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A SOMATOSTATIN ANALOGUE OCTREOTIDE INHIBITS PITUITARY ACTH CELLS IN RAT MALES

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The results on the effects of synthetic somatostatins on the secretion of ACTH have been rather conflicting, but some authors reported that somatostain is involved in the regulation of ACTH secretion in rats of both sexes. The inhibitory mechanism of ACTH secretion could operate through the inhibition of CRF release from the hypothalamus. In the present study, we have investigated the effects of Octreotide, a somatostain analogue given intracerebroventricularly (i.c.v.) in low doses, on morphometric characteristics of pituitary adrenocorticotropes (ACTH cells) in adult Wistar rat males. The animals were i.c.v. given three 1.0 μg doses of Octreotide dissolved in 10 μL saline every second day. The controls were treated in the same way with the same volume of

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saline. ACTH-producing cells were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Body weight, as well as absolute and relative pituitary weights were not significantly changed in comparison with the corresponding controls ($p>0.05$). The characteristics of immunohistochemicaly labelled ACTH cells in intact rat males were as follows: localization between the capillaries, stellate in shape with the cytoplasmatic processes among neighbouring cells. The nucleus follows the shape of the cell body. Small specific secretory granules were distributed mainly at the periphery of the cytoplasm. The shape, and localization of ACTH immunoreactive cells in Octreotide-treated animals were not significantly changed in comparison with the controls. Octreotide treatment significantly decreased all morphometric parameters measured, *i.e.* volume of ACTH cells and their nuclei by 44% and 18%, respectively, as compared to the controls ($p<0.05$). Volume densities were also significantly decreased (by 31%; $p<0.05$) comparing to the corresponding controls. These results indicate that Octreotide applied i.c.v. exerts a significant inhibitory effect on the immunohisto-chemical and morphometric characteristics of ACTH cells in rat males.

Key words: Octreotide, pituitary, ACTH-cells, central nervous system, rat males

INTRODUCTION

The somatostatin (SRIH) is a cyclic neuropeptide with two different biologically active forms SRIH-14 and SRIH-28, derived from a 92-amino acid precursor, presomatostatin (REICHLIN, 1983). SRIH is extensively distributed in human body including the central and peripheral nervous system, the gastrointestinal tract and various exocrine and endocrine glands (REUBI, 1997). A short synthetic somatostatin analogue Octreotide, consisting of eight amino acid residues (BAUER *et al.* 1982) was introduced for the treatment of GH-secreting adenoma because of its relatively long half life (approximately 110 min) (WASS, 1990). Octreotide is in clinical use for cancer therapy and gastrointestinal disorders. It interacts primarily with $sstr_2$ receptor and is much more stable than native somatostain (SCARPIGNATO, 1996).

In the present study, we have investigated the effects of low Octreotide doses applied intracerebroventricularly (i.c.v.) on morphometric characteristics of ACTH (adrenocorticotropic) cells in adult rat males.

MATERIAL AND METHODS

Adult Wistar rat males (210-230 g) were used. They were implanted with a headset later serving for i.c.v. injections. A minimum recovery time of 5 days was permitted before the onset of experiments. The animals were divided into two experimental groups each including five individuals. Those from the first group

were i.c.v. given three 1.0 μg doses of octreotide dissolved in 10 μL saline every second day. The second group serving as a control was treated in the same way and by the same schedule with physiological saline. All animals were sacrificed in deep anaesthesia by decapitation, 5 days after the last injection. The pituitary glands were excised, weighed in air, fixed in BOUIN'S solution and embedded in paraffin. Serial 5 μm thick tissue sections 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 after WEIBEL (1979). For the calculations of the cellular and nuclear volumes the formula of WEIBEL (1979) was used. Morphometric data obtained from each group was 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 as statistically significant.

RESULTS

Data on body weight, absolute and relative weights of the pituitary gland in Octreotide- treated group and the controls is summarized in Table 1. As seen, body weight, absolute and relative pituitary weights were not significantly changed in comparison with corresponding controls ($p>0.05$). The characteristics of the immuno-histochemically labelled ACTH cells in intact rat males were as follows: localization between the capillaries, stellate in shape with the cytoplasmatic processes among neighbouring cells. The nucleus followed the shape of the cell body. Small, specific secretory granules were distributed mainly at the periphery of the cytoplasm. The shape, and localization of ACTH immunoreactive cells in Octreotide-treated rat males were not significantly changed in comparison with the controls. The morphometric parameters, *i.e.* the volume of the ACTH cells and their nuclei, as well as volume density of these cells are shown in Fig. 1a, b, c. Volumes of ACTH cells and their nuclei were significantly decreased in Octreotide-treated rats by 44% and 18%, respectively, in comparison with the controls (p 0.05). Volume density of ACTH cells was also significantly decreased by 31% in comparison with the control ($p < 0.05$).

Table 1. - The effects of Octreotide on body weight, absolute and relative pituitary weight in adult rat males

Experimental group	Body weight (g)	Absolute pituitary weight (mg)	Relative pituitary weight (mg%)
Control	234 ± 15.1	12.1 ± 2.3	4.1 ± 0.7
Octreotide-treated	228 ± 8.3 (-3%)	10.6 ± 0.6 (-12%)	3.9 ± 0.2 (-5%)

The values represent the means ± SD (n=5/group)

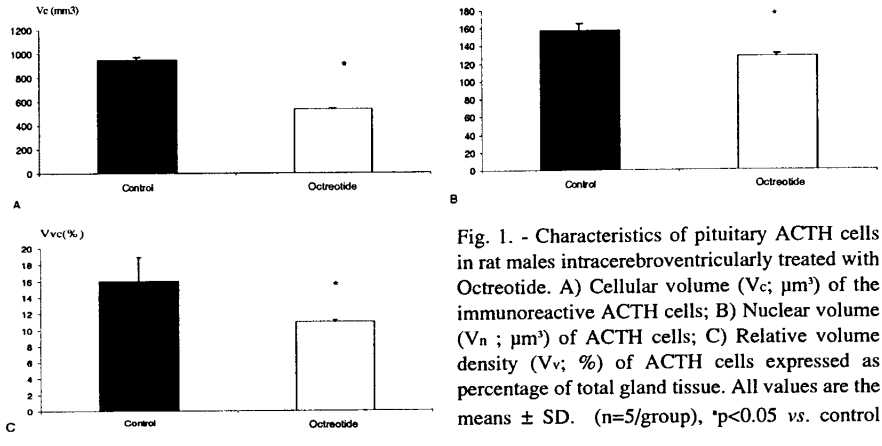

Fig. 1. - Characteristics of pituitary ACTH cells in rat males intracerebroventricularly treated with Octreotide. A) Cellular volume (V_c; μm^3) of the immunoreactive ACTH cells; B) Nuclear volume (V_n ; μm^3) of ACTH cells; C) Relative volume density (V_v; %) of ACTH cells expressed as percentage of total gland tissue. All values are the means ± SD. (n=5/group), *p<0.05 *vs.* control

DISSCUSION

Previously reported data on the effects of somatostatin on ACTH-secreting cells is somewhat conflicting (RICHARDSON, 1981). We have shown previosly that i.c.v. treatment of adult rat males with either SRIH-14 or SRIH-28 did not result in significant changes of either morphometric parameter of ACTH cells, or in plasma concentration of ACTH, as compared to the corresponding controls (MILOŠEVIĆ *et al.*, 1994; MILOŠEVIĆ, 1999). The results obtained throughout the present study demonstrate that repeated i.c.v. bolus injection of Octreotide significantly decreased all morphometric and immunocytochemical parameters of ACTH cells in adult rat males. Octreotide primarily interacts with sstr2 and sstr5 somatostatin receptor subtypes, inhibiting the adenylate cyclase system via type 2 of somatostatin receptors (PATEL and SRIKANT, 1994).

The mechanism of inhibition of ACTH secretion was hypothesized by several authors (RICHARDSON, 1983; BROWN *et al.*, 1984; SHIBASAKI *et al.*, 1988) to proceed via the inhibition of CRF release from the hypothalamus. Also, LITVIN *et al.* (1986) observed that SRIH inhibits CRF-induced ACTH secretion from the At20 cells *in vitro*. The action of SRIH was shown to be dose-dependent and to result in a reduced maximal ACTH secretion (RICHARDSON, 1983). Octreotide may directly inhibit growth of SRIH receptor-positive tumours by triggering signal transduction pathways that negatively control cell growth (HOFLAND *et al.*, 1992). Indirect effects of Octreotide may involve suppression of growth factors and hormones that stimulate tumour growth (SERRI *et al.*, 1992)

In conclusion, our results indicate that i.c.v.-applied Octreotide, exerts significant inhibitory effects on the immunohistochemical and morphometric characteristics of ACTH cells in adult rat males.

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EFEKTI OKTREOTIDA NA ACTH ĆELIJE HIPOFIZE MUŽJAKA PACOVA

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Izvod

Postoje veoma različita, a često i suprostavljena mišljenja o efektima sintetskih somatostatina na lučenje adrenokortikotropnog hormona (ACTH) iz hipofize. Mogući mehanizam inhibicije lučenja ACTH je preko inhibicije lučenja CRF iz hipotalamusa. U ovom radu su ispitivani efekti intracerebroventrikularno (i.c.v.) ubrizganog analoga somatostatina, Oktreotida, na rast ACTH ćelija adenohipofize mužjaka Wistar pacova. Sve eksperimentalne životinje su primile tri doze od po 1.0 μg Oktreotida rastvorenog u 10 μL fiziološkog rastvora, svakog drugog dana. Kontrole su tretirane na isti način fiziološkim rastvorom. Pacovi su žrtvovani pet dana posle poslednje injekcije. ACTH ćelije su imunocitohemijski bojene metodom peroksidaza-antiperoksidaza (PAP). Telesna masa, apsolutna i relativna masa hipofize nisu bile statistički značajno promenjene u poređenju sa odgovarajućom kontrolom ($p>0.05$). Karakteristike imunohistohemijski obojenih ACTH ćelija kontrolnih pacova su: lokalizacija između kapilara, zvezdast oblik sa citoplazmatičnim produžecima između drugih ćelija. Male specifične sekretorne granule raspoređene su po periferiji citoplazme. Oblik i lokalizacija ACTH ćelija životinja tretiranih Oktreotidom nisu bili značajno promenjeni u odnosu na kontrolu. Međutim, oktreotid je izazvao značajno smanjenje svih morfometrijskih parametara ovih ćelija ($p>0.05$). Zapremina ćelija je bila smanjena za 44%, jedara za 18%, a volumenska gustina za 31% ($p<0.05$) u poređenju sa odgovarajućom kontrolom. Na osnovu dobijenih rezultata može se zaključiti za Oktreotid deluje inhibitorno na rast ACTH ćelija adenohipofize mužjaka pacova.

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