Arch. Biol. Sci., Belgrade, 59 (3), 43P-44P, 2007. DOI:10.2298/ABS070343PT

EFFECTS OF SRIH-14 ON ACTH CELLS IN MALE RATS.

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Key words: somatostatin, ACTH cells, male rats

UDC 577.17 : 577.175.3 : 599

Somatostatin (SRIH), a regulatory peptide, is produced by neuroendocrine, inflammatory, and immune cells in response to ions, nutrients, neuropeptides, neurotransmitters, thyroid and steroid hormones, growth factors, and cytokines (P a t e l, 1999). Somatostatin has been demonstrated to play an important role as an endogenous inhibitor of cell proliferation in various normal and neoplastic tissues (S c h a l l y, 1988). The diverse biological effects of SRIH are mediated by a family of seven transmembrane domain G-protein-coupled receptors that comprise five distinct subtypes, 1-5 (sst_{1-5}) (P a t e l, 1999). The pituitary is the main target for physiological actions of SRIH, and different amounts of sst are present in the pituitary secretary cells. Somatostatin and its analogs are now being used for the treatment of different human tumors (R e u b i and L a i s s u e, 1995).

It therefore seemed worthwhile to examine the effect of SRIH-14 on morphofunctional parameters of pituitary corticotropes (ACTH cells).

Adult male Wistar rats were bred in the Institute for Biological Research in Belgrade, Serbia. The rats were housed in a controlled environment at 22±2°C under conditions of a 12 h of light/12 h of darkness schedule. The animals were allowed food and water *ad libitum*. Food for laboratory rats was prepared in the Veterinary Institute (Subotica, Serbia). Experimental protocols were ones 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.). Adult male rats were divided into four experimental groups. Rats of the first group were injected *s.c*. twice a day with 20 µg of SRIH-14 *per* 100 g b. w. for 5 consecutive days (multiple treatment). Males of the second group received 20 µg of SRIH-14 per 100 g b.w. for 28 consecutive days (chronic treatment). The control groups (third and fourth groups) received physiological saline for 5 or 28 consecutive days. All animals were sacrificed by decapitation under deep anesthesia 12 h after the last injection, at which time pituitary glands were excised. Pituitary glands were fixed in Bouin's solution, embedded in paraffin, and sliced through three tissue levels of the *pars distalis*. Five-µm-thick immunocytochemically stained pituitary sections were used for morphometric examinations (M i l o š e v i ć et al., 1994). The cell volumes (V_c), nuclear volumes (V_n), and volume densities (V_v) of ACTH cells were estimated under a light microscope (Carl Zeiss, Germany) at 1000x magnification using the M_{42} multipurpose test system (W e i b e l, 1979). The volumes of ACTH-positive cells are expressed in μm^3. Volume densities are presented as percentages (%) of total pituitary cells. Plasma levels of ACTH were determined by the chemiluminiscent enzyme immunometric assay IMMULITE ACTH (DPC, Los Angeles). The Duncan test was used for statistical analyses.

After multiple treatment, the absolute weights of the pituitary gland, volume of ACTH cells, and volume density decreased insignificantly ($p>0.05$) by 8, 3 and 3%, respectively, in comparison with the controls (Table 1). In animals chronically treated with SRIH-14, absolute weight of the pituitary gland and volume density of ACTH cells decreased significantly ($p<0.05$) by 22 and 11%, respectively, in comparison with the controls (Table 1). The volume of ACTH cells and their nuclei changed insignificantly ($p>0.05$) in comparison with the corresponding controls (Table 1). The plasma levels in both treated

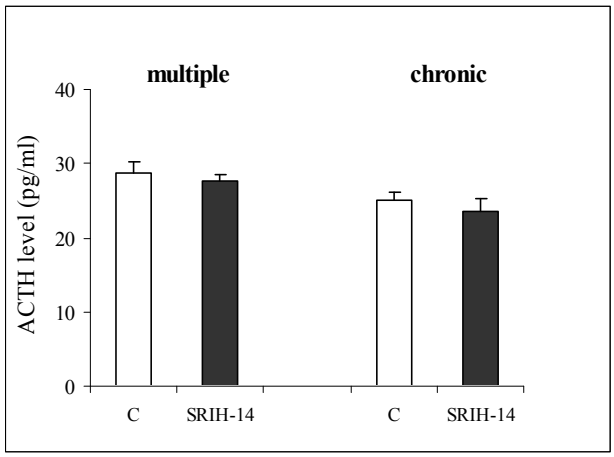
Table 1. Effects of multiple and chronic treatment with SRIH-14 on absolute pituitary weight, volume density of ACTH cells, and volumes of ACTH cells and their nuclei in male rats. (mean ± SD; n=5); $^{*}p < 0.05$ vs. controls

Groups		Pituitary weight (mg)	Volume density of ACTH (%)	Volume of ACTH cells (μm^3)	Volume nuclei of ACTH (μm^3)
Multiple treatment	C	10.3 ± 1.2	17 ± 1	1056 ± 11	147± 11
	SRIH-14	9.5 ± 2.1	16.5 ± 1.5	1024 ± 91	143± 10
Chronic treatment	C	9 ± 0.1	17 ± 1.3	1044 ± 40	144± 3
	SRIH-14	7 ± 1*	16.5 ± 1.5 *	996± 55	141± 4

groups decreased insignificantly ($p>0.05$) (Fig. 1) in relation to the corresponding controls.

The results of this experiment suggest that multiple and chronic administration of SRIH-14 causes decrease in morphometric parameters of ACTH cells and lowering of the ACTH blood level. Statistically significant reductions of absolute pituitary weights and volume density of the ACTH cells were observed in the case of chronic treatment with SRIH-14. An earlier study showed that decrease of absolute pituitary weights might be a result of decrease in volume density of somatotropes and lactotropes, which together comprise more than 60% of pituitary cells (M i l o š e v i ć et al., 1998). The absence of significant changes of stereological parameters after both treatments can be attributed to low expression of sst in pituitary ACTH cells (O ′ C a r r o l l and K r e m p e l s, 1995). Up-regulation of sst can account for greater reduction of stereological parameters after chronic than after multiple treatment (H u k o v i c et al., 1996).

Fig. 1. Effects of multiple and chronic treatments with SRIH-14 on blood ACTH concentrations in male rats (mean ± SD; n=5).



It can be concluded that s.c. injection of SRIH-14 can induce a low degree of changes in morphofunctional characteristics of ACTH cells.

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