PORCINE PEPTIDE HAVING N-TERMINAL HISTIDINE AND C-TERMINAL ISOLEUCINE AMIDE (PHI)

Vasoactive intestinal peptide (VIP) and secretin-like effects in different tissues from the rat

D. BATAILLE, C. GESPACH, M. LABURTHE, B. AMIRANOFF, K. TATEMOTO*, N. VAUCLIN, V. MUTT* and G. ROSSELIN

Institut National de la Santé et de la Recherche Médicale (INSERM) U.55, Hôpital Saint-Antoine, 75571 Paris Cedex 12, France and **Department of Biochemistry II, Karolinska Institute, S-104 01 Stockholm 60, Sweden*

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1. Introduction

We have suggested that the structurally related intestinal peptides vasoactive intestinal peptide (VIP) and secretin were effective on some of their target tissues through discrete binding sites coupled to a membrane-bound adenylate cyclase (EC 6.4.1.1) [1–3]. In [4], we have shown that a novel peptide, the 'porcine peptide having N-terminal histidine and C-terminal isoleucine amide' (PHI) (formerly named PIHIA [4]), isolated from the porcine duodenum according to a specific chemical feature [5,6], possesses VIP-like activities. We have investigated in vitro the effect of PHI on cellular and plasma membrane preparations from the rat that are known to display either VIP-receptors (liver [1] and intestine [2]), secretin-receptors (stomach [3]), or both (fat [1,7]). The interest of this work was further supported by the recent observation that PHI possesses structural similarities with the peptides of the glucagon–VIP–secretin family [6].

We show here that, when tested on the production of 3′,5′-cyclic adenosine monophosphate (cyclic AMP), PHI was 20–40% as potent as VIP in tissue preparations that are primarily sensitive to VIP and 2–5% as potent as secretin in a preparation in which the latter peptide displays the predominant effect. In all cases, the effectiveness of PHI was correlated to the presence of either or both VIP (and secretin) receptors.

2. Materials and methods

2.1. *Peptides*

All peptides were of porcine origin. PHI was isolated as in [5,6]. The samples used were essentially free of foreign protein material, as attested by chemical analysis; of particular interest was the absence of any detectable amount of VIP or secretin; it must also be noticed that: (i) PHI did not display any measurable interaction with labeled glucagon in its binding to hepatic receptors or to several anti-glucagon antibodies; (ii) the cross-reaction of PHI in VIP-radioimmunoassay [8] was 0.02%.

VIP was the natural peptide isolated as in [9]. Secretin was the synthetic peptide generously supplied by Professor E. Wünsch (Abteilung für Peptidchemie, Martinsried). VIP and PHI were labeled with ^{125}I at specific radioactivities ranging from 150–300 Ci/g (i.e., 0.3–0.6 atom iodine/molecule) using techniques in [1,10].

2.2. *Experimental procedures*

All tissues were obtained from Wistar rats. Binding studies were conducted with liver [11] and intestinal epithelial cell membranes [12] according to [1,10]. Intestinal epithelial cells [2], epididymal fat cells [1,7] and gastric glands from the fundus [3] were isolated and incubated as described. The cyclic AMP production was measured by radioimmunoassay [13,14] either directly in fat cells [1,7] or after succinylation [15] in intestinal cells or gastric glands [2,3], to increase the sensitivity of the assay procedure.

3. Results

3.1. *Binding experiments*

PHI was extremely effective in inhibiting the binding of [^{125}I]VIP to its receptors present either in liver membranes (fig.1, upper left) or in intestinal epithelial cell membranes (fig.1, upper right). In both cases, the potency of PHI, estimated in the experimental conditions used at concentrations of peptides that produced half-maximal inhibition of the binding, was ~20–25% of that of VIP. It must be noticed that this apparent affinity of PHI for the VIP-receptors is much higher than that of secretin, ~1% [1,16,17], the only natural peptide hitherto known to display a cross-reaction with the VIP-receptors. When the same type of experiment was performed using [^{125}I]PHI instead of [^{125}I]VIP, a similar pattern was observed: PHI was less potent than VIP in inhibiting the binding of [^{125}I]PHI to liver (fig.1, lower left) or intestinal (fig.1, lower right) membranes. The potency of PHI relative to that of VIP observed under these conditions (~20% and 10% in liver and intestinal membranes, respectively) was similar to that observed when VIP was used as the tracer (fig.1, upper panels). This clearly shows that these preparations did not contain 'PHI-specific' receptors and that the binding of PHI to these membranes may be entirely accounted for by the affinity of PHI for the VIP-receptors.

3.2. *Stimulation of cyclic AMP*

Whether the affinity of PHI for these receptors reflects its ability to modulate a biological process was tested by comparing the effect of PHI and VIP on cyclic AMP production in liver membranes (table 1) and in isolated intestinal epithelial cells (fig.2, left). Indeed, the potency of PHI relative to that of VIP was at least as high as that observed in the binding experiments, ~30–40% in both systems. It must also be noticed that the efficacy of PHI, i.e., its ability to stimulate maximally the cyclic AMP production, was identical to that of VIP (table 1, fig.2, left).

The picture is very different in isolated gastric glands (fig.1, right), where a cyclic AMP system highly sensitive to secretin and 200-times less sensitive to VIP has been discovered [3]: PHI was 2–5% as potent as secretin and 3–10-times more potent than VIP; the dilution curve did not parallel that of secretin or VIP and the efficacy of PHI was ~70% of that of either peptide.

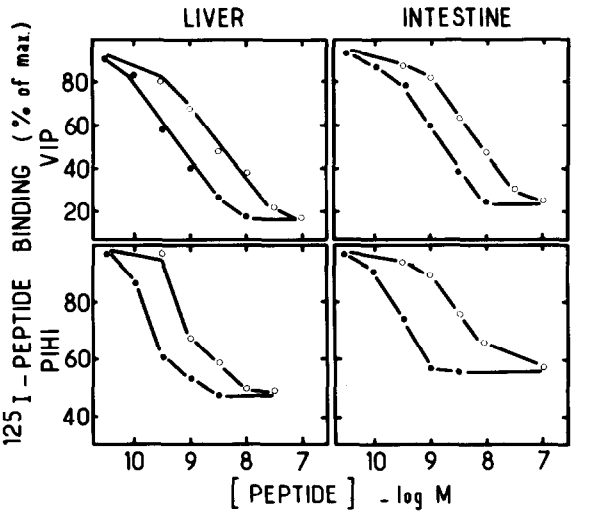

Fig.1. Effect of VIP (●——●) and PHI (○——○) on the binding of [^{125}I]VIP (upper panels) and [^{125}I]PHI (lower panels) to liver (left) and intestinal (right) membranes. Results are the means of duplicate determinations. Similar data were obtained in 6 expt. (upper left) and in 2 expt. (other panels).

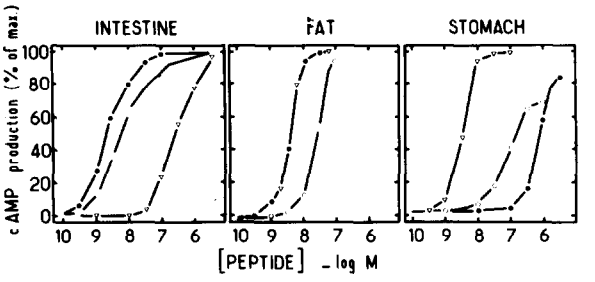

Fig.2. Effect of VIP (●——●), PHI (○——○) and secretin (△——△) on the cyclic AMP accumulation in isolated intestinal epithelial cells (left), fat cells (center) and gastric glands from the fundus (right). Each point is the mean of 2 expt., each performed in triplicate.

Table 1
Effect of serial dilutions of PHI on the activity of liver membrane-adenylate cyclase

PHI dilutions	VIP equivalent (%)		
	1	1/2	1/4
Expt. 1	50 ± 14	32 ± 4	26 ± 3
Expt. 2	29 ± 3	33 ± 2.5	31 ± 6

Results are expressed as VIP equivalent, in percent, and are the means ± SEM of 3 determinations. Initial concentrations of PHI were $3 \cdot 10^{-9}$ M and $4 \cdot 10^{-10}$ M in expt. 1 and 2, respectively and corresponded to the PHI-concentrations that display that maximal effect obtainable with either VIP or PHI

In isolated fat cell preparations previously referred to as 'type II' [7] that contain both VIP and secretin receptors and that, accordingly, are equally sensitive to VIP and secretin (fig.2, center), PHI was 10–15% as potent as VIP or secretin, with the same efficacy as either peptide (fig.2, center).

4. Discussion

These data clearly demonstrate that a new peptide, PHI, is almost as active as VIP in tissue preparations which are responsive to VIP: indeed, only 2.5–5-times higher concentrations of PHI were necessary to observe the same effect as with VIP. It is also clear that, in the tissues tested, this activity is mediated by a common receptor which is shared by VIP and PHI and which has been first described as VIP-receptor [1,16]. The secretin-like action of PHI in gastric glands is probably mediated by the secretin receptors as suggested by the high doses of PHI necessary to obtain a response (fig.2, right) and by the absence of additivity between the effects of PHI and secretin (not shown). In fat cells that contain both VIP and secretin receptors, the action of PHI is close to that which would be expected if only VIP receptors were present.

PHI is a novel 27 amino acid intestinal peptide which, in spite of some chemical [6] and biological (as shown here) similarities with VIP and secretin, displays many structural differences from all other peptides hitherto isolated [6]. The physiological status of this peptide is, so far, largely speculative. The relatively high concentrations necessary to obtain a response in gastric glands is not in favour of a physiological role of PHI in stomach, at least through its cyclic AMP-mediated secretin-like action. However, it must be kept in mind that all peptides used were of porcine origin, while the tissue preparations originate from the rat; the picture might be different in an homogeneous model. This holds true for the VIP-like effect of PHI; but, in any case, the close similarity between the potency of PHI and VIP observed in VIP-sensitive target tissue cannot be considered as the result of a simple cross-reaction with no physiological meaning. On the contrary, it allows to speculate that VIP and PHI may regulate the same physiological events via a common receptor–cyclic AMP system through different neural and/or endocrine pathways. On the other hand, whether PHI has, apart from its VIP and secretin-like effects, specific biological action(s) will await further in vivo and in vitro studies, and particularly the search for eventual specific PHI-receptors.

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