g*. The resulting supernatant was centrifuged for 20 min at 20,000 *g* and the pellet was recentrifuged after resuspension. The final pellet was suspended in the 20 mM Tris–HCl buffer (pH 7.4) as a membrane suspension of which (about 3 mg/mL) aliquots were stored at −80° until used. Protein concentration was determined by the modified Lowry method [14].

Binding of ^{125}I-VIP to hepatic membranes. VIP

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† Abbreviations: VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase activating peptide; PHI, peptide histidine isoleucine; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); GDPβS, guanosine 5′-*O*-(2-thiodiphosphate).

was radioiodinated by the chloramine T method and purified as described previously [15, 16]. The specific activity of ^{125}I-VIP was 23–42 TBq/mmol. All binding studies were carried out in 20 mM Tris–HCl buffer (pH 7.4) containing 2 mM $MgCl_2$, 0.05 mM phenylmethyl sulphonyl fluoride, 0.25 mg/mL bacitracin and 10 mg/mL bovine serum albumin. ^{125}I-VIP and unlabelled peptide were added to the incubation buffer, of which the final volume was 0.25 mL. Non-specific binding of ^{125}I-VIP was determined by adding 1 μM VIP. Incubation times at 17° were 30 and 45 min in the dog and rat hepatic membranes, respectively, to allow equilibrium binding. The termination of the incubation was made by rapid filtration on a GF/B glass–fibre filter (Whatman), pre-soaked with 0.1% polyethylenimine. The filter was washed twice with 20 mM Tris–HCl buffer (pH 7.4) and counted with an auto well γ-counter (Aloka, Japan).

Radioimmunoassay of VIP in plasma. The experiments were carried out on six mongrel dogs weighing 8–12 kg after overnight fast. Dogs were anaesthetized with α-chloralose (50 mg/kg) and urethane (100 mg/kg) after induction with pentobarbitone (30 mg/kg). The abdominal cavity was opened through a midline incision, and the blood was collected from the hepatic vein, hepatic artery and portal vein with syringes containing heparin and put into plastic tubes containing aprotinin (500 U), bacitracine (0.5 mg), protamine (0.5 mg) and EDTA (12.5 μmol) on ice. The blood collected was centrifuged for 10 min at 3000 rpm at 4° and the plasma was stored at −20° until assayed. The concentrations of VIP in the venous and arterial plasma were determined by radioimmunoassay as described previously [15, 16].

Materials. VIP and secretin were obtained from Protein Inc. (Osaka, Japan). PHI was purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). PACAP-27 and PACAP-38 were the gift of Dr C. Kitada (Tsukuba Research Labs, Takeda Chemical Industries Ltd, Japan). Adenine nucleotides, guanine nucleotides and related compounds were all obtained from Boehringer (Mannheim, Germany). All other reagents used were of the highest grade available.

RESULTS

^{125}I-*VIP binding to canine liver membranes*

The effect of temperature on the specific binding of ^{125}I-VIP to canine liver membrane was examined at 4°, 17° and 30°. At 17°, the binding equilibrium was achieved within 5 min and was stable from 5 to 30 min (Fig. 1A). Although ^{125}I-VIP binding occurred at 30°, a level comparable to that observed at 17°, it decreased with incubation time, probably because of enzymatic degradation of the peptide. Binding of ^{125}I-VIP also occurred at 4°, but only at a level half that at 17°. All following experiments, therefore, were performed at 17°, at which the binding of ^{125}I-VIP was stable for at least 1 hr. ^{125}I-VIP bound to canine liver membranes reversibly. Unlabelled VIP dissociated labelled VIP with a half-time of about 5 min. Half of the total binding corresponded to specific binding to liver membranes. The addition of

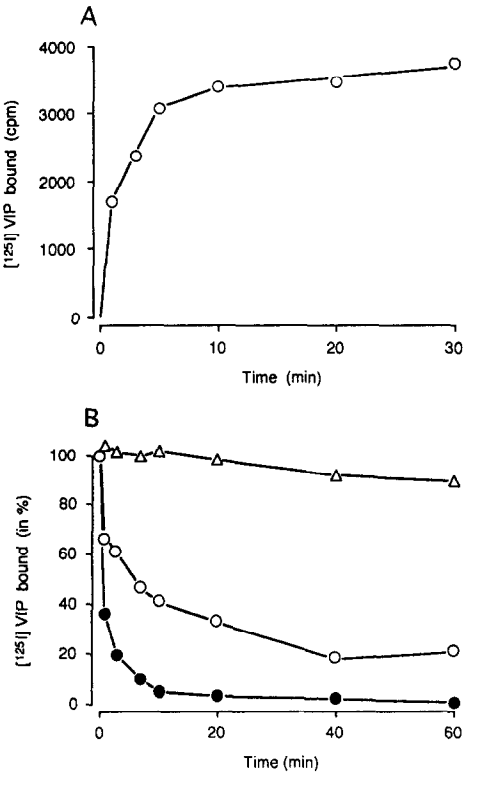

Fig. 1. Time course of ^{125}I-VIP specific binding (A) to and dissociation rate (B) from canine liver membranes. (A) Membranes (20 μg protein) were incubated with ^{125}I-VIP at 17°. Non-specific binding was determined in the presence of 1 μM VIP. (B) After a 20-min incubation, dissociation was induced by adding 1 μM VIP in the presence (●) and absence (○) of 10 μM GTP. No dissociation (△). Symbols indicate the means of three experiments done in duplicate.

10 μM GTP decreased the half-time of dissociation to less than 1 min (Fig. 1B).

Saturation curves were obtained by incubating canine and rat liver membranes with increasing concentrations of ^{125}I-VIP. Scatchard analysis of the curves allowed calculation of a K_d value of 0.75 ± 0.09 nM and a binding capacity of 732 ± 79 fmol/mg protein for canine liver membranes (N = 5) and, respectively, 0.13 ± 0.01 nM and 537 ± 31 fmol/mg protein for rat liver membranes (N = 4).

Effects of guanine nucleotides on ^{125}I-*VIP binding*

Since GTP increased the rate of dissociation of ^{125}I-VIP, we examined the effect of guanine nucleotides on VIP binding. The specific binding of ^{125}I-VIP was dose-dependently decreased by guanine nucleotides. As shown in Fig. 2, GTP, GTPγs and GDP at concentrations from 0.1 to 10 μM inhibited the binding of ^{125}I-VIP to canine liver membranes with similar potency. GDPβs and GMP were less effective in inhibiting ^{125}I-VIP binding. On the other hand, ATP did not affect the binding of ^{125}I-VIP to membranes even at concentrations as high as 1 mM.

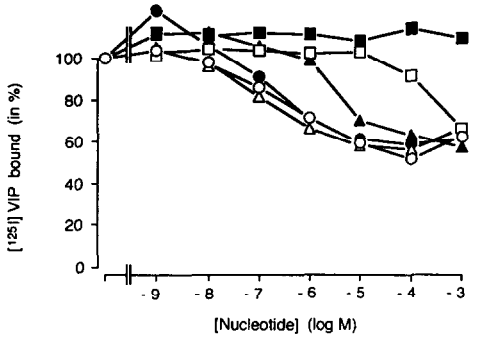

Fig. 2. Effect of guanine nucleotides on ^{125}I-VIP specific binding to canine liver membranes. Membranes (20 μg protein) were incubated with ^{125}I-VIP and increasing concentrations of GTP (○), GTPγs (●), GDP (△), GDPβs (▲), GMP (□) and ATP (■). Values are expressed as the percentage of specific binding in the absence of nucleotides. Symbols indicate the means of three experiments performed in duplicate.

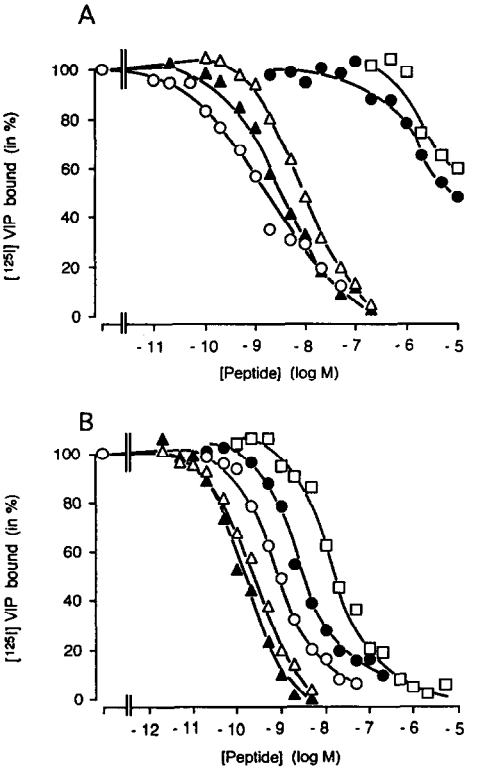

Fig. 3. Competitive inhibition of ^{125}I-VIP specific binding to canine (A) and rat (B) liver membranes by native VIP and structurally related peptides. Membranes (20 μg protein) were incubated with ^{125}I-VIP and increasing concentrations of VIP (○), PHI (●), PACAP-38 (△), PACAP-27 (▲) and secretin (□). Values are expressed as the percentage of specific binding in the absence of unlabelled peptides. Symbols indicate the means of four experiments performed in duplicate.

Specificity for ^{125}I-VIP binding

The specificity of the VIP binding in liver membranes of the dog and rat was studied by determining the ability of various peptides structurally related to VIP to compete with the binding of ^{125}I-VIP. All peptides used inhibited the binding of ^{125}I-VIP (see Fig. 3). The concentrations of unlabelled VIP and its related peptides required for half-maximal dissociation of ^{125}I-VIP binding are shown in Table 1. The order of potency was: VIP > PACAP-27 > PACAP-38 ⩾ PHI = secretin in canine liver membranes and PACAP-27 > PACAP-38 > VIP > PHI > secretin in rat liver membranes. Both PACAP-27 and -38 were more potent than VIP in the rat but vice versa in the dog. PHI and secretin were much less effective in displacing labelled VIP bound to liver membranes of the dog than in the rat.

VIP concentrations in the portal and hepatic vein

To estimate the removal of VIP released from the gastrointestinal tract by the canine liver, we measured VIP concentrations in the portal and hepatic vein and the hepatic artery. There was no difference between plasma VIP concentrations in the hepatic vein (39.2 ± 4.1 pM, N = 5) and artery (44.6 ± 10.4 pM, N = 3). On the other hand, plasma VIP concentration in the portal vein varied between 433 and 69 pM (190.9 ± 72.4 pM, N = 5) and was higher than that in the hepatic vein, indicating that immunoreactive VIP was removed during its passage through the liver. The mean ratio of VIP concentration in the hepatic/portal vein was 0.32 ± 0.08 (N = 5).

DISCUSSION

The present experiments characterized VIP binding sites in canine liver membranes. The interaction of ^{125}I-VIP with binding sites of canine liver membranes was rapid, reversible, saturable and of high affinity and thus fulfilled the criteria for the VIP receptor binding reaction as was the case in other VIP-binding systems [17]. There appears to be two classes of VIP binding sites in rat liver membranes, one with a K_d of around 0.1 nM and another with a K_d of around the 10 nM range [6, 11, 12]. The K_d values of ^{125}I-VIP binding to liver membranes in the dog and rat were closely related to high affinity VIP binding sites of corresponding membranes. It seems likely that concentrations of ^{125}I-VIP were too low to detect low affinity binding sites.

The VIP receptor system has been proposed to be modulated by GTP-regulatory proteins [18]. The present experiments also provide evidence for this because ^{125}I-VIP binding was inhibited by guanine nucleotides in a dose-dependent manner but not by ATP. The results suggest that VIP receptors are coupled to some GTP-binding proteins in canine liver membranes as is the case for rat liver membranes [18].

The order of potency estimated from analysis of the inhibition curves of ^{125}I-VIP binding by

Table 1. Comparison of the competitive binding of VIP and structurally related peptide to canine and rat liver membranes

Peptide	Dog		Rat	
	IC_{50}	Potency	IC_{50}	Potency
VIP	1.58 ± 0.27 nM	1.00	0.81 ± 0.16 nM	1.00
PACAP-27	3.30 ± 0.16 nM	0.48	0.13 ± 0.01 nM	6.23
PACAP-38	9.53 ± 2.56 nM	0.17	0.26 ± 0.02 nM	3.12
PHI	8.98 ± 2.74 μM	0.0002	2.23 ± 0.52 nM	0.36
Secretin	10.48 ± 3.12 μM	0.0002	37.13 ± 2.18 nM	0.02

The IC_{50} values (mean ± SEM) were derived from the competitive binding curves shown in Fig. 4. Potency is the ratio of IC_{50} value for the peptide to that for VIP.

unlabelled VIP and its related peptides was: VIP > PHI > secretin in canine and rat liver membranes, as has been shown in other tissues [19–21], with the exception of the submandibular gland [22]. Although the IC_{50} value for PHI was slightly higher than that for VIP in the liver membranes of the rat, PHI and secretin were 5000 times less effective in displacing ^{125}I-VIP bound to canine liver membranes than VIP. It is unlikely that this difference in affinity is due to species differences in VIP receptors between the dog and rat, because secretin has been shown to be only 100 times less effective than VIP in the small intestinal mucosa of the dog [21]. It seems therefore likely that VIP receptors located in canine liver membranes are unique in binding peptides structually related to VIP. PACAP-27 has been shown to bind to VIP receptors with higher affinity than VIP itself in rat liver membranes [6]. This was confirmed by the present experiments. However, both PACAP-27 and PACAP-38 were less potent in inhibiting ^{125}I-VIP binding than VIP in canine liver membranes. It therefore appears that the high affinity binding sites for VIP in canine liver membranes distinguish PACAP from VIP much more precisely than those in the rat.

The physiological role of VIP binding sites of the liver is still open to question. In the rat liver, activation of adenylate cyclase induced by VIP has been shown to be less potent than that induced by glucagon [23]. Although VIP stimulates adenylate cyclase at low concentrations comparable to K_d values of high affinity binding sites [10, 24], much higher concentrations are required for glycogenolysis in the liver [25, 26]. Plasma VIP concentration in the portal vein seems to be too low to elicit metabolic effects in the liver. It has been proposed that receptor binding mediates degradation of VIP by pig liver [13]. Most of the immunoreactive VIP released from the gastrointestinal tract is removed during its passage through the liver and thus VIP binding sites in canine liver may play a role in the removal of VIP released from the gastrointestinal tract.

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