Arch. Biol. Sci., Belgrade, 59 (2), 13P-14P, 2007. DOI:10.2298/ABS070213PM

INHIBITORY EFFECT OF SRIH-14 ON ACTH CELLS IN NEONATAL FEMALE RATS.

Verica Milošević[1], Milica Terzić[2], Milica Manojlović-Stojanoski[1], Nataša Nestorović[1], Milka Sekulić[1] and Vesna Starčević[2]. *[1]Siniša Stanković Institute for Biological Research; [2]Institute of Physiology, School of Medicine, University of Belgrade,* 11000 Belgrade, Serbia

Key words: SRIH-14, ACTH cells, inhibitorz effect, rats

UDC 619 : 612.432.018.08

Somatostatin (somatotropin release-inhibiting hormone, SRIH) was originally discovered (K r u l i c h et al., 1968) during purification of growth hormone-releasing hormone from rat hypothalamus and was subsequently isolated and characterizated in 1972 from ovine hypothalamus (B r a z e a u et al., 1973). Two active forms of SRIH were described: SRIH-28 and SRIH-14. The neuropeptide somatostatin, widely expressed in both the peripheral and the central nervous system (CNS) (R e i c h l i n, 1983), has multiple functions in higher organisms. It regulates endocrine and exocrine secretion, possesses antiproliferative properties, and acts as a neurotransmitter/neuromodulator (R e u b i, 1997). These diverse physiological effects are mediated by a family of G-protein-coupled cell surface receptors, the somatostatin receptors, named sstr1 to sstr5 (Ö s a p a y and Ö s a p a y, 1998).

In view of the above, the purpose of this study was to examine the effects of administration of SRIH-14 on morphological and stereological parameters of adrenocorticotropic (ACTH) 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 water freely available. Female pups 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 100 g b.w. for five consecutive days starting from the 3rd to 7th day of life (the first group). Female pups 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 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.). Pituitary glands were excised, fixed in Bouin's solution and embedded in paraffin. Serial tissue sections 5 µm thick were deparaffinized. Pituitary ACTH cells were localized by the peroxidase-antiperoxidase-complex (PAP) method of Sternberger *et al*. (1970). Measurements were performed on the widest portion of the pituitary gland and immunocytochemically-labelled ACTH cells were analyzed by the M_{42} test system. For calculations of cell and nuclear volumes, the formula of W e i b e l (1979) was used. Morphometric data obtained from each rat were averaged *per* experimental group and the standard deviation of the mean (SD) was calculated. The Student *t-test* was used to evaluate differences between the two groups and a probability value of 5% or less was considered statistically significant.

Data on body weight and absolute and relative weight of the pituitary in the SRIH-treated and control groups are summarized in Table 1. As can be seen, body weight and absolute and relative pituitary weights were not significantly ($p>0.05$) changed in SRIH-treated animals compared with the controls. ACTH-immunoreactive cells of control neonatal female rat pituitaries were stellate in shape with the cytoplasmatic processes among neighboring cells and localization between the capillaries. The nucleus followed the shape of the cell body. Small, specific secretory granules were distributed mainly on the periphery of the cytoplasm (Fig. 1a). In SRIH-treated rats, these cells were smaller, irregularly shaped, with more intensely stained secretory granules (Fig. 1b). All morphometric parameters were decreased in both SRIH-treated groups compared to

Table 1. Effects of SRIH-14 on body weight and absolute and relative pituitary weight in neonatal female rats. Values are means ± S.D. (n = 5).

Experimental group	Body weight (g)	Absolute pituitary weight (mg)	Relative pituitary weight (mg%)
Control	17.8 ± 0.7	2.0 ± 1.0	11.2 ± 5.4
SRIH-14	16.1 ± 1.8 (-10%)	1.8 ± 0.8 (-10%)	11.6 ± 5.3 (+4%)

Table 2. Morphometic parameters of ACTH cells in female rats after neonatal treatment with SRIF-14. Values are means ± S.D. (n = 5), * p < 0.05.

Groups	Volume of ACTH cells (µm³)	Volume of ACTH nuclei (µm³)	Relative volumetric density of ACTH cells (%)
Control	1242 ± 25.5	137.5 ± 3.5	16.5 ± 2.2
SRIH-14	757.0 ± 45.2* (- 39 %)	115.0 ± 14.1* (- 16 %)	11.0 ± 1.1* (-33%)

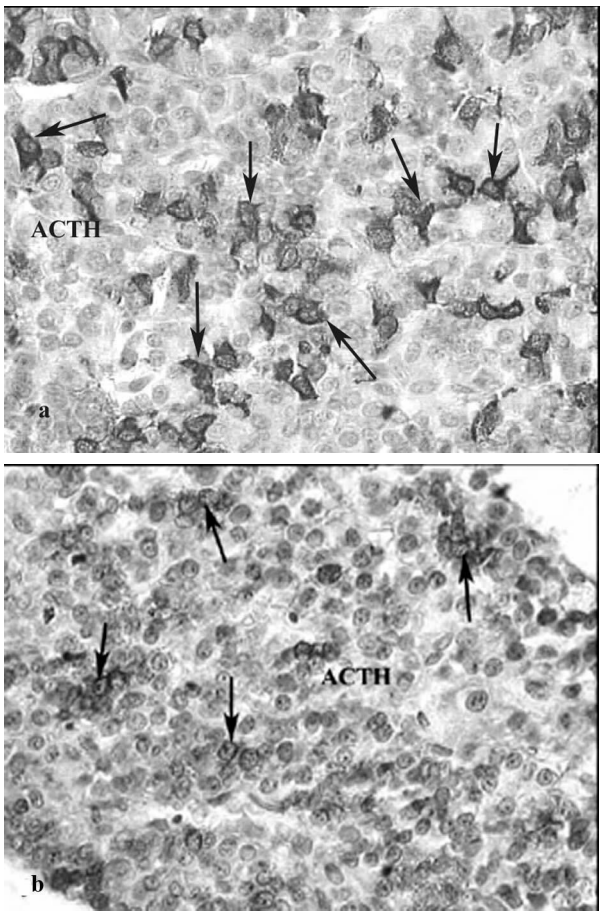

Fig. 1. Imunohistochemically labelled ACTH cells in: a) control rats, b) SRIH-14 treated females (PAP, 1256 X).

saline-treated rats (Table 2). The volume of ACTH cells and their nuclei were significantly decreased ($p<0.05$) by 39% and 16%, respectively, in comparison with the corresponding controls. The volumetric density of ACTH cells in neonatally treated rats was significantly decreased ($p<0.05$) by 33% in comparison with the controls (Table 2).

The obtained results demonstrate that SRIH-14, applied multiply in the neonatal period, exerted an inhibitory influence on ACTH cell morphology in neonatal female rats. These data are in accordance with our previous observation (Starčević et al. 2000) that intracerebroventricular (i.c.v.) administration of SRIH-28 to rat females exerts significant inhibitory action on the function and morphometric charateristics of ACTH cells. Moreover, Octreotide applied i.c.v. exerts significant inhibitory effects on the immunohistochemical and morphometric characteristics of ACTH cells in both female and male rats (Milošević et al. 2001, 2003). The mechanism of inhibition of ACTH secretion may be through inhibition of CRF release from the hypothalamus (Shibasaki et al., 1988). Litvin et al. (1986) asserted that SRIH inhibits CRF-induced ACTH secretion from At20 cells *in vitro*. The action of SRIH is dose-dependent with a half-maximal effect at 1×10^{-9} M and results in decreased maximal ACTH secretion (Richardson, 1983).

In conclusion, systemic application of SRIH-14 by the s.c. route produced a marked decrease in stereological parameters of the pituitary ACTH cells.

Acknowledgment– This work was supported by the Ministry of Science and Environment Protection of the Republic Serbia (Grants Nos. 143007 and 145003).

References: - Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and R. Guillemin (1973). *Science,* **79**, 77-79. – Krulich, L., Dhariwal, S., and S. McCann (1968). *Endocrinology,* **83**, 783-790. – Litvin, Y., Leiser, M., Fleischer, N., and J. Erlichman (1986). *Endocrenology,* **119**, 737-745. – Milošević, V., Brkić, B., Nestorović, N., Velkoski, S., and V. Starčević (2001). *Acta Vet.* **51**, 283-290. – Milošević, V., Manojlović, M., Kostić, N., Brkić, B., Velkovski, S., Šošić-Jurjrvić, B., Trifunović, S., and V. Starčević (2003). *Jugoslav. Physiol. Acta,* **39**, 25-30. – Ösapay, G., and K. Ösapay (1998). *Exp. Opin. Ther. Patents* **8**, 855-870. – Reichlin, S. (1983). *N. Eng. J. Med.* **309**, 1495-1501, 1556-1563. – Reubi J.C. (1997). *Endocrine Pathol,* **8**, 11-20. – Richardson, I. (1983). *Life Sci.* **33**, 1981-1988. – Shibasaki, T., Kim, Y., Yamauchi, N., Masuda, A., Imaki, T., Hotta, M., Demura, H., Wakabayashi, I., Ling, N., and K. Shizume (1988). *Life Sci.* **42**, 329-334. – Starčević, V., Milošević, V., Brkić, B., and W. Severs (2000). *Pharmacology,* **60**, 203-207. – Sternberger, L.A., Hardy, P.H.J., Cuculius, J.J., and H.G. Meyer (1970). *J. Histochem. Cytochem.* **18**, 315-333. – Weibel, E. R. (1979). *Practical Methods for Biological Morphometry,* New York: Academic Press, 415 pp.