Interaction of Gila monster venom with VIP receptors in intestinal epithelium of human

A comparison with rat

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Gila monster venom (1–300 μg/ml) is shown to inhibit completely the binding of [^{125}I]VIP to human and rat intestinal epithelial cell membranes. In both models, the venom inhibits [^{125}I]VIP binding and stimulates adenylate cyclase with a maximal efficiency that is similar to that of VIP and a potency that is 10000–50000 times lower than that of the peptide, on a weight basis. At maximal doses, VIP and Gila monster venom do not exert an additive effect on adenylate cyclase, suggesting that the activation of the enzyme by the venom occurs through VIP receptors. As is the case for VIP, adenylate cyclase activation by Gila monster venom requires the presence of GTP in the incubation medium. Finally, no VIP-like immunoreactivity was detected in the venom using an antiserum raised against mammalian VIP. All these data suggest the presence in the venom of the Gila monster, of a new substance which behaves as a VIP agonist in human as well as rat intestine.

Gila monster venom *VIP receptor* *Intestinal epithelial membrane* *Adenylate cyclase*

1. INTRODUCTION

Many peptides have been discovered and/or detected in non-mammalian species and found to be active in mammalian biological systems [1]. This includes the pancreatic and/or intestinal secretagogues present in amphibian skin [2] or in scorpion venom [3,4]. In a recent report, the venom of Gila monster (family Helodermatidae) was shown to stimulate amylase secretion and cyclic AMP production in guinea pig pancreatic acini [5]. This action was ascribed to the interaction of a peptide present in the venom with cell surface receptors for vasoactive intestinal peptide and/or secretin. However, as the two types of receptors are present in guinea pig pancreatic acinar cells, the site of the venom action remains uncertain. We here use intestinal epithelial cells, a system that contains VIP but not secretin receptors [6], to clearly demonstrate that Gila monster venom stimulates adenylate cyclase activity through its interaction with VIP receptors. The study was performed by using both human colonic epithelial crypt membranes and rat small intestinal epithelial membranes, since a great species specificity of VIP receptors in discriminating among the natural VIP agonists was previously demonstrated [7,8].

Abbreviation: VIP, vasoactive intestinal peptide

2. MATERIALS AND METHODS

Gila monster venoms (*Heloderma horridum* and *Heloderma suspectum*) were obtained from Sigma (St Louis, MO). VIP was purified from porcine duodenum [9]. EGF was a kind gift of Novo Research Institute (Copenhagen). Monocompetent

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pork insulin was purchased from Novo. Membranes were prepared as in [10] from isolated rat small intestinal epithelial cells [11] or isolated human colonic epithelial crypts [12]. [^{125}I]VIP [13], [^{125}I]EGF [14] and [^{125}I]insulin [15] were prepared by the chloramine T method at a specific activity of 250 Ci/g, 160 Ci/g and 200 Ci/g, respectively. Studies of binding of [^{125}I]VIP to membranes were conducted as in [16]. Binding of [^{125}I]EGF and [^{125}I]insulin were studied as in [14,15]. Adenylate cyclase was assayed as in [10]. The radioimmunoassay of VIP was conducted as in [17].

3. RESULTS

The Gila monster venom (*H. horridum* and *H. suspectum*) inhibits the specific binding of [^{125}I]VIP to epithelial cell membranes prepared from human colon (fig.1, bottom). The dose-response of venom (1–300 μg/ml concentration range; ED_{50} = 20 μg/ml) parallels that of VIP, suggesting a competitive inhibition of labelled VIP binding by a substance present in the venom. At maximal venom concentration, the binding of [^{125}I]VIP to human colonic membranes is completely suppressed. Fig.1 (top) shows that the venom inhibits also the binding of [^{125}I]VIP to rat intestinal membranes with an ED_{50} of 10 μg/ml similar to that observed in human intestine. On a weight basis, 10000–50000 times as much Gila monster venom (from both sources) as pure porcine VIP are thus needed to achieve an identical effect.

Rat intestinal epithelial cells were previously shown to exhibit specific insulin and epidermal growth factor receptors besides VIP receptors [14,18]. Gila monster venom (1–300 μg/ml) does not alter the specific binding of [^{125}I]insulin nor [^{125}I]EGF to their respective receptors (not shown), indicating the specificity of the venom action on VIP receptors.

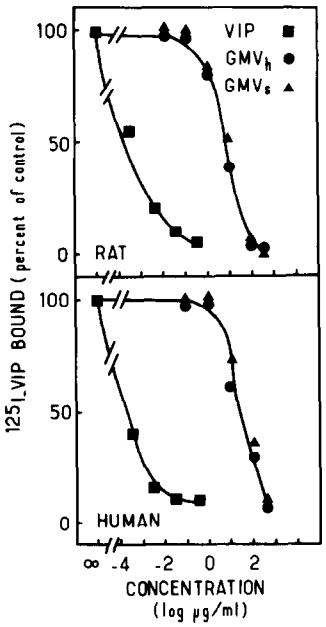

Fig.1. Competitive inhibition of specific [^{125}I]VIP binding to human colonic epithelial crypt membranes (bottom) and rat small intestinal epithelial membranes (top) by VIP, Gila monster venom from *H. horridum* (GMVh) and from *H. suspectum* (GMVs). Results are expressed as the percentage of radioactivity specifically bound in the absence of addition. Each point is the mean of triplicate determinations. Two other experiments gave similar results.

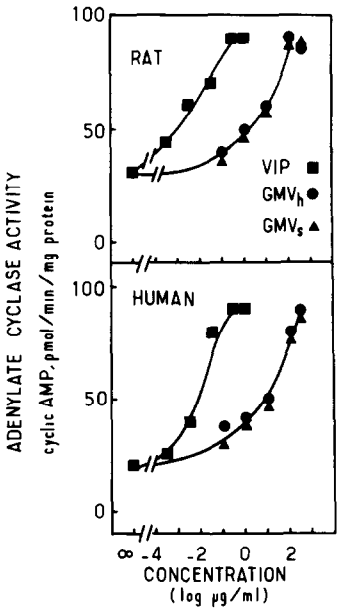

Fig.2. Adenylate cyclase activity in human colonic epithelial crypt membranes (bottom) and rat small intestinal epithelial membranes (top) in response to increasing concentrations of VIP, Gila monster venom from *H. horridum* (GMVh) and from *H. suspectum* (GMVs). Each point is the mean of triplicate determinations. Two other experiments gave similar results.

Table 1

Adenylate cyclase activity in human intestinal epithelial cell membranes

Addition	Adenylate cyclase activity cAMP pmol · min^{-1} · mg protein^{-1}
0	17 ± 1
Gila monster venom (100 μg/ml)	95 ± 3
VIP (10^{-7} M)	114 ± 10
Gila monster venom (100 μg/ml) + VIP (10^{-7} M)	119 ± 5

Membranes are incubated as in section 2. Results are the mean ± SEM of triplicate determinations. Another experiment gave similar results

As shown in fig.2, the Gila monster venom from both sources is able to stimulate adenylate cyclase activity in human (bottom) and rat (top) intestinal membranes. In both species, the dose-responses of Gila monster venom in stimulating the enzyme are similar with an ED_{50} of 20 μg/ml. Likewise, the Gila monster venom and VIP act with the same efficiency, in rat and human tissue. The dose-responses of Gila monster venom in inhibiting [^{125}I]VIP binding and in stimulating adenylate cyclase activity in intestinal membranes are very similar suggesting that a substance present in the venom acts on the enzyme through its interaction with VIP receptors. This is further supported by the fact that:

(i) Gila monster venom like VIP [19] requires the presence of GTP to stimulate intestinal cyclase activity (not shown);

(ii) Gila monster venom and VIP, tested at maximally active doses do not elicit an additive stimulatory effect on cyclase activity (table 1). Such data strongly suggest that Gila monster venom contains a VIP-like bioactive substance which behaves as a VIP agonist. However, as tested using an antibody raised against mammalian VIP, the venom is devoid of VIP-like immunoreactivity (fig.3).

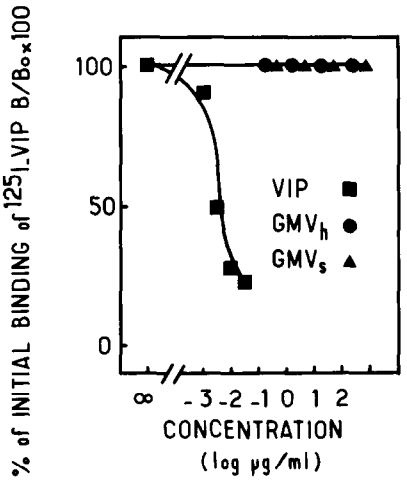

Fig.3. Effect of Gila monster venom from *H. horridum* (GMVh) and from *H. suspectum* (GMVs) on the binding of [^{125}I]VIP to an antibody raised against mammalian VIP as in [17]. Results are expressed as the percentage of initial binding of [^{125}I]VIP to the antibody. Each point is the mean of triplicate determinations. Another experiment gave similar results.

4. DISCUSSION

The present data indicate that the venom from *H. horridum* and *H. suspectum* contains a substance that interacts specifically with VIP receptors in human and rat intestinal epithelium. This substance appears to be a full agonist of VIP since it is as efficient as VIP in inhibiting binding of [^{125}I]VIP to VIP receptors and in stimulating adenylate cyclase. Since the venom was shown to contain some degradative activities such as phospholipase A_2 and proteolytic activities [20], the question whether it interacts with VIP receptors by destroying them has to be evoked. Our data, indicating that the venom does not alter the binding of [^{125}I]insulin and [^{125}I]EGF to their specific receptors make such hypothesis very unlikely. The venom thus may contain a VIP-like bioactive substance. Its high sensitivity to trypsin digestion strongly suggests that this substance is of proteic nature [5]. The comparison of the Gila monster venom effect in rat and human intestine supports the conclusion that this substance does not resemble the natural VIP agonists actually characterized including PHI, secretin and growth hormone-releasing factor [8]. Indeed, while rat and human VIP receptors present great species specificity regarding their ability to discriminate between those different peptides [8], Gila monster

venom appears to be recognized similarly by the two receptors. In that respect, the active substance is not yet identified but appears to be a new agonist of VIP. The absence of VIP-like immunoreactivity in the venom indicates that this VIP agonist does not possess the same antigenic determinant(s) as mammalian VIP. Whether it is a lizard VIP different from mammalian VIP, or a new peptide synthesized specifically in the venom sacs remains to be determined. Likewise, whether the interaction of this substance with VIP receptors may participate in the toxicity of the venom is not yet determined. It is clear, however, that some toxic effects of Gila monster venom, i.e., hypotension and reduction of blood flow [21], resemble the well-known actions of VIP [22]. A definite answer to those questions has to wait for the purification and structural analysis of this new VIP-like bioactive substance.

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