RAPID COMMUNICATION

In Vitro Effects of Synthetic Somatotropin-Release Inhibiting Factor on Cyclic AMP Level and GH Release in Rat Anterior Pituitary Gland

TOSHIO KANEKO, HIROSHI OKA, SHIRO SAITO*, MASAHIDE MUNEMURA,**
KIMITAKA MUSA*, TOSHITSUGU ODA, NOBORU YANAIHARA*** AND
CHIZUKO YANAIHARA***

*The First Department of Medicine, University of Tokyo Faculty of Medicine, Tokyo,
Department of Clinical Laboratory, University of Tokushima School of Medicine,
Tokushima*, Department of Obstetrics and Gynecology, Kumamoto University,
Kumamoto** and Department of Bioorganic Chemistry, Shizuoka College of
Pharmacy, Shizuoka****

Synopsis

Synthetic somatotropin-release inhibiting factor (10 ng/ml) decreased significantly basal level of cyclic AMP concentration and showed marginal inhibition of GH release in rat anterior pituitary gland *in vitro*. Higher dose of synthetic somatotropin-release inhibiting factor was needed to produce significant inhibition of GH release from the gland. Results obtained in the experiments suggest that somatotropin-release inhibiting factor as well as hypothalamic releasing hormones may act through adenyl cyclase-cyclic AMP system in the anterior pituitary gland.

Recently, Guillemin and his coworkers (Brazeau *et al.*, 1973) reported that tetradecapeptide (H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH) isolated from ovine hypothalamus inhibits the secretion of immunoreactive pituitary growth hormone in rat and in monolayer cultures of dispersed cells of rat adenohypophysis, and that the synthetic replicate was biologically active. As hypothalamic releasing hormone (LH-RH, TRH) stimulation of adenyl cyclase activity and cyclic AMP production in the anterior pituitary gland were confirmed (Borgeat *et al.*, 1972; Kaneko *et al.*, 1973), it is quite interesting to investigate whether or not hypothalamic hormone-release inhibiting factor affects cyclic AMP level in the gland.

Present studies were undertaken to examine the effects of synthetic somatotropin-release inhibiting factor (SRIF) on cyclic AMP for-

mation and GH release in rat anterior pituitary gland *in vitro*.

Whole rat anterior pituitary was incubated at 37°C in Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose, 1 mg/ml of albumin, 10^{-2} M theophylline and the substance to be tested. After incubation, tissue was immediately frozen and homogenized in 0.1N HCl. Cyclic AMP was assayed in neutralized supernatant by competitive protein binding assay (Gilman, 1970) using purified rat liver protein kinase (Kumon *et al.*, 1970). GH contents in the medium were determined by radioimmunoassay kit for rat GH provided by NIAMDD. Synthetic SRIF was prepared by conventional peptide synthesis. Rat hypothalamic extract used in the experiments was generous gift of NIAMDD.

Table 1 shows the effects of synthetic SRIF on cyclic AMP concentration and GH release in rat anterior pituitary gland. Synthetic SRIF decreased significantly the basal level of cyclic

Table 1. The effects of synthetic SRIF on cyclic AMP level and GH release in rat anterior pituitary *in vitro*.

Incubation Time	Dose/ml	cyclic AMP (mμmoles/g)	GH release (μg/Pit.)
		10 min	120 min
Control		0.83 ± 0.03	3.36 ± 0.39
SRIF	1 ng	0.76 ± 0.10	—
SRIF	10 ng	0.66 ± 0.08**	2.67 ± 0.33
SRIF	100 ng	0.57 ± 0.07**	2.59 ± 0.14
SRIF	1 μg	0.55 ± 0.04*	2.60 ± 0.29
SRIF	5 μg	—	2.23 ± 0.12***
SRIF	10 μg	0.42 ± 0.06*	—
SRIF	25 μg	—	1.98 ± 0.28***

Values represent the mean ± SEM.

* p < 0.01, ** p < 0.025, *** p < 0.05.

Table 2. The effect of synthetic SRIF on prostaglandin E₁(PGE₁) stimulation of cyclic AMP production and GH release in rat anterior pituitary for 30 min incubation.

	Dose/ml	cyclic AMP (mμmoles/g)	GH release (μg/Pit.)
Control		1.18 ± 0.03	3.69 ± 0.67
PGE ₁	10 μg	40.25 ± 10.34*	6.62 ± 0.96**
PGE ₁	10 μg	40.25 ± 10.34	6.62 ± 0.96
PGE ₁ + SRIF	10 μg + 50 μg	24.35 ± 3.56*	3.36 ± 1.24**

Values represent the mean ± SEM.

* p < 0.01 ** p < 0.05.

AMP in the tissue and the minimum effective dose of SRIF was 10 ng/ml for 10 min incubation. The significant inhibition of GH release by SRIF was found at the incubation longer than 30 min. The results on SRIF inhibition of GH release from the gland for 120 min incubation were shown in Table 1. Smaller dose of SRIF showed marginal inhibition of GH release from the pituitary gland, but higher dose of SRIF decreased significantly GH release.

The inhibitory effects of synthetic SRIF on prostaglandin E₁ stimulation of cyclic AMP formation and GH release in the pituitary gland are demonstrated in Table 2. Cyclic AMP concentration and GH release in rat anterior pituitary were markedly increased with prostaglandin E₁ (10 μg/ml) for 30 min incubation, but the stimulating effects of

prostaglandin E₁ were significantly inhibited in the presence of synthetic SRIF (50 μg/ml).

Cyclic AMP production in rat anterior pituitary gland was also stimulated markedly by rat hypothalamic extract. When synthetic SRIF (5 μg/ml) was added to the test tube or the gland was transferred to a fresh buffer containing synthetic SRIF after incubation for 3 min with rat hypothalamic extract (0.8 mg/ml), increased cyclic AMP concentration in the tissue due to rat hypothalamic extract was significantly decreased after following 7 min incubation in both experimental series (Fig. 1).

In the results reported by Guillemin and his coworkers, GH release into the medium from cultured monolayer rat pituitary cells was inhibited significantly by synthetic SRIF at a concentration of 1.0 nM. In our experimental

Fig. 1 Effect of synthetic SRIF on rat hypothalamic extract stimulation of cyclic AMP formation.

In the first incubation, the glands of each experimental group were incubated for 3 min in KRB-buffer containing glucose and albumin with theophylline and rat hypothalamic extract (RAT-HE). In the second following 7 min incubation, the glands indicated as RAT-HE were incubated in the same mixture, the glands indicated as GIF (SRIF) were incubated in the same buffer with theophylline and SRIF instead of RAT-HE, and the glands indicated as RAT-HE, GIF were incubated in the same buffer with theophylline, SRIF and RAT-HE. Brackets represent the mean $\pm$ SEM.

system, 10 ng/ml (about 7.0 nM) of synthetic SRIF decreased significantly basal level of cyclic AMP in the tissue and showed marginal inhibition of GH release, but it was needed higher dose of synthetic SRIF to get the significant inhibition of GH release from the gland.

These results confirmed that the tetradecapeptide corresponding to amino acid sequence proposed by Guillemin and his coworkers had the inhibitory action for GH secretion from the pituitary gland, and suggest that SRIF as well as hypothalamic releasing hormones may act through adenyl cyclase-cyclic AMP system

in rat anterior pituitary gland. Because Peake *et al.*, (1972) reported that GRF stimulated both cyclic AMP and cyclic GMP formation of the anterior pituitary gland, the effect of synthetic SRIF on cyclic GMP concentration in the tissue are under investigation.

Acknowledgments

We would like to thank the National Institute of Arthritis, Metabolic and Digestive Diseases, National Institute of Health for supplying the radioimmunoassay kit for rat GH and the rat hypothalamic extract.

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