Effect of somatostatin on the mass accumulation of inositol-1,4,5-trisphosphate in rat hypothalamus, striatum, frontoparietal cortex and hippocampus

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Abstract

Somatostatin-14 (SS) significantly increased inositol-1,4,5-trisphosphate (IP_3) accumulation in rat hypothalamic, striatal, frontoparietal cortical and hippocampal slices. However, this stimulation of IP_3 accumulation by SS was highest in the frontoparietal cortex and hippocampus. The effect was already significant with 0.01 μM in the frontoparietal cortex ($P < 0.05$) and hippocampus ($P < 0.05$) and the maximal accumulation was evident with 0.1 μM SS, in all areas studied. A concentration of 1 μM SS, lacked this effect in hypothalamus and striatum. SS rapidly increased IP_3 accumulation in all brain areas studied. This effect was maximal at 15 s of incubation and decreased subsequently. At 60 s incubation, levels were still elevated in frontoparietal cortex and hippocampus but had returned to basal values in hypothalamus and striatum. Somatostatin-28 (SS-28) and the SS analogues, D-Trp^8-D-Cys^{14} and SMS 201–995, also significantly stimulated IP_3 accumulation although the effect of SMS 201–995 was greater than that of SS in the striatum in comparison with controls ($P < 0.001$ and $P < 0.01$, respectively). These results suggest that SS action at the hypothalamus, striatum, frontoparietal cortex and hippocampus is mediated at least in part by the accumulation of IP_3, which may initiate intracellular processes responsible for some biological SS effects.

Keywords: Somatostatin; Inositol-1,4,5-trisphosphate; Brain; Rat

The tetradecapeptide somatostatin-14 (SS), is widely distributed through the central nervous system (CNS) [22]. Its highest concentration is found in the hypothalamus [22]. SS acts in the CNS as a neurotransmitter or neuromodulator, in both an excitatory and inhibitory manner [7]. The actions of SS are mediated through its specific receptors (SSTRs) [6,7,19,26]. Up to now, five subtypes of SSTRs have been cloned (SSTR1 to SSTR5) [9,10]. SSTRs have been reported to be coupled to several intracellular effector systems, including adenylate cyclase [23] and ion channels [30,31]. Recent studies suggest that inositol-1,4,5-trisphosphate (IP_3) is also involved in the mechanism of action of SS [1,12,13,15,16,18,25]. The SSTRs in hypothalamus and striatum, two specific areas for SS action [7,8,28], are coupled to phospholipase C

(PLC) [1]. The facts that SS stimulates phosphoinositide (PI) hydrolysis in the hippocampus [12,25] and that the hippocampus and striatum are closely associated structures [27], suggest that SS can also modify IP_3 accumulation in this brain area. However, to date the effect of SS on accumulation of IP_3 in the hypothalamus and striatum is unknown. The present study was undertaken to determine the effect of SS on accumulation of IP_3 in rat hypothalamic and striatal slices. In order to compare with other brain areas, the frontoparietal cortex and hippocampus were also studied.

SS and somatostatin-28 (SS-28) were purchased from Universal Biologicals Ltd. (Cambridge, UK); SMS 201–995 from Sandoz (Basel, Switzerland); IP_3 and inositol-1,2,3,4,5,6-hexaphosphate (IP_6) from Calbiochem (San Diego, CA, USA); [^{3}H]IP_3 from Du Pont (Boston, MA, USA); and DL-dithiothreitol (DTT) from Sigma (St Louis, MO, USA).
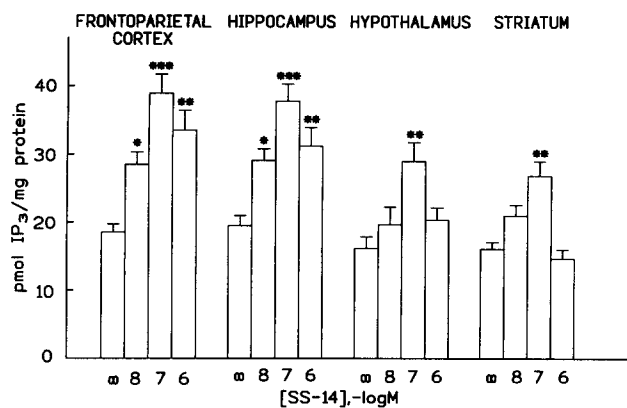
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Fig. 1. Concentration-dependent effect of SS-14 on the accumulation of IP₃ in frontoparietal cortex, hippocampal, hypothalamic and striatal slices. Brain slices were incubated for 15 s at 37°C with the different concentrations of SS-14. Values are expressed as the mean ± SEM of five separate experiments. Statistical comparison versus control: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Cross-chopped brain slices (350 × 350 μm) were prepared from male Wistar rats (230–250 g) and preincubated for 45 min at 37°C in Krebs bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 11.7 mM glucose, pH 7.4, saturated with O_2/CO_2 (95:5)) with buffer replacement and gassification every 15 min [4]. Slices were allowed to sediment and 50 μl of packed slices were added to 220 μl Krebs buffer; these samples were gassed regularly, capped and incubated. All additions of test substances were made in a volume of 30 μl. Incubations were terminated by addition of 300 μl 15% trichloroacetic acid (TCA). Acidified samples were left on ice for 15 min and centrifuged for 5 min at 2000 × g. The supernatants (500 μl) were left for 15 min at room temperature and then TCA was removed by five successive washings with 2 vols. of water-saturated diethyl ether and the addition of 125 μl 30 mM EDTA and 30 μl 6 mM $NaHCO_3$. The protein concentration was determined in the tissue pellets according to the method of Lowry et al. [14].

The specific binding of [³H]IP₃ to a particulate preparation of bovine adrenal cortex was used as a radioreceptor assay to determine the concentration of IP₃ in these slices by the method described by Challis et al. [4]. Aliquots of the binding protein obtained previously [4] were dissolved in 0.05% sodium azide, 5 mM EDTA, 5 mM EGTA, 50 mM TAPS (pH 8.6), at a protein concentration of 1.5 mg/ml. Then 3.5 × 10^{-2} μCi of [³H]IP₃ was added per ml of solution to prepare the receptor-tracer solution. Assays were performed in a final volume of 500 μl. Receptor-tracer solution (400 μl) was added to 100 μl of unknown samples or 100 μl of standard samples containing IP₃ (0.6–24 pmol in 1 mM EDTA, 1 mM EGTA, pH 7.5) or IP₆ (1% w/v in 20 mM EDTA, 20 mM EGTA, 0.05% sodium azide, pH 7.5) to define non-specific

binding. All tubes were incubated for 1 h at 4°C. Separation of bound and free IP₃ was achieved by centrifugation at 12 000 × g for 10 min. After aspiration of the supernatant, 50 μl of 0.15 M NaOH was added to each tube and the pellet dissolved by shaking. The radioactivity was determined by liquid scintillation spectrometry. The IP₃ content was determined by comparing the extent of the inhibition of [³H]IP₃ binding with a calibration curve obtained with known amounts of IP₃. Non-specific binding was about 13% of the total binding.

Statistical comparisons of all the data were analyzed by ANOVA and the Newman–Keuls t-test. Means between groups were considered significantly different when the P value was less than 0.05. Each individual experiment was performed in duplicate.

SS significantly increased IP₃ accumulation in all brain areas investigated and this increase was highest in the frontoparietal cortex and hippocampus (Fig. 1). The effect was already significant with 0.01 μM in the frontoparietal cortex ($P < 0.05$) and hippocampus ($P < 0.05$) and the maximal accumulation was evident at 0.1 μM SS, in all areas studied (2.1, 1.9, 1.8 and 1.7-fold increase over basal values in frontoparietal cortex, hippocampal, hypothalamic and striatal slices, respectively). A concentration of 1 μM SS lacked this effect in striatum and hypothalamus. Since there are five SS receptor subtypes, SSTR1–5, in the CNS [10], and their number, affinity for SS [10] and ability to activate PLC [1] all vary, an explanation for this result could be that IP₃ accumulation occurs with higher affinity receptors. Occupation of lower affinity receptors by SS might reduce accumulated IP₃.

SS rapidly increased IP₃ accumulation in all brain areas studied (Fig. 2). This effect was maximal at 15 s of incubation and decreased subsequently. After 60 s the levels in frontoparietal cortex and hippocampus were still elevated whereas those in hypothalamus and striatum returned to control values.
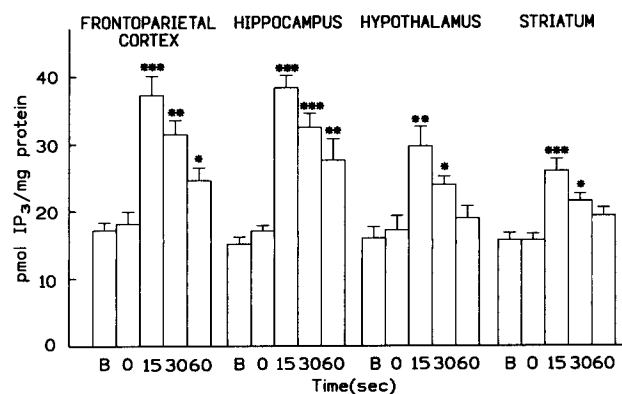

Fig. 2. Time-course effect of 10^{-7} M of SS-14-induced IP₃ accumulation in frontoparietal cortex, hippocampal, hypothalamic and striatal slices. Values are expressed as the mean ± SEM of five separate experiments. Statistical comparison versus control: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. B, basal.
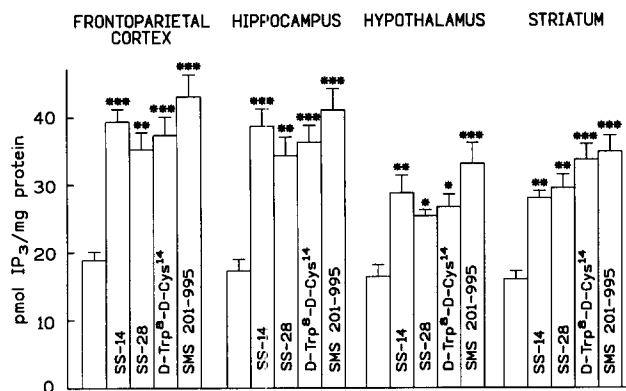
Fig. 3. Effect of various SS analogues on IP$_3$ accumulation in fronto-parietal cortex, hippocampal, hypothalamic and striatal slices. Brain slices were incubated for 15 s at 37°C with 10^{-7} M of the different SS peptides. Values are expressed as the mean ± SEM of five separate experiments. Empty bars correspond to the basal values. Statistical comparison versus control: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

The effects of SS-28 and two SS analogues, D-Trp[8]-D-Cys[14] and SMS 201–995, on IP$_3$ accumulation were also studied. All peptides significantly stimulated IP$_3$ accumulation although the effect of SMS 201–995 was higher than that of SS in the striatum in comparison with controls ($P < 0.001$ and $P < 0.01$ respectively) (Fig. 3). In the hypothalamus the effect of SMS 201–995 also seems to be greater than SS but this difference is not statistically significant (Fig. 3).

A similar effect of SS on IP$_3$ accumulation has already been observed in cerebral cortex [12], hippocampus [12,15], pituitary gland [12], bovine adrenal gland [18], SSTR1-expressing Chinese hamster ovary (CHO) cells [11] and COS-7 cells transfected with human SS receptors (hSSTR) [29]. The regional pattern of SS response may be accounted for by the regional distribution of SSTRs [6,26] or may be due to a regional difference in the efficiency of the coupling mechanism, perhaps via nucleotide-regulated protein [5] between the SSTRs subtypes and PLC.

The effect of SS on IP$_3$ accumulation seems to be a direct consequence of SSTR occupancy. Recently, it has been reported that cloned hSSTR subtypes 1–5 activate PLC and mobilize Ca^{2+} in transfected COS-7 cells [1]. Activation of PLC by SS is in the order hSSTR5 > hSSTR2 > hSSTR3 > hSSTR4 > hSSTR1 [1]. The hypothalamus shows a very high level of SSTR5 expression and moderate levels of other subtypes of SSTR coupled to PLC [10]. The fact that SS does not increase further IP$_3$ accumulation in the hypothalamus could be due to the high endogenous SS content [22]. Expression of these SSTR subtypes in striatum and other brain areas has been reported [10]. However, since cyclic AMP (cAMP) has been shown to inhibit PI breakdown in a number of cell systems [20] and SS decreases cAMP levels [23], it is possible that the IP$_3$ accumulation induced by SS could also occur at the cAMP level.

The SS concentration eliciting the maximal IP$_3$ accumulation was about two orders of magnitude higher than the corresponding binding affinities obtained in previous studies [6,19,26]. This discrepancy can be explained by the different experimental conditions used in each type of experiment. Our previous binding studies were obtained at equilibrium after an incubation of 60 min at 30°C [19]. In contrast, IP$_3$ accumulation is a transient process that must be measured 15 s after exposing brain slices to SS. Therefore, this very short time may not be sufficient to reach binding equilibrium. The fast and transitory character of IP$_3$ formation is compatible with a function of this molecule as a second messenger.

Whether or not the SS-evoked IP$_3$ accumulation in the slices is derived from neuronal cells is unknown. In this regard, SS is known to potentiate de-α_1-adrenergic activation of PLC in striatal astrocytes [16]. Therefore, the possibility that the SS-evoked increase in IP$_3$ accumulation in the studied brain slices could be derived from glial cells cannot be excluded. SS directly activates serotonergic and dopaminergic nerve terminals to release serotonin [28] and dopamine [8], respectively, and this is additional support for the notion that SS acts, at least in part, on IP$_3$ signaling in the hypothalamic, striatal, hippocampal and cortical neurons.

The robust IP$_3$ response produced by SMS 201–995 may be due to the activation of SSTR subtypes that have very high affinity (SSTR2, SSTR5) or some affinity (SSTR3) for this SS analogue [10] and that powerfully activate the PLC-Ca^{2+} system [1].

The accumulation of IP$_3$, which is known to release calcium from internal stores [2] may provide a mechanism for the SSTRs-mediated cellular effects of SS. A few studies have reported that SS activates PLC followed by Ca^{2+} mobilization in native SS receptor-expressing cells such as striatal astrocytes [16] and NG 108–15 neuroblastoma × glioma hybrid cells (NG 108–15 cells) [21]. A requirement for Ca^{2+}-dependent phospholipase A$_2$ activation has also been postulated in SS actions in hippocampal cells [24]. SS facilitates neurotransmitter release [8,28], long-term potentiation [17] and neurite growth [3]. All these responses may be secondary to receptor-mediated PI hydrolysis and subsequent mobilization of intracellular Ca^{2+} [2].

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