Inhibitory effect of somatostatin on basal and vasoactive intestinal peptide-stimulated cyclic AMP production and cyclic AMP-dependent protein kinase activity in HeLa cells

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1. INTRODUCTION

Both vasoactive intestinal peptide (VIP) and somatostatin are present in central nervous system as well as in specialized cells or nerves which are located in close proximity to the endocrine or exocrine cells on which they are presumed to act [1,2]. Therefore the notion has arisen that these peptides may act together or separately with a dual role as paracrine or neurocrine agents on target tissues.

It is well accepted that the interaction of VIP with target cells induces a cascade of events, including the activation of adenylate cyclase [1,3–7] and cyclic AMP-dependent protein kinase [8,9]. On the contrary, the involvement of the cyclic AMP system in the mechanism of action of somatostatin has been questioned [1011]. Nevertheless, the inhibitory role of somatostatin on cyclic AMP production [12–14] and cyclic AMP-dependent protein kinase [15,16] has been shown in a variety of cell preparations.

To understand further the molecular mechanism of somatostatin action, we have simultaneously studied here the effect of this peptide on cyclic AMP production and cyclic AMP-dependent protein kinase activity in HeLa cells. This malignant cell line derives from a human carcinoma of the uterine cervix and possesses high-affinity binding sites for VIP and a VIP-dependent adenylate cyclase system [17] coupled to cyclic AMP-dependent protein kinase [18]. We have also determined the antagonistic relationship between VIP and somatostatin on the regulation of both cyclic AMP production and cyclic AMP-dependent protein kinase, 2 parameters of great importance in cellular processes such as the cell cycle and development associated with growth, proliferation, differentiation and hypertrophy of cells.

2. MATERIALS AND METHODS

Procedures for growth in culture, collection and washing of HeLa cells have been described in [17,18].

Cyclic AMP production was studied as in [17]. The incubation medium (0.5 ml final vol.) consisted of 35 mM Tris–HCl buffer (pH 7.5), 50 mM NaCl, 1.4% (w/v) bovine serum albumin, 1 mg bacitracin/ml, 0.2 mM 3-isobutyl-1-methylxanthine (IBMX) and, when indicated, somatostatin and/or VIP. The addition of 2×10^5 cells/ml was followed by 45 min incubation at 15°C. Then, 2.5 ml methanol was added and, after centrifugation, the supernatant was evaporated and cyclic AMP determined by a protein binding assay [21].

Experiments on cyclic AMP-dependent protein kinase activity were conducted as in [18]. The assay was performed in the same medium as above, supplemented with 5 mM $MgCl_2$ (0.25 ml final vol), at the indicated concentrations of somatostatin and/or VIP. The reaction was initiated by the addition of 10^6 cells/ml followed by 15 min incubation at 15°C. Then, 0.1 ml cold Triton X-100 (0.4%) in 35 mM Tris–HCl buffer (pH 7.5) was added. After sonication the lysate was centrifuged at 10 000 $\times$ *g* for 2 min. Protein kinase activity in the resulting supernatant was determined as in [22] and expressed as activity ratio, i.e., the activity determined

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without added cyclic AMP (–) vs the activity determined in the presence of an excess (10^{-5} M) of cyclic AMP (+).

Values are mean ± SEM. Statistical analysis was carried out by Student's *t*-test. Synthetic somatostatin and VIP were from Peninsula Labs; bacitracin, bovine serum albumin and cyclic AMP from Sigma; and IBMX from Aldrich.

3. RESULTS

As expected, VIP stimulated cyclic AMP production in HeLa cells in a dose-dependent manner (fig.1). Half-maximal stimulation was observed at 2.5 ± 0.3 nM VIP and maximal stimulation (3.5-times above basal level) at > 10 nM peptide.

Somatostatin, at 1 μM, significantly inhibited basal and VIP-stimulated cyclic AMP levels (fig.1) at 0.1–300 nM added VIP. In this condition, half-maximal stimulation of cyclic AMP production was elicited by 1.9 ± 0.3 nM VIP (not significantly different from the value obtained in the absence of somatostatin), and maximal stimulation was again 3.5-times above basal level. Basal cyclic AMP was inhibited by 40% at 1 μM somatostatin whereas the increment produced by a maximally effective VIP concentration was reduced by 60%.

Table 1

Effects of VIP and somatostatin on cyclic AMP-dependent protein kinase activity

	Specific activity ratio (– cyclic AMP/ + cyclic AMP)
Basal	0.51 ± 0.01
30 nM VIP	0.82 ± 0.07
1 μM Somatostatin	0.47 ± 0.02
+30 nM VIP	0.60 ± 0.06

HeLa cells (10^6 cells/ml) were incubated at 15°C for 15 min in 0.25 ml medium containing either no effector, VIP, somatostatin or both peptides. Results are the mean ± SEM (n = 4) of the protein kinase activity ratio, each assay being made in triplicate without and with 10 μM cyclic AMP. Differences with respect to basal value are all statistically significant ($p < 0.05$ or lower)

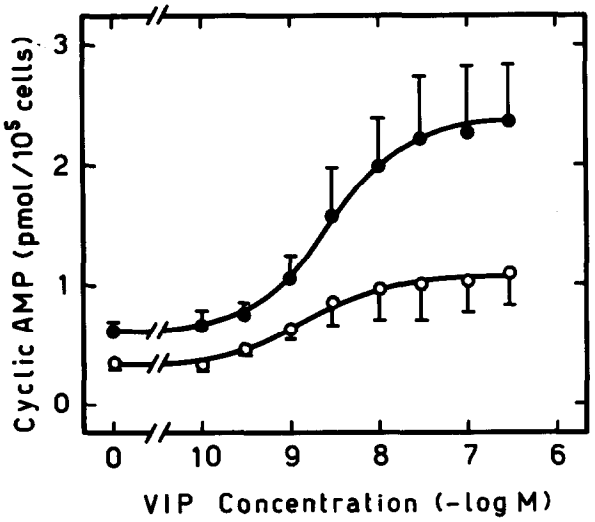

Fig.1. Ability of increasing doses of VIP to stimulate cyclic AMP production in the absence (•) or presence (○) of 1 μM somatostatin. Each point is the mean ± SEM of 3 expt, each performed in triplicate. When comparing both curves, differences were statistically significant ($p < 0.05$ or lower) throughout the whole range of VIP concentrations as well as in the absence of this peptide.

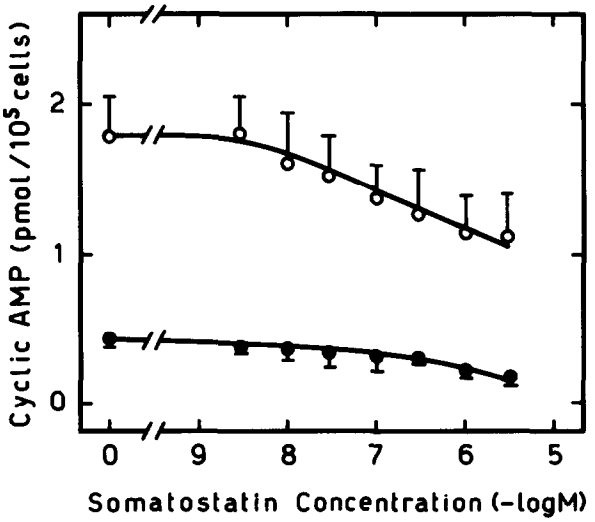

Fig.2. Ability of increasing doses of somatostatin to inhibit cyclic AMP levels in the absence (•) or presence (○) of 30 nM VIP. Each point is the mean ± SEM of 3 expt, each performed in triplicate. Somatostatin at ≥ 1 μM elicited a statistically significant ($p < 0.05$) inhibition of either basal or VIP-stimulated cyclic AMP levels.

The inhibitory effect of somatostatin was dose-dependent (fig.2). Both basal and VIP (30 nM)-stimulated cyclic AMP production were progressively inhibited by increasing concentrations of somatostatin, significant decreases being first observed at 1 μM. We did not test somatostatin doses > 3 μM.

The ability of somatostatin to inhibit cyclic AMP-dependent protein kinase in HeLa cells is shown in table 1. A slight but significant effect on basal activity could be defined. Furthermore, 1 μM somatostatin inhibited by 60% the increase in the activity ratio elicited by 30 nM VIP.

4. DISCUSSION

These data demonstrate a somatostatin induction in HeLa cells of decreased cyclic AMP levels related to other cellular signals such as cyclic AMP-dependent protein kinase activity in basal as well as in VIP-stimulated conditions. Furthermore, these results suggest that HeLa cells must have specific receptors for somatostatin. However, it is not clear whether this peptide acts by first interacting with a membrane site or after penetrating into the cell. In fact, peptides of the size of somatostatin or larger can enter into cells [23].

The observed inhibitory effect of somatostatin on cyclic AMP production in HeLa cells appears to be related to a decreased activity of adenylate cyclase more than to an increased activity of cyclic nucleotide phosphodiesterase. In fact, experiments were performed in the presence of a concentration of phosphodiesterase inhibitor that blocks the action of the enzyme in this cell preparation [17].

These results indicate that somatostatin antagonizes the known [17,18] stimulatory effect of VIP on cyclic AMP production and cyclic AMP-dependent protein kinase activity in HeLa cells. Somatostatin appears to impair the efficiency (the magnitude of the induced stimulation) of VIP on adenylate cyclase without altering the potency (referred to the concentration of effector used) of this peptide. In fact, half-maximal stimulation of cyclic AMP production was observed at a similar VIP concentration both in the absence or presence of somatostatin. Furthermore, the observed inhibitory effect of somatostatin alone on cyclic AMP levels as well as on cyclic AMP-dependent protein kinase activity cannot explain the results obtained in the simultaneous presence of VIP and somatostatin.

With respect to the biological significance of the action of VIP and somatostatin in HeLa cells, it remains a matter of conjecture. Since proliferation and differentiation of cells appear to be related to cyclic AMP levels, cyclic AMP-dependent protein kinase activity and protein phosphorylation [19,20], HeLa cells provide a valuable model to study the role of both peptides in such processes. In another context, it should be remembered that these malignant cells derive from the epithelium of the uterine cervix, a site where VIP and somatostatin have been identified as neuropeptides [24]. Then, they could be liberated in situ at high levels and participate in the local regulation of some physiological functions. Studies on normal cells should permit to learn the modifications of somatostatin and VIP actions during the malignant transformation as well as the involvement of both peptides in functions of the genito-urinary tract.

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