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SOMATOSTATIN ACTS BY INHIBITING PITUITARY TSH CELLS IN FEMALE RATS

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The effects of intracerebroventricularly (i.c.v.) administered synthetic somatostatins (SRIH-14 and SRIH-28) on pituitary TSH cells of adult female rats were studied. The animals were i.c.v. injected with three 1.0 μg (5 μl) doses of SRIH-14 or SRIH-28 every second day and sacrificed five days after the last dose. The controls received an equivalent volume of saline in the same manner according to the same schedule. The pituitary glands were excised and used for immunohistochemical and morphometric evaluation. The results obtained demonstrated a somatostatin-related decrease in relative pituitary weight. The morphometric analyses showed decreased volumetric density of TSH-immunoreactive cells in SRIH-treated groups, but this difference was more expressed in SRIH-14- treated animals than in those receiving SRIH-28. On the basis of these results it can be concluded that centrally administered somatostatins inhibit TSH cells.

Key words: female rats, pituitary, TSH cells, immunohistochemistry, somatostatin

INTRODUCTION

Somatostatin (SRIH) is synthesized as a prohormone which undergoes tissue-specific processing resulting in either a 14 amino acid peptide (SRIH -14) or a 28 amino acid, N-terminally extended form (SRIH-28) which are present in the hypothalamus in the ratio of 4:1 (Gillies, 1997). SRIH is widely distributed in higher organisms and expresses inhibitory effects on the secretion of various polypeptide hormones. Epelbaum et al. (1987) suggested that SRIH-14 was 8 times more potent than SRIH-28 with regard to TSH secretion. Five specific subtypes of somatostatin receptors have been cloned, each of them having a characteristic tissue-specific pattern of expression (Coy and Rossowski, 1995). They have been described and characterized in rat pituitary gland, the central nervous system, adrenal cortex and pancreatic acini (Aguilera and Parker, 1982; Srikant and Patel, 1985). In the adenohypophysis SRIH-binding sites were identified on the three target cell types: somatotrophs, thyrotrophs and lactotrophs

(Epelbaum et al., 1987; Day et al., 1995). Originally, somatostatin was shown to inhibit growth hormone (GH) secretion as a neuroendocrine hormone (Milošević et al., 1998). However, it is less well known, but nonetheless important, that SRIH plays a physiological role in the regulation of the hypothalamo-pituitary-thyroid axis (Singer, 1990). It has been reported that TSH secretion is stimulated by TRH, while somatostatin inhibits TSH synthesis and release (Theodoropoulos, 1985). SRIH inhibition of TRH-stimulated TSH secretion has been demonstrated in neonatal 3 day old rats, but no effect on basal TSH levels was observed during the first 60 days of life (Oliver et al., 1982). The inhibitory action of somatostatin was confirmed by discrete lesions of SRIH neurons in the rat periventricular nucleus of the preoptic-anterior hypothalamus leading to transient elevation in GH and TSH secretion (Critchlow et. al., 1981).

Huffman et al. (1990) suggested that somatostatin did not affect thyroid hormone or plasma calcium levels, while plasma TSH was decreased in rats. Recent data of Roussel et al. (1997) showed that SRIH inhibits TSH response to physiological concentrations of TRH in primary cultures of rat anterior pituitary cells in a dose-dependent manner. Similarly increased hypothalamic SRIH levels induced by oral glucose administration were found to suppress TRH-stimulated TSH response in man (Yang et al., 1996).

James et al. (1997) showed a synergistical action of thyroid hormone (T_4) and somatostatin to control TSH secretion, expressing both anti-secretory and anti-proliferative effects on pituitary thyrotroph tumours in mice. Somatostatin decreased pituitary TSH secretion, caused tumour shrinkage when used for treatment of human pituitary thyrotropinomas (Comi et al., 1987) and also suppressed proliferation of a human differentiated thyroid carcinoma cell line (Ain et al., 1997).

All these data prompted us to examine possible effects of i.c.v. applied SRIH-14 or SRIH-28 on morphometric characteristics of pituitary TSH cells in adult rat females.

MATERIAL AND METHODS

Adult two-month-old female Wistar rats were used. They were kept under a 12/12 h light-dark cycle at 22±2 C, and fed commercial rat chow.

Surgical procedures were performed under ether anaesthesia. A headset was implanted into the rats and used later for intracerebroventricular (i.c.v.) injections. The headset consisted of a Silastic-sealed 20-gauge cannula (Starčević et al., 1988), implanted into a lateral cerebral ventricle, 1.0 mm posterior and 1.5 mm lateral to the bregma and 3 mm below the cortical surface. A small stainless steel anchor screw was placed at a remote site on the skull. The cerebroventricular cannula and screw were cemented to the skull with dental acrylic resin (Simgal; ICN, Yugoslavia).

After recovery from the surgical procedure (5 days), the rats were divided into three experimental groups, each consisting of 5 animals. Two groups were given i.c.v. three 1.0 μg doses of SRIH-14 or SRIH-28 (S 9129 and S 6135,

respectively; Sigma, St. Louis, MO, USA) dissolved in 5 μl saline every second day. All animals were sacrificed under deep ether anaesthesia by decapitation, 5 days after the last injection.

The pituitary glands were immediately excised, measured, fixed in Bouin's solution for 48 h and embedded in paraffin. Serial 4 μm thick sections were mounted on gelatine-coated glass slides. TSH-producing cells were studied using the peroxidase-antiperoxidase (PAP) method as already described (Sekulić et al., 1995). TSH β immunoreactive cells in the pituitary pars distalis were stereologically analyzed by simple point counting (Weibel, 1979). Morphometric parameters (mean volumes of TSH cells and the nuclei and relative volume density of these cells) were measured exactly as previously (Sekulić et al., 1995).

The data obtained for each rat were averaged per group and the standard error of the mean (SEM) was calculated. The the statistical significance of differences between mean values was evaluated by using Student's t-test.

RESULTS

Body weight of somatostatin-treated rats decreased in comparison with the values recorded at the onset of the experiment, but the difference was not statistically significant. Relative pituitary weight of the rats treated with SRIH-14 or SRIH-28 decreased by 9% and 5% respectively compared to the controls (Table 1).

Table 1. Effects of intracerebroventricularly applied SRIH-14 or SRIH-28 on body and relative pituitary weight in adult female rats

Experimental group	Body weight (g)	Relative pituitary weight (mg%)
Control	238.0 ± 6.4	4.3 ± 0.1
SRIH-14	234.0± 4.0	3.9 ± 0.23
SRIH-28	220.0 ± 12.2	4.1 ± 0.4

The values are the mean SEM; n=5

TSH immunoreactive cells of control adult female rats were localized in the anteromedial portion of the anterior pituitary gland. They had a round or irregular shape with long cytoplasmic elongations, situated singly or in clusters, and were strongly immunostained (Fig. 1a). The average TSH cell volume was 1113 μm^3 (Fig. 2a) and they made up 10% of the pituitary volume (Fig. 2c).

TSH immunoreactive cells in SRIH-14-treated animals were small, often pycnotic and darkly stained (Fig. 1b). A significant decrease of both cellular and nuclear volume of TSH cells (24% and 23%, respectively) was observed (Figs. 2a b).

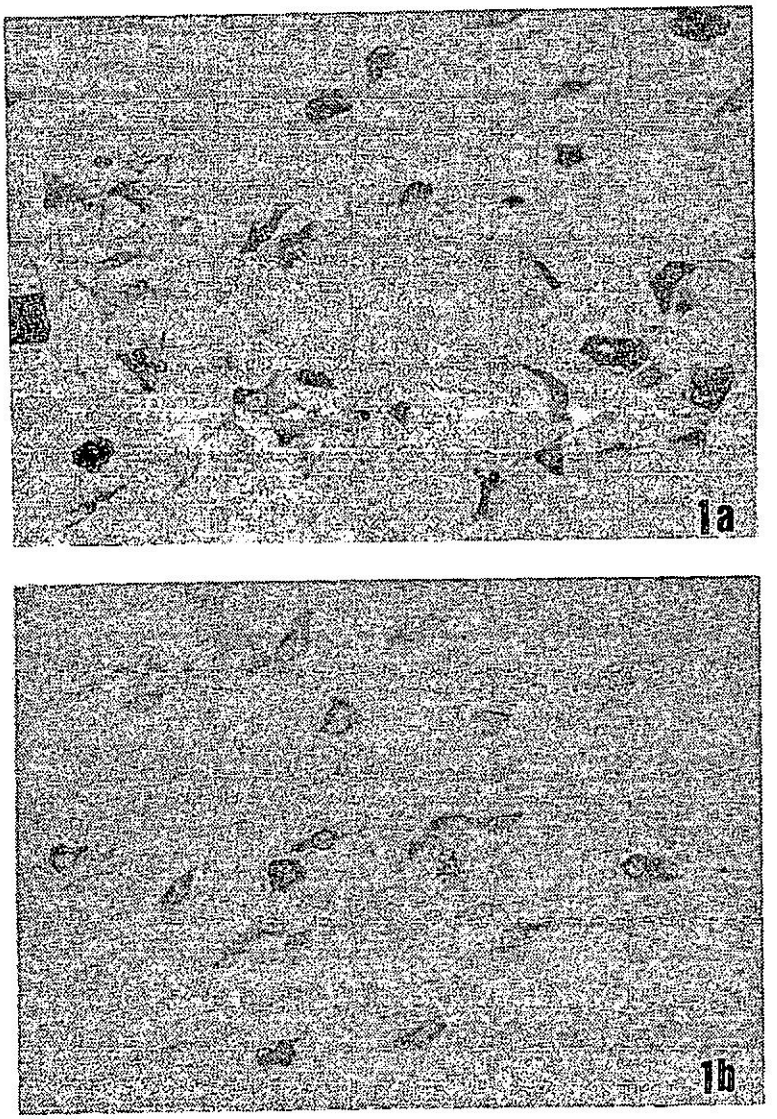

Figure 1. a. Immunoreactive TSH cells in pituitary pars distalis of a control adult rat female. b. Rarely distributed small TSH cells in an SRIH-14-treated animal. (PAP; X 700).

Most vacuolated TSH cells were noticed in rats administered SRIH-28. Some pycnotic cells were also seen and cytoplasmic elongations were seldom. Cell volume and nuclear volume of TSH cells were unchanged (Fig. 2a).

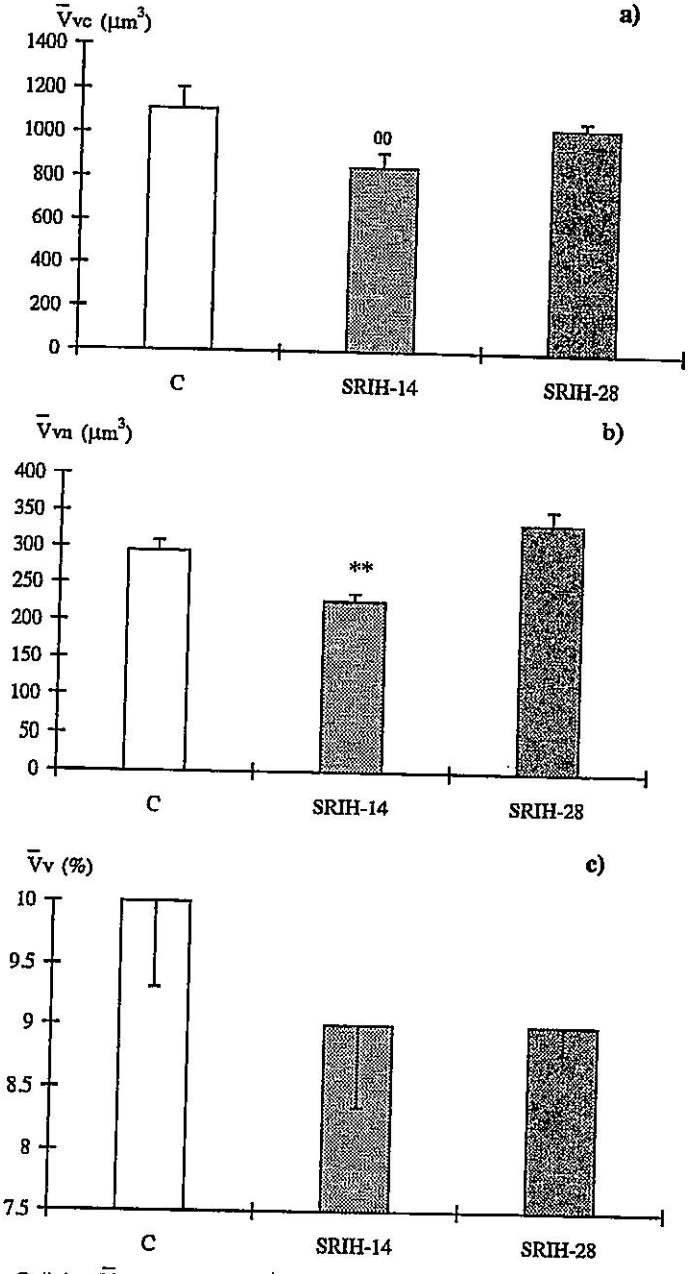

Figure 2. a. Cellular ($\bar{V}_{vc}$) volume (μm^3) of the immunoreactive TSH cells b. Nuclear ($\bar{V}_{vn}$) volume (μm^3) of TSH cells; c. Relative volume density ($\bar{V}_v$) of cells expressed as percentages of total gland tissue. C - control, SRIH-14 and SRIH-28- somatostatin-treated adult rat females. All values are the means ± SEM, $^0P<0.025$; **P<0.005; ***P<0.001.

DISCUSSION

The results of the present study demonstrate that i.c.v. administered somatostatin, at first isolated on the basis of its capacity to inhibit GH secretion (Reichlin, 1983), and especially SRIH -14, expressed inhibitory effects on TSH cell structure as well as their volume in adult female rats.

It is generally accepted that somatostatin, a hypothalamic tetradecapeptide, directly inhibits anterior pituitary secretion of TSH, and may also inhibit TRH secretion. Based on its tonic inhibitory effect it was assumed that in perinatal rats somatostatin represents a physiological regulator of TSH secretion operating before TRH (Theodoropoulos, 1985). SRIH and dopamine, acting at the pituitary level as neurohormones and neurotransmitters, can contribute to CNS modulation of TSH release (Fisher and Polk, 1989). A modulatory role for SRIH in TSH control of adult rats was suggested by Oliver et al. (1982) who found that the administration of SRIH antiserum increased basal TSH level and potentiated TSH response to cold. Recent data of Roussel et al. (1997) showed that SRIH inhibited TSH response to TRH in a dose-dependent manner, but the mechanism(s) underlying this effect is still unclear. Crichlow et al. (1981) confirmed the inhibitory role of SRIH in controlling TSH secretion, since discrete lesions in the periventricular zone of the anterior hypothalamus caused transient elevation in GH and TSH secretion.

Biological actions of SRIH-14 are receptor -mediated, and besides the CNS, SRIH-14-specific receptors have been characterised in the pituitary (Srikant and Patel, 1985). Coy and Rossowski (1995) suggested the presence of several subtypes of somatostatin receptors and successfully cloned five of them.

TSH cells are a target for SRIH and, for that reason, the aim of our study was to examine the effects of low doses of i.c.v. injected SRIH-14 or SRIH-28 on immunoreactive TSH cells. Morphometric and quantitative changes in TSH pituitary cells were more obvious in SRIH-14- than in SRIH -28-treated animals. A significant decrease of TSH cells volume by 24% was observed in the SRIH-14-treated group, while after SRIH -28 this parameter was decreased by 7% only, in comparison with the controls. Cytological features of immunoreactive TSH cells in the SRIH-14-treated group indicate clear signs of inhibitory changes, seen as pycnotic involutive cells. Our results revealed less inhibition of TSH cells after SRIH-28 and additional examinations are necessary to explain the action of this hormone. The differences in morphometric parameters of TSH immunoreactive cells between SRIH-14- and SRIH-28-treated animals, could be explained in terms of the 8 times higher potency of SRIH - 14 as an inhibitor of TSH secretion, as suggested by Epelbaum et al. (1987).

The decrease of relative pituitary weight in somatostatin-treated female rats observed in our study corresponded well with decreased body weight but these differences were not statistically significant, probably due to the short period of treatment, i.e. the time from the onset to the end of the experiment.

It has been demonstrated that the effect of SRIH on the secretion of GH, PRL and TSH from anterior pituitary of female rats is mediated through the activation of a single class of membrane receptors (Epelbaum et al. 1987). However, the inhibition of hormone secretion by SRIH cannot be explained solely by adenylate cyclase inhibition, and some other mechanism(s) could operate. Roussel et al. (1997) suggested that SRIH attenuates Ca^{2+} influx into rat pituitary cells thus reducing TSH secretion in primary cell cultures.

The potential use of SRIH analogues in the treatment of clinical conditions ranging from human cancers to Alzheimer's and Parkinson's diseases is of great interest. DeRosa et al. (1983) found that somatostatin significantly reduced the TSH response to TRH in normal or hypothyroid subjects and in patients with TSH secreting pituitary adenoma, while it did not influence basal TSH level in normal or hypothyroid subjects. These results indicate a potential therapeutic use of somatostatins in thyroid diseases. SRIH and its analogues have been shown to inhibit the proliferation of human thyroid carcinoma cell lines, as well (Ain et al., 1997).

Somatostatin-binding sites on the cell surface of the thyrotropic tumour cells and thyroid hormones play a pivotal role in regulating somatostatin receptors in murine thyrotrope tumours. Thyroid hormones influence the somatostatinergic system and control antisecretory and antiproliferative somatostatin effects in thyrotropic cells. In fact, thyroid hormones and somatostatin act synergistically to control TSH secretion (James et al. 1997).

It is well established that SRIH acts on somatotrophs in the anterior pituitary gland via its G protein-coupled, transmembrane receptor domain to reduce cAMP formation and to inhibit the secretion but not the synthesis of GH (Gillies, 1997). It is possible that the same mechanism of SRIH action on TSH is operational and that its inhibitory effects are directly expressed at the level of the thyrotrophs.

In summary, our results indicate that centrally administered SRIHs inhibit TSH cell structure. Additional studies will be necessary to explain the mechanism of their action on thyrotropic function.

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INHIBITORNO DELOVANJE SOMATOSTATINA NA TSH ĆELIJE HIPOFIZE U ŽENKI PACOVA

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SADRŽAJ

Ispitivani su efekti intracerebroventrikularno (i.c.v.) injiciranih sintetičkih somatostatina (SRIH-14 i SRIH-28) na hipofizne TSH ćelije adultnih ženki pacova. Životinje su i.c.v. injicirane sa tri doze od po 1 μg SRIH-14 ili -28 rastvorenog u 5 μl fiziološkog rastvora svaki drugi dan, a žrtvovane 5 dana posle poslednje injekcije. Kontrole su tretirane odgovarajućom dozom fiziološkog rastvora. Hipofize su izolovane i pripremljene za imunocitohemijska i morfometrijska ispitivanja. Oba somatostatina izazivaju smanjenje relativne težine hipofize. Morfometrijska analiza pokazuje da je u obe tretirane grupe životinja volumenska gustina TSH-imunoreaktivnih ćelija smanjena a te razlike su značajnije izražene u grupi životinja tretiranih sa SRIH-14 u odnosu na one tretirane sa SRIH-28. Na osnovu dobijenih rezultata možemo zaključiti da centralno aplikovani somatostatini inhibiraju TSH ćelije hipofize.