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MORPHOMETRIC AND FUNCTIONAL CHANGES OF FEMALE RAT PITUITARY SOMATOTROPES AFTER CENTRAL APPLICATION OF SOMATOSTATIN

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This study examined the effects of intracerebroventricularly (i.c.v.) administered somatostatin (SRIH-14 or SRIH-28) on growth and function of pituitary somatotropes (GH cells). Female rats were subjected to intracerebroventricular administration of three 1g doses of SRIH-14 or SRIH-28 every second day. Blood samples were collected for hormone analyses and pituitaries were removed for histological and morphometric evaluation, five days after the last injection. Compared to control animals, SRIH treatment decreased ($p<0.05$) all morphometric measurements obtained for GH cells. The concentration of serum growth hormone (GH) in SRIH-14- or SRIH-28-treated groups was lower ($p<0.05$) by 69% and by 61% respectively, in comparison to control rats. These findings suggest that centrally administered somatostatin is specifically involved in the control of growth and secretory activity of GH cells. Thus, pharmacological manipulation of SRIH receptors reached from cerebrospinal fluid may alter the systemic effects of GH.

Key Words: GH cells, GH, SRIH-28, SRIH-14, female rats.

INTRODUCTION

Somatostatin (somatotropin release-inhibiting hormone; SRIH), is a neuropeptide initially described as inhibiting growth hormone release from the pituitary gland (Brazeau et al. 1973). There are two major forms of somatostatin, a cyclic tetradecapeptide somatostatin-14 (SRIH-14), originally isolated from bovine hypothalamic extracts, and an N-terminally elongated form consisting of 28 amino acid residues, octacosapeptide somatostatin-28 (SRIH-28) (Reichlin 1983). Both somatostatins are widely distributed in the central and peripheral nervous system (Raulf et al. 1994). In the central nervous system SRIH acts as a neurotransmitter and/or neuromodulator and affects locomotor activity, cognitive functions and behavioral processes (Epelbaum 1986). Both peptides are also present in non-

neuronal tissues such as the gastrointestinal tract and endocrine tissues including the pancreas, thyroid and adrenal gland (Reichlin 1983). Somatostatin inhibits the secretion of several non-pituitary hormones such as insulin, glucagon, gastrin, secretin and aldosterone (Epelbaum et al. 1995).

It is well known that SRIH inhibits the release of growth hormone (GH) from somatotropes in male rats (Milošević et al. 1998) via separate receptors in the plasma membrane (Wehrenberg et al. 1982). Hyphothalamic SRIH also inhibits the secretion of luteinising hormone (Lovren et al. 1998), prolactin (PRL) (Milošević et al. 1998), thyrotropin-stimulating hormone (TSH) (Epelbaum 1994) from the anterior pituitary.

This study was designed to evaluate the effects of intracerebroventricular (i.c.v.) administration of low doses of SRIH-14 and SRIH-28 on the morphology and secretory activity of somatotropes in pituitary glands of female rats.

MATERIAL AND METHODS

The study was performed on adult female Wistar rats (210-230 g), bred in the Institute for Biological Research in Belgrade. Rats were kept under a 12:12 h light-dark cycle, at 22 ± 2 C. They were fed with special food for laboratory rats (prepared by D.D.Veterinarski Zavod Subotica, Subotica, Yugoslavia). Food and water were available to the rats ad libitum.

Animal preparation. Surgical procedures were performed under ether anesthesia (aether ad narcosis Ph. lug. III. produced by "Lek", Ljubljana, Slovenia). The rats were implanted with a headset later used for i.c.v. injections. A minimum recovery time of 5 days was permitted before the onset of experiments. The headset consisted of a silastic-sealed 20-gauge cannula (Starčević et al. 1998), implanted into the lateral cerebral ventricle, 1 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 cannula and screw were cemented to the skull with dental acrylic (Simgal; ICN Galenika, Belgrade, Yugoslavia).

Treatment of animals. After the rats recovered from surgery they were divided into three experimental groups of five animals per group. The first and second groups consisted of rats which were given (i.c.v.) three 1-μg doses of SRIH-14 (S 9129; Sigma, St. Louis, Mo., USA) or SRIH-28 (S 6135; Sigma, St. Louis, Mo., USA) dissolved in 5 μL saline. The third group was a control group, comprised of rats treated in the same manner, except that they received only 5 μL of saline i.c.v. All animals were sacrificed by decapitation during deep anesthesia 5 days after the last injection.

Light microscopy and immunocytochemistry. Pituitary glands were excised, fixed in Bouins solution for 48 h and embedded in paraffin. Serial 5μm thick tissue sections were deparaffinized in xylol and serial alcohol dilutions. Pituitary hormones were localized by the peroxidase-antiperoxidase-complex (PAP) method of Sternberger et. al. (1970). Endogenous peroxidase activity was blocked by

incubation in 9 mmol/L hydrogen peroxide in methanol for 30 min at ambient temperature. Before application of specific primary antisera, nonspecific background staining was minimized by incubation of the sections with non-immune, porcine serum diluted with phosphate buffered saline pH 7.4 (PBS) for 60 min. Sections were then overlaid with the appropriate dilutions of the specific primary antibodies (hGH-antisera, Dako A/S, Glostrup, Denmark) for 24 h at 4 °C. After washing in PBS, sections were incubated for another 60 min with the second antibody-swine-antirabbit IgG for 45 min, rinsed again with PBS for 10 min and incubated with rabbit PAP serum for 45 min. Antibody localization was visualized by incubating the sections in Tris-HCl buffered saline (0.05 mol/L, pH 7.4) supplemented with 3,3-diaminobenzidine tetrachloride (DAB) (Serva, Heidelberg, Germany) and 9 mmol/L hydrogen peroxide. Slides were throughly washed under running tap water, counterstained with hematoxylin and mounted in Canada balsam (Alkaloid, Skopje, Macedonia). Control sections were incubated without primary antisera or by substituting non-immune rabbit serum for the primary antiserum.

Morphometry. Measurements were performed on the widest portion of the pituitary gland and immunocytochemically-labelled GH cells were analyzed by the M_{42} test system of Weibel (1979). The formula of Weibel and Gomez (1962) was used for calculation of the cell and nuclear volumes

Hormone assay. Serum concentrations of GH in control and experimental rats were measured by the Delfia method (hGH-Delfia kits, LKB, Turky, Finland).

Statistical analyses. Biochemical and morphometric data obtained from each group were averaged, and the standard deviation of the mean was calculated. A one-way analysis of variance (ANOVA), followed by the multiple range test of Duncan (Pharmacological Calculation System, 1986) was used for statistical comparisons between groups. A probability value of 5% or less was considered statistically significant.

RESULTS

Data for the body weight, absolute and relative weight of the pituitary in SRIH-treated groups and controls are summarized in Table 1.

Table 1. The effects of intracerebrovenricularly administered SRIH-14 or SRIH-28 on body weight and absolute and relative weight of the pituitary in adult female rats. (means ± SD; n=5)

Experimental group	Body weight (g)	Absolute pitutary weight (mg)	Relative pitutary weight (mg/100g)
Control	238 ± 6.4	10.5 ± 0.4	4.3 ± 0.1
SRIF-14	234 ± 4.0	9.2 ± 0.6	3.9 ± 0.2
SRIF-28	220 ± 12.2*	10.0 ± 0.4	4.1 ± 0.4

The values are the means ± S.D. for five animals. * $p<0.05$.

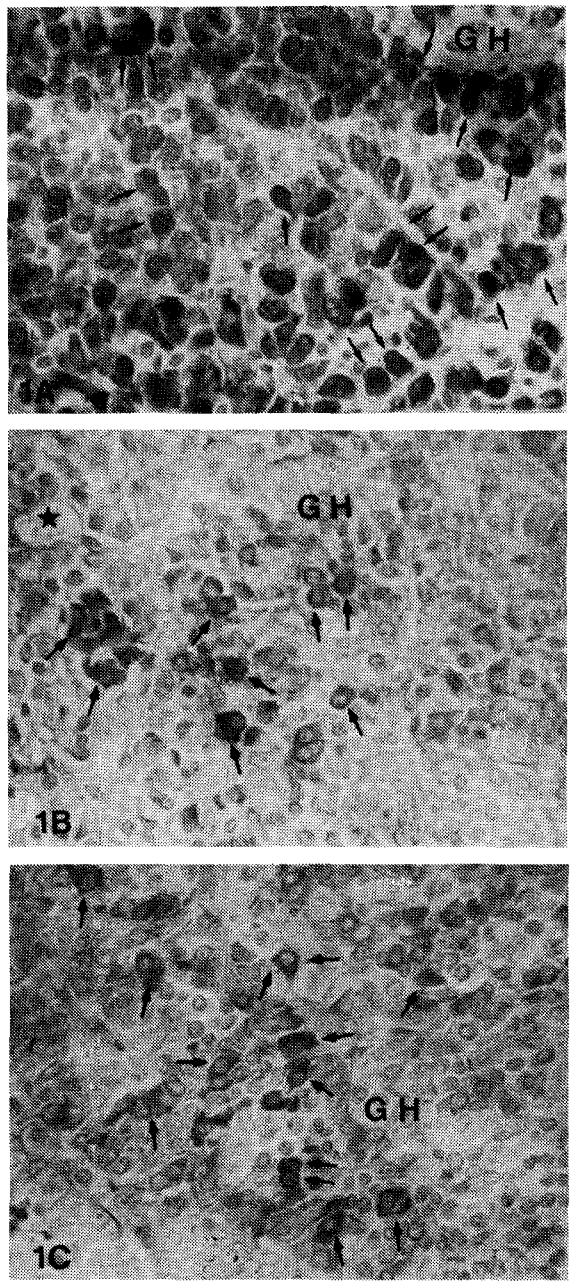
As can be seen, a significant ($p<0.05$) decrease of body weight by 10% was observed only in rats treated with SRIH-28 in comparison with controls. Absolute and relative pituitary weights were not significantly ($p<0.05$) decreased in either group of SRIH-treated animals compared with corresponding controls. Somatotropes were the predominant cell type in the anterior pituitary lobe of the adult female rat (Table 2).

Table 2. Morphometric parameters of the GH cells of the pituitary after intracerebroventricular administration of SRIH-14 or SRIH-28 in female rats. (means ± SD; n=5)

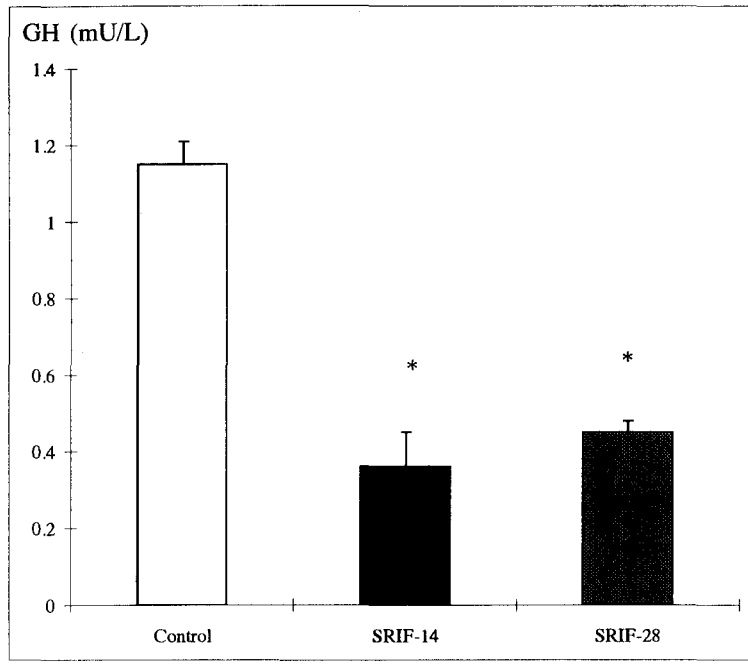
Experimental group	Volume of the GH cells (μm^3)	Volume of the GH nucelei (μm^3)	Volume density of the GH cells (%)
Control	1433±31.9	137±13.4	42±1.5
SRIF-14	1044±44.5*	104±4.2*	32±0.1*
	(-27%)	(-24%)	(-24%)
SRIF-28	751±42.4*	75±4.2*	28±1.4*
	(-48%)	(-45%)	(-33%)

* $p<0.05$, in comparison with controls

Immunocytochemically identified GH cells in control rat pituitaries ranged from ovoid to pyramidal in shape, with a spherical centrally located nucleus. GH cells were usually situated along sinusoids (Fig. 1A). In SRIH-treated pituitaries, GH cells were smaller, irregularly shaped, with more intensely stained secretory granules (Figs. 1B, 1C). Blood capillaries were dilated (Fig. 1B). All morphometric parameters were significantly ($p<0.05$) decreased in both SRIH-treated groups compared with controls (Table 2). The serum concentration of GH was signifi-



Fgure 1. Immunohistochemically labeled GH cells in : A control rats, B SRIH-14 and C SRIH-28 intracerebroventrculary rats (PAP, 1256 x). →indicate cells, * indicates blood camplaries.



Fgure 2. Serum concentrations of GH after intracerebroventricular administration of SRIH-14 or SRIH-28 in adult female rats. Data are expressed as mean values ± S.D.(n=5), *p<0.05 in comparison with controls.

cantly decreased ($p<0.05$) by 69% and by 61% respectively after i.c.v. SRIH-14 or SRIH-28 treatment, in comparison to controls (Fig. 2)

DISCUSSION

The presented results clearly demonstrate that repeated i.c.v. administration of SRIH-14 or SRIH-28 significantly decreased all measured morphometric indices of somatotropes. The secretion of anterior pituitary hormones is regulated mainly by hypothalamic releasing and inhibiting hormones (Meyerhof et al. 1992). It is well know that SRIH inhibits release of GH from somatotropes via separate receptors in the plasma membrane (Wehrenberg et al. 1982; Epelbaum et al. 1987). Control of hormone secretion involves many cell surface receptors and activation of multiple cellular pathways such as mobilization of calcium, cyclic AMP, diacylglycerol (Chen and Clarke 1992). Local application of somatostatin peptides induces hyperpolarization of somatotropes and inhibition of adenylate cyclase (Chen et al. 1994) with modulation of calcium currents (Meyerhof et al. 1992).

A number of physiological and biochemical studies *in vivo* and *in vitro* have been performed to examine the role of both somatostatins on the mechanism of GH secretion (Epelbaum 1986; Epelbaum et al. 1987; Blanchard et al. 1988; Tannenbaum 1988). By contrast, only a few studies have investigated morphological features of somatotropes during inhibition or stimulation of GH secretion (Shimada and Tosaka-Shimada 1989; Shimada et al. 1990). Shimada et al. (1990) suggest that inhibition of GH release by SRIH involves a change in the distribution of microfilaments rather than microtubules. These authors found microfilament bundles running parallel to the plasmalemma in the space between granules at 2 and 5 minutes after injection of SRIH.

We have previously observed that both types of SRIH given i.c.v. to male rats led to a decrease in the number of GH cells, accompanied by a reduction in both their cellular and nuclear volumes in comparison with controls (Milošević et al. 1998).

In summary, an intermittent exposure of female rats to i.c.v. SRIH-14 or SRIH-28 reduces serum GH concentrations with corresponding changes in morphology of pituicytes that secrete these hormones. Pharmacological manipulation of these central SRIH receptors may then alter GH physiology.

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MORFOMETRIJSKE I FUNKCIONALNE PROMENE SOMATOTROPNIH ĆELIJA HIPOFIZE ŽENKI PACOVA POSLE CENTRALNE APLIKACIJE SOMATOSTATINA

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SADRŽAJ

Ispitivani su efekti intracerebroventrikularno (i.c.v.) ubrizganih somatostatina (SRIH-14 ili SRIH-28) na rast i funkciju somatotropnih ćelija hipofize ženki pacova. Sve eksperimentalne životinje su primale po 1μg SRIH-14 ili SRIH-28 rastvorenog u 5μL fiziološkog rastvora, svaki drugi dan. Kontrole su na isti način

injecirane fiziološkim rastvorom. Životinje su žrtvovane petog dana po poslednjoj primljenoj dozi. Krv je sakupljana za odredjivanje koncentracije hormona rasta (GH) u serumu, a hipofize su pripremane za histološku i morfometrijsku analizu. Dobijeni rezultati pokazuju da SRIH tretirane životinje pokazuju značajno smanjenje ($p<0.05$) svih ispitivanih morfometrijskih parametara (volumen ćelija i jedara, volumenska gustina) u odnosu na kontrolu. Koncentracija GH u serumu SRIH-14 i SRIF-28 tretiranih ženki takodje je značajno smanjena ($p<0.05$) za 69% odnosno 61% u poredjenju sa kontrolnim ženkama. Na osnovu ovako dobijenih rezultata može se zaključiti da oba aplikovana somatostatina inhibiraju rast i sekretornu aktivnost somatotropnih ćelija hipofize.