Arch. Biol. Sci., Belgrade, 58 (2), 11P-12P, 2006.

REDUCED GROWTH OF THE ADRENAL ZONA GLOMERULOSA AFTER NEONATAL TREATMENT OF FEMALE RATS WITH SRIH-14.

Verica Milošević, Svetlana Trifunović, and Milka Sekulić. *Institute for Biological Research*, 11 060 Belgrade, Serbia and Montenegro

UDC 591.134:577.112.8
591.8

Somatostatin 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). At the periphery, SRIH inhibits the secretion of several non-pituitary hormones such as insulin, glucagon, gastrin, secretin and aldosterone (Reichlin, 1983; Milošević, 1999). Within the nervous system, SRIH acts as a neuromodulator, affecting neuroendocrine, motor, and cognitive functions (Reichlin, 1983; Epelbaum *et al.*, 1994). Because of its ability to inhibit secretory functions of various organs, its therapeutic value in clinical conditions is recognized and used.

In view of the above, the purpose of this study was to examine the effects of administration of SRIH-14 on morphological and stereological features of adrenal zona glomerulosa (ZG) cells in neonatal female rats.

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 (product of Veterinarski zavod Subotica, Subotica, Serbia and Montenegro) and water freely available. Female pups of the first group were injected s.c. twice a day (8 am and 8 pm) with 20 μg of SRIH-14 (S9129, Sigma, St. Louis, Mo., USA) per 100g b.w. for five consecutive days (from the 3rd to 7th day of life). Those of the second group served as the control, and received physiological saline. All animals were sacrificed by decapitation under deep anesthesia 12 h after the last treatment. Experimental protocols were ones 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.).

The left adrenal gland was excised, fixed in Bouin's solution, embedded in paraffin, and serially cut into sections 5 μm thick. The sections were stained with hematoxylin-eosin and stereologically analyzed using the M_{42} multipurpose test system inserted into the ocular of a Zeiss light microscope. The volume densities of nuclei and cytoplasm of parenchymal cells were estimated at a total magnification of x 1000. For each adrenal gland, a single paraffin section containing medulla was chosen and 30 test areas of the ZG were stereologically analyzed. On the basis of earlier karyometric studies (Malendowicz, 1974), the β shape coefficient was assumed to be 1.500 for ZG cells. It relates N_v (number of cells counted *per* unit of 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 (1979).

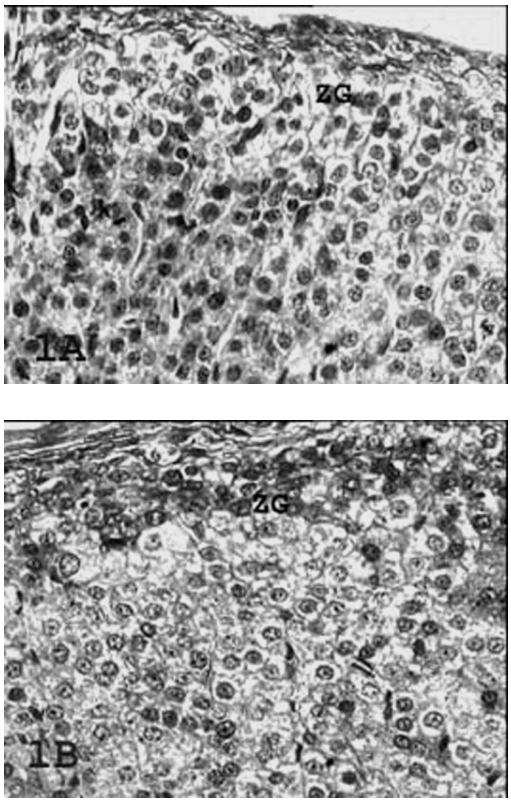

Fig. 1. Histochemically labelled ZG cells in adrenal cortex of female rats in: A. control rats, B. after multiple treatment with SRIH-14. (H&E, objective magnification x 40).

The ZG cells were arranged in closely packed ovoid clusters. The cells were relatively small, columnar or pyramidal in shape. Their nuclei were round or oval, with an evidenced nucleolus. In these cells lipid droplets were observed. The shape of ZG cells in animals neonatally treated with SRIH-14 was not significantly changed, but the cytoplasm of these cells was darkly stained in comparison to the controls (Fig. 1A, B). Absolute and relative volumes of the ZG were significantly ($p <$

Tab. 1. Absolute and relative volume of the adrenal cortex and ZG in female rats after neonatal treatment with SRIF-14. Values are means ± S.D. (n = 5), * $p < 0.05$.

Groups	Absolute volume cortex (mm^3)	ZG (mm^3)	Relative volume cortex (%)	ZG (%)
Control	3.5 ± 0.3	1.1 ± 0.02	92 ± 1.4	29.5 ± 1.9
SRIH-14	2.5 ± 0.07* (- 30 %)	0.7 ± 0.01* (- 36 %)	85 ± 1.4* (- 8 %)	23.5 ± 0.7* (- 20%)

Tab. 2. Morphometic parameters of adrenal zona glomerulosa in female rats after neonatal treated with SRIF-14. Values are means ± S.D. (n = 5), * $p < 0.05$.

Groups	Volume of ZG cells (µm³)	Volume of ZG nuclei (µm³)	Total number of ZG cells (1 x 10^6)
Control	1160.0 ± 0.1	109.0 ± 9.9	926.0 ± 23.6
SRIH-14	1080.0 ± 14.1* (- 7 %)	93.0 ± 0.9* (- 15 %)	627.0 ± 23.6* (-32%)

0.05) reduced by 36% and 20%, respectively, in comparison with control values (Table 1). The volumes of ZG cells and their nuclei were significantly ($p < 0.05$) reduced by 7% and 15%, respectively, in comparison with control values.

Our results strongly suggest that neonatal treatment of female rats with SRIH-14 suppresses growth of the adrenal ZG and its cells. These results are in accordance with earlier observations of Mazzocchi *et al.* (1985) and Robba *et al.* (1986), who showed that SRIH-14 has a direct inhibitory effect on growth and functional capacity of the adrenal ZG. Pawlikowski *et al.* (1990) also reported a reduced basal proliferation rate of these cells, probably owing to blocked basal trophic action of angiotensin-II (Aguilera *et al.*, 1981). Rebuffat *et al.* (1994) found that SRIH-induced atrophy of the ZG and its parenchymal cells affected the mitochondrial and smooth endoplasmic reticulum cell compartments, while Kong *et al.* (1994) showed that the rat adrenal gland manifested a high level of SRIH-receptor mRNA, implying direct somatostatine influence on the adrenal gland. However, earlier studies of Milošević *et al.* (1996, 1997) and Näsman *et al.* (1995) showed that intracerebroventricular application of SRIH reduced size of the ZG and the volume of its cells by influencing the regulatory hypothalamic-pituitary axis.

Based on these results, it can be concluded that neonatal application of SRIH-14 inhibits growth of the adrenal ZG and its cells in female rats.

Acknowledgement – This work was supported by the Ministry for Science and Environment Protection of the Republic Serbia (Grant 143007).

References: Aguilera, G., Harwood, J., Catt, K. (1981). *Nature*. **292**, 262-263. – Epelbaum, J., Dournaud, P., Fodor, M., Viollet, C. (1994). *Crit Rev Neurobiol,* **8**, 25-44. – Florio, T., Yao, H., Carey, K., Dillon, T., Stork, P. (1999) *Mol. Endocrinol.* **13,** 24-37. – Kong, H., De Paoli, A., Breder, C., Yasuda, K., Bell, G., Reisine T. (1994). *Neuroscience.* **59**, 175-184. – Malendowicz L. (1974). *Cell Tissue Res.* **151**, 525-536. – Mazzocchi, G., Robba, C., Rebuffat, P., Gottardo, G., Nussdorfer, G. (1985). *J Steroid Biochem.* **23,** 353-356. – Milošević, V., Velkovski, S., Brkić, B., Sekulić, M., Lovren, M., Starčević, V., Severs, W. (1996). *Pharmacology.* **53**, 369-375. - .Milošević, V., Brkić , B., Velkovski, S., Lovren, M., Sekulić, M., Starčević, V. (1997). *Jugoslov. Med. Biohem.* **16,** 89-94. - Milošević, V. (1999). Zadužbina Andrejević, Beograd, pp. 1-71. - Näsman B, Olsson T, Viitanen M, Carlström K. (1995). *Psychoneuroendocrinology.* **20**, 211-220. – Pawlikowski, M., Lewinski, A., Sewerynek, E., Szkudlinski, M., Kunert-Radek, J., Wajs, E. (1990). *Biochem Biophys Res Comm* **166**, 1171-1175. – Rebuffat, P., Belloni, A., Musajo, F., Rocco, S., Markowska, A., Mazzocchi, G., Nussdorfer, G. (1994). *J. Steroid Biochem. Mol. Biol.*. **48**, 353-360. – Reichlin, S. (1983). *N. Engl. J. Med.* **309**, 1495-1501; 1556-1563. - Weibel, E. R. (1979). *Practical Methods for Biological Morphometry,* New York: Academic Press, 1-415.