Interaction of GRF with VIP receptors and stimulation of adenylate cyclase in rat and human intestinal epithelial membranes

Comparison with PHI and secretin

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GRF (10^{-8}–10^{-5} M) is shown to inhibit competitively the binding of [^{125}I]VIP to human and rat intestinal epithelial membranes. The affinity of GRF for VIP receptor is 700–800-times lower than that of VIP in both species. The order of affinity of different peptides is VIP > PHI > secretin > GRF in rat, and VIP > GRF > PHI > secretin in man. The important species specificity of VIP receptors in recognizing PHI and secretin does not occur in the case of GRF. GRF stimulates adenylate cyclase through its interaction with VIP receptors in rat and human membranes. However, while GRF behaves as a VIP agonist in human tissue, it is a partial agonist/antagonist of VIP in the rat.

VIP receptor *Intestinal epithelial membrane* *Adenylate cyclase* *GRF* *PHI* *Secretin*

1. INTRODUCTION

The growth hormone releasing factor was recently isolated from a human pancreatic islet tumor [1]. The analysis of the sequence of this 44 amino acids peptide revealed close structural similarities with the members of the so-called glucagon-VIP-secretin family [1]. GRF possesses 12, 9, 8, 6 and 5 amino acids in common with the porcine gut peptide, PHI [2], VIP [3], glucagon [4], secretin [5] and gastric inhibitory polypeptide, GIP [6], respectively. Peptides of this structural family exhibit great overlapping biological activities in various tissues [7]. This was shown to be due in some cases to cross-reactivity at the receptor level. In particular, we have shown that secretin and PHI are able to bind to VIP receptors with a low affinity [8].

The sequence homology between GRF and VIP prompted us to investigate the possible interaction of GRF with VIP receptors. Here, we show that GRF binds to VIP receptors in intestinal epithelial membranes and stimulates adenylate cyclase activity. The effect of GRF is compared to that of secretin and PHI. It is analyzed both in rat and human intestine since marked species specificity has been previously noted for VIP receptors [9].

Abbreviations: GRF, growth hormone releasing factor; PHI, porcine intestinal peptide having N-terminal histidine and C-terminal isoleucine amide; VIP, vasoactive intestinal peptide

2. MATERIALS AND METHODS

Synthetic human GRF-44-NH_2 was prepared by

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solid-phase techniques [10] and was shown to have the full biological activity of native GRF [1]. PHI was isolated from porcine duodenum [2]. Synthetic porcine secretion was prepared as in [11]. VIP was purified from porcine duodenum [3]. Membranes were prepared as in [12] from isolated rat small intestinal epithelial cells [13] or isolated human colonic epithelial crypts [14]. [^{125}I]VIP was prepared by the chloramine T method at a spec. act. of 250 Ci/g [15]. It displays the same activity as native VIP in stimulating cyclic AMP accumulation in a cultured cell line (HT-29) which is highly and specifically sensitive to VIP [16]. Studies of binding of [^{125}I]VIP to membranes were conducted as in [17]. Adenylate cyclase was assayed as in [12].

3. RESULTS

GRF inhibits competitively the specific binding of [^{125}I]VIP to epithelial cell membranes prepared from rat small intestine (fig.1, top) and human colon (fig.1, bottom). The inhibition is observed for GRF from 10^{-8}–3×10^{-6} M (fig.1), half-maximal inhibition being obtained at a very similar GRF concentration in the two species; i.e., 280 nM. This contrasts markedly with the very different affinity of the human and rat receptors for two other peptides; i.e., secretin and PHI (fig.1). Indeed, while half-maximal inhibition of [^{125}I]VIP binding is obtained for 4.7 nM PHI and 31 nM secretin in rat, much higher concentrations of peptides are needed to obtain the same effect in man; e.g., 660 and 2200 nM for PHI and secretin, respectively. These observations indicate that the great species specificity of VIP receptors with respect to PHI and secretin recognition does not concern the recognition of GRF. Neither does it concern the recognition of VIP itself since the affinity of VIP receptors for VIP is very similar in human and rat intestine (fig.1).

Fig.2 shows the dose–response of GRF, PHI, secretin and VIP in stimulating adenylate cyclase activity in rat (top) and human (bottom) intestinal membranes. As far as rat membranes are concerned, the order of potency of the different peptides in stimulating adenylate cyclase (fig.2, top) and in

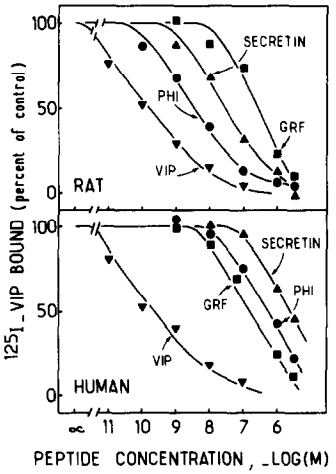

Fig.1. . Competitive inhibition of specific [^{125}I]VIP binding to rat small intestinal epithelial membranes (top) and human colonic epithelial crypt membranes (bottom) by GRF, PHI, secretin and VIP. Results are expressed as the percentage of radioactivity specifically bound in the absence of added unlabeled peptide. Each point is the mean of 3 separate experiments; in each of the experiments determinations were made in quadruplicate. For the sake of clarity, standard errors are not indicated. They are always below 9% of the mean values.

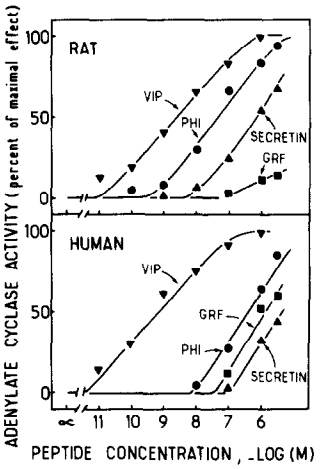

Fig.2. Adenylate cyclase activity in rat small intestinal epithelial membranes (top) and human colonic epithelial crypt membranes (bottom) in response to increasing concentrations of GRF, PHI, secretin and VIP. Each point is the mean of 3 separate experiments; in each of the experiments determinations were made in triplicate. For the sake of clarity, standard errors are not indicated. They are always below 8% of the mean values.

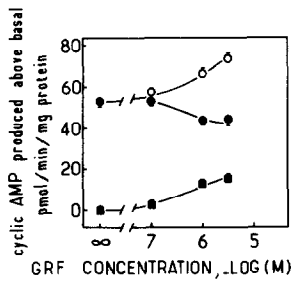

Fig.3. Effect of increasing concentrations of GRF on the VIP-stimulated adenylate cyclase activity in rat small intestinal epithelial membranes. Each point is the mean ± SE of 3 experiments: (■) GRF alone; (●) GRF + VIP (10^{-8} M), experimental values; (○) GRF + VIP (10^{-8} M), values expected if the effects of VIP and GRF were additive.

inhibiting [^{125}I]VIP binding (fig.1, top) is the same; i.e., VIP > PHI > secretin > GRF. However, while PHI and secretin appear to be full agonists of VIP, GRF behaves as a partial agonist with low intrinsic activity (fig.2, top). Indeed, at the maximal concentration tested (3×10^{-6} M), GRF completely inhibits [^{125}I]VIP binding (fig.1, top) but poorly stimulates adenylate cyclase activity (fig.2, top). This observation suggests that GRF may antagonize the VIP effect in rat membranes. Indeed, as shown in fig.3, GRF antagonizes partially and in a dose-dependent fashion the VIP (10^{-8} M)-stimulated adenylate cyclase activity in rat intestinal membranes. In human membranes, GRF stimulates adenylate cyclase activity with a dose–response curve parallel to those observed with VIP, PHI and secretin (fig.2, bottom). However, it must be noticed that the orders of potency of the different peptides in stimulating adenylate cyclase activity (VIP > PHI > GRF > secretin) and in inhibiting the binding of [^{125}I]VIP (VIP > GRF > PHI > secretin) are slightly different.

4. DISCUSSION

Here, we demonstrate that GRF is able to bind with a low affinity to VIP receptors in human and rat intestinal membranes. The concentrations of GRF needed to interact with VIP receptors and to stimulate adenylate cyclase through VIP receptors are high (10^{-8}–10^{-5} M) in both species. This makes it very unlikely that GRF may elicit some physiological response through its interaction with VIP receptors. Indeed specific GRF actions as the stimulation of growth hormone release in vitro are observed for very low doses of peptide between 10^{-12}–10^{-9} M [1]. Nevertheless, the interaction of GRF with VIP receptors provides new features to analyze the structure–function relationship of peptides of the VIP-secretin family. It is clear, both from these results and from those [18,19] and other laboratories [20] that an important species specificity exists in the ability of VIP receptors to discriminate the different peptides of this structural family. While PHI and secretin have a much higher affinity for VIP receptors in rat than in man, the apparent affinity of GRF for VIP receptors appears to be low but very similar in the two species. This suggests that GRF does not contain the structural domain(s) involved in the determination of species specificity at the recognition site of VIP receptors. Despite its low affinity, GRF is able to completely inhibit the binding of [^{125}I]VIP to human or rat receptors. Previous works have shown the importance of the N-terminal portion of VIP in the binding to receptors [18,21]. As far as this domain is concerned, GRF has 5 amino acids in common with VIP in the 10 first amino acids at the N-terminal while 4 other amino acids are in common in the remainder of the molecule. In particular, the aspartate, phenylalanine and threonine residues, respectively, located in position 3, 6 and 7, are identical in VIP, PHI, secretin and GRF and have been shown to be very important for binding to receptors [18]. Although these amino acids are certainly involved in the recognition of GRF by VIP receptors, the resemblances in the remainder of the peptide sequence cannot be neglected. Indeed, the entire length of the VIP sequence is probably necessary to the process of binding, since VIP fragments have a very low affinity, if any, for the receptors [15]. The presence of a N-terminal tyrosine in GRF instead of a histidine in VIP and related peptides, is very probably a cause of the low affinity of GRF for VIP receptors. Indeed, modifications of this residue are associated with a marked loss of affinity of secretin analogues for VIP receptors [18]. Such modification is even more drastic for the biological activity as shown with secretin analogues [22] and may be responsible for the low intrinsic activity of GRF to stimulate

adenylate cyclase activity, at least in rat intestinal membranes. It may also be the reason why GRF behaves as a partial VIP antagonist in rat membranes. Indeed, the modification of the N-terminal histidine of glucagon was similarly shown to result in the formation of a partial glucagon antagonist [23].

In conclusion, this paper indicates that GRF binds to human and rat VIP receptors with a low affinity and may behave as a partial VIP agonist/antagonist in rat and as a VIP agonist in man. However, the concentrations of GRF needed to interact significantly with VIP receptors rule out the possibility that GRF may exert its physiological effect through VIP receptors.

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