CHANGES OF SOMATOTROPES IN FEMALE RATS AFTER MULTIPLE AND CHRONICAL TREATMENT WITH SOMATOSTATIN. Verica Milosevic1, Nataša Nestorović1, Milica Terzić2 and Vesna Starčević2, 1Institute for Biological Research, 2Institute of Physiology, School of Medicine, University of Belgrade, 11000 Belgrade, Yugoslavia.

The neuropeptide somatostatin (SRIH) originally isolated from the hypothalamus as a growth hormone (GH) release-inhibiting hormone, is widely distributed both in the central nervous system (Reichlin 1983) and in the peripheral tissues (Hulkes 1994). It is well known that SRIH inhibits GH release from somatotropes in male and female rats (Milosević et al. 1998) via separate receptors in the plasma membrane (Wehrenberg et al. 1982). Hypothalamic SRIH also inhibits secretion of luteinizing hormone (Lovren et al. 1998), prolactin (PRL) (Milosević et al. 1998) and thyrotropin-stimulating hormone (TSH) (Sekulić et al. 2000; Milosević et al. 2000) from the anterior pituitary in rats. In many animal tumor models and cultured tumor cell lines, somatostatin and somatostatin analogs inhibit tumor growth (Reubi and Laisse 1995), probably, because the highest incidence of somatostatin receptors was observed in neuroendocrine tumors (Reubi 1997).

This study was designed to evaluate the effects of multiple and chronic subcutaneous (s.c.) treatment with SRIH on the stereology and morphology of somatotropes in pituitary glands of the female rats.

The study was performed using adult female Wistar rats (210-230 g). The rats were divided into four experimental groups of five animals per group. The first group received two 100 μg/kg b.w. SRIH doses per day (Rebuffat et al. 1984), for five consecutive days (75-79th day of life; multiple treatment). Females of the second group received two 20 μg/100 g b.w. SRIH doses per day, from the 23rd day till the 71st day of life (chronical treatment). The somatostatin used was cyclic somatostatin-14 (Bachem, P50M10, USA). The third and the fourth group were the corresponding controls. Females of these groups were treated multiply or chronically with saline only. All animals were sacrificed under deep anesthesia by decapitation during 12 h after the last injection. Pituitary glands were excised, fixed in Bouin's solution and embedded in paraffin. Pituitary GH cells were immunocytochemically localized by the peroxidase-antiperoxidase-complex (PAP) method of Sternberger et al. (1970). Measurements were performed on the widest portion of the pituitary gland. Immunopositive GH cells were analyzed by the M42 test system after Wibe1 (1979). For the calculations of the cell and nuclear volumes the formula of Wibe1 (1979) was used. Morphometric data obtained from each group were averaged, and the standard deviation of the mean was calculated. A 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.
rats (Figs. 1-3). The nuclear volume of GH cells was insignificantly decreased (p>0.05; by 15%) after both multiple and chronic SRIH-14 treatment in comparison with the corresponding controls (Fig. 1). The cellular volume of GH cells was significantly decreased (p<0.05) in both multiply and chronically treated animals, by 39% and 56%, respectively (Fig 2), in relation to the corresponding controls. After chronic SRIH-14 treatment, the GH cells volume was lower by 22% (p<0.05) than after multiple SRIH-14 treatment. The volume density of GH cells in both SRIH-treated groups was decreased by 29% in the first and by 45% in the second group (p<0.05) in relation to the corresponding controls (Fig. 3). The volume density of GH cells in chronically treated rats was significantly decreased (p<0.05) by 28% in comparison with multiply SRIH-treated rats (Fig. 3).

![Graph](image)

Fig. 3. Relative volume density (Vv; %) of GH cells expressed as percentages of total gland tissue. All values are the means ± SD. (n=5/group), *p<0.05 vs corresponding control; †p<0.05 vs multiply SRIH-treated.

The results of the present study demonstrate that multiply or chronically applied SRIH-14 expressed an inhibitory influence on GH cells morphology in female rats. Morphometric and quantitative changes of GH pituitary cells were more obvious in animals chronically treated with SRIH-14 than after multiple SRIH-14 treatment. This data is in accordance with our previous observations that SRIH-14 and SRIH-28 given intracerebroventricularly to male and female rats led to a decrease in the number of GH cells, accompanied by a reduction in both their cellular and nuclear volumes in comparison with the controls (Milos et al. 1998, 2000). The inhibition of GH release by SRIH involves a change in the distribution of microfilaments rather than microtubules (Shimada et al. 1990). These authors found microfilament bundles running parallel to the plasmamembrane in the space between the granules after injection of SRIH.

In conclusion, systemic application of SRIH-14 by s.c. route induced marked changes in immunocytochemical and morphometric characteristics of pituitary GH cells. The volume and volume density of GH cells were significantly reduced after multiple and chronic SRIH-14 treatment. However, in chronically treated females a more markedly expressed inhibitory effect of SRIH-14 on GH cells were found than in multiply treated animals.