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# Inhibitory effect of somatostatin on prostaglandin $E_2$ synthesis by primary neonatal rat glial cells

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ABSTRACT

Glial inflammation plays an integral role in the development of neurodegenerative disease. Although somatostatin is known to be a local anti-inflammatory factor in the periphery, evidence of a similar function in the brain is scarce. The aim of the present study was to investigate the effect of somatostatin on prostaglandin $E_2$ synthesis in primary neonatal rat glial cells. The data shows that high concentrations of somatostatin ($10^{-5}$–$10^{-4}$) significantly increased prostaglandin synthesis. By contrast, when used at physiologically relevant concentrations ($10^{-9}$–$10^{-7}$ M), somatostatin and somatostatin receptor agonists decreased prostaglandin $E_2$ synthesis in non-stimulated glial cells as well as in lipopolysaccharide-induced prostaglandin synthesis. The inhibitory effect of somatostatin in lipopolysaccharide-treated cells could be mimicked by protein kinase A inhibitor and was prevented by forskolin.

These observations suggest the presence of a novel neuro-immune feedback pathway through which somatostatin inhibits glial prostaglandin synthesis, and thus may prove to play a role in brain inflammation. This action of somatostatin may have a therapeutic potential in pathological conditions of the brain, where an inflammatory response is involved.



## 1. Introduction

During the development of neurodegenerative diseases such as Alzheimer's disease, brain inflammation is frequently observed. One of the major characteristics of the inflammatory response in the brain is the activation of the resident immune cells of the brain, the microglia and astrocytes, through a process termed reactive gliosis [1,2]. Reactive microglia and astrocytes cluster at sites of amyloid β deposition. Once activated, these cells initiate a cycle of events including the synthesis and release of a variety of proinflammatory and cytotoxic factors. The glia-released factors include interleukin-1β, tumor necrosis factor α, complement proteins, acute phase proteins, nitric oxide and prostaglandins [3–6].

Prostaglandins are inflammatory mediators that are produced via the metabolism of arachidonic acid by cyclooxygenase and prostaglandin synthase enzymes. Cyclooxygenase-2 protein levels have been reported to be increased in brains of Alzheimer's disease patients and correlate with levels of amyloid β peptide [4]. According to epidemiological studies, non-steroidal anti-inflammatory drugs, the principle target of which is cyclooxygenase, reduce the incidence and progression of Alzheimer's disease [7]. Importantly, elevated prostaglandin $E_2$ levels were found in the cerebrospinal fluid from Alzheimer's disease patients, compared with controls [8].

Modifications in the concentrations and activity of various neuropeptides also occur in brains of Alzheimer's disease patients. One of these peptides, somatostatin, is a tetradecapeptide found at high concentrations in the hypothalamus, where it serves to inhibit the secretion of several anterior pituitary hormones, most notably growth hormone. Several pieces of evidence indicate that somatostatin may also affect memory and learning [9,10]. In Alzheimer's patients, there is marked reduction in somatostatin levels in the cerebral cortex and cerebrospinal fluid [11].

Besides its well known neuroendocrine functions, somatostatin exhibits other properties, including immunomodulatory and anti-inflammatory activities. Although somatostatin has been shown to be a local anti-inflammatory factor in the periphery [12], evidence of a similar function in the brain is scarce.

Somatostatin receptors were detected in neurons and in glia [13,14]. In rat glia, somatostatin receptor subtypes 1, 2, 3 and 4 were detected and shown to be functionally active [13,14]. In a detailed study of somatostatin receptor subtypes in Alzheimer's disease brain, Kumar [15] reported that all five somatostatin receptors were differentially expressed in cortical neurons with significant variations in control vs. Alzheimer's disease brain. Alzheimer's disease cortex showed a marked reduction in neuronal expression of somatostatin receptors 4 and 5 and a modest decrease in somatostatin receptor 2-like immunoreactivity without any changes in somatostatin receptor 1 immunoreactive neurons. In contrast,

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somatostatin receptor 3 was the only receptor subtype that increased in Alzheimer's disease cortex. In Alzheimer's disease cortex, somatostatin receptors 1, 3 and 4-like immunoreactivities were strongly expressed in glial cells but not somatostatin receptors 2 and 5. Subtype-selective changes in the expression of somatostatin receptors at protein levels in Alzheimer's disease cortical regions suggest that somatostatin and somatostatin-containing cells are pathologically involved in Alzheimer's disease [15]. Our working hypothesis is that somatostatin may be involved in the regulation of glial production of inflammatory mediators such as prostaglandin $E_2$ and thus in the pathogenesis of Alzheimer's disease.

## 2. Materials and methods

### 2.1. Primary rat glial cell cultures

Primary glial cell cultures were prepared from whole brains of 1-day old Wistar rats, according to well established protocols [16,17]. Cells were grown in 75 $cm^2$ flasks or 24-well plates covered by poly-L-lysine at 37 °C in 5% $CO_2$. High glucose (4.5 mg glucose/ml) Dulbecco's modified Eagle's medium, supplemented with 10% fetal calf serum, 0.2 mM L-glutamine, 0.2 U/ml penicillin, 100 μg/ml streptomycin and 100 U/ml insulin was used as the culture medium. Immunocytochemistry studies as previously described [16] revealed that these cultures contain about 80% astrocytes and about 20% microglia.

Prior to an actual experiment, the cells were incubated in 1 ml serum-free medium for 24 h. The test agents were added to serum-free medium containing 0.1% bovine serum albumin and 10 mM HEPES. The length of incubation with test agents is indicated for each experiment. At the end of each experiment, cells were harvested with trypsin, and counted using Z1 Coulter counter (Coulter electronics, Miami, Fl, USA).

The following agents were used: lipopolysaccharide from *Escherichia coli*, protein kinase A inhibitor H-89 and Octreotide (Sigma Chemical Co., St Louis, MO, USA), somatostatin (Calbiochem, San-Diego, CA, USA). Forskolin (Biomol Research Laboratories, PA, USA). BIM 23926, a specific somatostatin receptor 1 agonist and BIM 23120, a specific somatostatin receptor 2 agonist were kindly donated by Dr. Michael D. Culler (Endocrine research IPSEN Group, MA, USA). L-796,778, a specific somatostatin receptor 3 agonist and L-803,087, a specific somatostatin receptor 4 agonist were kindly donated by Dr. Susan Rohrer (Department of Medical Chemistry, Merck Research Laboratories, NJ, USA).

### 2.2. Prostaglandin $E_2$ assay

The prostaglandin $E_2$ that had accumulated in the medium was measured in unextracted samples of medium by single antibody radioimmunoassay with dextran-coated charcoal precipitation. Medium did not interfere with the assay. The assay was performed in duplicate for each sample. Rabbit antibody to prostaglandin $E_2$ was obtained from Sigma (Sigma Chemical Co., St Louis, MO, USA), and tritium-labeled prostaglandin $E_2$ (160 Ci/mmol) was supplied by the Radiochemical Center (Amersham, UK). Radioactivity was measured on a β-scintillation counter (TriCarb 2100, Packard, Meriden CT USA). The sensitivity of the assay was 0.07 ng/ml.

### 2.3. Statistics

Results are expressed as means + S.E.M. for each experiment. Statistical analysis of the results was performed using one way ANOVA followed by Tukey (multiple comparison post test) test if the ANOVA's $P<0.05$. Values of $P<0.05$ of Tukey test were considered statistically significant.

## 3. Results

As shown in Fig. 1A, somatostatin ($10^{-9}$–$10^{-7}$ M) significantly decreased basal prostaglandin $E_2$ synthesis while higher concentration of somatostatin ($10^{-5}$, $10^{-4}$ M) significantly increased the basal production of prostaglandin $E_2$ (Fig. 1A inset). Octreotide, a synthetic long acting analogue of somatostatin, which selectively binds to somatostatin receptors 2, 3 and 5, decreased basal prostaglandin $E_2$ synthesis in a dose-dependent manner as well (Fig. 1B). Fig. 2 shows that the specific agonists for somatostatin receptors 1–4, namely BIM 23926 (Agonist 1), BIM 23120 (Agonist 2), L-796,778 (Agonist 3) and L-803,087 (Agonist 4) also significantly decreased basal prostaglandin $E_2$ synthesis.

Lipopolysaccharide, an inflammatory inducer, dose-dependently induced prostaglandin $E_2$ synthesis several fold. Somatostatin and octreotide ($10^{-9}$–$10^{-7}$ M) each attenuated the lipopolysaccharide-

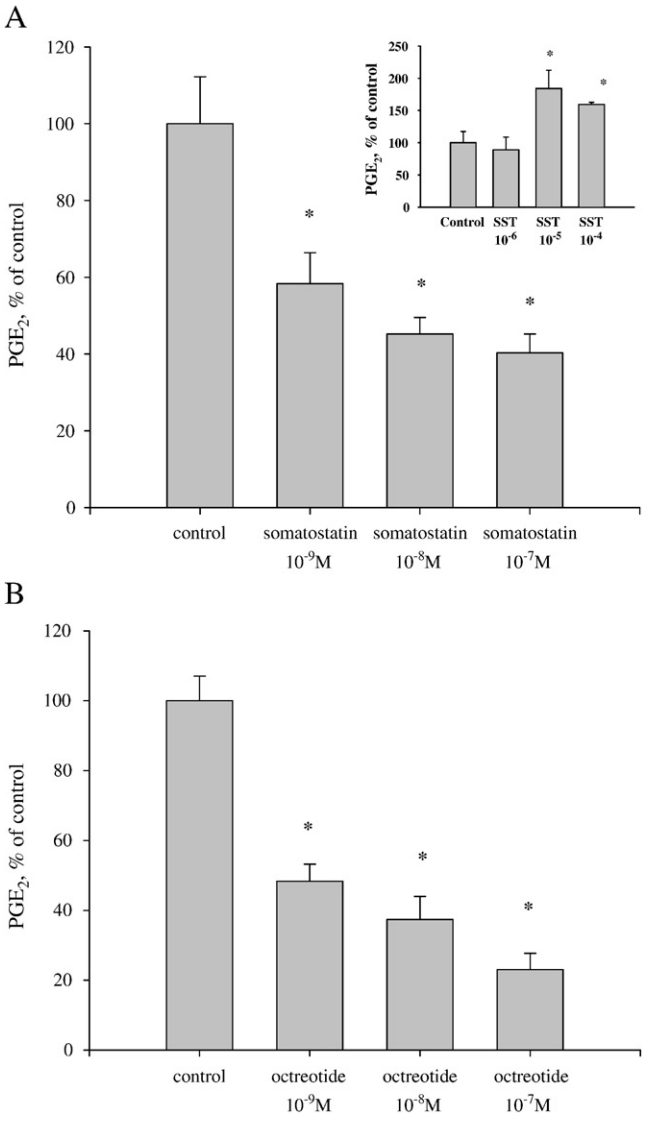


**Fig. 1.** Effect of somatostatin and octreotide on prostaglandin $E_2$ ($PGE_2$) synthesis in non-stimulated glial cells. Cells were incubated with somatostatin (A) or octreotide (B) at different concentrations ($10^{-9}$–$10^{-7}$ M or $10^{-6}$–$10^{-4}$ M (1A. inset)) for 1 h. Thereafter, the media were collected and assayed for $PGE_2$. These results were obtained in three separate experiments ($n=6$, each) in which cell cultures were derived from different animals. Results are means + S.E.M. of $n=18$, $^*P<0.05$ compared with control.