EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN HUMAN PROLACTIN (PRL) SECRETING PITUITARY ADENOMAS

Stimulation of PRL release and activation of adenylate cyclase

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1. Introduction

Vasoactive intestinal polypeptide (VIP) might be involved in the regulation of pituitary function [1–4]. Some of the peptide action is probably effected at the level of pituitary cells, as suggested by its presence at high concentration in the hypophyseal portal blood [5] and by the existence of a VIP-sensitive adenylate cyclase in rat pituitary gland [6,7]. In particular, although the available experimental evidence is still incomplete and controversial, a physiological role of VIP in the direct control of prolactin (PRL) release has been envisaged [1,3].

These results support this hypothesis and give some insight on the mechanisms of action of VIP on pituitary mammotroph cells. In fact, working on human PRL-secreting adenomas, i.e., on homogeneous populations of mammotroph cells, we demonstrate that:

(1) VIP stimulates the release of PRL from tissue fragments incubated in vitro;
(2) A VIP-sensitive adenylate cyclase is present in these pituitary tumors.

2. Experimental

2.1. *Materials*

Natural porcine VIP was kindly supplied by Professor Mutt, Karolinska Institutet, Stockholm. Adenosine-5′-triphosphate (ATP), adenosine-3′,5′-cyclic monophosphate (cAMP), creatine phosphate, creatine phosphokinase, guanosine-5′-triphosphate (GTP) and ethylene glycol-bis (2-aminoethylether) *N,N,N′,N′*-tetraacetic acid (EGTA) were purchased from Sigma, St Louis, MO. [8-^{14}C]ATP and cyclic [8-^{3}H]AMP were purchased from the Radiochemical Center, Amersham. PRL radioimmunoassay kit was supplied by Biodata-Serono, Rome.

2.2. *PRL release*

Pituitary adenomas were surgically removed from patients through the transsphenoidal route, placed in oxygenated Krebs Ringer bicarbonate medium containing glucose and amino acids (pH 7.4) (KRB) [8], and quickly cut into small fragments of ~1 mm^3. Incubations were carried out at 37°C in a shaking bath first for two 1 h periods in plain KRB (6 fragments/ml) and then for 30 min in KRB with or without VIP (10^{-7} M). At the end of the final incubation, the media were collected, centrifuged at 105 000 × *g* for 60 min and stored at −20°C until radioimmunoassay of PRL was performed according to [9]. Adenoma fragments were homogenized and their protein content assayed according to [10].

2.3. *Adenylate cyclase assay*

Adenoma fragments, frozen immediately after surgery, were stored up to 10 days at −20°C. Homogenization was performed in 20 vol. 1 mM Tris–maleate buffer (pH 7.4) by a glass–Teflon Potter homogenizer. The enzyme assay was carried out either on the total homogenate, filtered through a nylon gauze, or on the crude membrane preparation obtained by centrifuging the homogenate at 20 000 × *g* for 10 min and resuspending the pellet in 1 mM Tris–maleate

buffer at 1–25 mg protein/ml. The assay mixture contained: Tris-maleate buffer (50 mM (pH 7.4)) [8-^{14}C]ATP (0.15 mM, 40 dpm/pmol), cyclic [8-^{3}H]-AMP (1 mM, 350 dpm/nmol), creatine phosphate (7 mM), creatine phosphokinase (6.2 U/ml), $MgSO_4$ (5 mM), EGTA and GTP (0.3 and 0.01 mM, respectively, unless specified differently), VIP, pituitary homogenate or membranes to 0.1 ml final vol. Isolation and measurement of cyclic [8-^{3}H, ^{14}C]AMP was performed according to [11]. These experimental conditions permitted correction for the effect of phosphodiesterases which in any case was almost negligible [12].

3. Results

The effect of 10^{-7} M VIP on in vitro PRL release was investigated in 3 different human prolactinomas. In all cases a significant stimulation was observed, with increases over the basal levels of 70–170% (table 1).

The effect of VIP on adenylate cyclase was studied in 6 adenomas. In all of them the enzyme activity was stimulated, although the degree of this stimulation was quite variable (20–119% at 10^{-6} M VIP) (table 2). VIP was equally effective on both total homogenates and crude membrane preparations. Fig.1 illustrates the dose response relationship in one pituitary adenoma. Half-maximal stimulation occurred at 4.5×10^{-7} M and the highest enzyme activation at 3×10^{-6} M VIP. At a higher concentration (10^{-5} M) the peptide elicited an inhibitory effect. The curves

Table 1
In vitro effect of VIP on PRL release from human PRL-secreting pituitary adenomas[a]

Case	μg PRL/mg protein		% increase	P
	basal	10^{-7} M VIP		
G.A.	3.32 ± 0.45	5.77 ± 0.15	74	<0.05
P.C.	3.10 ± 0.20	8.37 ± 0.42	170	<0.01
I.B.	2.61 ± 0.10	6.32 ± 0.30	142	<0.01

[a] Values given are the means (± SE) of 2 determinations each carried out on duplicate incubation media

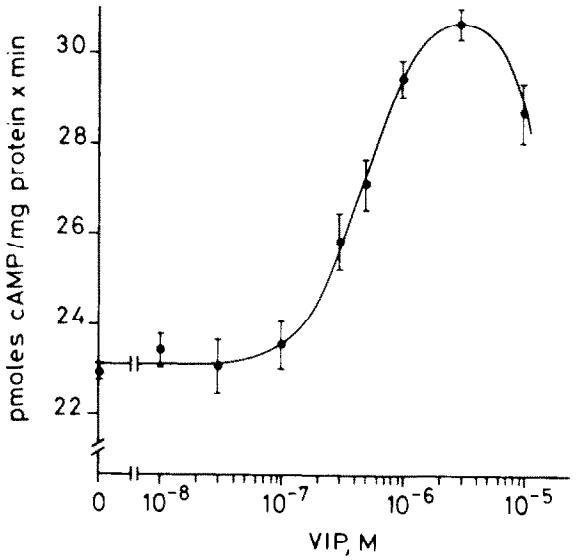

Fig.1. Effect of different doses of VIP on adenylate cyclase activity in the crude membrane preparation (case I.B.). Values given are the means of 3 determinations ± SE.

Table 2
VIP-sensitive adenylate cyclase in human PRL-secreting pituitary adenomas[a]

Case	Enzyme prep.	pmol cAMP/mg protein × min		% stimulation	P
		Basal	10^{-6} M VIP		
E.B.	H[b]	13.65 ± 0.52	19.10 ± 0.43	39.9	<0.005
G.A.[c]	H[b]	8.47 ± 0.19	10.68 ± 0.31	26.1	<0.01
M.L.	H[b]	8.56 ± 0.44	14.10 ± 0.43	64.7	<0.005
	M[d]	22.90 ± 2.22	40.00 ± 1.40	74.7	<0.005
C.A.	M[d]	13.87 ± 2.00	30.36 ± 0.92	118.9	<0.005
P.C.	M[d]	38.90 ± 1.20	46.64 ± 0.12	19.9	<0.001
I.B.	M[d]	22.95 ± 0.11	29.39 ± 0.45	28.1	<0.005

[a] Values given are the means of 3 determinations ± SE
[b] H, total homogenate
[c] In this case EGTA was 5×10^{-4} M
[d] M, crude membrane preparation

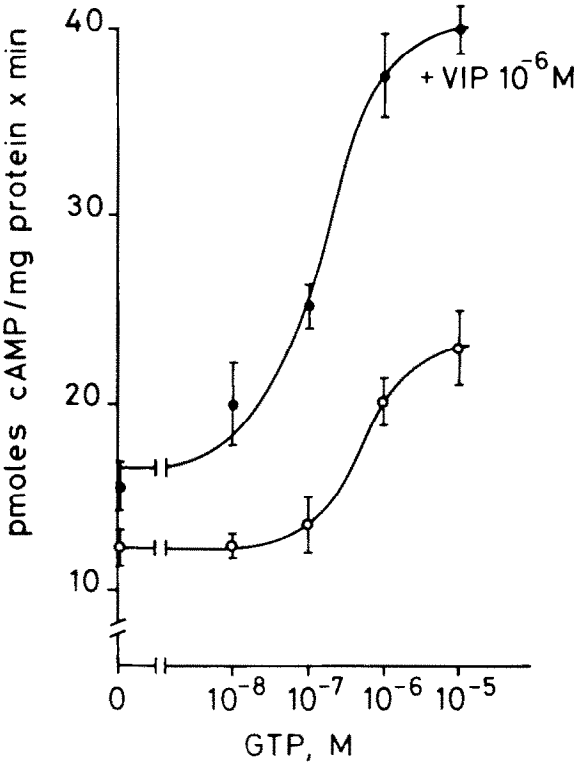

Fig.2. Effect of GTP on basal (○——○) and VIP-stimulated (●——●) adenylate cyclase activity in the crude membrane preparation (case M.L.). Values given are the means of 3 determinations ± SE.

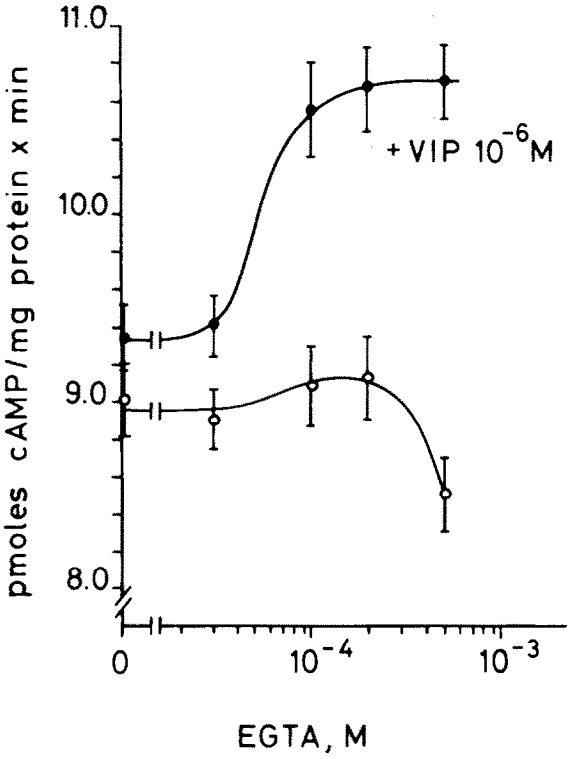

Fig.3. Effect of EGTA on basal (○——○) and VIP-stimulated (●——●) adenylate cyclase activity in the total homogenate (case G.A.). Values given are the means of 3 determinations ± SE.

obtained with the other tumors showed similar patterns.

The effect of various doses of GTP on the basal and VIP-stimulated adenylate cyclase of a crude membrane preparation obtained from one tumor is shown in fig.2. GTP increased the basal activity considerably and the VIP-stimulated activity to an even higher degree. GTP was maximally effective at 10^{-5} M.

When EGTA was added to the total homogenate, the basal activity remained almost unchanged at up to 2×10^{-4} M and was inhibited at 5×10^{-4} M. In contrast, the VIP-sensitive activity was markedly stimulated even by lower concentrations of EGTA (fig.3). This result suggests a role of Ca^{2+} in the regulation of the cyclase by the peptide.

4. Discussion

The results we have obtained clearly demonstrate that VIP promotes a significant increase of PRL release from human PRL-secreting adenomas through a direct action on the tumor mammotrophs, in agreement with results in non-human experimental models (normal rat hemipituitaries [1] and GH3/B6 rat PRL-secreting cell line [3]). The adenoma tissue we used consisted essentially of a homogeneous PRL-secreting cell population; the homogeneity was routinely checked by histological examination and SDS–polyacrylamide gel electrophoresis of homogenates (data not shown). Since we have demonstrated here that VIP activates adenylate cyclase in such a tissue, we can conclude that the VIP-sensitive enzyme is located in the adenomatous PRL-secreting cell. This conclusion is also in line with a single prolactinoma study [13].

The VIP-sensitive enzyme shows GTP and Ca^{2+} dependence. The same biochemical characteristics are displayed by the VIP-stimulated cyclase detected in normal rat pituitary gland [6].

The demonstration of a VIP-sensitive adenylate cyclase in the tumor mammotrophs raises the question as to whether the stimulating effect of the peptide on PRL release is mediated by an increase in the intracellular concentration of cAMP. The fact that a concentration of VIP (10^{-7} M) which has a marked effect on PRL release does not stimulate adenylate cyclase might appear to be in contradiction with this hypothesis. However, this is not necessarily the case, because the same discrepancy has been observed in other systems where the mediation of cAMP in eliciting the biological response is well established [14,15]. In contrast, the importance of the cAMP–adenylate cyclase system in PRL secretion is suggested on one hand, by the consistency of our findings in all the tumors investigated, on the other hand, by our previous finding that dibutyryl-cAMP and theophylline markedly enhanced PRL release from adenoma fragments (unpublished results and [16]).

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