# STIMULATION OF GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE ACCUMULATION IN RAT ANTERIOR PITUITARY GLAND IN VITRO BY SYNTHETIC SOMATOSTATIN

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Summary. Synthetic somatostatin stimulated cyclic GMP accumulation with dose dependency (10 ng/ml - 10 μg/ml in a dose examined) in rat anterior pituitary gland in vitro. The stimulation of cyclic GMP levels in the gland was observed after 2 min incubation with somatostatin. Cyclic AMP production induced by TRH or $PGE_1$ was supressed by this GH release inhibiting factor, while cyclic GMP concentration in the gland was elevated. The present results seem to suggest that inhibitory effect on GH release by somatostatin in anterior pituitary gland is mediated through change in concentration of cyclic AMP and cyclic GMP in the target cells.

Recently, the inhibition of basal adenosine 3',5'-cyclic monophosphate (cyclic AMP) and prostaglandin $E_1$ ($PGE_1$) induced cyclic AMP accumulation in rat anterior pituitary gland by somatostatin has been demonstrated and a marked inhibition of both growth hormone (GH) and thyrotropin release by this release-inhibiting factor was observed with lowering of cyclic AMP in the gland (1,2), following the structure determination of somatostatin (3). Recent evidence implicated guanosine 3',5'-cyclic monophosphate (cyclic GMP) as a chemical mediator antagonistic to the metabolic effects of cyclic AMP in certain biological systems (4,5). Our interest has been focused upon investigation of possible participation of cyclic GMP in the metabolic event in in vitro somatostatin-GH release system of the anterior pituitary gland. The present communication describes the effect of synthetic somatostatin on cyclic GMP concentration in rat anterior pituitary gland. Under the same condition, cyclic AMP concentration was also examined.

Table 1. Effects of synthetic somatostatin on cyclic nucleotide levels in rat anterior pituitary gland in vitro

| | somatostatin ng/ml | cyclic AMP* pmoles/g.pit. | cyclic GMP* pmoles/g.pit. |
|---|---|---|---|
| Exp. 1 | 0 | 1120 ± 25 | 21.8 ± 1.3 |
| | 1 | 996 ± 58 | 39.6 ± 4.1 |
| | 10 | 844 ± 25 | 40.9 ± 1.7 |
| | 100 | 727 ± 30 | 62.1 ± 2.5 |
| | 1000 | 680 ± 41 | 68.6 ± 3.1 |
| | 10000 | 635 ± 17 | 108.4 ± 3.0 |
| Exp. 2 | 0 | 1200 ± 28 | 12.4 ± 0.8 |
| | 100 | 1033 ± 10 | 22.6 ± 0.9 |
| | 5000 | 830 ± 24 | 83.5 ± 1.2 |

Anterior pituitary glands were incubated for 10 min at 37°C.
*Values represent the mean ± SEM of triplicate determinations.
Other experimental conditions are described in the text.

## Materials and methods

The male rat anterior pituitary gland was obtained as described previously (6). Whole intact anterior pituitary (5-6 glands/test tube) was incubated at 37° C in Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose, 1 mg/ml of bovine serum albumin, $10^{-2}$M theophylline and appropriate substance to be tested. The gas phase was 95%$O_2$-5%$CO_2$. Following the final incubation, the tissue was immediately homogenized in TCA and extracted with water-saturated ether. The neutralized sample was applied on AG-1-X8 (200-400 mesh, Bio-Rad Lab.) and eluted with formic acid by the method of Murad et al., (7). Each fraction containing cyclic AMP and cyclic GMP was lyophylized and dissolved in a small amount of 0.2M acetate buffer, pH 4.0. The concentration of cyclic AMP and that of cyclic GMP were determined by the competitive protein binding assay, using purified rat liver cyclic AMP binding protein and silk moth fat body cyclic GMP binding protein, respectively. The recovery of each nucleotide

was over 80%. Somatostatin (cyclized form) was synthetized by peptide synthesis in solution (8), and purified extensively by gel filtration on Bio-Gel P2 before it's use. $PGE_1$ was a generous gift of Ono Pharmaceutical Co. Japan.

Results and discussion

The data in table 1 demonstrated that synthetic somatostatin increased cyclic GMP concentration with the lowering of cyclic AMP concentration with dose dependency between 10 ng/ml and 10 μg/ml in rat anterior pituitary gland. Previously, we reported that 10 ng/ml of synthetic somatostatin was the minimum effective dose for producing significant decrease of cyclic AMP concentration and GH release into the incubation medium of anterior pituitary gland (2). As shown in table 2, lowering of cyclic AMP concentration and stimulation of cyclic GMP accumulation were observed after 2 min of incubation with somatostatin in rat anterior pituitary gland. The effects of this peptide were found to be more remarkable at 5 or 10 min incubation. Table 3 shows that

Table 2. Time course of the effects of synthetic somatostatin on cyclic nucleotide levels in rat anterior pituitary in vitro

| | somatostatin μg/ml | incubation time (min) | cyclic AMP* pmoles/g.pit. | cyclic GMP* pmoles/g.pit. |
|---|---|---|---|---|
| Exp. 1 | 0 | 0 | 1279 ± 35 | 26.4 ± 1.4 |
| | 10 | 0 | 1266 ± 29 | 25.9 ± 1.8 |
| | 0 | 5 | 1292 ± 10 | 24.3 ± 1.5 |
| | 10 | 5 | 814 ± 24 | 112.2 ± 1.6 |
| | 10 | 5 | 763 ± 39 | 108.2 ± 2.5 |
| | 0 | 10 | 1421 ± 59 | 27.8 ± 1.4 |
| | 10 | 10 | 749 ± 23 | 72.6 ± 1.5 |
| Exp. 2 | 0 | 0 | 1162 ± 21 | 44.2 ± 1.6 |
| | 0 | 2 | 1169 ± 17 | 45.8 ± 1.7 |
| | 10 | 2 | 782 ± 16 | 72.6 ± 1.6 |
| | 10 | 5 | 763 ± 14 | 105.6 ± 2.7 |
| | 0 | 10 | 1305 ± 33 | 48.3 ± 1.2 |
| | 10 | 10 | 764 ± 41 | 103.2 ± 0.9 |

* Values represent the mean ± SEM of triplicate determinations. Other experimental conditions are described in the text.

Table 3. Effects of synthetic somatostatin on thyrotropin releasing hormone stimulation of cyclic AMP production of rat anterior pituitary in vitro

| | cyclic AMP* pmoles/g.pit. | cyclic GMP* pmoles/g.pit. |
|---|---|---|
| control | 667 ± 53 | 42.6 ± 1.9 |
| TRH 1 μg/ml | 1463 ± 95 | 45.3 ± 1.9 |
| TRH 1 μg/ml + somatostatin 5 μg/ml | 790 ± 86 | 64.0 ± 1.5 |

Anterior pituitary glands were incubated for 10 min at 37° C.
*Values represent the mean ± SEM of triplicate determinations.
Other experimental conditions are described in the text.

Table 4. Effects of synthetic somatostatin on $PGE_1$ stimulation of cyclic AMP production in rat anterior pituitary in vitro

| | cyclic AMP* pmoles/g.pit. | cyclic GMP* pmoles/g.pit. |
|---|---|---|
| control | 1140 ± 55 | 33.3 ± 1.1 |
| $PGE_1$ 1 μg/ml | 37800 ± 1720 | 36.0 ± 2.4 |
| somatostatin 10 μg/ml | 820 ± 24 | 57.3 ± 1.1 |
| $PGE_1$ 1 μg/ml + somatostatin 10 μg/ml | 28000 ± 1604 | 56.0 ± 0.9 |

Anterior pituitary glands were incubated for 10 min at 37° C.
*Values represent the mean ± SEM of triplicate determinations.
Other experimental conditions are described in the text.

synthetic somatostatin inhibits increment of cyclic AMP accumulation produced by thyrotropin releasing hormone (TRH) in vitro, but stimulates accumulation of cyclic GMP in the gland. Hall et al.(9), reported TRH stimulation of TSH secretion in man was inhibited during infusion of synthetic somatostatin.

PGE has been well known to stimulate cyclic AMP production and GH release in rat anterior pituitary gland (10). Synthetic somatostatin inhibited such effect of $PGE_1$ to some extent and , in turn, stimulated the production of cyclic GMP in the gland (table 4).

GH secretion from the anterior pituitary gland is thought to be controlled by hypothalamic GH releasing and inhibiting factors. Although Peake et al., (11) reported that cyclic GMP is potent GH secretagogue and can serve as an intracellular mediator of stimulation of GH release,our results showed that synthetic somatostatin in a dose of inhibition of GH release inhibits cyclic AMP production and stimulates cyclic GMP accumulation in the anterior pituitary gland.

These observations seem to be suggesting that GH release from the anterior pituitary gland is controlled by cyclic AMP and cyclic GMP of the gland and that somatostatin inhibits GH release through change in concentration of the cyclic nucleotides in the target cells.

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