Short Communication

Centrally Applied Somatostatin Influences Morphology of Pituitary FSH Cells But Not FSH Release

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Abstract. Effects of i.c.v. administered somatostatins on morphology and function of pituitary FSH cells were examined in adult male Wistar rats. The animals were given three 1 μg doses of SRIH-14 or SRIH-28 in 5 μl saline every second day. Controls were given the same volume of saline only. Both SRIH treatments lead to a significant decrease in absolute pituitary weight and volume of FSH cells in comparison with controls. Relative pituitary weight was significantly decreased only after SRIH-28 treatments, while FSH secretion was insignificantly decreased by both SRIH treatments. Our results indicate that i.c.v. applied somatostatins have significant inhibitory effect on absolute pituitary weight and on the volume of FSH cells, without affecting the hormone secretion in male rats.

Key words: FSH cell — FSH — Rat — Somatostatin

Somatostatin exists as a 14 amino acids (somatostatin-14; SRIH-14) or 28 amino acids (somatostatin-28; SRIH-28) long peptide, present mainly in the central nervous system (Reubi 1997), the hypothalamo-pituitary system, the gastrointestinal tract, the exocrine and endocrine pancreas, and the immune system (Reichlin 1983a). It inhibits the secretion of several non-pituitary hormones such as insulin, glucagon, gastrin, secretin and aldosterone (Epelbaum 1986; Milošević 1999). It is well known that SRIH inhibits the release of growth hormone (GH) from somatotrophes *via* separate receptors in the plasma membrane (Reichlin 1983b). Hypothalamic SRIH also inhibits the secretion of thyrotropin-stimulating hormone (TSH), luteinising hormone (LH), and prolactin (PRL) from the anterior pituitary (Milošević 1999; Lovren et al. 2000; Sekulić et al. 2000). Earlier, Reichlin (1983b) indicated that somatostatins do not affect the release of gonadotropin hormones

(LH and FSH). However, McCann (1982) hypothesized that sufficiently large so-matostatin doses could block the release of all pituitary hormones. The present study was designed to evaluate the effects of intracerebroventricular (i.c.v.) admin-istration of SRIH-14 or -28 in nanomolar amounts on morphometric and functional characteristics of gonadotrophic follicle-stimulating hormone (FSH) cells in the pi-tuitary gland of male rats.

Adult male Wistar rats (210–230 g), kept individually in cages at $22 \pm 2\,^\circ\text{C}$ were used. They were implanted a headset, later serving for i.c.v. injections, under aether anaesthesia. A minimum recovery time of 5 days was permitted before the onset of experiments. All animals underwent adaptation to i.c.v. injections (kin-dling) and were randomly divided into three experimental groups, each including five animals. Rats in the first and second group were given (i.c.v.) three single 1-μg doses of SRIH-14 (S9129; Sigma, St. Louis, MO., USA) or SRIH-28 (S6135; Sigma) dissolved in 5 μl saline, every second day. The third, control group, comprised rats treated in the same manner with an equal volume of saline. The animals were al-lowed to move freely prior to and after the injection. All animals were sacrificed by decapitation during deep anaesthesia 5 days after the last i.c.v. injection. Pituitary glands were excised, weighed in air, fixed in Bouin's solution and embedded in paraffin. Serial, 5 μm thick tissue sections were deparaffinized. Pituitary FSH cells were localized by the peroxidase-antiperoxidase complex (PAP) method of Stern-berger et al. (1970). Measurements were performed on the whole central section surface of the widest portion of the pituitary gland. FSH cells were immunocy-tochemically labelled with specific anti-rat antibodies to FSH β-subunit (diluted $1:300$ with phosphate buffer solution) and analyzed using the Weibel's M_{42} mul-tipurpose test system (Weibel 1979). Serum concentrations of FSH in control and experimental rats were measured by a radio immunoassay (FSH-RIA kits, INEP, Serbia and Montenegro) method. Morphometric and hormonal level data obtained from each group were averaged and standard deviations of the means were calcu-lated. A one-way analysis of variance (ANOVA), followed by the Duncan multiple range test was used for statistical comparisons between the groups. A probability value of 5 % or less was considered statistically significant.

Table 1. Effects of i.c.v. applied SRIH-14 or -28 on absolute and relative pituitary weight and morphometric parameters of FSH cells in adult male rats

Group	Absolute pituitary weight (mg)	Relative pituitary weight (mass %)	Volume of FSH cells $(\mu\text{m})^3$	Volume density of FSH cells (%)
Control	11.5 ± 0.3	5.5 ± 0.3	819 ± 11	15 ± 0.7
SRIH-14	9.7 ± 0.2*	5.3 ± 0.2	670 ± 59*	14 ± 0.9
	(-16%)	(-3%)	(-18%)	(-7%)
SRIH-28	8.0 ± 0.6*	4.5 ± 0.3*	759 ± 43*	14 ± 0.8
	(-30%)	(-18%)	(-7%)	(-7%)

All values are mean $\pm$ SD; ($n = 5$ in each group); * $p < 0.05$ in comparison with controls.

The data on absolute and relative weights of the pituitary in the SRIH-treated groups and in controls are summarized in Table 1. The absolute pituitary weight was significantly lower ($p < 0.05$) in both treatment groups, while the relative pituitary weight was significantly ($p < 0.05$) lower only in SRIH-28-treated animals compared to controls.

FSH immunoreactive cells in the pituitary *pars distalis* of control adult male rats were localized in close contact with blood capillaries. They had an ovoid or irregular shape with prominent, often eccentrically located nuclei (Figure 1A). FSH immunoreactive cells in SRIH-treated animals were smaller and more intensely stained (Figure 1B and C) compared to controls.

The volume of FSH cells was significantly ($p < 0.05$) decreased by 18 % in SRIH-14- and by 7 % in SRIH-28-treated animals compared to controls. Volume density (fraction of FSH cells count out of total cells count) of these cells in both SRIH-treated groups was insignificantly decreased by about 7 % compared to controls (Table 1). Serum concentration of FSH in both SRIH-treated groups did not change significantly in comparison with control rats (Figure 2).

The present results clearly demonstrate that i.c.v. administered SRIH-14 or -28 significantly decrease absolute pituitary weight compared to saline-treated controls. Somatostatin has already been demonstrated to decrease food intake in animals (Lieverse et al. 1995), which could be the mechanism of decrease in both body weight and absolute pituitary weight in the SRIH-treated animals (Starčević et al. 2000, 2002). The decrease in pituitary weight was accompanied by a significant decrease in all morphometric parameters of somatotropes, lactotropes and thyrotropes, measured previously (Milošević et al. 1998, 2000).

There are a number of conflicting data about somatostatin effects on pituitary FSH cells in male and female rats (Yu et al. 1997; Lovren et al. 2000). It is obvious that SRIH effects are mediated through interaction with somatostatin receptors (SSTR) 1–5 (Patel and Srikant 1994). The presence of somatostatin receptors on FSH cell membrane has been demonstrated (O'Carroll and Krempels 1995). The same authors suggested that FSH cells express low amounts of all five SSTR mRNAs in contrast to the high amounts of SSTR expressed in GH or TSH cells. Señarís et al. (1996) demonstrated that SSTR1 and SSTR3 mRNA levels in the anterior pituitary exhibit sexual dimorphism. These authors showed that the levels of SSTR1 mRNA in female rats are higher than in male rats and this can explain the gender differences in the secretory response of FSH cells to SRIH administration. Our results are in accordance with results of Yu et al. (1997) showing that somatostatin has no significant effect on FSH release in *in vitro* studies in the presence of LH-releasing hormone in male rats. Nestorović et al. (2001, 2004) reported that multiple subcutaneous SRIH-14 treatments of female rats decreased all measured morphometric parameters of LH cells and, to a lesser extent, of FSH cells. The morphometric changes of FSH cells may be explained either as a consequence of failed follicle-pituitary feedback control and/or as a direct effect of SRIH on FSH cells *via* SSTR. Our recently reported data on the effects of i.c.v. somatostatin on pituitary LH cells showed that treatments with both SRIH-14 and -28 significantly
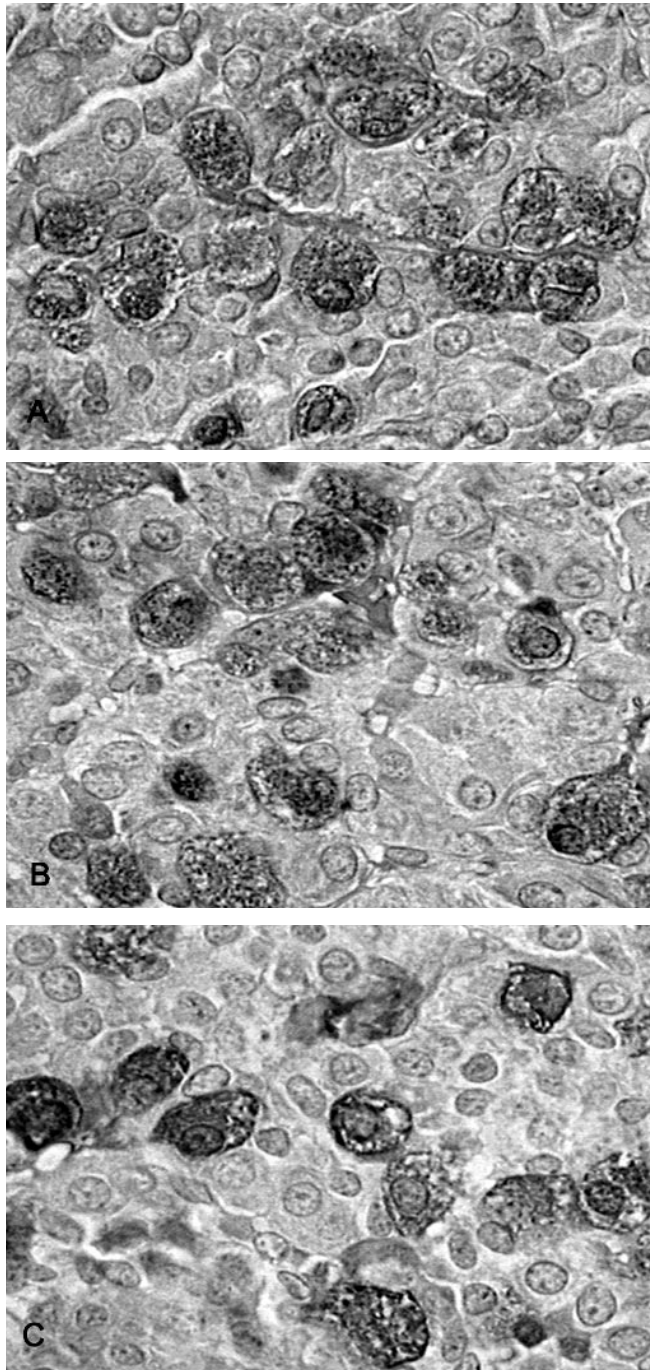
Figure 1. Immunopositive FSH cells in: **A.** control rats; **B.** SRIH-14-treated rats; **C.** SRIH-28-treated rats. (Peroxidase-antiperoxidase complex method of Sternberger, × 1256).

FSH(mU/l)
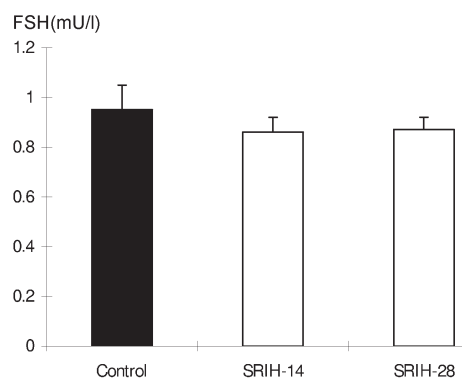
Figure 2. Serum concentration of FSH after i.c.v. administration of SRIH-14 or -28 in adult male rats. All values are mean $\pm$ SD ($n = 5$ in each group).

reduced all morphometric parameters and secretory activity of LH cells in male rats (Starčević et al. 2002).

In conclusion, our results indicate that i.c.v. applied somatostatins significantly reduce absolute weight of the pituitary and volume of the FSH cells. The relative pituitary weight was significantly reduced only by SRIH-28. The SRIH treatment also induced morphometric changes of FSH cells, but did not affect serum FSH concentration.

References

Epelbaum J. (1986): Somatostatin in the central nervous system, physiological and pathological modifications. Prog. Neurobiol. **27**, 63—100

Lieverse R. J., Jansen J., Masclee A., Lamers C. (1995): Effects of somatostatin on human satiety. Neuroendocrinology **61**, 112—116

Lovren M., Sekulić M., Milošević V., Radulović N. (2000): Effect of somatostatins on gonadotrophic cells in female rats. Acta Histochem. **100**, 329—335

McCann S. M. (1982): Physiology and pharmacology of LHRH and somatostatin. Annu. Rev. Pharmacol. Toxicol. **22**, 491—515

Milošević V. (1999): The Effects of Somatostatins on the Pituitary and Adrenal Gland (Ed. K. Andrejević), Zadužbina Andrejević, Belgrade

Milošević V., Brkić B., Velkovski S., Sekulić M., Lovren M., Starčević V., Severs W. (1998): Morphometric and functional changes of the rat pituitary somatotropes and lactotropes after central administration of somatostatin. Pharmacology **7**, 27—34

Milošević V., Sekulić M., Brkić B., Lovren M., Starčević V. (2000): Effect of centrally administered somatostatin on pituitary thyreotropes in male rats. Histochem. J. **32**, 565—569

Nestorović N., Lovren M., Sekulić M., Filipović B., Milošević V. (2001): Effects of multiple somatostatin treatment on rat gonadotrophic cells and ovaries. Histochem. J. **33**, 695—702

Nestorović N., Lovren M., Sekulić M., Negić N., Šošić-Jurjević B., Filipović B., Milošević V. (2004): Chronic somatostatin treatment affects pituitary gonadotrophs, ovaries onset of puberty in rats. Life Sci. **74**, 1359—1373

O'Carroll A. M., Krempels K. (1995): Widespread distribution of somatostatin receptor messenger ribonucleic acids in rat pituitary. Endocrinology **136**, 5224—5227

Patel Y. C., Srikant C. B. (1994): Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors. Endocrinology **135**, 2814—2817

Reichlin S. (1983a): Somatostatin. N. Engl. J. Med. **309**, 1495—1501

Reichlin S. (1983b): Somatostatin (second of two parts). N. Engl. J. Med. **309**, 1556—1563

Reubi J. C. (1997): Relevance of somatostatin receptors and other peptide receptors in pathology. Endocr. Pathol. **8**, 11—20

Sekulić M., Lovren M., Milošević V. (2000): Somatostatin acts by inhibiting pituitary TSH cells in rat females. Acta Vet. (Beogr.) **50**, 3—12

Señarís R., Lago F., Dieguez C. (1996): Gonadal regulation of somatostatin receptor 1, 2 and 3 mRNA levels in the anterior pituitary. Mol. Brain Res. **38**, 171—175

Starčević V., Milošević V., Brkić B., Severs W. (2000): Effects of centrally applied somato-statin on pituitary adrenocorticotropes in female rats. Pharmacology **60**, 203—207

Starčević V., Milošević V., Brkić B., Severs W. (2002): Effects of somatostatin on mor-phology and secretion of pituitary LH cells in male rats. Life Sci. **70**, 3019—3027

Sternberger L. A., Hardy P. H. J., Cuculius J. J., Meyer H. G. (1970): The unlabelled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish perox-idase) and its use in identification of spirochetes. J. Histochem. Cytochem. **18**, 315—333

Weibel E. R. (1979): Stereological methods: Practical Methods for Biological Morphom-etry. Vol. 1, Academic Press, New York

Yu W. H., Kimura M., McCann S. M. (1997): Effect of somatostatin on the release of gonadotropins in male rats. Proc. Soc. Exp. Biol. Med. **214**, 83—86