EFFECT OF SOMATOSTATIN ON LABELED 5-HYDROXYTRYPTAMINE RELEASE FROM RAT SPINAL CORD AND METHIONINE ENKEPHALIN ANTAGONISM IN VIVO

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Brazeau et al. (1) first isolated the tetradecapeptide, somatostatin, from bovine hypothalamus as a potent inhibitory factor of pituitary hormone secretion. This substance has been found to be widely distributed in the central nervous system (2-4) and has also been considered as a neurotransmitter (5, 6). In addition to its neuroendocrine role, the stimulating effects of somatostatin on the release of acetylcholine from rat hippocampal synaptosomes (7), the release of nor-epinephrine from rat cerebral cortex (8), the turnover rate of DOPA, 5-hydroxytryptophan and 5-hydroxyindoleacetic acid in rat brain (9), and the secretion of histamine from rat mast cells (10) were reported. This study was carried out to determine the effect of somatostatin on 5-hydroxytryptamine (5-HT) release and the relationship in 5-HT release between somatostatin and methionine enkephalin (met-EK) in the spinal cord.

The effect of somatostatin on 5-HT release from the spinal cord was determined by spinal perfusion. Male Sprague Dawley rats, weighing between 200-250 g, anesthetized with urethane (1.2 g/kg, i.p.) were used. The rat's brain was fixed on a stereotaxic instrument. A stainless steel cannula as a pull-tube was inserted into the cisterna magna, and a polyethylene tube (no. 10) as a push-tube was also inserted intrathecally from the lumbosacral enlargement. Oxygenated Krebs-bicarbonate solution (composition in mM: NaCl, 120; KCl, 5; NaHCO₃,

15; CaCl₂, 1.5; MgSO₄, 1.0; glucose, 10), which contained 7.5×10^{-5} M pargyline to prevent the metabolism of 5-HT, was used as a perfusate. The spinal cord was first perfused circularly with the Krebs-bicarbonate solution containing ³H-5-HT (1 μ Ci, specific activity: 32.1 Ci/mmol) for 40 min at 37°C. After the incubation, the spinal cord was continuously perfused for 20 min with Krebs-bicarbonate solution containing 7.5×10^{-5} M pargyline and 3.75×10^{-5} M desipramine. Then, the perfused samples were collected at every 5 min interval (0.25 ml/min) to the end of the experiment through the pull-tube.

Neuropeptides (somatostatin: 10 and 30 μ M, met-EK: 40 μ M) were added into the perfusate medium, and their effects on the release of ³H-5-HT were observed. The radioactivity in each fraction was measured in a liquid scintillation spectrometer (Aloka, LSC-673) and the release of 5-HT was expressed as dpm $\times 10^2$ /tube. Statistical analysis of the difference was carried out in a pair experiment using Student's *t*-test. It was checked in another experiment using a Sephadex G-10 column that approx. 40% of the total radioactivities measured in the perfusate was due to 5-HIAA, a metabolite of 5-HT and others. The 5-HT release evoked by 20 mM KCl was also examined. The 5-HT release from the spinal cord was dose-dependently increased by somatostatin (Fig. 1). This effect of somatostatin (30 μ M) was significantly antagonized

by 40 μ M met-EK, which alone showed no considerable change in 5-HT release (Fig. 2).

Enkephalin-containing interneurons are rich in the substantia gelatinosa on the spinal

cord, suggesting that EK has a physiological role in the transmission of nociceptive information in the central nervous system (11). Somatostatin located in small neuronal cell bodies in the spinal ganglia and somatostatin-positive fibers were also found in the spinal dorsal horn, Lissauer's fasciculus and adjacent area of the lateral funiculus (12, 13). Somatostatin was considered as a neuromodulator, which was examined on both pre and postsynaptic sites in frog spinal cord (14). The physiological role of somatostatin in the spinal cord is not clearly known. Recently, however, some reports have shown that it may have a role as an inhibitory transmitter substance in the spinal sensory pathways (15).

Many studies have shown that one of the most important descending pain modulatory pathways is a serotonergic system which originates from nucleus raphe magnus to the spinal dorsolateral funiculus (16–18).

In this study, we found that 5-HT release from the spinal cord was evoked by somatostatin, but not by met-EK; and moreover, the effect of somatostatin on 5-HT release was antagonized by met-EK. As we already found in an *in vitro* experiment (19) that 5-HT release from rat spinal slices was evoked by somatostatin, and it was not suppressed by met-EK at all, it may suggest with the present finding that met-EK seems to act on sites further up from the spinal cord.

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Fig. 1. Effect of somatostatin on 5-HT release in rat spinal cord. Each result shows the mean $\pm$ S.E.M. of 4 experiments. Solid line represents 10 μ M somatostatin, and the broken line represents 30 μ M. * $P < 0.05$, ** $P < 0.01$.

Fig. 2. Antagonism of met-enkephalin on 5-HT release evoked by 30 μ M somatostatin in rat spinal cord. Each result shows the mean $\pm$ S.E.M. of 4 experiments. The broken line shows 30 μ M somatostatin only, and the solid line shows 30 μ M somatostatin plus 40 μ M met-enkephalin. * $P < 0.05$, ** $P < 0.01$.

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