Volume 218, number 1, 73–76 FEB 04781 June 1987

Somatostatin inhibits VIP- and isoproterenol-stimulated cyclic AMP accumulation in rat prostatic epithelial cells

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Received 8 April 1987

The dual regulation of cyclic AMP accumulation was studied in rat prostatic epithelial cells incubated with somatostatin, vasoactive intestinal peptide (VIP), and the β-adrenergic agent isoproterenol. Somatostatin noncompetitively inhibited the stimulatory effect of VIP and isoproterenol, but it did not alter basal cyclic AMP levels. In addition to the multifactorial regulation of the cyclic AMP system in rat prostatic epithelium, these results suggest that somatostatin may play a physiological role at this level.

Somatostatin; VIP; Isoproterenol; cyclic AMP

1. INTRODUCTION

The prostate is considered to be controlled primarily by androgens although it seems to become increasingly evident that some peptide hormones and neuromolecules could also be involved at this level [1]. Previous studies from our laboratory have shown that the prostatic cyclic AMP system is activated by a variety of agonists including classical neurotransmitters such as β-adrenergic agonists [2] as well as more complex agents such as the neuropeptide vasoactive intestinal peptide (VIP) [3]. In contrast, the regulatory role of hormones or other factors negatively coupled to adenylate cyclase has not been as extensively investigated since only muscarinic receptors are known to lead to negative responses in this gland [4].

Recent immunohistochemical studies have demonstrated the presence of somatostatin endocrine-paracrine cells in the prostate gland [5,6] thus making possible a physiological involvement of this hormone in prostatic growth and functions. Somatostatin is a well known inhibitor of cyclic AMP synthesis stimulated by a number of agents including VIP [7] and catecholamines [8] in other tissues. The present study examined the action of somatostatin, both alone or in combination with VIP and the β-adrenergic agonist isoproterenol, upon cyclic AMP accumulation in isolated epithelial cells of rat ventral prostate. Consequently, the results extend present knowledge on the dual regulation of prostatic cyclic AMP and represent the first indication of a direct action of somatostatin in this gland.

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2. MATERIALS AND METHODS

Synthetic rat VIP was from Peninsula (St. Helens, England), 3-isobutyl-1-methylxanthine from Aldrich (Milwaukee, WI), and DL-isoproterenol, bacitracin and bovine serum albumin from Sigma (St. Louis, MO). Somatostatin was a generous gift from Serono (Madrid, Spain).

The procedures for preparing epithelial cells from the ventral lobes of rat prostate [3,9] and for incubating the cells in order to study cyclic AMP accumulation [3] have been described. Cell-associated endogenous materials that could in-

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00145793/87/$3.50

terfere with results were removed by preincubating the isolated cells for 30 min at 37°C in 9% NaCl, followed by washing. More than 90% of the cells were viable as measured by exclusion of trypan blue dye. Protein determination was performed using bovine serum albumin as a standard [10]. The basal medium used in all experiments consisted of 35 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1.4% (w/v) bovine serum albumin, 1 mg/ml bacitracin, and 0.2 mM 3-isobutyl-1-methylxanthine. Cyclic AMP levels were determined after a 60-min incubation of cells (0.3 mg protein/ml) at 15°C in the absence or presence of VIP, isoproterenol and somatostatin at a total volume of 0.5 ml. The reaction was stopped by the addition of 2.5 ml cold methanol, and the suspension was centrifuged for 10 min at 2000 × *g*. The supernatant was evaporated for the determination of cyclic AMP [11]. The data shown represent means ± SE of experiments performed in triplicate.

3. RESULTS

As shown in fig.1, 100 nM somatostatin inhibited in a non-competitive manner the stimulatory effect of VIP upon cyclic AMP accumulation in rat prostatic epithelial cells without altering the basal level. The effect of a maximally active (100 nM) VIP concentration was reduced by about 60% in the presence of somatostatin. Half-maximal stimulation (ED_{50}) of cyclic AMP was elicited by about 1 nM VIP both in the absence and presence of the inhibitory hormone.

A qualitatively similar pattern was obtained when studying the action of somatostatin on the cyclic AMP response to the β-adrenergic agent isoproterenol (fig.2). The ED_{50} was observed at about 100 nM isoproterenol and did not vary with the addition of 100 nM somatostatin whereas this concentration of the inhibitory hormone resulted in a 75% decrease of the cyclic AMP response at the maximally effective dose (100 μM) of the β-adrenergic agonist.

The data in fig.3 show that somatostatin did not alter basal cyclic AMP levels in rat prostatic epithelial cells throughout the whole range of concentrations studied (up to 3 μM). However, the inhibitory role of this hormone was shown to depend on the dose used upon both VIP- and isoproterenol-stimulated cyclic AMP levels. The stimulatory effect of an intermediate concentration (10 nM) of VIP was completely abolished by high concentrations of somatostatin, half-maximal inhibition

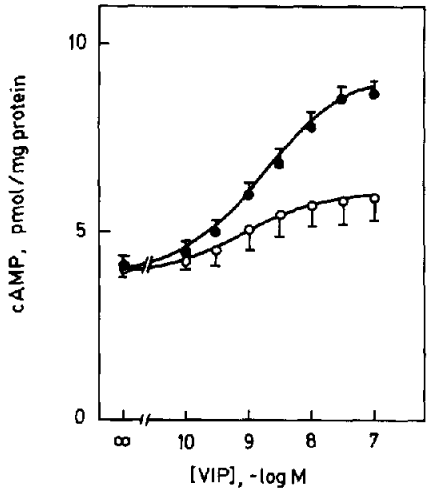

Fig.1. Cyclic AMP accumulation in rat prostatic epithelial cells incubated with increasing concentrations of VIP in the absence (●) or presence (○) of 100 nM somatostatin. Values are the means ± SE of six triplicate experiments.

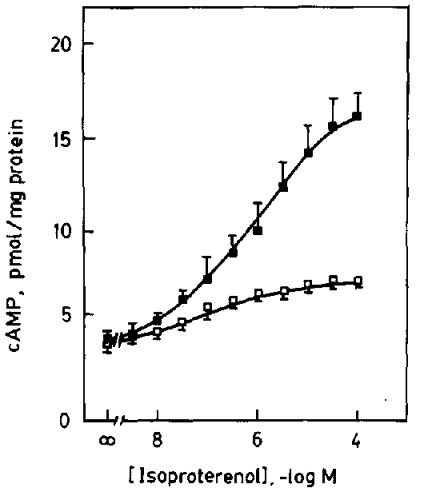

Fig.2. Cyclic AMP accumulation in rat prostatic epithelial cells incubated with increasing concentrations of isoproterenol in the absence (■) or presence (□) of 100 nM somatostatin. Values are the means ± SE of six triplicate experiments.

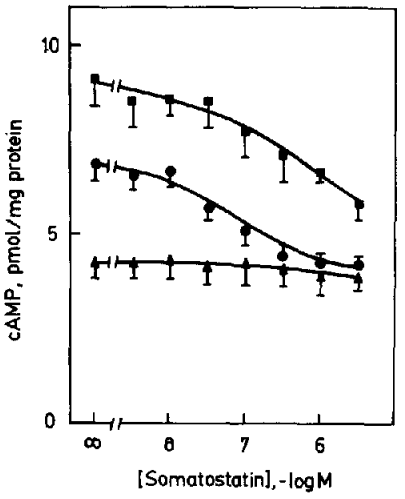

Fig.3. Cyclic AMP accumulation in rat prostatic epithelial cells incubated with increasing concentrations of somatostatin in the absence (▲) or presence of either 10 nM VIP (●) or 1 μM isoproterenol (■). Values are the means ± SE of six triplicate experiments.

(IC_{50}) being observed at about 70 nM. In concert with these results, increasing doses of somatostatin caused a continuous decrease of isoproterenol (1 μM)-stimulated cyclic AMP levels (IC_{50} = 1 μM) although absolute blocking of the stimulatory response could not be obtained at a somatostatin concentration as high as 3 μM.

4. DISCUSSION

This study indicates that somatostatin inhibits both VIP- and isoproterenol-stimulated cyclic AMP accumulation in rat prostatic epithelial cells by a noncompetitive mechanism without altering the basal cyclic AMP content. It represents the first description of the involvement of somatostatin in the cyclic AMP system of the prostatic epithelium. The present data together with previous results on the characterization of somatostatin-containing cells in the prostate gland [5,6] suggest a physiological involvement of the hormone acting in a local manner at this level. Furthermore, the increase of the number of hormones and neurotransmitters evoking a response from the cyclic AMP system [2–4] implies that the control of prostatic epithelial function may be exceedingly complex, involving not only androgens but also many other factors.

The characteristics of the inhibition by somatostatin of the VIP- and β-adrenergic-induced cyclic AMP accumulation in rat prostatic epithelial cells are similar to those previously described in other systems such as pituitary tumor cells [7] and gastric epithelial glands [8]. Somatostatin was without effect on basal cyclic AMP levels whereas VIP and isoproterenol exerted a permissive role upon the somatostatin inhibitory response. Furthermore, somatostatin decreased maximal stimulation of cyclic AMP accumulation by both VIP and isoproterenol but did not alter their potencies. On the other hand, the observed changes in cyclic AMP levels are likely to result from modulation of adenylate cyclase activity since the experiments were performed in the presence of a high concentration of phosphodiesterase inhibitor. In fact, accumulating evidence indicates that regulation of adenylate cyclase may be the main mechanism for somatostatin actions in many cell types [12]. Therefore, the present results in rat prostatic epithelial cells suggest the existence of somatostatin receptors coupled to adenylate cyclase in an inhibitory manner, probably through a guanosine nucleotide-binding protein [13]. However, further studies are required to understand the precise role of cyclic AMP in the action of somatostatin at this level since a cyclic AMP-independent mechanism may also be involved in some somatostatin effects in various tissues [7]. Finally, the exact nature of somatostatin receptors in prostatic epithelium remains to be established since biologically active somatostatin receptors appear to exist in both plasma membrane and intracellularly in various systems [14]. Thus, at the present time, we cannot determine whether somatostatin exerts the studied effects by first interacting with a membrane site or after penetrating into the prostatic epithelial cells.

ACKNOWLEDGEMENTS

This research was supported by grants from the Comisión Asesora de Investigación Científica y Técnica and the Fondo de Investigaciones Sanitarias.

REFERENCES

[1] Webber, M.M. (1981) in: The Prostatic Cell: Structure and Function, Part B (Murphy, G.P. et al. eds) pp.63–68, A.R. Liss, New York.
[2] Carmena, M.J., Sancho, J.I. and Prieto, J.C. (1986) Biochem. Int. 13, 479–485.
[3] Carmena, M.J. and Prieto, J.C. (1983) Biochim. Biophys. Acta 763, 414–418.
[4] Carmena, M.J. and Prieto, J.C. (1985) Biosci. Rep. 5, 791–797.
[5] Sant'Agnese, P.A. and Jensen, K.L.M. (1984) Arch. Pathol. Lab. Med. 108, 693–696.
[6] Pekary, A.E., Yamada, T., Sharp, B., Bhasin, S., Swerdleff, R.S. and Hershman, J.M. (1984) Life Sci. 34, 939–945.
[7] Dorflinger, L.J. and Schonbrunn, A. (1983) Endocrinology 113, 1551–1558.
[8] Boige, N., Dupont, C., Chenut, B., Gespach, C. and Rosselin, G. (1984) Eur. J. Clin. Invest. 14, 42–48.
[9] Jung-Testas, I., Groyer, M.T., Bruner-Lorand, J., Hachter, O., Baulieu, E.E. and Robel, P. (1981) Endocrinology 109, 1287–1289.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Gilman, A.G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[12] Koch, B.D. and Schonbrunn, A. (1984) Endocrinology 114, 1784–1790.
[13] Dolphin, A.C. (1987) Trends Neurosci. 10, 53–58.
[14] Steiner, C., Dahl, R., Sherman, N., Trowbridge, M., Vatter, A., Robbins, R. and Draznin, B. (1986) Endocrinology 118, 766–772.