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THE ADRENAL CORTEX IN MALE RATS AFTER CENTRALLY APPLIED SOMATOSTATIN (SRIF-28): STEREOLOGY AND HORMONE CONCENTRATIONS

VERICA MILOŠEVIĆ*, BRANISLAVA BRKIĆ**, S.VELKOVSKI***, MILKA SEKULIĆ*, MIRJANA LOVREN*, GORDANA UŠĆEBRKA**** and VESNA STARČEVIĆ***

*Institute for Biological Research "Siniša Stanković", Belgrade, **Clinical Centre "Dr Dragiša Mišović", Belgrade, ***Institute of Physiology, School of Medicine, University of Belgrade, Belgrade, ****Faculty for Agriculture, University of Novi Sad, Novi Sad, Yugoslavia

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The effects of intracerebroventricular (i.c.v.) administration of somatostatin (SRIF-28) on zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) of the adrenal cortex and on the blood concentrations of adrenocorticotropic hormone (ACTH), prolactin (PRL), growth hormone (GH), aldosterone, cortisol, progesterone, estradiol, Na+ and K+ in male rats were studied. Each experimental animal was given three 1 μg doses of SRIF-28 dissolved in 5 μl saline by the i. c. v. route every second day. Controls were treated in the same way with the same volume of saline. The rats were sacrificed by decapitation five days after the last SRIF-28 dose. The left adrenal gland oF each animal was dissected out and prepared for morphometric analyses. Blood samples were collected for hormone determinations. SRIF-28 led to significant reduction ($p<0.05$) of the absolute volume of the adrenal gland and of the volume of the cortex and ZG in comparison with the controls. Total number and volume of ZG cells and their nuclei was also significantly reduced ($p<0.05$) in SRIF-28-treated rats as compared to the controls. There were no significant changes in morphometric parameters of ZF and ZR. At the same time, significant decreases ($p<0.05$) of aldosterone, GH, PRL and Na+ levels were observed, while the concentrations of ACTH, cortisol, progesterone, and estradiol were almost unaltered in comparison with control values. It can be concluded that centrally applied SRIF-28 inhibits both the secretory activity and growth of adrenal ZG cells but insignificantly affects ZF and ZR cells.

Key Words: Adrenal cortex, SRIF-28, ACTH, PRL, GH, aldosterone, cortisol, progesterone, estrdiol, male rats.

INTRODUCTION

The octacosapeptide somatostatin-28 (SRIF-28), an N-terminally elongated form of SRIF-14 that consists of 28 amino acid residues (Meyerhof et al., 1992) is widely distributed in the central and peripheral nervous system (Epelbaum et al., 1994; Carpentier et al., 1996).This peptide is also present in nonneuronal tissues such as: gastrointestinal tract, endocrine pancreas (Reichlin, 1983), thyroid (Bordi et al., 1996), adrenals (Morel et al., 1990; Gillies, 1997), thymus (Campbell and Scanes, 1995) and ovaries (Holst et al., 1995). Hypothalamic SRIF is responsible for the inhibition of growth hormone (GH) (Milošević et al., 1994), thyrotropin-stimulating hormone (TSH) and prolactin (PRL) secretion from the anterior pituitary (Epelbaum et al., 1994). Also, somatostatin inhibits the secretion of several nonpituitary hormones such as insulin, glucagon, gastrin, secretin (Francis et al., 1990) and aldosterone (Mazzocchi et al., 1985; Robba et. al., 1986; Aguilera, 1993; Milošević et al., 1997) and possesses potent antiproliferative properties (Lamberts et al., 1991).

It has been reported that SRIF inhibits growth and secretory activity of the adrenal cortex zona glomerulosa (ZG) (Mazzocchi et al., 1985; Rebuffat et al., 1994). In addition to this direct inhibitory effect, SRIF can influence the adrenal cortex through the hypothalamic-pituitary axis (HPA) (Ganong et al., 1987). This might represent a physiological, indirect effect of hypothalamic somatostatin on the growth and function of ZG-cells in male rats.

In view of the above-stated hypothesis, the purpose of this study was to examine the effects of intracerebroventricular administration of very low doses of SRIF-28 on the morphology and secretory activity of all three zones of the adrenal cortex in adult male rats.

MATERIAL AND METHODS

Adult male Wistar rats (200-220 g), bred at the Institute for Biological Research, Belgrade were used. They were kept under a 12/12 h light-dark cycle, at 22 ± 2^{0}C and fed commercial rat chow (D.D. "Veterinarski zavod "Subotica, Yugoslavia). Food and water were available *ad libitum.*

Animal preparation. Surgical procedures were performed under ether anesthesia (aether ad narcosis Ph. Iug. III. produced by "Lek", Ljubljana, Slovenia). A headset implanted into the rats was used later 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., 1988), implanted into a lateral cerebral ventricle, 1mm 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. Cannula and screw were cemented to the skull with dental acrylic (Smgal; ICN Galenika, Belgrade, Yugoslavia).

Treatment of animals. After the rats had recovered from surgery they were divided into 2 groups each consisting of five animals. Animals from the first group were given (at 10.00 a.m.) three 1 μg doses of SRIF-28 (Sigma No. S 9129, U. S. A) dissolved in 5 μL saline every second day. The second group (control) of rats

were treated in the same manner with 5 μ L salinei. c. v. All animals were sacrificed by decapitation during deep anesthesia, at 10.00 a. m. (5 days after the last injection).

Light microscopy. Left adrenal glands were excised, fixed in Bouin's soution, embedded in paraffin and serially cut into 5 μ m thick sections which were stained with hematoxylin-eosin and examined under a light microscope(Opton).

Morphometry. Stage 1: Zonation of the adrenal gland. In order to evaluate the volume densities of the adrenocortical zones, every thenth section of the gland was analyzed using 125x magnification and the multiporose test system M_{42} (Weibel and Gomez, 1962). The absolute volume of the glands was calculated on the basis of their weight, assuming an average specific gravity of the adrenal of 1.039 g/cm^3 (Swinyard, 1938).

Stage 2: Size and number of adrenocortical cells. The volume densities of nuclei and cytoplasm of parenchymal cells were estimated on a screen at 1000x magnification using the multiporose test system M42 (Weibel and Gomez, 1962). For each adrenal gland, a single paraffin section containg zona medullaris was chosen and 30 test areas of ZG and 50 test areas of ZF and ZR were analyzed. On the basis of earlier karyometric studis (Malendowicz, 1974), the shape coefficient β was assumed to be 1.382 for the ZF and 1.500 for the ZG. It relates N_v (number of cells counted *per unit* volume) to N_a (number of cells counted *per* mm^2) and V_v (volume density)and depends on the axial ratio of estimated nuclei. The number of adrenocortical cell nuclei *per* mm^3 was calculated according to the method of Weibel and Gomez (1962). Since the rat adrenocortical cells are mononucleated, the numerical density of nuclei corresponds to the number of cells *per* mm^3.

Biochemical analyses. Plasma levels of adrenocorticotropic hormone (ACTH) in control in control and SRIF-28-treatd rats were measured by radioimmunoassay (ACTH RIA kit, Vinča, Belgrade, Yugoslavia). Serum levels of aldosterone were also determined by radioimmunoassay (ALDO RIA kit, Biodata, Rome, Italy). The Delfia method (hGH, hPRL, cortisol, estradiol, and progesteorne Delfa kits, LKB, Turku, Finland) was employed to estimate GH, PRL, cortisol, estradiol and progesterone levels in serum. The serum concentrations of sodium (Na^+) and potassium (K^+) were measured using flame photometry (LKB, Stockholm, Sweden).

Statistical analyses. Biochemical and morphometric data obtained from each group were aweraged and the standard deviation of the mean was calculated. One-way analysis of variance (ANOVA), followed by the multiple-range test of Duncan was used for statistical comparisons between groups. Values of Pless than 0.05 were considered to indicate statistical significance.

RESULTS

Body weight, absolute and relative weight of the adrenal gland

Body weight and absolute weight of the adrenal gland were significantly decreased ($p<0.05$) in SRIF-28-treated animals, while relative weight of the adrenals remained unchanged (Table 1).

Table 1. Effects of i. c. v. administred SRIF-28 on the body weight and absolute and relative weights of the adrenal gland in adult male rats.

	Body weight (g)	Absolute adrenal weight (mg)	Relative adrenal weight (mg/100g)
Controls	217.5 ± 12.6	26.6 ± 1.6	12.2 ± 0.7
SRIF-28	180 ± 12.2*	21.4 ± 2.1*	11.9 ± 1.3

Values are means ± S. D. (n=5),* P<0.05.

Morphometry of the adrenal cortex

Application of SRIF-28 led to a significant decrease in the volume of the adrenal glands (22%) as compared to the controls (Figure 1). Also, the absolute and relative volumes of adrenal cortex of the former group were significantly decreased (by 21% and 2%, respectively) as seen in Figures 1 and 2. All three cortical zones of the adrenal gland in all examined preparations were clearly visible: ZG, ZF and ZR.

Zona glomerulosa. Both absolute and relative volumes of ZG were

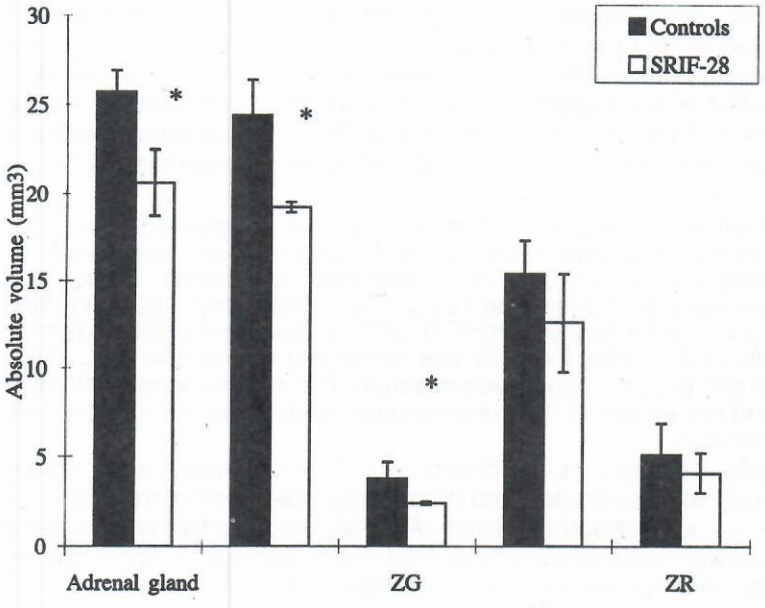

Figure 1. Absolute volumes of adrenal gland, cortex, ZG, ZF, and ZR after i. c. v. administration of SRIF-28 to male rats. Values are means æ S. D. (n=5), *p<0.05.

decreased (p<0.05) by 37% and 20%, respectively (p<0.05) in the rats that received SRIF-28, as compared to the controls (Figures 1 and 2). The ZG-cell volume and their nuclear volume were significantly (p<0.05) decreased by 11%

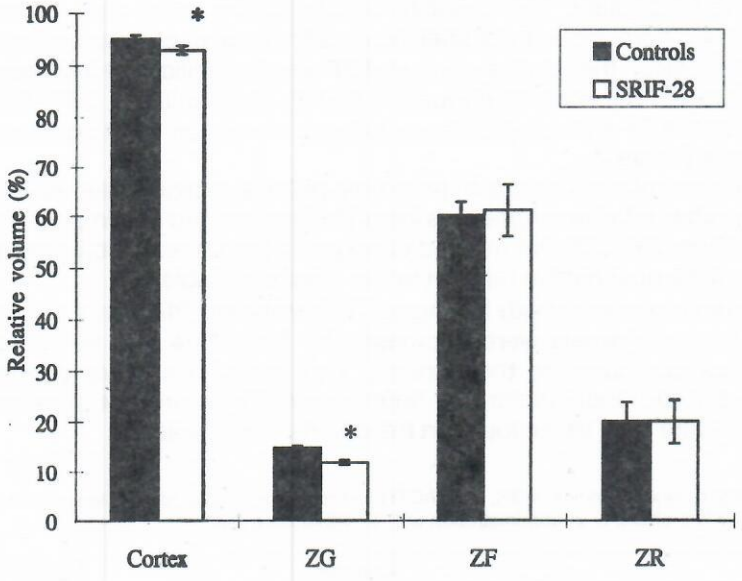

Figure 2. Relative volumes of adrenal cortex, ZG, ZF and ZR after i. c. v. administration of SRIF-28 to male rats. Values are means æ S.D. (n=5), *p<0.05.

and 16% respectively after i. c. v. administrationof SRIF-28 in relation to the controls. Total number of ZG-cells was significantly decreased in comparison with the controls (Table 2).

Table 2. Effects of i. c. v. administered SRIF-28 on the morphometic parameters of adrenal cortex zones in adult male rats.

	Controls	SRIF-28
ZG		
Volume of cells (μm^3)	995 ± 46.5	883 ± 34.4*
Volume of nuclei (μm^3)	115 ± 5.5	97 ± 7.9*
Total number of cells ($1x10^6$)	3.488 ± 0.1	2.507 ± 0.1*
ZF		
Volume of cells (μm^3)	1798 ± 35.6	1773 ± 60.0
Volume of nuclei (μm^3)	179 ± 3.6	177 ± 6.0
Total number of cells ($1x10^6$)	6.686 ± 0.7	6.325 ± 0.7
ZR		
Volume of cells (μm^3)	1172 ± 27.6	1132 ± 29.9
Volume of nuclei (μm^3)	117 ± 1.8	118 ± 3.6
Total number of cells ($1x10^6$)	3.947 ± 0.4	3.391 ± 0.4

Values are means ± S.D. (n=5), * p<0.05.

Zona fasciculata. The absolute volume of this zone was decreased by 18%,but the difference was not statistically significant in comparison with saline-treated rats. Also, the relative volume of ZF was insignificantly increased by 2% as compared to the controls (Figures 1 and 2). No significant SRIF-28-induced changes of the ZF stereological parameters were observed in comparison with the controls (Table 2).

Zona reticularis. The absolute volume of ZR was slightly decreased by 20% ($p>0.05$), while relative volume was insignifantly changed in comparison with the controls (Figs. 2 and 3). No marked changes of the stereological parameters of this zone were observed in relation to the controls (Table 2).

Serum levels of growth hormone (GH),prolactin (PRL) and aldosterone in SRIF-28-treated animals were decreased by 30%, 31% and 80%,respectively ($p<0.05$),as compared to the controls. The levels of cortisol, progesterone, estradiol and adrenocorticotropic hormone (ACTH) were not significantly different ($p>0.05$) from those found in the control rats (Table 3).

Table 3. Blood concentrations of GH, PRL, ACTH, aldosterone, cortisol, progesterone, estradiol, Na+ and K+ after i. c. v. administration of SRIF-28 to male rats.

	Controls	SRIF-28
GH (mU/L)	0.50 ± 0.05	0.32 ± 0.03*
PRL (ng/ml)	10.4 ± 1.5	7.2 ± 0.9*
ACTH (pmol/L)	19.6 ± 4.0	23.1 ± 4.1
aldosterone (nmol/L)	0.84 ± 0.04	0.17 ± 0.01*
cortisol (nmol/L)	229.0 ±17.4	235.0 ± 23.0
estradiol (pmol/L)	52.0 ± 2.3	55.2 ± 7.6
progesterone (nmol/L)	9.6 ± 1.1	11.4 ± 0.4
Na^+ (mmol/L)	133.8 ± 4.7	124.0 ± 4.0*
K^+ (mmol/L)	5.0 ± 0.1	6.2 ± 0.1*

Values are means ± S. D. (n=5). *$p<0.05$.

Sodium ion (Na+) concentration was decreased by 7% ($p<0.05$) in comparison with the corresponding controls, while the opposite change of potassium ion (K+) level which was increased by 24% ($p<0.05$) was recorded (Table 3).

DISCUSSION

The present findings provide strong evidence that repeated intracerebrovntricular (i. c. v.) bolus injctions of very low SRIF-28 doses suppress both the growth and secretory capacity of the rat adrenal ZG. Previous reports have shown that SRIF directly inhibits growth and steroidogenic capacity of rat ZG cells (Mazzocchi et al., 1985; Robba et al., 1986) and decreases their basal prolifration rate (Pawlikowski et al., 1990), probably by blocking the basal trophic action of angiotensin-II (Aguilera et al., 1981). The SRIF-induced atrophyu of ZG and its parenchymal cells is coupled with a decrease in the volume of the

mitochondrial compartment and smooth endoplasmic reticulum (SER) (Rebuffat et al., 1994). The enzymes of aldosterone synthesis are located in both mitohondria and SER (Nussdorfer, 1986; Morel et al., 1990), and the changes in the surface area per cell mitochondrial cristae and SER tubules are tightly coupled with corresponding changes in the activity per cell of some of these enzymes (Nussdorfer, 1986). Several studies indicate that the ZG of rats possess specific SRIF receptors (Maurer and Reubi, 1985; Srikant and Patel, 1985; Morel et al., 1990; Lee et al., 1994; Epelbaum et al., 1995; Yamaguchi et al., 1995). Kong et al. (1994) showed that rat adrenal gland expresses high levels of SRIF-receptor mRNA. However, regulatory mechanisms and pathways by which the central somatostatinergic system affects the rat adrenal ZG remain to be elucidated. Two pathways are possible: 1) via the hypothalamic pituitary axis (Näsman et al., 1995; Milošević et al., 1997) and 2) through an altered sympathetic tone to the chromaffin cells in the adrenal medulla which exerts aparacrine control of the cortical function, especially in the ZG (Cooper et al., 1991; Einer-Jensen and Carter 1991; Feldman and Weidenfeld, 1995; Nussdorfer, 1996). The latter possibility is unlikely, because central SRIF has not been shown to interact with the adrenergic system. However, there is an interaction with the cholinergic system i. e. an increased responsiveness to acetylcholine (Neri et al., 1996). Moreover, it is well known that the main regulatory control of the secretory activity of the adrenal cortex is essentially hormonal and/or humoral (Mazzocchi et al., 1986; Chiodini et al., 1991). Accordingly, i. c. v. administration of SRIF may decrease the secretion of GH (Milošević et al., 1994) and PRL by the anterior pituitary via periventricular structures and hypothalamic somatostatinergic pathways. It has been established that GH and PRL stimulate growth and especially aldosterone secretion of ZG cells (Aguilera, 1993). A decrease in the secretion of these hormones as a result of intracerebroventricular SRIF -administration would be expected to suppress growth and secretion of ZG cells. The present results clearly demonstrate that i. c. v. SRIF-28 significantly decreases serum concentrations of Na+ and significantly increases serum concentrations of K+, as a logical consequence of aldosterone deficiency.

Our results did not show statistically significant changes of the ZF and ZR cells. These results are in accordance with those of other authors (Mazzocchi et al., 1985; Robba et al., 1986; Rebuffat et al., 1994; Milošević et al., 1997). The serum concentration of cortisol, progesterone and estradiol, the main hormonal products of rat ZF and ZR were insignificantly increased in comparison with controls. In fact, the synthesis and the secretion of these hormones from the ZF and ZR cells are controlled by ACTH (Nussdorfer, 1986). The secretion of ACTH was modestly increased, but not significantly in comparison with the control rats. This slight increase in plasma ACTH levels may have some clinical implications. Glucocorticoids are well known to inhibit growth and GH secretion in humans and animals through an increase in hypothalamic somatostatin (Guistina and Wehrenberg, 1992).

Acute glucocorticoid administration, often used for treatment purposes, may increase the hypothalamic somatostatin tone and thus suppress GH secretion (Guistina et al., 1990). Since SRIF is widely distributed in all brain regions,

further research is necessary to shed more light on the specific mechanisms by which centrally administered SRIF affects adrenocortical function. It can be concluded that centrally applied SRF-28 inhibits both the secretory activity and growth of adrenal ZG cells only, while ZF and ZR cells were insignificantly affected.

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KORA NADBUBREŽNE ŽLEZDE MUŽJAKA PACOVA POSLE CENTRALNO UBRIZGANOG SRIF-28: Sterološka i hormonska analiza

VERICA MILOŠEVIĆ,BRANISLAVA BRKIĆ, S. VELKOVSKI, MILKA SEKULIĆ, MIRJANA LOVREN, GORDANA UŠĆEBRKA i VESNA STARČEVIĆ

SADRŽAJ

Ispitivani su efekti intracerebroventikularno (i. c. v.) ubrizganog somatostatina (SRIF-28) na zona glomerulosa (ZG), zona fasciculata(ZF) i zona raticularis (ZR) kore nadbubrežne žlezde mužjaka pacova. Uporedo je određivana koncentracija ACTH, PRL, GH, aldosterona, koritozola, progesterona i estradiola u krvi po tretmanu sa SRIF-28. Sve eksperimentalne životinje su primile tri 1 μg doze SRIF-28 rastvorenog u 5 μL fiziološkog rastvora, svaki drugi dan. Kontrole su tretirane na isti način fiziološkim rastvorom. Pacovi su žrtvovani petog dana po poslednjoj primljenoj dozi SRIF-28. Leva nadbubrežna žlezda je izvađena i pripremljena za histološku i morfometrijsku analizu. Istovremeno je uzimana krv za određivanje koncentracije hormona. U SRIF-28 tretiranih pacova značajno su smanjeni ($p<0.05$) apsolutni volumen cele žlezde, kore i ZG u poređenju sa kontrolom. Ukupan broj ćelija ZG, zapremina ovih ćelija i njihovih jedara takođe su značajno smanjeni ($p<0.05$) u ovoj grupi u odnosu na kontrolu. U ZF i ZR nisu nađene značajne promene morfometrijskih parametara. Koncentracije ALDO, PRL., GH u krvi značajno su smanjene ($p<0.05$) u odnosu na kontrolu, dok koncentracije ACTH, kortizola, progesterona i estradiola ne pokazuju značajne promene u poređenju sa kontrolnom. Na osnovu toga može se zaključiti da SRIF-28 inhibira rast i sekretornu aktivnost ćelija ZG, dok ne ispoljava značajan uticaj na ćelije ZF i ZR.