ADENYLATE CYCLASE OF RAT PITUITARY GLAND

Stimulation by vasoactive intestinal polypeptide (VIP)

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1. Introduction

Like several other peptides, vasoactive intestinal polypeptide (VIP) occurs in gastrointestinal and nervous tissues [1]. In the central nervous system, VIP has a discrete distribution, being most abundant in cerebral cortex and hypothalamus, including, in human brain, the median eminence [2,3]. VIP is also concentrated in nerve endings from which it can be released on depolarization [2,4]. These and other data [5] favour a neurotransmitter or neuromodulator role for the peptide.

Recently, immunoreactive VIP was found in portal hypophyseal blood in concentrations 19-times those in peripheral blood [6], suggesting release of the peptide from the hypothalamus and raising the possibility that it may control some aspects of pituitary function. To examine the latter possibility we investigated the ability of VIP to stimulate adenylate cyclase activity in the rat pituitary, since activation of this enzyme correlates with hormonal secretion in this gland [7].

2. Materials and methods

VIP was the natural porcine polypeptide prepared in the laboratory of Professor V. Mutt, Karolinska Institutet, Stockholm. Synthetic fragments VIP_{6-28} and VIP_{14-28} were prepared by Professor M. Bodanszky, Cleveland, OH.

8-[^{14}C]ATP and 8-[^{3}H]cAMP were from The Radiochemical Center, Amersham. ATP, cAMP, norepinephrine, haloperidol, D,L-propranolol, creatine phosphate and creatine phosphokinase were purchased from Sigma, St Louis, MO. [Arg^{8}]-vasopressin was obtained from Sandoz, AG, Basel and GTP from Boehringer-Mannheim Gmbh.

2.1. *Preparation of homogenates*

Male rats (Sprague-Dawley 150–200 g) were killed by decapitation; pituitaries were rapidly dissected, washed in ice-cold Tris–maleate buffer (50 mM, pH 7.4) containing 0.33 mM EGTA, and homogenized in the same medium (1:60, w/v). The homogenate was either used as such (2–3 mg protein/ml) or centrifuged at 12 000 × *g* at 0°C for 15 min. The pellet was resuspended in Tris–maleate (50 mM, pH 7.4) to give 1.5–2 mg protein/ml.

2.2. *Adenylate cyclase assay*

The adenylate cyclase assay was performed essentially as in [8]. Incubation was carried out at 30°C for 5 min, unless stated otherwise. The standard assay mixture contained Tris–maleate buffer (50 mM, pH 7.4); 8-[^{14}C]ATP (0.15 mM, 50 dpm/pmol); 8-[^{3}H]-cAMP (0.15 mM, 300 dpm/nmol); creatine phosphate (7 mM), creatine phosphokinase (6.2 U/ml); $MgSO_4$ (5 mM); EGTA (0.1 mM, added with the homogenate); and substances to be tested, dissolved in the Tris–maleate buffer. Isolation and detection of 8-[^{3}H,^{14}C]cAMP was performed according to [9]. Protein was assayed according to [10].

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3. Results

VIP activated the adenylate cyclase in homogenates of rat pituitary in a concentration-dependent fashion, with an app. $K_d = 2 \times 10^{-7}$ M (fig.1).

The accumulation of cAMP, at the maximally effective concentration (10^{-6} M) of the peptide, was a linear function of time, over the period of observation (fig.2), and was not influenced by bacitracin, an inhibitor of peptidases (data not shown).

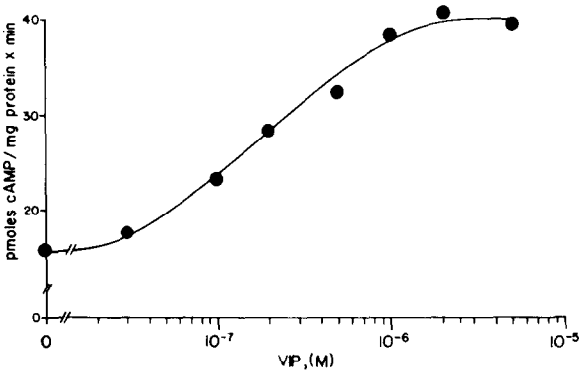

Fig.1. VIP-induced, concentration-dependent activation of adenylate cyclase in homogenate of pituitary gland. Each point is the mean of 3–6 values.

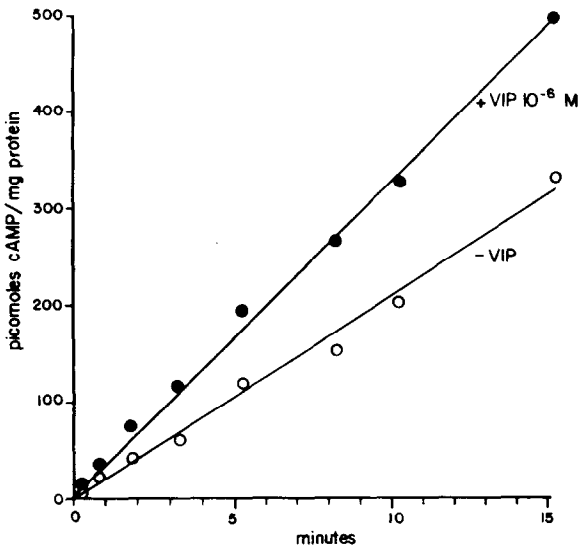

Fig.2. Time-course of basal (open circles) and VIP-stimulated (closed circles) cyclic AMP accumulation in homogenate of pituitary gland. Each point is the mean of 3–6 values.

The magnitude of VIP (10^{-6} M)-induced stimulation of adenylate cyclase was comparable to that obtained by NaF (10^{-2} M) and higher (2-fold) than that elicited by PGE_2 (2×10^{-6} M) (table 1). Other peptides, [Arg^8]-vasopressin (10^{-6} M), and the synthetic VIP fragments, VIP_{6-28} and VIP_{14-28} (5×10^{-5} M in each case), were completely devoid of stimulatory activity (table 1). Other experiments (not reported here) showed that the VIP-induced stimulation of adenylate cyclase was unaffected by the addition of the VIP synthetic fragments (both at 5×10^{-5} M), or propranolol, a β-adrenergic antagonist (10^{-6} M). In addition, neither dopamine (10^{-7}–10^{-5} M) nor haloperidol, a dopamine-receptor blocker (10^{-6} M) modified the extent of VIP activation.

Both PGE_2 and norepinephrine promoted the formation of cAMP in pituitary homogenates, being maximally effective at 10^{-5} M; addition of VIP (10^{-6} M) to the incubation medium increased the formation of cAMP elicited by either agent (fig.3). On the other hand, VIP had no influence on the pituitary cyclase already exposed to the maximally effective concentration of NaF (10^{-2} M) (fig.3).

To test the influence of Ca^{2+} and GTP on adenylate cyclase, these factors were added to resuspensions of particulate fractions obtained by centrifuging pituitary homogenates (12 000 × *g* for 15 min). Ca^{2+} at $\leq 10^{-6}$ M increased moderately (2-fold) the basal

Table 1
Rat pituitary adenylate cyclase: Stimulation by various agents

Additions		pmol cAMP/mg protein × min (mean ± SD, n = 3–6)	Stimulation (-fold)
None		29.6 ± 3.3	–
VIP	10^{-6} M	120.2 ± 8.0	4.06
PGE_2	2×10^{-6} M	63.0 ± 6.8	2.13
NaF	10^{-2} M	131.1 ± 6.6	4.43
[Arg^8]-vasopressin	10^{-6} M	26.9 ± 4.3	0.89
VIP_{6-28}	5×10^{-5} M	28.1 ± 4.4	0.95
VIP_{14-28}	5×10^{-5} M	27.2 ± 2.8	0.92

cyclase activity; higher concentrations of this ion (10^{-5}–10^{-8} M) resulted in marked inhibition of basal adenylate cyclase activity and complete abolition of VIP stimulation (data not shown). GTP (10^{-7}–10^{-5} M) stimulated the rat pituitary cyclase and strongly enhanced the stimulant effect of VIP on this enzyme (fig.4).

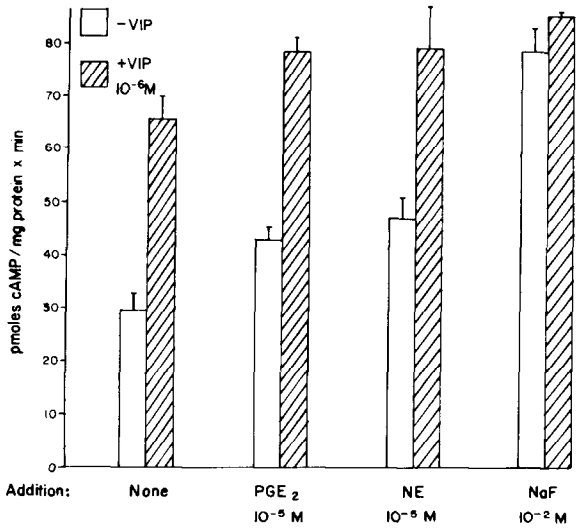

Fig.3. Effect of PGE_2, norepinephrine (NE) and NaF on basal (open bars) and VIP-stimulated (hatched bars) adenylate cyclase in homogenate of pituitary gland. Values are means of 3 determinations, ± SD.

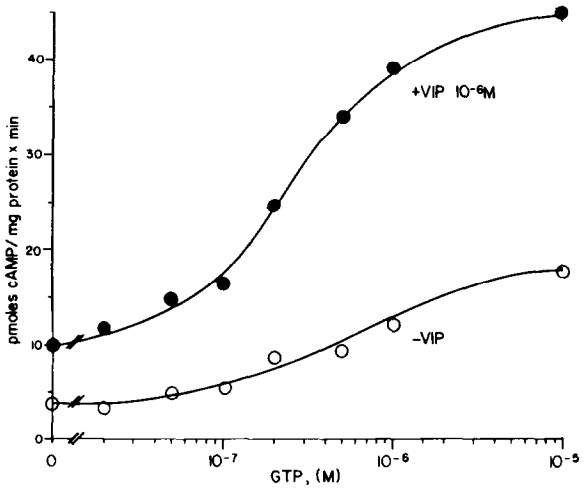

Fig.4. Effect of GTP on basal (open circles) and VIP-stimulated (closed circles) adenylate cyclase in particulate fraction (pellet at 12 000 × *g*) of pituitary gland. Each point is the mean of 3–6 values.

4. Discussion

Earlier studies established the ability of VIP to stimulate adenylate cyclase activity in homogenates of brain and several other organs of rat and other species [8,11–16]. The present results show that the peptide also activates this enzyme in rat pituitary. This activation seems to occur via a specific receptor, distinct from that responsible for the action of PGE_2 or norepinephrine, since the effects of either of these agents with VIP were additive (fig.3). Further, the β-blocker, propranolol, was without effect on this VIP action, indicating that the VIP receptor is distinct from the β-receptor in this organ, as it is in other organs and tissues [17].

Dopamine has been demonstrated to have an inhibitory action on prolactin release [18,19], and on the adenylate cyclase from human pituitary adenoma [20]. It was tempting, therefore, to speculate that VIP and dopamine may act through the same receptor (to cause opposite effects). This does not appear to be the case, however, since neither dopamine nor a dopamine receptor-blocker (haloperidol) affected VIP-stimulation of adenylate cyclase.

In agreement with work on rat brain homogenates [8], the partial sequences of VIP, VIP_{6-28}, and VIP_{14-28}, were inactive as agonists or antagonists of VIP. These data suggest that the N-terminal 5 amino acids of the molecular sequence are required for binding or for recognition by VIP receptors in this preparation. Recent studies on [^{125}I]VIP binding to brain membranes have reached similar conclusions [21].

As observed in similar studies on rat cerebral cortex [8], Ca^{2+}, which had a biphasic effect on basal pituitary adenylate cyclase, completely abolished VIP stimulation of this enzyme. Conversely, this action of VIP was markedly potentiated by GTP, in a manner consistent with the controlling influence of this compound on cyclase stimulation by hormones and neurotransmitters [22].

VIP stimulated the pituitary adenylate cyclase in a concentration range (10^{-7} M) comparable to that observed for other hypothalamic peptides [23]. Other tissues, particularly enterocytes, respond at lower concentrations of VIP [24]. The reasons for the lower sensitivity of the pituitary enzyme are not clear, but could be related to different sensitivities of different cell types represented in the pituitary homogenates.

VIP has been reported to stimulate the secretion of prolactin from the rat pituitary [25–27]. In two of these reports, the VIP effect was obtained only in vivo, while in the third a direct action on the pituitary was observed. Strong evidence points to a close relationship between the accumulation of cAMP elicited by neural peptides and the secretion of pituitary hormones. In the instances in which the activation of the enzyme and the hormonal secretion were measured simultaneously the correlation has been excellent [7]. The secretion of prolactin is tonically inhibited by the activity of the tuberoinfundibular dopaminergic neurons, but is probably controlled also by positive hypothalamic influences [28]. Thus, prolactin-releasing activity has been detected in the median eminence of the rat hypothalamus [29]. The presence of VIP immunoreactivity and immunofluorescence in these areas, together with the stimulation of pituitary adenylate cyclase reported here, makes this peptide a possible candidate for the postulated hypothalamic positive influence on prolactin secretion.

Acknowledgements

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Note

While this work was in progress S.I.S. found that Dr E. Frandsen was obtaining similar results.

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