VASOACTIVE INTESTINAL PEPTIDE (VIP) STIMULATES PROLACTIN (PRL) RELEASE AND cAMP PRODUCTION IN A RAT PITUITARY CELL LINE (GH3/B6). ADDITIVE EFFECTS OF VIP AND TRH ON PRL RELEASE

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1. Introduction

The recent localization of VIP [1] (a peptide initially discovered in the gastrointestinal tract) within the hypothalamus, the hypothalamo-hypophyseal portal blood and the adenohypophysis [2–6], strongly suggested its possible regulatory role on the pituitary gland. Kato et al. [7] and Vijayan et al. [8] have shown that VIP was actually able to stimulate the release of several adeno-hypophyseal hormones including prolactin (PRL) in vivo, but not in vitro. They concluded, therefore, that VIP was acting on the secretion of pituitary hormones by means of indirect mechanisms [7,8]. Ruberg et al. nevertheless, demonstrated a direct stimulating effect of VIP on PRL release by incubated rat hemi-pituitaries [9].

We have investigated the responsiveness to VIP of a rat prolactin-secreting cell line, the GH3/B6. The hormonal secretion of these cells, or of related strains, has already been shown to be sensitive to various physiological modulators of PRL secretion in vivo, including thyroliberin (TRH) [10] (for recent reviews see refs. [11,12]). Such an homogeneous population of target cells provides, therefore, a suitable model for investigating a hypothetical effect of VIP on prolactin cells at the cellular and subcellular levels. Here, we show that the GH3/B6 cells are highly responsive to VIP which stimulates in a concomitant manner both their PRL release and 3′,5′-cyclic adenosine monophosphate (cAMP) production. These effects of VIP were compared to those of TRH, a well-established hypothalamic PRL-stimulating factor [10–12]. The effect of VIP and TRH were found to be additive on PRL release, but not on cAMP production.

2. Experimental

2.1. *Cells and experimental schedules*

The GH3/B6 strain, a subclone of the GH3 rat pituitary tumor cell line [13], were routinely grown as monolayer in Ham's F 10 medium enriched with heat-inactivated horse serum (15%), fetal calf serum (2.5%) and antibiotics as previously described [14]. In these conditions, they continuously secrete PRL and growth hormone as does the parent cell line [15]. The cells used for the present work had been subcultured 12–17 times. For the experiments, 150 000 cells/2 ml of medium/3.5 cm diameter-culture dishes were seeded and grown for 6 days at 37°C. Prior to the incubation, the medium was discarded, the cells rinsed with warm Ham's F 10 solution and 900 μl of complete culture medium (cf. above), containing or not 0.2 mM iso-butyl-methyl-xanthine (IBuMeXan), was added. Incubations were performed at 25°C ± 1°C. After 5 min, 100 μl of medium containing or not VIP, or TRH, or VIP plus TRH were introduced under gentle stirring. The medium of each dish was collected after a 15-min incubation and one ml of methanol immediately added to the cells. The medium was aliquoted for PRL assay and the rest (700 μl) was gathered with the cells scraped in a total amount of 4 ml methanol. Parallel dishes were saved for cell number and protein assay determinations [16].

2.2. *Prolactin assay*

PRL was radioimmunoassayed using the kit kindly provided by the NIAMDD, Rat Prolactin Distribution Program and polyethylene glycol as the precipitating agent. Samples were run in duplicate and estimated in terms of RP-1 equivalents.

2.3. *cAMP assay*

One ml of the supernatant of the methanol extract was evaporated, then succinylated in order to increase the sensitivity of the assay [17] and submitted to radioimmunoassay [18] using reagents and technical procedures developed in our laboratory [19]. Each sample was run in one assay at two or three different dilutions.

2.4. *Reagents*

Highly purified natural porcine VIP was a generous gift from Pr. V. Mutt (Karolinska Institute, Stockholm, Sweden). Synthetic TRH was purchased from Calbiochem (USA). Culture media, sera and reagents were purchased from Eurobio (Paris, France) and culture dishes from Corning or Falcon (USA). All other chemicals were of analytical grade from Merck (Darmstadt, GFR) or Prolabo (Paris, France).

3. Results

Figure 1 (upper pannel) shows that VIP was able to increase the release of PRL within 15 min of incubation at 25°C, in a dose-dependent manner. The apparent half-maximum stimulation occurred for ca. 2.8×10^{-10} M. The maximal effect (3 times the basal level) was reached for 10^{-8} M VIP, while higher concentrations led to a lower stimulating effect. Concomitantly, an augmentation of cell plus medium-cAMP was elicited by VIP. Although the cAMP increase at 10^{-11} and 10^{-10} M VIP was very limited, higher doses of VIP induced a highly significant enhancement of the cAMP production by the GH3/B6 cells. It reached 170% of the basal cAMP level for 10^{-8} M, corresponding to the optimal PRL response, and 230% of the basal production at 10^{-7} M, when the induced PRL release appeared sub-maximal.

Figure 1 (lower pannel) illustrates the dose-dependent effect of TRH observed in parallel experiments. Although TRH appeared less potent than VIP, the

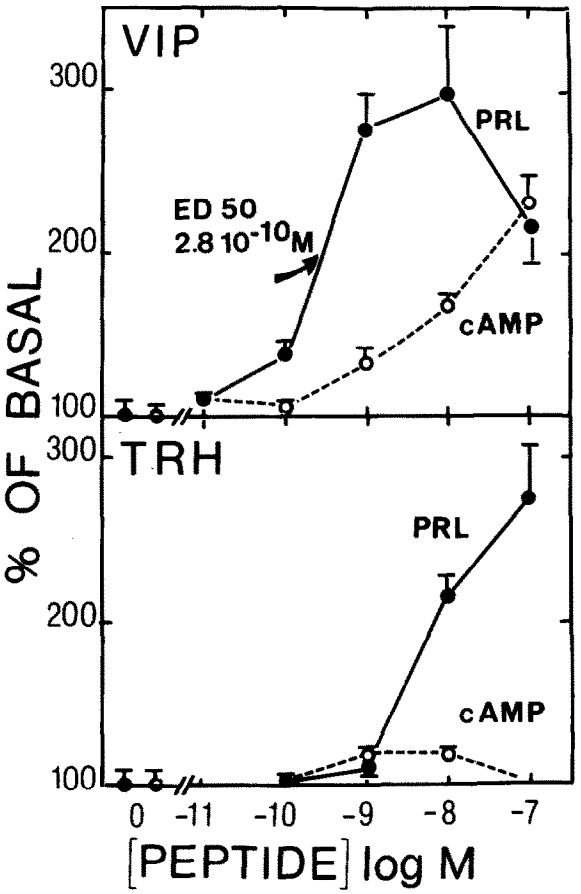

Fig.1. Dose-dependent effects of VIP (upper panel) and TRH (lower panel) on PRL release (solid lines) and cAMP production (interrupted lines). Experimental conditions are described in section 2. Each point represents the mean ± S.E.M. of 7 determinations from two experiments for PRL, and of 4 determinations from one experiment for cAMP. Each dish contained ca. 1.2×10^6 cells (350 μg protein) which produced under basal conditions 9.7 ± 0.85 ng of PRL and 6.25 ± 0.61 pmol of cAMP within 15 min.

optimal increase of PRL release was of the same order of magnitude with either peptide. In contrast, TRH stimulated very poorly the cAMP production: the cAMP increment did not exceed 20% above control and did not evoluate in a dose-dependent manner. In addition, increasing the TRH concentration from 10^{-8} to 10^{-7} M induced a further enhancement in the PRL release contrasting with the disappearance of the minute increase of cAMP production.

When cells were simultaneously exposed to VIP

Table 1
Combined effects of VIP (10^{-8} M) and TRH (10^{-8} M) on PRL release and cAMP production

	Basal	VIP	VIP + TRH	THR
PRL ng/dish	9.5 ± 0.5	28.0 ± 2.8	47.6 ± 3.4	24.4 ± 1.85
% stimulation	–	+ 194%	+ 400%	+ 154%
cAMP pmole/dish	5.5 ± 0.80	9.8 ± 0.28	9.8 ± 0.55	6.4 ± 0.40
% stimulation	–	+ 78%	+ 78%	+ 17%

Each point is the mean ± S.E.M. of 6 determinations from two experiments each performed in triplicate

(10^{-8} M) and TRH (10^{-8} M), an additive effect was observed on the PRL release (table 1). In contrast, no additive effect was observed concerning the cAMP production (table 1).

4. Discussion

Several lines of conclusions can be drawn from the present data.

(1) VIP is actually a direct and powerful PRL releasing factor in GH3/B6 rat pituitary cells, in a range of doses which includes the VIP concentrations recently measured in the rat hypothalamo-hypophyseal portal blood, i.e., 3×10^{-10} M [5]. This cell line appears highly sensitive and responsive to VIP in terms of PRL release as compared to incubated rat hemi-pituitaries [9]. In addition, in the present experimental conditions, the VIP potency was higher than that of TRH.

(2) VIP is an efficient stimulating peptide for cAMP production by prolactin-secreting cells. Moreover, as shown in table 2, the addition of 0.2 mM IBuMeXan to the incubation medium led to a higher augmentation of the VIP-induced cAMP production, raised the basal PRL release, but partially masked the VIP-dependent hormonal release. It is noteworthy that, in all VIP-sensitive target tissues tested, an augmentation of cAMP production and/or adenylate cyclase stimulation was observed: liver and fat cell membranes [20,21], human colonic carcinoma cells [22], rat enterocytes [23], isolated guinea-pig pancreatic cells [24], subcellular particles from rat brain [25] and from a human prolactin-secreting pituitary

Table 2
Influence of IBuMeXan (0.2 mM) on VIP (10 nM)-induced cAMP production and PRL release by GH3/B6 cells

	With IBuMeXan		Without IBuMeXan	
	cAMP pmoles/dish	PRL ng/dish	cAMP pmoles/dish	PRL ng/dish
Basal	15.9 ± 1.08	69.1 ± 2.19	10.2 ± 0.47	47.2 ± 2.71
VIP	42.5 ± 1.41	102.0 ± 3.81	20.0 ± 3.76	95.8 ± 3.00
% of Basal	267%	147%	196%	200%

Each point is the mean of triplicate ± S.E.M.

adenoma [26]. Our results thus suggest that the final biological response induced by VIP (i.e., PRL release) is under the control of a cAMP-dependent process. The observed shift of responsiveness between the increase of cAMP production and PRL release (fig.1) is not incompatible with such an hypothesis [27,28]. Nevertheless, further experiments must be performed to validate this interpretation. As far as the effect of TRH on PRL release is concerned, the direct involvement of cAMP is still controversial in spite of numerous experimental approaches [29–31]. In our experiments, the cAMP production appeared barely dependent on TRH, and no additive effect on cAMP production was observed when VIP and TRH were applied simultaneously; that contrasts with the additive effect observed on PRL release (table 1). These results strongly suggest the hypothesis that these two neuropeptides which finally generate the same hormonal response, trigger on the same target cell different subcellular mechanisms.

The present work is the first demonstration that VIP stimulates directly the PRL release from PRL-secreting cell line. Together with other findings [2–9] it supports the hypothesis that VIP is of physiological importance in the control of PRL secretion.

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