The effects of chronic SRIH-14 and octreotide administration on the pituitary-adrenal axis in adult male rats

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The effects of chronic treatments with SRIH-14 and octreotide on pituitary corticotropes (ACTH cells) and on the adrenal cortex of male Wistar rats were examined. Adult males received two daily s.c. injections of 20 μg/100 g of body weight of either SRIH-14 or octreotide for 28 consecutive days. ACTH cells were studied using a peroxidase-antiperoxidase immunocytochemical procedure. Morphometry was used to evaluate the changes in cell and nuclear volumes (μm^3) and volume densities (%) of ACTH-immunoreactive cells. The adrenal cortex was analyzed by histological and morphometric methods. A significant ($p<0.05$) decrease in body weight and in the absolute weights of the pituitary and adrenal glands was observed in both treated groups. Morphometric parameters of ACTH cells in both treated groups were not significantly ($p>0.05$) different than in control rats. The absolute volumes of the adrenal gland and adrenal cortex were significantly ($p<0.05$) decreased in both treated groups. The absolute and relative volumes of the zona glomerulosa (ZG), as well as the cellular and nuclear volumes of the ZG were significantly ($p<0.05$) decreased in the both treated groups. In rats treated with SRIH-14 and octreotide, the absolute and relative volumes of the zona fasciculata (ZF) and zona reticularis (ZR), as well as their stereological parameters, did not change significantly ($p>0.05$). The aldosterone levels in the SRIH-14 and ocreotide-treated groups were significantly ($p<0.05$) decreased – by 13% and 19%, respectively. The concentration of ACTH and corticosterone did not change significantly. Together, these findings show that SRIH-14 and octreotide administration affected the morphological characteristics of the adrenal ZG in a similar manner, and brought about a decrease in plasma aldosterone concentration. These treatments did not affect pituitary ACTH cells or adrenal ZF and ZR functioning.

Key words: SRIH-14, octreotid, ACTH cells, adrenal gland, stereology.

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Somatostatin (somatotropin release-inhibiting hormone; SRIH) is a regulatory peptide produced by neuroendocrine, inflammatory and immune cells (Patel, 1999). It acts as an endogenous regulator of the secretory and/or proliferative responses of target cells (Patel, 1999). It affects growth hormone secretion by the pituitary (Brazeau *et al.*,1973), as well as the secretion of insulin, glucagon, gastrin and secretin (Arnold and Linkisch, 1980). SRIH was detected in different regions of the brain, in the pituitary, the gastrointestinal tract and endocrine pancreas, the thyroid and adrenal glands and kidneys (Epelbaum, 1994; Reichlin, 1983a,b).

SRIH is synthesized as part of a large precursor molecule which is enzymatically cleaved into a prohormone form that is further processed to yield two active forms – SRIH-14 and SRIH-28 (Reichlin, 1983a,b). Hormone action in target cells is mediated by a family of G-protein-coupled receptors that contain seven transmembrane domains comprised of five distinct subtypes, named somatostatin receptors 1-5 (sst_{1-5}). Different experimental approaches showed that different sst receptors are expressed in normal and in tumor tissues (Patel, 1999).

Octreotide is a synthetic somatostatin analogue that has found clinical use in cancer therapy and in treatments of gastrointestinal and hormonal hypersecretory disorders. Octreotide is a cyclic octapeptide that is more powerful than endogenous SRIH because of its extended half-life (Bauer *et al.*, 1982; Lamberts *et al.*, 1991). Octreotide binds with high affinity to sst_2 and sst_5 receptors, with a moderate affinity to subtype 3, but it does not bind to either subtypes 1 or 4 (Rossowski and Coy, 1994).

SRIH receptors were detected in all types of hormone-producing cells of the pituitary gland (Day *et al.*, 1995). SRIH decreases the release of hormones from somatotropes (Brazeau *et al.*,1973; Milošević *et al.*, 1994), lactotropes (Bjoro *et al.*, 1988; Milošević *et al.*, 1998), thyrotropes (Milošević *et*

al., 2000) and gonadotrophic LH cells (Starčević *et al.*, 2002). It also exerts a negative effect on the morphometric features of gonadotrophic FSH cells (Nestorović *et al.*, 2001; Milošević *et al.*, 2004).

The role of SRIH in the modulation of corticotropes (ACTH cells) is still unclear. In pathological states such as Addison's disease and ACTH-producing tumors, it suppresses elevated ACTH levels (Patel, 1999). However, in healthy male adult rats intracerebroventricular (ICV) application of SRIH does not affect either the morphometric features of pituitary ACTH cells or the plasma level of ACTH (Milošević *et al.*, 1994). On the contrary, ICV treatment with octreotide exerts an inhibitory effect on the morphometric characteristics of ACTH cells (Milošević *et al.*, 2003).

The adrenal gland could also be a potential target of direct SRIH action. Somatostatin receptors have been detected both in the rat adrenal cortex and medulla (Aguilera *et al.*,1982; Maurer and Reubi, 1986). *In vitro* studies showed that SRIH influences the secretion of aldosterone by blocking its response to angiotensin II but not to ACTH (Boscaro *et al.,* 1982). Decreases of aldosterone levels were demonstrated after octreotide administration to rats with portal hypertension (Albillos *et al.*, 1993) and in cirrhotic patients (Sabat *et al.*, 1998; Kalamokis *et al.*, 2005), suggesting that octreotide regulates angiotensin II and aldosterone secretions in certain conditions.

Bearing in mind the important clinical uses of the SRIH analog octreotide, its different actions compared to SRIH, as well as the significance of adrenal gland signaling during states of stress, the aim of this study was to investigate and compare the effects of chronic SRIH-14 and octreotide treatments on the morphofunctional features of pituitary ACTH cells and the adrenal cortex in adult male rats.

Materials and Methods

Experimental animals

Wistar adult male rats were bred in the Institute for Biological Research in Belgrade. The rats were housed in a controlled environment at 22±2°C, with a 12 h light/12 h dark schedule. The animals were allowed food and water ad *libitum*. Food for laboratory rats was prepared in the Veterinarski zavod (Subotica, Srbija).

Experimental protocol

All experimental protocols were approved by the Local Animal Care Committee. They conformed to the recommendations provided in the *Guide for the Care and Use of Laboratory Animals* (1996, National Academy Press, Washington D.C.). The rats were divided into three experimental groups consisting of five animals per group. Rats from the first group were injected subcutaneously (s.c.) twice a day with 20 mg SRIH-14 (N°S 9129; Sigma, St. Louis, Mo., USA) per 100 g b.w. for 28 consecutive days (between the ages of 32 and 59 days). The dose regimen selected for SRIH-14 was based on that of Rebuffat *et al.* (1984), except that SRIH was administered every 12 h instead of every 8 h. Rats from the second group received twice a day a s.c. injection of 20 μg octreotide (Sandostatine, Novartis, Switzerland) per 100 g b.w. for 28 consecutive days (between the ages of 32 and 59 days). The third, control group was comprised of rats that were treated only with saline. All animals were killed under ether anesthesia 12 h after the last injection. The pituitary and adrenal glands were excised, fixed in Bouin's solution for 48 h, dehydrated and embedded in paraffin.

Immunocytochemistry of pituitary corticotropes

Five μm thick pituitary sections were immunocytochemically stained. Pituitary hormones were localized by the peroxidase-anti-peroxidase complex (PAP) method of Sternberger *et al.* (1970). Endogenous peroxidase activity was blocked by incubation in 9 mmol hydrogen peroxide solution in methanol for 30 min at ambient temperature. Before application of a specific primary antiserum, nonspecific background staining was achieved by a 60 min incubation of the sections with a non-immune, normal porcine serum diluted with phosphate buffered saline (PBS) pH 7.4. The sections were then overlaid with the appropriate dilutions of specific primary antibodies (hACTH antisera, Dako A/S, Glostrup, Denmark) for 24 h at room temperature. After washing in PBS, the sections were incubated for 60 min with the second antibody and swine-anti-rabbit IgG, for 45 min, rinsed with PBS for 10 min and incubated with rabbit PAP serum for 45 min. Antibodies were visualized by incubating the sections in Tris-HCl-buffered saline (0.5 mol/l, pH 7.4), supplemented with 3,3-diaminobenzidine tetrachloride (DAB, Serva, Heidelberg, Germany) and 9 mmol/l hydrogen peroxide. Slides

were thoroughly washed under running tap water, counterstained with hematoxylin, and mounted in Canada balsam (Alkaloid, Skopje, FYROM). Control sections were incubated either without a primary antiserum or with a nonimmune rabbit serum.

Morphometric measurements in pituitary corticotropes

Immunocytochemically stained sections of pituitaries that were cut through three tissue levels of the pars distalis were used for morphometric examinations. The cell volumes (V_c), nuclear volumes (V_n) and volume densities (V_v) of ACTH cells were estimated under the light microscope (Carl Zeiss, Germany) at 1000x magnification on 5 μm thick sections, using the M_{42} multipurpose test system (Weibel, 1979). The volumes of ACTH-positive cells were expressed in μm^3. The volume densities were presented as percentages of total pituitary cells in %.

Morphometric measurements in the adrenal gland

The absolute volume of the glands was calculated on the basis of their weight, assuming an average specific gravity of 1.039 g cm^{-3} (Swinyard, 1938). In order to evaluate the volume densities of the adrenocortical zones, every tenth section (5 μm thick) of the gland was analyzed at 125x magnification with the multipurpose test system M_{42} (Weibel, 1979).

The nuclear and cytoplasmic volumes of parenchymal cells were estimated under the light microscope at 1000x magnification on 5 μm thick sections with the M_{42} multipurpose test system (Weibel, 1979). For each adrenal gland, a single paraffin section containing the zona medullaris was chosen and 30 test areas of the ZG and 50 test areas of both the ZF and ZR were analyzed.

Hormone assays

The plasma levels of adrenocorticotropic hormone in the control, SRIH-14-treated and octreotide-treated rats were measured with the chemiluminiscent enzyme immunometric assay IMMULITE ACTH (DPC, Los Angeles). Serum aldosterone levels were determined with the enzyme immunoassay Aldosterone ELISA (DRG International, Inc. USA). Serum corticosterone levels were determined with the Corticosterone Immunoassay (R&D Systems Inc. USA).

Statistical analyses

The biochemical and morphometric data for each group were averaged and the SD of the means were calculated. One-way analysis of variance (ANOVA), followed by the multiple-range test of Duncan, were used for statistical comparisons between the groups. Values of p less than 0.05 were considered statistically significant.

Results

Measurements of body weight and absolute and relative pituitary and adrenal weights

Significant ($p<0.05$) decreases in body weight by 19% and 16% were observed after the last administration of SRIH-14 and octreotide, respectively (Table 1). The absolute weights of the pituitary glands were significantly ($p<0.05$) decreased – by 22% and 19% in SRIH-14- and octreotide-treated animals, respectively (Table 1). The absolute weights of the adrenal glands were significantly ($p<0.05$) decreased – by 23% and 21% in the SRIH-14- and octreotide-treated groups, respectively (Table 1). The relative weights of the pituitary and adrenal glands did not differ significantly between the SRIH-14- and octreotide-treated groups (Table 1).

Immunopositive ACTH cells

In control males, ACTH cells immunostained intensely, either individually or in groups located between the capillaries in the pituitary pars distalis. The ACTH cells were of irregular shape. They possessed eccentrically placed, ovoid nuclei and cytoplasmic processes between neighboring cells.

Table 1. Effects of chronic treatment with SRIH-14 and octreotide on body weight, apsolute pituitary and adrenal weight, relative pituitary and adrenal weight in male rats.

Groups	Body weight, (g)	Absolute weight, (mg)		Relative weight, (mg %)	
		pituitary	adrenal	pituitary	adrenal
Controls	269.1±22.9	9.1±0.1	18.1±0.5	3.4±0.3	6.7±0.5
SRIH-14	217.2±19.2[a] -19%	7.1± 0.6[a] -22%	13.7±0.6[a] -23%	3.3±0.6	6.2±0.5
Octreotide	225.6±12[a] -16%	7.3±0.7[a] -19%	14.3±1.2[a] -21%	3.2±0.3	6.5±0.3

(mean ± SD; n=5); [a], p<0.05 vs. controls

The localization and shape of ACTH immunoreactive cells did not change in either treated or control rats (Figure 1 A, B, C).

The morphometric parameters are shown in Table 2. The volumes of ACTH cells and their nuclei in the SRIH-14- and ocreotide-treated groups were not significantly ($p>0.05$) decreased compared to the control. Compared to the control, the volume density of the ACTH cells in the SRIH-14-treated group decreased significantly ($p<0.05$) by 11%, whereas in the octreotide-treated group the observed decrease was not significant ($p>0.05$).

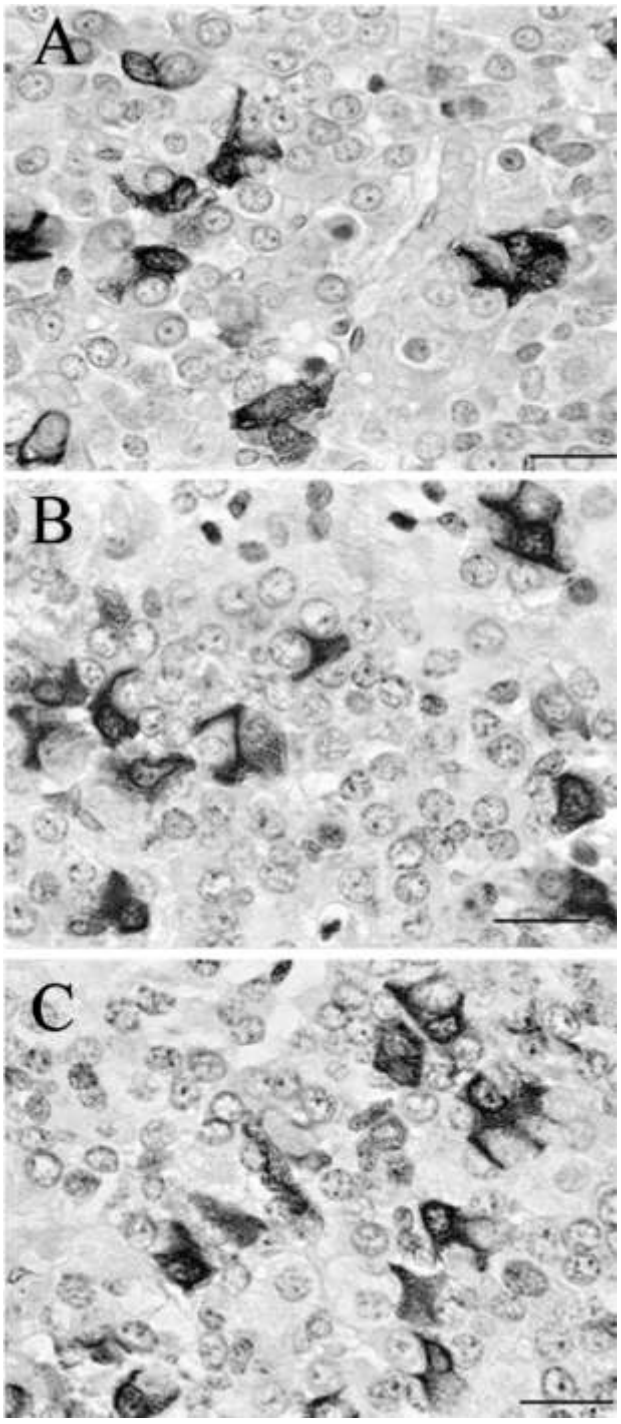

Figure 1. ACTH cells in A) control rats; B) rats treated with SRIH-14; C) rats treated with octreotide. Bar, 20 μm.

The measured decrease in plasma ACTH levels with respect to the control was not statistically significant ($p>0.05$) in either experimental group (Figure 2).

The morphometric parameters of the ACTH cells, as well as ACTH levels, did not differ significantly between the SRIH-14 and octreotide-treated groups.

The adrenal gland

The absolute volumes of the adrenal glands decreased significantly ($p<0.05$) – by 24% and 20% in the SRIH-14- and octreotide-treated groups, respectively (Figure 3). Compared to the control, the absolute volumes of the adrenal cortices were significantly ($p<0.05$) decreased – by 13% and 17% in the SRIH-14- and octreotide-treated groups, respectively.

The relative volumes of the adrenal cortices remained unchanged in both of the treated groups (Figure 4). The absolute volumes of the adrenal glands and the adrenal cortices did not differ significantly between the SRIH-14- and octreotide-treated groups.

The adrenal cortex – histological and morphometric findings

Zona glomerulosa (ZG)

The ZG is arranged in closely packed ovoid-shaped cell clusters. The cells of the ZG are relatively small, columnar or pyramidal, with oval nuclei.

Table 2. Effects of chronic SRIH-14 and octreotide treatments on the morphometric parameters of ACTH cells in male rats.

Groups	*Volume of the ACTH cells, (μm^3)*	*Volume of the ACTH nuclei, (μm^3)*	*Volume density of the ACTH cells, (%)*
Controls	1044±40	144±3	17±1
SRIH-14	996±55	141±4	15±1[a] -11%
Octreotide	1033±31	139±6	16±1

(mean ± SD; n=5); [a], p<0.05 vs. controls

The overall shape of ZG cells in both treated groups did not change (Figure 5 A, B, 5C).

The absolute volumes of the ZG decreased significantly ($p<0.05$) – by about 19% and 25% in the SRIH-14- and octreotide-treated groups, respectively (Figure 3).

The relative volumes of the ZG in both treated groups were significantly decreased ($p<0.05$) – by about 13% and 16% in the SRIH-14- and octreotide-treated groups, respectively (Figure 4).

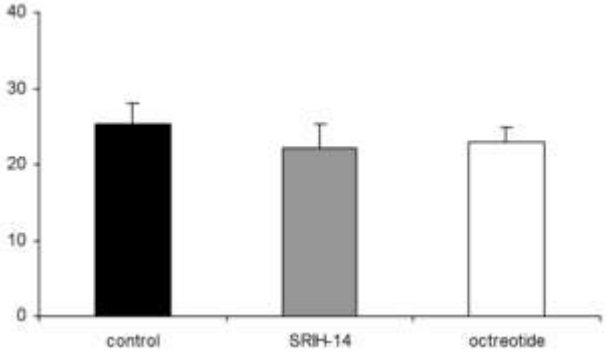

Figure 2. The effects of treatments with SRIH-14 and octreotide on blood ACTH concentrations (pg/mL) in male rats. (mean ± SD; n=5).

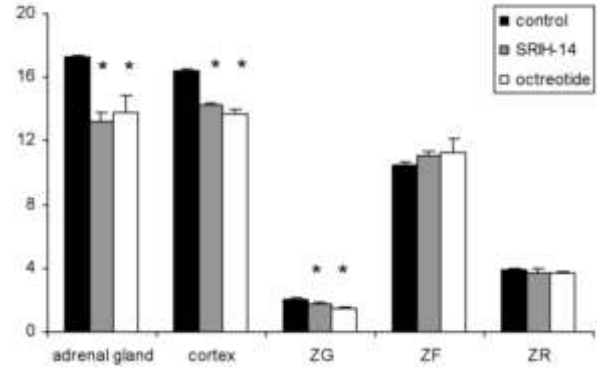

Figure 3. The absolute volume (cm^3) of the adrenal cortex, zona glomerulosa (ZG) and zona fasciculata (ZF) after treatments with SRIH-14 and octreotide in male rats. (mean ± SD; n=5); [a], $p<0.05$ *vs.* controls.

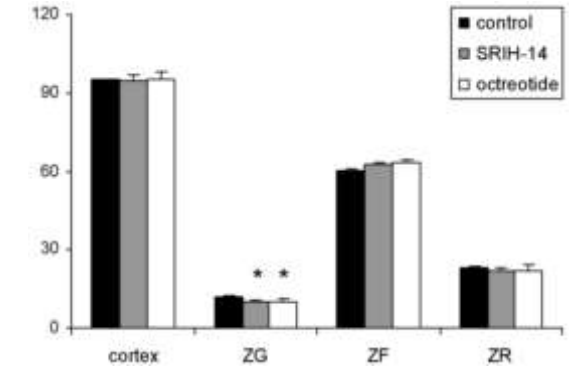

Figure 4. The relative volume (%) of the adrenal cortex, zona glomerulosa (ZG) and zona fasciculata (ZF) after treatments with SRIH-14 and octreotide in male rats. (mean ± SD; n=5); [a], $p<0.05$ *vs.* controls.

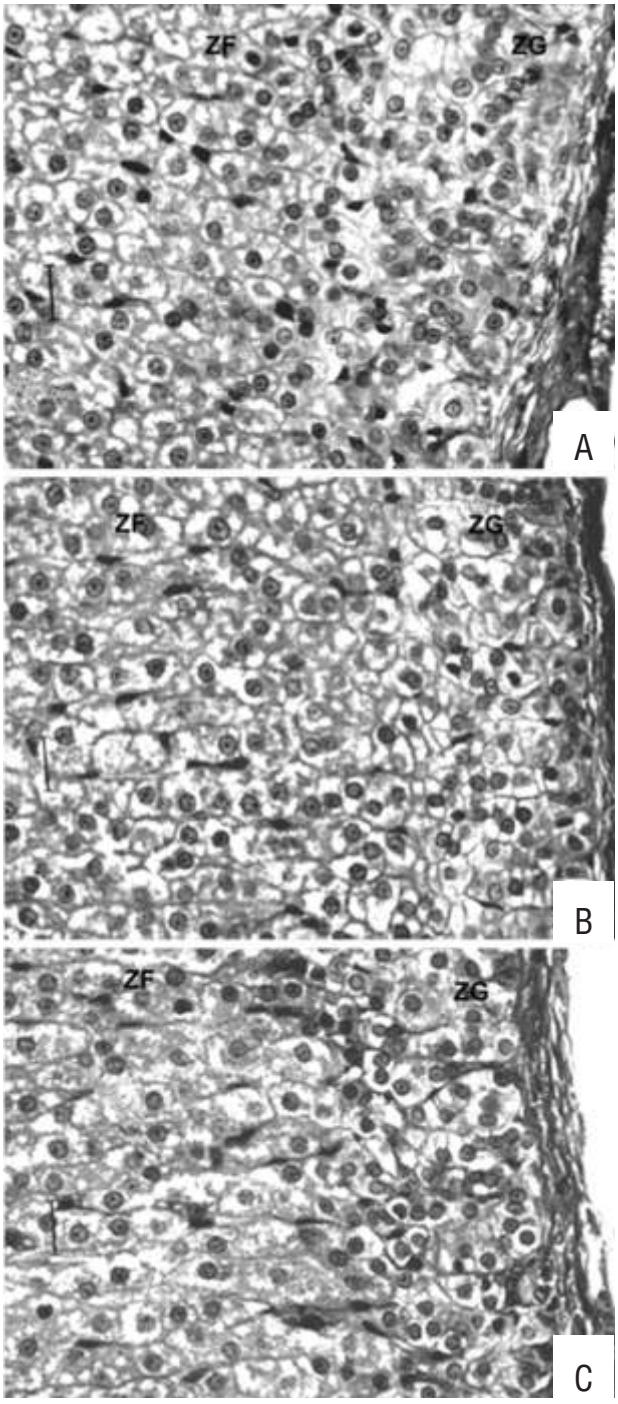

Figure 5. ZG and ZF in A) control rats; B) rats treated with SRIH-14; C) rats treated with octreotide. Bar, 20 μm. ZG – zona glomerulosa; ZF – zona fasciculata.

Stereological analysis showed that the volumes of the ZG cells and their nuclei in both treated groups were significantly ($p<0.05$) decreased in comparison with the control (Table 3).

The serum levels of aldosterone significantly ($p<0.05$) decreased – by 13% in the SRIH-14-treated group and by 19% in the octreotide-treated group (Figure 6).

Zona fasciculata (ZF) and zona reticularis (ZR)

The ZF is large and it is made up of polyhedral cells. The cells are arranged in long, straight, one to two cells thick cords separated by sinusoidal capillaries. The shapes and positions of the cells did not change after chronic treatments with either SRIH-14 or octreotid (Figure 5A, B, 5C). The absolute and relative volumes and stereological parameters (cell and nuclear volumes) of the ZF and the ZR did not change (Figures 3 and 4; Table 3). Neither the SRIH-14- or octreotide-treated groups demonstrated significant ($p>0.05$) increases in corticosterone serum levels (Figure 7).

The morphometric parameters of the ZG, ZF and ZR cells and the aldosterone and corticosterone concentrations did not differ significantly between the SRIH-14- and octreotide-treated groups.

Discussion

The aim of this study was to examine the effects of chronic SRIH-14 and octreotide administration on the structure and function of the pituitary-adrenal axis in adult male rats.

Under our experimental conditions, a significant lowering of the body weight was detected after both treatments. These results are in agreement with our previous findings obtained after ICV administration of SRIH (Miloševic *et al.*, 1996). Other authors have shown that SRIH reduces food intake both in man and in the rat (Lieverse *at al.* 1995; Scalera and Tarozzi, 1998).

A decrease of absolute pituitary weight after SRIH-14 and octreotide administration was observed whereas its relative weight remained unchanged. This result could be due to the significant decrease in volume density of somatotropes and lactotropes which together contribute to more than 60% of pituitary cells (Milošević *et al.*, 1998). The ratio of the pituitary weight to the relative body weight did not change since both the absolute pituitary weight and the body weight decreased after the treatments.

The relative volume density of pituitary corticotrophes decreased significantly after the treatment with SRIH-14, while octreotide did not affect this stereological parameter. Other examined stereological parameters after both treatments were not significantly changed, and neither were the mean

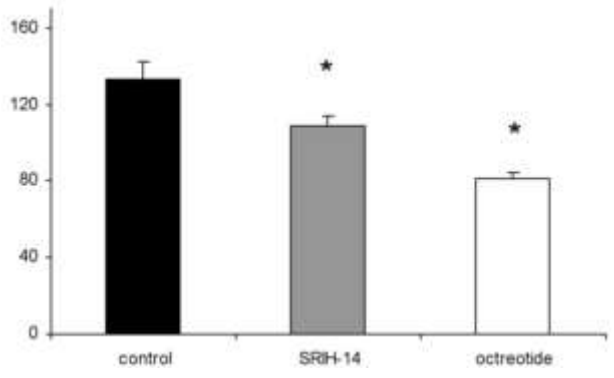

Figure 6. The effects of treatments with SRIH-14 and octreotide on aldosterone blood concentrations (pg/mL) in male rats. (mean ± SD; n=5); [a], $p<0.05$ *vs.* controls

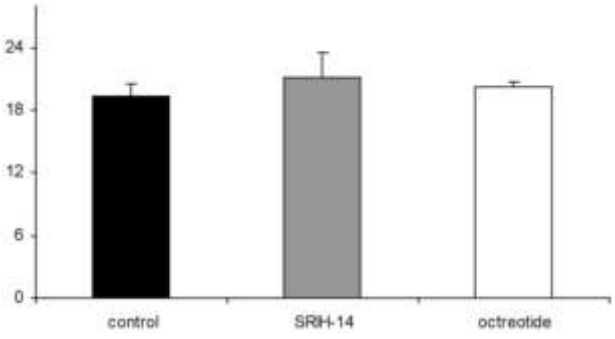

Figure 7. The effects of treatments with SRIH-14 and octreotide on corticosterone blood concentrations (ng/mL) in male rats. (mean ± SD; n=5).

Table 3. Effects of chronic SRIH-14 and octreotide treatments on the morphometric parameters of male rat zona glomarulosa (ZG), zona fasciculate (ZF) and zona reticulata (ZR).

Groups	*Volume of cells,(μm³)*			*Volume of nuclei,(μm³)*		
	ZG	*ZF*	*ZR*	*ZG*	*ZF*	*ZR*
controls	909±43.6	1521±40.3	1038±34	100±1.5	140±8.2	130±4
SRIH-14	825±30.4[a] -10%	158±14.7	996±33	87±1.8[a] -13%	140±1	123±5
octreotide	823±16.1[a] -9%	1585±34.8	980±40	85±0.5[a] -15%	139±3.7	120±1

(mean ± SD; n=5); [a], p<0.05 vs. controls.

plasma ACTH levels, although a tendency towards decrease was observed. *In vitro* studies showed that native SRIH either did not have any effect or inhibited basal and CRH-induced ACTH release from cultured pituitary cells obtained from normal rats (Brown *et al.*, 1984; Nicholson *et al.*, 1984). However, in At-T20 mouse and 7315 rat pituitary tumor cell lines SRIH exerted an inhibitory effect (Richardson and Schonbrunn, 1981; Lamberts *et al.* 1986). Octreotide did not affect basal and CRH-induced ACTH release in cultured normal rat pituitary cells (Lamberts *et al.*, 1989), whereas chronic administration of octreotide inhibited the growth of ACTH-PRL-secreting pituitary tumors (Lamberts *et al.*, 1986). It is well known that somatostatin activity is mediated through all five somatostatin receptor subtypes, while octreotide actions are mediated through only sst_2 and sst_5 (high affinity) and sst_3 (moderate affinity) receptors. O'Carroll and Krempels (1995) showed that in corticotropes the expression of all five sst mRNAs was low. Therefore, the weak and negative effect of SRIH-14 and the absence of octreotide-promoted effects under our experimental conditions could be due to the low expression of sst in pituitary ACTH cells.

At the level of adrenal gland, SRIH-14 and octreotide treatments brought about a significant decrease of all of the examined stereological parameters in the ZG and of the serum level of aldosterone. However, in the ZF and ZR, the stereological parameters and the serum corticosterone levels were not affected by the treatments.

These findings are in agreement with the results obtained after multiple ICV treatments with SRIH-14 and SRIH-28 which caused the ZG cells to atrophy (Milošević *et al.* 1996). Somatostatin can exert an inderect effect on ZG cells by influencing the rennin-angiotensin regulating system (in particular angiotensin II-induced aldosterone secretion; Aguilera *et al.*, 1981). In addition, it is very likely that both SRIH and octreotide decreased the secretion of pituitary GH and PRL hormones, the endogenous stimulators of ZG cell growth and secretion (Mazzocchi *et al.*,1986; Rebuffat *et al.*,1986; Bjoro *et al.,* 1988; de Bruin *et al.,* 1992; Patel, 1999; Andersen *et al.,* 1995). Since sst_1 sst_2 sst_4 and sst_5 mRNAs were detected in the ZG, and as the expression of sst is extremely low in the ZF and ZR (O'Carroll, 2003), somatostatin probably directly affected the adrenal ZG as well.

In conclusion, our results showed that the administration of both SRIH-14 and octreotide affected the morphological features of the adrenal ZG in the same manner – by decreasing the total volume, as well as the cell and nuclear volumes. The treatments significantly lowered the plasma level of aldosterone. They did not change the structure and functioning of pituitary ACTH cells. The structure and functioning of adrenal ZF and ZR cells remained unchanged.

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