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CHARACTERIZATION OF VIP-SENSITIVE ADENYLATE CYCLASE IN GUINEA PIG BRAIN

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1. Introduction

Biologically active peptides, like substance P [1], somatostatin [2], enkephalins [3], the C-terminal octapeptide of pancreozymin [4,5] and vasoactive intestinal polypeptide (VIP) [6,7] have been found in several discrete areas of the mammalian brain. More specifically, their concentration and localization in nerve endings suggest a possible involvement in neurotransmission. VIP, found essentially in the cortical gray matter and in the hypothalamus, has been detected chiefly in the synaptosomal fraction – which also contains dopamine – during subcellular fractionations [8]. A further argument in favor of VIPs suspected role in neurotransmission would be the detection of biological activity at the cerebral cell level.

As VIP is a potent activator of an adenylate cyclase from both liver [9] and pancreas [10], we tested the hypothesis that VIP could also activate a brain adenylate cyclase. This paper demonstrates that VIP activates an adenylate cyclase from a synaptosomal fraction of guinea pig brain. This activation was not potentiated by guanyl triphosphate nucleotides, and was unaffected by α- and β-adrenergic blockers and by atropine. Furthermore, peptides related to VIP, like secretin, glucagon and somatostatin, were devoid of significant agonistic or antagonistic activity. EGTA was also without effect on basal and VIP-stimulated activities while calcium at concentrations higher than 10^{-5} M inhibited both activities.

2. Materials and methods

2.1. *Brain membrane preparation*

For each preparation, two adult female guinea pigs were killed by decapitation and the brains, weighing approx. 3 g, were homogenized in 30 ml ice-cold 0.32 M sucrose, using a glass homogenizer with a Teflon pestle. Subcellular fractionation was conducted by a modification of the Whittaker procedure [11]. The homogenate was centrifuged at $1000 \times g$ for 10 min. The supernatant was centrifuged at $12\,000 \times g$ for 20 min. The resulting pellet was resuspended in 15 ml of ice-cold distilled water, rehomogenized, and then diluted with an equal volume of a 25 mM Tris–HCl buffer, pH 7.4, containing 1 mM 2-mercaptoethanol and 2 mM $MgCl_2$. This suspension, 5 ml as layered on a discontinuous density gradient consisting of 5 ml aliquots of, respectively, 0.4 M, 0.6 M, 0.8 M, 1.0 M and 1.2 M sucrose, and centrifuged for 2 h at 25 000 rev/min in a rotor SW 27 in a Beckman Model L ultracentrifuge. The interfaces were collected, diluted in 20 vol. the abovementioned buffer and centrifuged at $50\,000 \times g$ for 30 min. The pellets, rehomogenized in the same buffer, were stored in liquid nitrogen and were stable for at least 3 weeks. The present data were obtained on the material collected at the 1.0 M – 1.2 M sucrose interface, which exhibited the highest adenylate cyclase activity.

2.2. *Adenylate cyclase activity*

Adenylate cyclase activity was determined accord-

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ing to Salomon et al. [12]. The standard adenylate cyclase reaction mixture (final vol. 60 μl) contained the following final concentrations: 30 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM EGTA, 1 mM cyclic AMP, 1 mM theophylline, 0.5 mM [α-^{32}P]ATP, 10 mM phospho(enol)pyruvate and 30 μg/ml pyruvate kinase. In all the test conditions, the kinetics remained linear for at least 9 min at 37°C.

2.3. *Protein estimation*

Proteins were estimated by the method of Lowry et al. [13] using bovine serum albumin as the standard.

3. Results

VIP- activated adenylate cyclase of a guinea pig synaptosomal fraction in a dose-dependent manner. The concentration giving half-maximal stimulation was approximately 5 × 10^{-7} M. The maximal VIP-stimulated cyclic AMP production was a 2.2-fold increase of the basal enzyme activity (fig.1).

The two guanyl triphosphate nucleotides, GTP and guanosine 5′(β,γ-imido)triphosphate (Gpp(NH)p), were potent activators of this brain adenylate cyclase but did not potentiate VIP stimulation. At any VIP concentration, the effect of a nearly maximal concentration of both nucleotides was purely additive (fig.2). Furthermore, the dose–response curves of each nucleotide in the absence or presence of a submaximal 10^{-6} M VIP concentration were almost parallel (fig.3).

The VIP-stimulated adenylate cyclase appeared highly specific as it was not influenced by either an α-adrenergic blocker (phentolamine), a β-adrenergic blocker (propranolol) or a muscarinic inhibitor (atro-

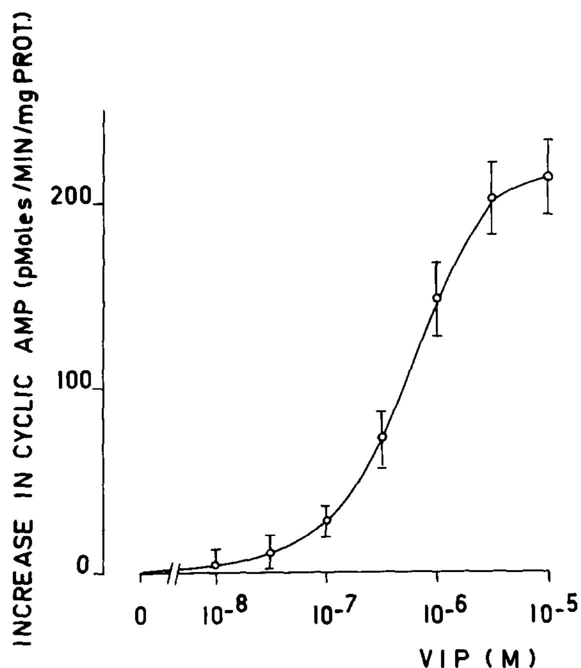

Fig.1. Dose–response curve of adenylate cyclase activation of a guinea pig brain synaptosomal fraction in presence of increasing concentrations of VIP. The results obtained in the standard assay conditions (7 min incubation at 37°C) are presented as increases in cyclic AMP over the basal value (189 ± 12 pmol/min/mg protein) and are the mean ± SEM of 6 determinations.

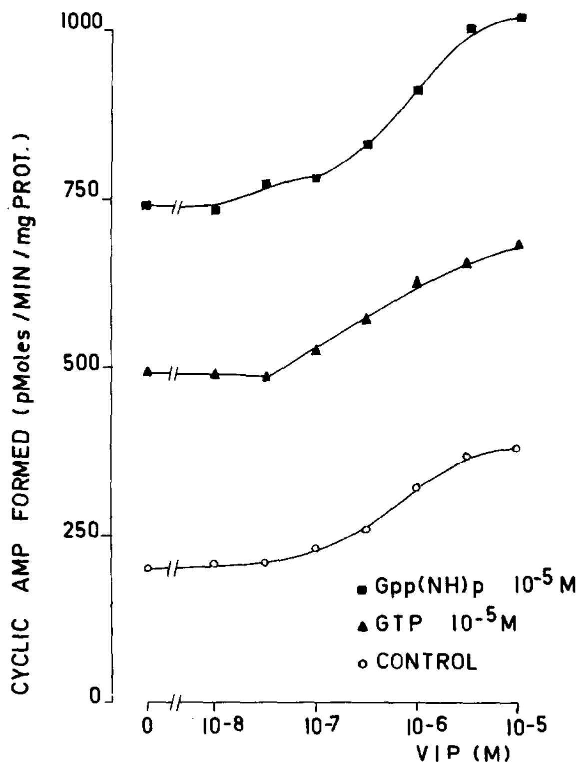

Fig.2. Dose–response curves of VIP-stimulated adenylate cyclase activity of a guinea pig brain synaptosomal fraction in absence (○——○) and presence of 10^{-5} M GTP (▲——▲) or 10^{-5} M Gpp(NH)p (■——■). The results obtained in the standard assay conditions (7 min incubation at 37°C) are the mean of three experiments.

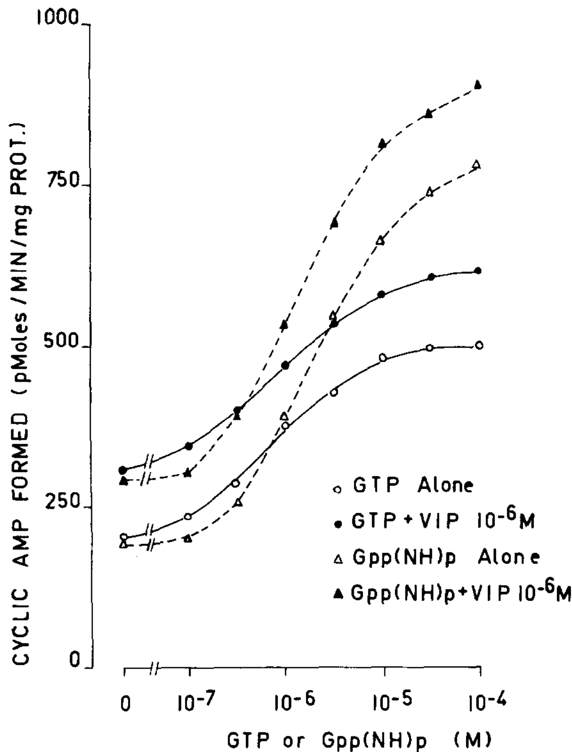

Fig.3. Dose–response curves of guanyl triphosphate nucleotide-stimulated adenylate cyclase activity of a guinea pig synaptosomal fraction in absence (open symbols) or presence (closed symbols) of 10^{-6} M VIP. The results for GTP (○,•) and Gpp(NH)p (△,▲) are the mean of three experiments.

pine). Furthermore, the hormonal peptides possessing structural similarities with VIP (secretin, glucagon and somatostatin) were devoid of any significant effect on basal or VIP-stimulated adenylate cyclase (table 1).

EGTA 0.5 mM moderately decreased the basal value but did not modify the VIP-stimulated activity. On the contrary, it systematically enhanced the stimulation by Gpp(NH)p. In the absence of EGTA, calcium at micromolar concentrations had no influence on either basal, guanyl triphosphate nucleotide- and VIP-stimulated adenylate cyclase. At concentrations higher than 10^{-5} M, calcium appeared to be an inhibitor of all kinds of adenylate cyclase activations (fig.4).

4. Discussion

Among the putative neurotransmitters of the central nervous system, some are thought to act via

Table 1

Effects of phentolamine, propranolol, atropine and peptides of the secretin family on basal and VIP-stimulated adenylate cyclase

Drug or peptide		Adenylate cyclase activity (pmol formed/min/mg protein)	
		Basal	+ 10^{-6} M VIP
Control		190	389
Phentolamine	10^{-3} M	190	382
Propranolol	10^{-3} M	179	412
Atropine	10^{-3} M	171	350
Secretin	10^{-5} M	195	392
Glucagon	10^{-5} M	183	378
Somatostatin	10^{-5} M	164	347

Incubations were performed under standard conditions (37°C, 7 min incubation) and the results are the mean of two experiments made in duplicate

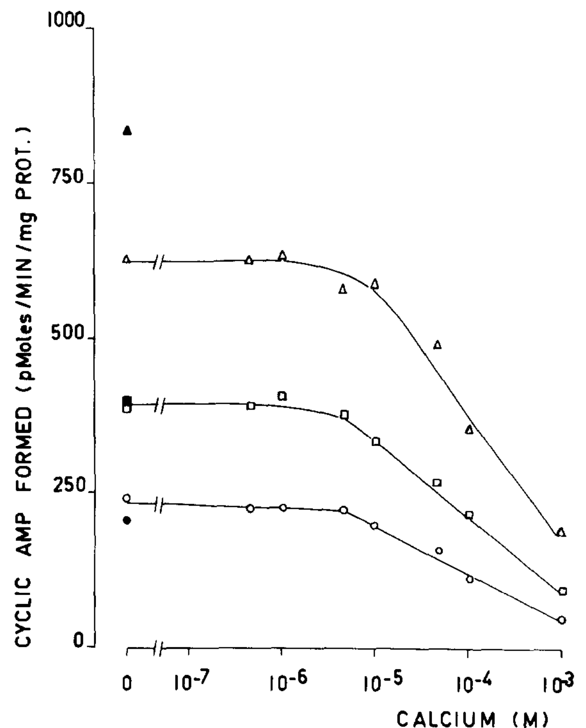

Fig.4. Effect of 0.5 mM EGTA (closed symbols) and increasing concentrations of calcium (open symbols) on basal (○,•); 3.10^{-6} M VIP (□,■); and 3.10^{-5} M Gpp(NH)p (△,▲) stimulated adenylate cyclase of a guinea pig brain synaptosomal fraction. The results are the mean of three experiments.

adenylate cyclase activation. This is the case for biogenic amines like norepinephrine, dopamine, serotonin and histamine [14], although these substances do not always perfectly satisfy Sutherland's four essential criteria [15]. The recently discovered brain peptides have been less extensively studied and only substance P has been reported to activate a particulate brain adenylate cyclase [16].

Our results clearly demonstrate that VIP must be added to the list of the brain substances activating an adenylate cyclase in brain extract.

With respect to adenylate cyclase activation, there were two main differences between brain VIP receptors and the VIP receptors of mammalian liver, pancreas and adipose tissue:

1. Brain receptors were highly specific: they did not recognize the parent hormones, glucagon and secretin. Brain VIP-stimulated adenylate cyclase, like the VIP-stimulated enzyme from rat liver [9], guinea pig pancreas [17] and rat adipose tissue [18] was not influenced by glucagon. But, the latter tissues are in fact influenced by secretin: guinea pig pancreatic acinar cells, for example, possess two distinct VIP receptor sites coupled with an adenylate cyclase, both of which are occupied to variable degrees by secretin [17]. The VIP-sensitive brain adenylate cyclase, however, remained unaffected by secretin.

   Somatostatin can be considered as a secretin-family peptide analog on the basis of a common tetrapeptide sequence [19]. Here again, somatostatin was a partial agonist of secretin-induced cyclic AMP accumulation in rat pancreas fragments [19], and had no effect on the brain VIP-sensitive adenylate cyclase.

2. Brain VIP-sensitive adenylate cyclase was not potentiated by guanyl triphosphate nucleotides: in contrast to liver [20] and pancreas [21], the effect of GTP and Gpp(NH)p on VIP-stimulated enzyme were purely additive, suggesting an independence of the hormonal and guanyl nucleotides sites.

In some brain adenylate cyclase preparations, calcium is reported to be an activator of the basal adenylate cyclase activity at low concentrations and to be an inhibitor at higher concentrations [22]. In our preparation, low micromolar calcium concentrations were without effect but concentrations higher than 10^{-5} M were inhibitory on basal, guanyl triphosphate nucleotide- and VIP-stimulated activity. Similar findings have been described for histamine-stimulated adenylate cyclase in guinea pig brain [23].

In conclusion, the presence of a highly specific VIP-sensitive adenylate cyclase in a guinea pig synaptosomal fraction supports the concept that the VIP present in brain cortical synaptosomes could be a neurotransmitter in the mammalian brain.

Acknowledgements

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