Vasoactive Intestinal Polypeptide Stimulation of Cyclic AMP Formation in Rat Central Nervous System

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Synopsis

Vasoactive intestinal polypeptide (VIP) stimulated cyclic AMP formation in the anterior pituitary, hypothalamus, pons and cerebral medulla of rat *in vitro*.

The concentration of VIP above 10^{-7} M significantly raised cyclic AMP level of these tissues with dose-dependency and the maximum stimulation of cyclic AMP formation was obtained at 5 min incubation. This stimulatory effect of VIP was not inhibited by propranolol or somatostatin.

However, no enhancement of hormone release from the anterior pituitary or hypothalamus into the incubation medium by VIP was observed.

These results suggest that VIP may act through adenylate cyclase in specific areas of rat central nervous system independent of β -adrenergic receptor, but it may not necessarily be related to hormone release from the anterior pituitary or hypothalamus.

Vasoactive intestinal polypeptide (VIP), originally isolated from the small intestine by Said and Mutt (1970, 1972), has been demonstrated to have potent and diverse effects on the cardiovascular and gastrointestinal systems. Based on the development of an immunoassay method for VIP, Bryant and co-workers (1976) reported first the presence of immunoreactive VIP in the central nervous system of man and pig. Said and Rosenberg (1976) also demonstrated VIP immunoreactivity in the clonal cell lines of neuronal and glial origin, and they suggested a possible dual role for VIP as a gastrointestinal hormone and a neurotrans-

mitter substance.

On the other hand, Schwartz *et al.* (1974), had reported that VIP stimulated cyclic AMP production in ileal mucosa and caused the net secretion of both sodium and chloride in stripped ileum of the rabbit.

These results prompted us to investigate the physiological role and mechanism of action of VIP in the central nervous system.

This paper presents the results related to the effect of VIP on the cyclic nucleotide concentration in various areas of the central nervous system *in vitro*.

Materials and Methods

This work was supported in part by grants (No. 457591 and 337003) from the Ministry of Education, Science and Culture, Japan.

VIP was synthesized in solution according to the method previously described (Yanaihara *et al.*, 1977)

and its purity was examined by thin layer chromatography, amino acid analysis and isoelectric focussing by comparing with natural porcine VIP.

The other peptides used in the experiments, porcine motilin, bombesin, substance P, human gastrin I, were also synthesized in solution and their physicochemical and biological characteristics examined.

Male Wistar rats weighing 150–170 g were killed by decapitation and the whole brain and pituitary gland were quickly removed and then each area was isolated. After preincubation at 37°C for 30 min in Krebs-Ringer bicarbonate buffer containing 0.1% glucose and gassed with 95% O₂–5% CO₂, the tissues were transferred into the same fresh buffer containing 5 mM theophylline, 0.1% bovine serum albumin and a suitable amount of VIP and were further incubated. The tissues were immediately frozen after the final incubation and homogenized with 6% TCA in a small conical homogenizer. The supernatant of the homogenate was extracted with water-saturated ether and lyophilized. The residue was dissolved in an appropriate volume of Tris-HCl buffer, pH 7.4 and used for the determination of cyclic AMP and cyclic GMP. Cyclic AMP was assayed by the competitive protein binding method of Gilman (1970), and cyclic GMP was determined by the specific radioimmunoassay.

Hormone released from the anterior pituitary or hypothalamus into the incubation medium was measured by the specific radioimmunoassay system.

Results

Table 1 shows the effect of VIP on cyclic AMP formation in various areas of the rat central nervous system. VIP stimulated cyclic AMP formation of the anterior pituitary, hypothalamus, pons and cerebral medulla, but it did not increase the cyclic AMP concentration of the posterior pituitary, cerebellum and cerebral cortex.

The other synthetic peptides tested in the experiments (porcine motilin, bombesin, substance P and human gastrin-I) did not change the cyclic AMP concentration in the rat anterior pituitary. In the hypothalamus and cerebral medulla, the same results were obtained. Although VIP stimulated cyclic AMP production in the rat anterior pituitary, it did not change the cyclic GMP level of the anterior pituitary (Table 2).

VIP at high concentrations above 10⁻⁷ M

Table 1. VIP (10⁻⁷ M) stimulation of cyclic AMP formation in various areas of rat central nervous system for 5 min incubation.

Tissue	Cyclic AMP (pmol/mg. prot.)	
	Control	VIP
Anterior pituitary	12.9±2.5	27.2±5.0*
Posterior pituitary	7.3±0.8	6.8±0.8
Hypothalamus	10.9±1.3	17.2±0.9*
Pons	3.4±0.3	4.7±0.7**
Cerebellum	3.2±0.3	2.8±0.2
Cerebral cortex	7.2±2.3	8.2±1.1
Cerebral medulla	2.4±0.3	4.6±0.6*

* p<0.01, ** p<0.05.

Table 2. Effects of various peptides on cyclic AMP and cyclic GMP levels in the rat anterior pituitary. Concentration of each peptide: 10⁻⁷ M, 5 min incubation.

	Cyclic AMP (pmol/mg. prot.)	Cyclic GMP (pmol/mg. prot.)
Control	7.7±0.5	0.46±0.04
VIP	12.3±0.5*	0.52±0.04
Motilin	7.1±0.6	0.46±0.03
Bombesin	7.8±0.6	0.42±0.04
Substance P	7.0±0.4	—
Gastrin	7.5±0.4	—

* p<0.01.

stimulated cyclic AMP formation significantly and at the maximum stimulation the peptide increased the cyclic AMP concentration to about four times the control value. This stimulatory effect of VIP reached its maximum at 5 min incubation and was then gradually reduced (Fig. 1).

The β -adrenergic blocking agent (propranolol, 10⁻⁴ M) did not inhibit the stimulation of cyclic AMP formation induced by VIP in the anterior pituitary and hypothalamus.

Somatostatin (7×10⁻⁶ M) decreased the basal and stimulated level of cyclic AMP induced by TRH, LH-RH or prostaglandin in the anterior pituitary as reported previously (Kaneko *et al.*, 1974), but it did not inhibit the stimulation of cyclic AMP

Fig. 1. Dose response and time course curve of the effect of VIP on cyclic AMP formation in rat anterior pituitary.

production by VIP in the gland (Fig. 2). The same phenomena were observed in the hypo- thalamus.

Table 3 demonstrates the effect of VIP on hormone release into the incubation medium from the anterior pituitary or hypothalamus. Under our experimental conditions, no significant enhancement of the hormone release from the anterior pituitary or hypothalamus by VIP was observed.

Discussion

Recently, the presence of many peptides isolated from the intestine has been demonstrated in the central nervous system by specific immunochemical methods, and pos-

Fig. 2. Effect of somatostatin (7×10^{-6} M) and propranolol (10^{-4} M) on VIP (10^{-7} M) stimulation of cyclic AMP production in rat anterior pituitary. GIF: somatostatin.

Table 3. Hormone release from the anterior pituitary or hypothalamus by VIP (10^{-7} M) for 60 min *in vitro*.

	Anterior pituitary			
	GH	TSH (μ g/pit)	LH	PRL
Control	37.1 $\pm$ 4.1	19.5 $\pm$ 1.7	8.6 $\pm$ 0.5	3.5 $\pm$ 0.4
VIP	41.9 $\pm$ 9.7	25.4 $\pm$ 2.9	8.0 $\pm$ 0.7	3.4 $\pm$ 0.8
	Hypothalamus			LHRH
	TRH (pg/mg. prot.)			
Control	15.4 $\pm$ 6.1			11.5 $\pm$ 3.3
VIP	14.7 $\pm$ 3.6			17.0 $\pm$ 8.7

sibility as a neurotransmitter with some peptides was suggested. However, the exact function and mechanism of action of these peptides in the central nervous system remain to be clarified.

The stimulatory effect of VIP on cyclic AMP formation in the intestine reported by Schwartz *et al.* prompted us to investigate the action of this peptide on cyclic nucleotide level of the various areas in the central nervous system.

As shown in table 1 and 2, VIP stimu-

lated significantly cyclic AMP formation in specific areas of the rat central nervous system, but had no effect on cyclic GMP concentration in these tissues. The synthetic peptides, porcine motilin, bombesin, substance P and human gastrin-I, did not show any stimulatory or inhibitory effect on cyclic AMP and cyclic GMP concentrations in the anterior pituitary or other tissues (not shown in the table), although species variation of the amino acid sequence of the peptide hormones examined may or may not exist. VIP raised the cyclic AMP level not only in the anterior pituitary, but also in the hypothalamus, pons and cerebral medulla. The stimulation of cyclic AMP formation induced by VIP in the rat anterior pituitary was observed with dose-dependency and peaked at 5 min incubation and then decreased gradually.

The β -adrenergic blocking agent (10^{-4} M of propranolol) did not inhibit a VIP effect on cyclic AMP in the gland as shown in Fig. 2, indicating that VIP does not act through the β -adrenergic receptor.

Somatostatin, which inhibits the secretion or action of many peptide hormones, lowered the basal and stimulated level of cyclic AMP induced by TRH, LH-RH or prostaglandin in the anterior pituitary as reported previously (Kaneko *et al.*, 1974), but it did not inhibit the stimulatory action of VIP on cyclic AMP formation in the anterior pituitary or in the hypothalamus. As TRH and LH-RH stimulated cyclic AMP formation and enhanced hormone release from the anterior pituitary respectively, these results suggest the possibility that VIP might stimulate the release of a hormone or hormones from the anterior

pituitary or hypothalamus through the activation of adenylate cyclase.

The results shown in the table 3 demonstrated the negligible effect of VIP on hormone release into the incubation medium from the anterior pituitary or hypothalamus for 60 min incubation. Under our experimental conditions, even after 60 min incubation or longer, VIP did not significantly stimulate the release of GH, TSH, LH, prolactin, or FSH and ACTH (not shown in the table) from the anterior pituitary. In the hypothalamus, no enhancement of secretion of TRH and LH-RH and also somatostatin (not shown in the table) by VIP was observed.

These results suggest that the action of VIP in stimulating cyclic AMP formation in the specific areas of the rat central nervous system is not through β -adrenergic receptor and may not necessarily be related to the hormone release from the tissues.

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