Arch. Biol. Sci., Belgrade, 60 (1), 41-47, 2008 DOI:10.2298/ABS0801041M

ADRENAL CORTEX IN PERIPUBERTAL AND ADULT FEMALE RATS AFTER NEONATAL TREATMENT WITH SRIH-14

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Abstract — Neonatal female rats (3-7 days old) were injected subcutaneously twice daily with 20 μg of SRIH-14/100g b.w. for five consecutive days. The animals were sacrificed in the peripubertal (38 days old) or adult (80 days old) periods of life. Control rats were treated in the same way with identical volumes of saline. SRIH-14 led to significant reduction ($p<0.05$) of the absolute and relative volumes of the ZG in peripubertal animals, by 11 and 22%, respectively in comparison with the controls. The total number and volume of ZG cells and their nuclei were also significantly decreased ($p<0.05$) in peripubertal SRIH-14-treated rats as compared to the controls. There were no significant ($p>0.05$) changes in the morphometric parameters of adult adrenal cortex after SRIH-14 treatment compared with control females. It can be concluded that subcutaneously applied SRIH-14 in the neonatal period inhibits growth of adrenal ZG cells in female rats only up to the peripubertal stage, while the adrenal gland cortex recovers before mature adulthood is reached.

Key words: Neonatal period, *zona glomerulosa, zona fasciculata, zona reticularis*, somatostatin, female rats

UDC 612.018.2:59.017.6:612.453

INTRODUCTION

Somatostatin (SRIH) is a circulating peptide hormone that displays an array of biological actions including the inhibition of hormone secretion, modulation of neural transmission, and regulation of cell growth (Florio et al., 1999). Two active forms have been described: SRIH-28 and SRIH-14. SRIH is heterogenously distributed in the central and peripheral nervous systems (Epelbaum et al., 1995). SRIH-28 and SRIH-14 are also present in non-neuronal tissues such as the gastrointestinal tract, endocrine pancreas, and thyroid (Reichlin, 1983); and the adrenals and thymus (Gillies, 1997). Hypothalamic SRIH is responsible for the inhibition of growth hormone (GH), thyrotropin-stimulating hormone (TSH), and prolactin (PRL), as well as for gonadotrophin secretion from the anterior pituitary (Milošević et al., 2000a, 2000b, 2003; Nestorović et al. 2006). Somatostatin also inhibits the secretion of several non-pituitary hormones such as insulin, glucagon, gastrin, secretin, and aldosterone (Aguilera, 1981; Milošević et al., 1997) and possesses potent antiproliferative properties (Lamberts et al., 1991).

Many lines of evidence indicate that SRIH plays an important physiological role in the modulation of adrenal mineralocorticoid secretion in mammals (Nussdorfer, 1996). SRIH-14 administered intracerebroventricularly (i.c.v.) inhibited growth and secretory activity of the adrenal ZG in the adult period of life (Milošević et al., 1996). Besides this direct inhibitory effect, SRIH can also act on the adrenal cortex through the hypothalamic-pituitary (HP) axis (Ganong, 1987).

It is known that the neonatal period is a critical interval during the life cycle when control of neuroendocrine regulation is established. The hypothalamic-pituitary-adrenal (HPA) axis is characterized by decreased sensitivity in that period,

which is known as the stress-hyporesponsive period (Yoshimura et al., 2002).

The aim of this study was to investigate whether multiple subcutaneous neonatal SRIH-14 treatment affects the adrenal cortex cells of female rats in the peripubertal and adult periods of life.

MATERIAL AND METHODS

Animals

Time-mated pregnant Wistar rats were housed individually and maintained in a controlled environment (12 h light : 12 h dark; 22 ± 2°C) with food (Subotica Veterinarski Zavod, Subotica, Serbia) and water freely available. Female pups were injected subcutaneously twice a day (8 AM and 8 PM) with 20 μg of SRIH-14 (S9129, Sigma, St. Louis, Mo., USA) per 100 g of body weight for five consecutive days (3rd – 7th day of life; neonatal SRIH-14 treatment). The dose regime for SRIH-14 was selected according to Rebuffat et al. (1984) and modified by administration every 12 h instead of every 8 h. Control female pups received the equivalent volume of physiological saline on the same schedule. The rats were sacrificed at 8 AM in the peripubertal (38th day) or adult (80th day) periods of life. Each age group comprised five females. The experimental protocols were approved by the Local Animal Care Committee and conformed to the recommendations given in "Guide for the Care and Use of Laboratory Animals" (1996, National Academy Press, Washington D.C.)

Light microscopy

Left adrenal glands were excised, fixed in Bouin's solution, embedded in paraffin and serially cut into 5 μm 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 of the adrenocortical zones, every 10th section of the gland was analyzed using the M_{42} multipurpose test system (Weibel, 1979) at 100x magnification. The absolute volume of the glands was calculated on the basis of their weight, assuming the average specific gravity of the adrenal to be 1.039 g cm^{-3} (Swinyard, 1938).

Stage 2. Size and number of adrenocortical cells. The volume densities of both the nuclei and the cytoplasm of parenchymal cells were estimated on a screen using the M_{42} multipurpose test system (Weibel, 1979) at 1000x magnification. For each adrenal gland, a single paraffin section containing the *zona medullaris* was chosen, and 30 test areas of the *zona glomerulosa* (ZG) and 50 test areas of the *zona fasciculata* (ZF) and *zona reticularis* (ZR) were analyzed. On the basis of earlier karyometric stud-

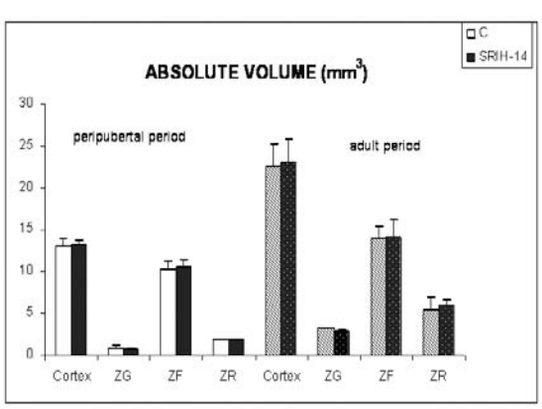

Fig. 1. Absolute volume of *zona glomerulosa* (ZG), *zona fasciculata* (ZF), and *zona reticularis* (ZR) in peripubertal and adult female rats after subcutaneous administration of SRIH-14 neonatally. Values are means ± SD (n = 5),*p< 0.05 vs. control.

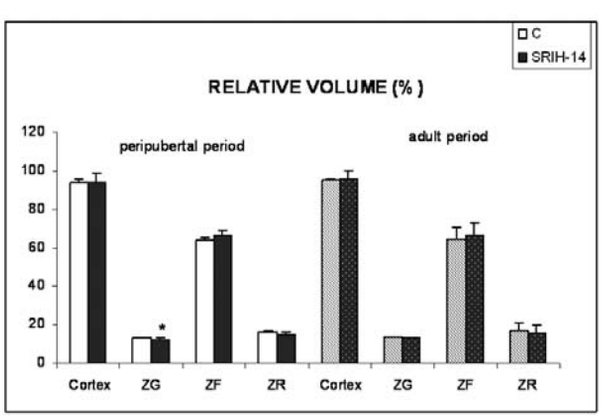

Fig. 2. Relative volume of *zona glomerulosa* (ZG), *zona fasciculata* (ZF), and *zona reticularis* (ZR) in peripubertal and adult female rats after subcutaneous administration of SRIH-14 neonatally. Values are means ± SD (n = 5), *p< 0.05 vs. control.

Table 1. Body weight and absolute and relative adrenal weight of control (C) and female rats in different periods of life after neonatal SRIH-14 treatment.

Groups		Body weight (g)	Absolute adrenal weight (mg)	Relative adrenal weight (mg%)
Peripubertal	C	117.4 ± 8.6	10.6 ± 0.9	4.8 ± 0.8
	SRIH-14	130.0 ± 0.7	13.4 ± 1.1	10.3 ± 0.9
Adult	C	255.0 ± 20.7	24.8 ± 2.3	11.7 ± 0.7
	SRIH-14	232.5 ± 20.5	25.0 ± 2.2	11.1 ± 1.4

All values are given as means ± SD.

ies, the shape coefficient β was assumed to be 1.382 for the ZF and ZR and 1.500 for the ZG. It relates Nv (number of cells counted per unit of volume) to Na (number of cells counted per mm^2) and Vv (volume density) and depends on the axial ratio of the estimated nuclei. The number of adrenocortical cell nuclei per mm^3 was calculated according to the method of Weibel (1979). Since rat adrenocortical cells are mononuclear, the numerical density of the nuclei corresponds to the number of cells per mm^3.

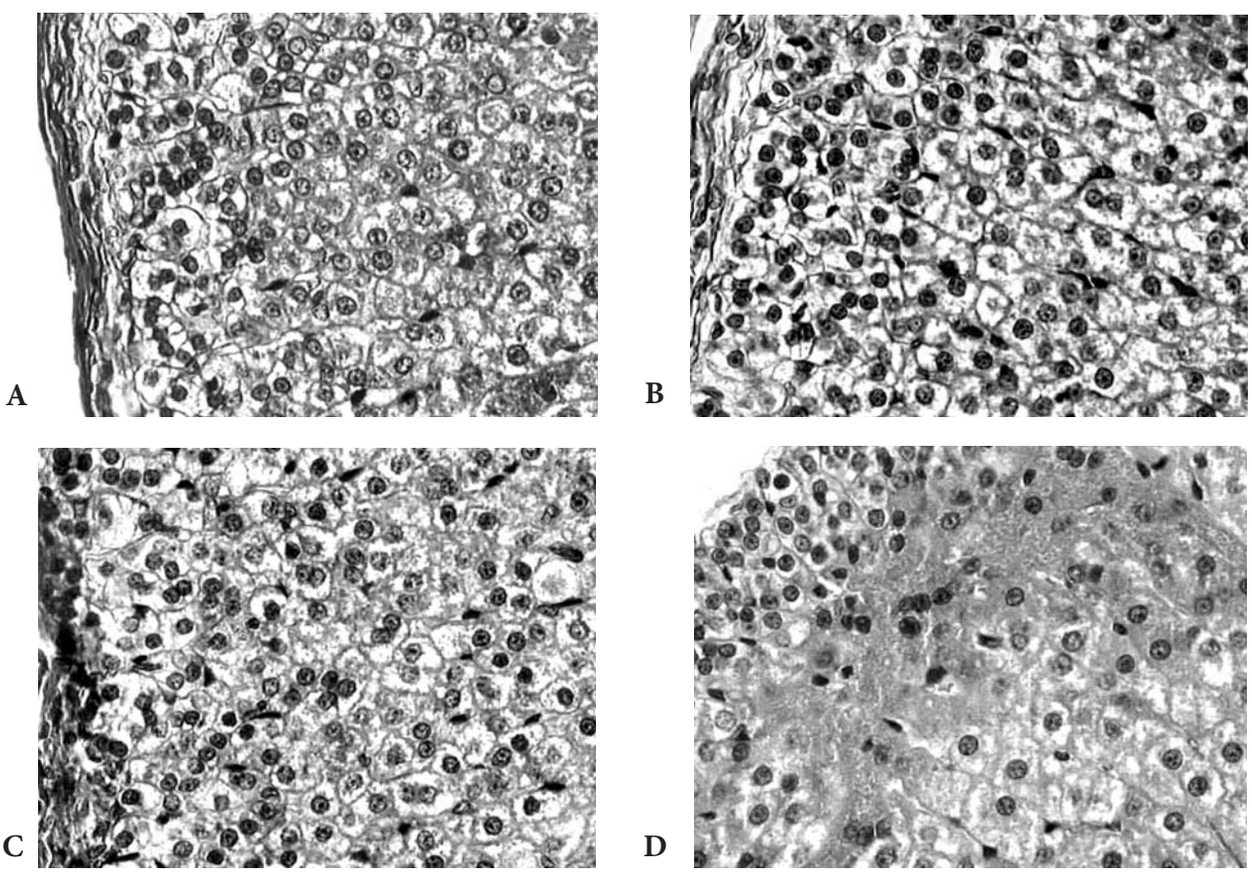

Fig. 3. Adrenal cortex of female rats in: A) control peripubertal rats; B) control adult rats; C) peripubertal rats after multiple treatment with SRIH-14; D) adult rats after multiple treatment with SRIH-14 (H&E, objective magnification x 40).

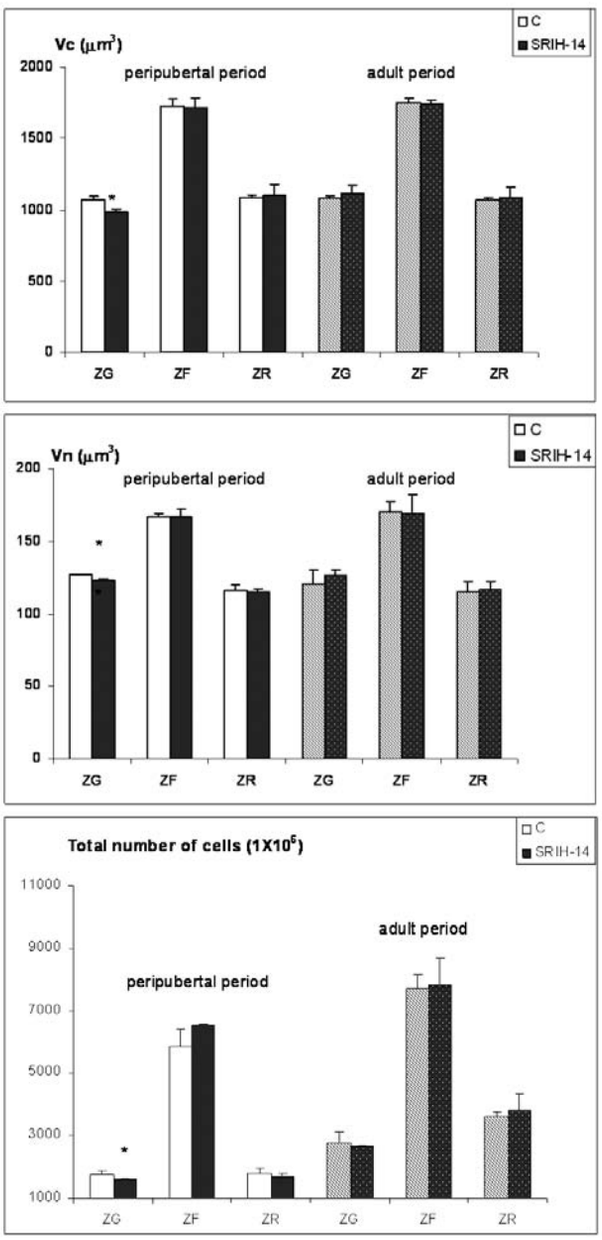

Fig. 4. A) Cellular volume (Vc; μm3) of *zona glomerulosa* (ZG), *zona fasciculata* (ZF) and *zona reticularis* (ZR) cells; B) Nuclear volume (Vn;μm3) of the *zona glomerulosa* (ZG), *zona fasciculata* (ZF), and *zona reticularis* (ZR); C) Total number of cells (1X106) in the *zona glomerulosa* (ZG), *zona fasciculata* (ZF), and *zona reticularis* (ZR). Values are means ±SD (n = 5/group), *p<0.05 vs. control.

Digital images were made on a DM RB Photomicroscope (Leica, Wetzlar, Germany) with a JVC TK 1280E Video Camera (Leica). For image acquisition, the Qwin program (Leica) was used.

Statistical analyses

The morphometric data for each group were averaged and the standard deviation (SD) 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 the groups. A probability value of 5% or less was considered statistically significant.

RESULTS

Body weight and absolute and relative weight of the adrenal gland

The data on body weight and absolute and relative adrenal weights in the control and SRIH-treated animals are summarized in Table 1. As can be seen, neonatal SRIH-14 treatment did not induce any significant changes ($p>0.05$) in these parameters in female rats at either age (Table 1).

Adrenal cortex

All three cortical zones of the adrenal gland (ZG, ZF, and ZR) were clearly visible in all examined preparations. The absolute and relative volumes of the adrenal cortex in SRIH-14-treated groups of rats were not significantly ($p>0.05$) different from those in the corresponding controls (Figs. 1 and 2).

Characteristics of the zona glomerulosa

The ZG was arranged in closely packed ovoid clusters, with relatively small columnar or pyramidal cells. The nuclei were round or oval with an evident nucleolus (Fig. 3A, B). The shape of ZG cells in the neonatally SRIH-14-treated animals was not significantly changed in the peripubertal period, but the cytoplasm in these cells was more intensively stained than in the corresponding controls (Fig. 3C, D). In adult animals, the *zona intermedia* was clearly visible after neonatal treatment with SRIH-14 (Fig. 4D). This zone consisted of small cells, each with a regularly ovoid nucleus and scarce cytoplasm.

Stereological measurements demonstrated that subcutaneous administration of SRIH-14 in the neonatal period led to a significant decrease ($p<0.05$) in absolute and relative volumes of the ZG in the peri-

pubertal period, by 11 and 22%, respectively, compared with the corresponding controls (Figs. 1 and 2). This was due to atrophy of ZG cells, manifested as a significant decrease in their volume, the volume of their nuclei, and the total number of ZG cells at the peripubertal stage when compared to the corresponding controls (Fig. 4A, B, C). In the rats sacrificed as adults, the absolute and relative volumes of the ZG were slightly decreased, by 4 and 12% ($p>0.05$), respectively, after SRIH-14 administration in comparison with the corresponding controls (Figs. 1 and 2). There were no significant differences in the volume of ZG cells and their nuclei and their total number ($p>0.05$) between the two groups of adult rats (Fig. 4A, B, C).

Characteristics of the zona fasciculata and zona reticularis

There were no significant changes in the histological appearance and stereological parameters of the ZF and ZR in peripubertal and adult female rats after neonatal SRIH-14 treatment (Figs. 1, 2, 4A, 4B, 4C).

DISCUSSION

Neonatal SRIH-14 treatment did not affect the body weight in either examined age group. These results are in accord with Starčević et al. (2000), who reported that intracerebroventricularly (i.c.v.) administered SRIH-14 did not inhibit body weight gain in female rats. Moreover, the synthetic somatostatin, an octreotide, applied i.c.v. likewise had no significant effect on body weight in male and female rats (Milošević et al., 2001a, 2001b). Thus, the absolute and relative adrenal weights in peripubertal and adult rats treated with SRIH-14 in the neonatal period were not significantly different from those of the controls. We have also shown previously that i.c.v. treatment with either SRIH-14 or SRIH-28 did not result in significant changes of absolute and relative adrenal weights in adult female rats (Milošević et al., 2001a).

However, treatment SRIH-14 in the neonatal period had an immediate and prolonged inhibitory effect on ZG cells in female rats sacrificed in the neonatal and juvenile periods of life (Milošević et al., 2006a, 2006b). The present results demonstrate that neonatally applied SRIH-14 has a prolonged inhibitory effect on the volume of ZG cells and their nuclei and the total number of these cells right up to the peripubertal period. This confirms the earlier observations of Mazzocchi et al. (1985), Robba et al. (1986), and Rebuffat et al. (1994), who showed that SRIH-14 directly inhibited growth and functional capacity of the adrenal ZG. There are specific SRIH receptors in the rat ZG (O'Caroll, 2003; Unger et al., 2004). Moreover, Kong et al. (1994) showed that the rat adrenal gland expresses high levels of SRIH receptor mRNA, while Pawlikowski et al. (1990) reported a decreased basal proliferation rate of ZG cells, probably caused by blocked basal tropic action of angiotensin-II (Aguilera et al., 1981). Regulatory mechanisms and pathways by which the central somatostatinergic system affects the rat adrenal ZG remain to be elucidated. They are realized via the hypothalamic-pituitary axis and/or through an altered sympathetic tone to the chromaffin cells in the adrenal medulla, which exerts paracrine control of cortical function, especially in the ZG (Nussdorfer, 1996; Einer-Jensen and Carter, 1995; Feldman and Weidenfeld, 1995).

On the other hand, our experimental data did not reveal statistically significant changes in the morphometric parameters of ZG, ZF, and ZR cells in adult animals neonatally treated with SRIH-14, which confirms previous data (Milošević et al., 2001a). The appearance of a visible *zona intermedia* in adults after neonatal SRIH-14 treatment probably represents increased proliferative activity of these cells, allowing recovery of the ZG in adult rats.

From these results, it can be concluded that neonatal application of SRIH-14 inhibits growth of the adrenal ZG and its cells only up to the peripubertal period in female rats, while the effect in adult females is negligible.

Acknowledgments — The authors are especially grateful to MSci. Goran Granić ("ProMedia", Zrenjanin, Serbia and MSci. Ivanka Milenković ("Olympus", Belgrade). This work was partially supported by the Ministry of Science of the Republic of Serbia (Grant No. 143007).

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КОРА НАДБУБРЕЖНЕ ЖЛЕЗДЕ ПЕРИПУБЕРТАЛНИХ И ОДРАСЛИХ ЖЕНКИ ПАЦОВА ПОСЛЕ НЕОНАТАЛНОГ ТРЕТМАНА СА SRIH-14

ВЕРИЦА МИЛОШЕВИЋ[1], МИЛИЦА ТЕРЗИЋ[2], Б. ФИЛИПОВИЋ[1], МИЛИЦА МАНОЈЛОВИЋ[1], БРАНКА ШОШИЋ-ЈУРЈЕВИЋ[1], МИЛКА СЕКУЛИЋ[1] и ВЕСНА СТАРЧЕВИЋ[2]

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Женке пацова од 3-7. дана старости (неонатални период) су субкутано третиране два пута дневно са SRIH-14 у дози од 20 μg на 100 г телесне масе током пет узастопних дана. Животиње су биле жртвоване у перипубертално (38. дана) или одраслом (80. дана) периоду живота. Контролне групе су на идентичан начин третиране физиолошким раствором. У поређењу са контролним вредностима третман са SRIH-14 је значајно смањио ($p<0.05$) апсолутни (-11%) и релативни (-22%) волумен зоне гломерулозе (ЗГ) у перипубертални животиња. Укупан број и запремина ћелија ZG и њихових једара такође су значајно смањени ($p<0.05$) у пацова третираних са SRIH-14 у перипубертални периоду живота у поређењу са контролним женкама. У кори надбубрежне жлезде одраслих женки пацова нису запажене ($p>0.05$) промене морфометријских параметрима после SRIH-14 третмана у поређењу са одговарајућом контролом. На основу добијених резултата можемо закључити да је субкутано третирање женки пацова у неонаталном периоду са SRIH-14 инхибирало раст зоне гломерулозе у перипубертални пацова.