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SOMATOSTATIN-14 INCREASES THE INOSITOL-1,4,5-TRISPHOSPHATE CONTENT IN VARIOUS AREAS OF THE BRAIN

Agnieszka Lachowicz[1], Marek Pawlikowski[1] and Tomasz Ochędalski[2]

[1]Institute of Endocrinology, Department of Experimental Endocrinology and Hormone Research, [2]Institute of Obstetrics and Gynaecology, Medical University of Łódź, Dr. Sterling Str. 3, 91-425 Łódź, Poland

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Summary: The effects of somatostatin-14 on inositol-1,4,5-trisphosphate (IP_3) content were examinated in rat pituitary, hippocampus and cortex homogenates. Somatostatin increased IP_3 concentration in all these investigated regions in a dose- and time-dependent manner. Maximal increase of IP_3 content was found in the pituitary homogenate, and in the hippocampal and cortex homogenates the IP_3 argumentation was slightly smaller. Time-response studies showed that this effect declined with extended time of incubation. These results suggest that IP_3 is involved in the mechanism of action of somatostatin in brain.

Somatostatin, a widely distributed neuropeptide also with a wide range of action, modulates many physiological and pathological functions, including cell proliferation and hormone secretion [1]. Molecular mechanism of action of this peptide is linked with calcium ions and cyclic nucleotides, mainly cAMP. Somatostatin has a partially inhibitor influence on cAMP production and intracellular Ca^{2+} concentration, via modulating kalium channels activity [1]. The pivotal role of inositol-1,4,5-trisphosphate (IP_3) as a calcium mobilizing second messenger for many hormonal, neurotransmitter and growth factor signals has been well demonstrated in a variety of cells, including brain [2]. This product of the phosphatidiloinositol-1,4,5-bisphosphate hydrolysis by phospholipase C can release calcium from its nonmitochondrial stores [2]. The role of IP_3 in somatostatin action has not been well documented yet. There is a rapidly accumulating evidence, that neuropeptides which control such

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a complicated process as proliferation of a variety of cell types, acting through distinct signals transduction pathways [3]. In our previous report we showed that somatostatin can modulate IP_3 content in the mouse spleen lymphocytes [4]. It was then of interest to examine the possible influence of somatostatin on the IP_3 concentration in the brain and especially in the pituitary gland, a specific site of somatostatin action.

MATERIALS AND METHODS

The experiments were performed in vitro. Male, Wistar rats weighing 180 - 220 g were used. The rats were kept in light- and temperature-controlled rooms with tap water and food available ad lib. After decapitation, the pituitary gland, hippocampus and cortex were immediately removed, weighed and homogenized at 0 - 4°C. Protein content in the samples was estimated according to the method of Ohnishi and Barr [5], with bovine serum as a standard.
Somatostatin was obtained from Sandoz, Basle, Switzerland. The dose-dependent relationship was investigated using peptide concentration from 10^{-11}M to 10^{-5}M. The time-response relationship was studied using incubation times of 3, 5, 15 and 30 min, with 10^{-7}M of somatostatin.

Extraction of the samples.

Homogenates were extracted by mixing with 20% ice-cold perchloric acid and then centrifugated at 200 g for 15 min. at 4°C. Supernatants were titrated to pH 7.5 with 10N KOH and kept ice-cold. All the assays were performed in triplicate.

Determination of IP_3 content.

Inositol-1,4,5-trisphosphate concentration was measured by using assay kits, obtained from Amersham International plc. The assay was based on the competition between unlabelled IP_3 and fixed quantity of ^{3}H-labelled IP_3 for binding sites of bovine adrenal binding protein preparation.

Statistical analysis.

The data were calculated as mean of values SD. Comparison between means was performed using ANOVA followed by Newman-Keul's test (statistical significance - $p < 0.05$). Basal levels of IP_3 was 22.43 $\pm$ 1.97 pmol/mg of protein.
The experiment was double repeated with similar results.

RESULTS

The results are shown in the figures (each point represents mean $\pm$SD). It is evident that the IP_3 content augments in all three investigated regions after the stimulation with somatostatin. The augumentation of the IP_3 concentration was dramatic in the pituitary homogenates (Fig. 1). In the hippocampus and cerebral cortex the increase of IP_3 concentration was smaller and at the same level in both studied homogenates (Fig. 2). In all studied areas of the rat brain the most stimulatory dose was 10^{-7}M of somatostatin.

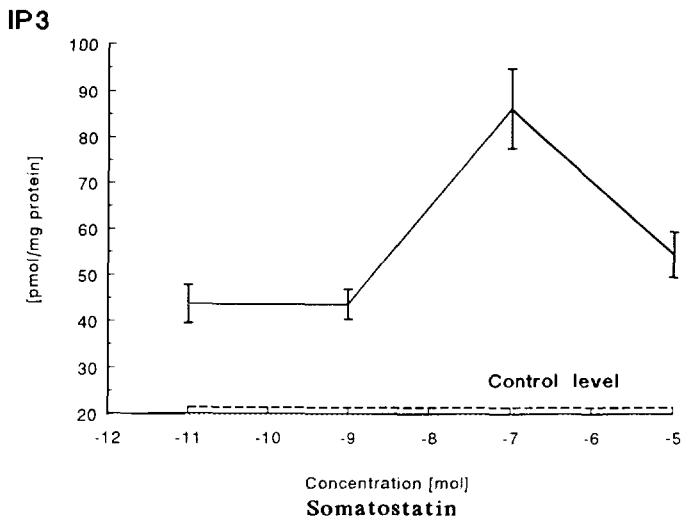

Fig. 1. The effect of somatostatin-14 on IP_3 concentration in pituitary homogenate. Dose-dependent relationship.

We also observed that the largest increase of the IP_3 content occurred in a short time of incubation (3 min.). After 5 minutes of incubation the level of IP_3 gradually decreased and after 15 min was similar to control (Fig. 3).

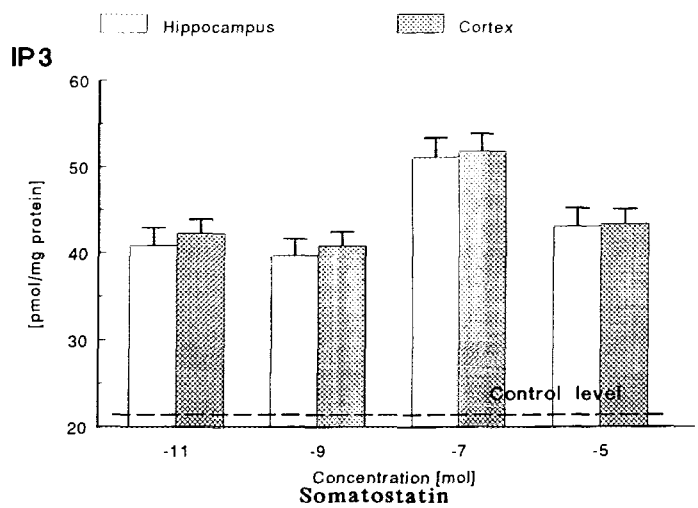

Fig. 2. The concentrations of IP_3 in cerebral cortex and hippocampus homogenates after various doses of somatostatin-14.

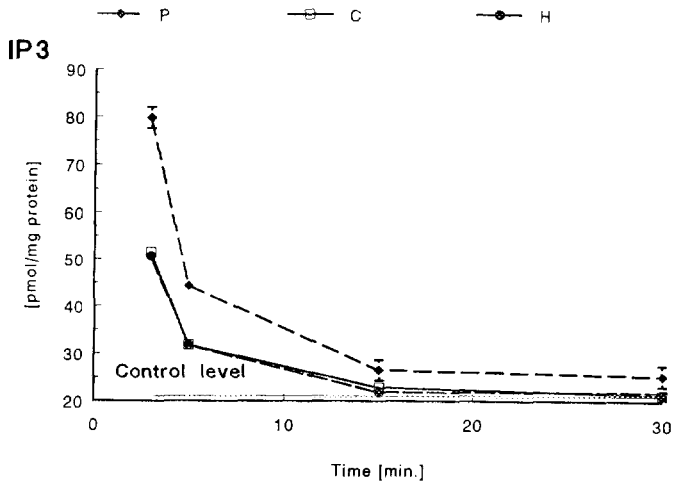

Fig. 3. The changes of IP_3 content in all studied areas (P - pituitary, C - cerebral cortex, H - hippocampus) at the various times of incubation. Concentration of somatostatin-14 used in this experiment - 10^{-7}M.

DISCUSSION

In our previous report we have shown the modulation of the inositol pathway by somatostatin in spleen lymphocytes [6]. In this previous study we have found that SST decreased IP_3 concentration and we have hypothesized that this process could contribute (at level in part) in the inhibitory action of somatostatin. In the present study we report that somatostatin increases the IP_3 concentration in the rat brain and anterior pituitary. Since somatostatin in the anterior pituitary has mainly inhibitory effect on hormone secretion as well as on cell proliferation, we have expected rather a decrease of phospatidiloinositol turnover. But it should be emphasized that there is a multiplicity of SST receptor subtypes [7]. The binding of somatostatin to these subtypes could result in different signals. Some of these subtypes are not at all coupled to G-proteins or coupled to different G-proteins [8]. Thus, we speculate that brain and anterior pituitary SST receptors differ to those present in lymphocytes not only in structure but also in signals detected by them. Intracellular Ca^{2+} concentration is submitted to various modulating processes and IP_3 is only one among numerous factors which are able to change it. Of course, the rise of the IP_3 cused by somatostatin may trigger the release of Ca^{2+} from the endoplasmic reticulum stores and subsequently cause a rise of cytosolic Ca^{2+} [2], but this phenomenon could be

have various functional consequences. First, a transient rise of cytosolic Ca^{2+} is indispensable, among others, for hyperpolarization of the plasma membrane through the activation of Ca-dependent kalium channels [1,9]. Hyperpolarization of plasma membrane leading to a closure of calcium channels is considered to be responsible for decrease intracellular calcium caused by somatostatin [9]. Another explanation of the observed phenomenon is provided by the quantal nature of IP_3-evoked Ca^{2+} release. Depending on its concentration, IP_3 can generate only local Ca^{2+} spikes or repetitive Ca^{2+} waves [10]. Thus, IP_3 concentration determines whether the cytosolic Ca^{2+} signals are local or global. It is difficult to decide whether IP_3 concentration observed in our study were sufficient to generate global or only local signals, because IP_3 level needed to evoke a particular kind of signal could change depending on various factors (e.g. intracellular concentration of various ions). Somatostatin is known to rapidly decrease the intracellular calcium concentration [1], so it is also possible that increase of IP_3 concentration showed by us represents a mechanism that counteract this process and protect the cell against the extensive drop of intracellular Ca_{2+} concentration.

Although the best known intracellular action of somatostatin is connected with cAMP pathway, it should to be emphasized that neurotransmitters that are coupled to the formation of cAMP can also inhibit or activate the hydrolysis of phosphatidiloinositol by phospholipase C, depending on the type of receptor and G-protein to which it couples [11]. A number of reports have evidence for biochemical and functional interactions occurring between IP_3 - Ca^{+2} and cAMP systems in various tissues [11, 12]. Phorbol esters and protein kinase C could alter the activity of adenylate cyclase in GH_3 cells, the cerebral cortex, erythrocytes, rat pinealocytes and smooth muscle [11]. On the other hand, cAMP may also influence IP_3 production, e.g. in dog sphincter, where low concentrations of carbachol increased the IP_3 production and, simultaneously, cAMP formation inhibition [11]. It is possible that the increase of IP_3 content observed in our study was caused by this "cross-talk" between cAMP and phosphoinositide systems. It may be a kind of feedback or an effect of an activation a specific G-protein, which regulates both cAMP and IP_3 formation processes.

The intracellular concentration of free calcium ions in cells fluctuates dramatically under a variety of stimuli. This Ca^{+2} concentration variability produces intracellular signals triggering a diversity of physiological, morphological and molecule-biological

events, including neurotransmitter action. Our results indicate that complex function of somatostatin as a modulator of many events needs the involvment of diverse systems of cellular signalling.

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