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THE EFFECTS OF CENTRALLY APPLIED OCTREOTIDE ON ACTH CELLS IN THE FEMALE RAT

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The effects of intracerebroventricular (i.c.v.) application of a somatostatin analogue, Octreotide, on growth of pituitary adrenocorticotropes (ACTH cells) were examined in adult female Wistar rats. The animals were subjected to i.c.v. administration of three 1.0 μg doses of Octreotide dissolved in 10 μl saline every second day. Controls were treated in the same way with the same volume of saline. ACTH-producing cells were studied using the peroxidase -antiperoxidase (PAP) immunohistochemical procedure. Octreotide treatment of rat females, significantly decreased ($p<0.05$) all morphometric parameters measured, i.e. volume of ACTH cells and their nuclei by 52% and 11%, respectively, as compared to the controls. The volume densities were also significantly decreased (by 47%; $p<0.05$) in comparison with the corresponding controls. These findings suggest that centrally administered somatostatin analogue (Octreotide) is specifically involved in the control of growth and secretory activity of ACTH cells in female rats.

Key words: octreotide, pituitary, ACTH-cells, adrenocorticotropic hormone, somatostatin, central nervous system, female rats.

INTRODUCTION

The neuropeptide somatostatin, widely expressed both in the periphery and in the central nervous system (CNS) (Reichlin, 1983) has multiple functions in higher organisms. It regulates endocrine and exocrine secretion, possesses antiproliferative properties and acts as a neurotransmitter/neuromodulator (Reubi, 1997). Two biologically active molecular forms deriving from a 116 amino acid precursor have been characterized, the tetradecapeptide somatostatin-14 (SRIH-14) and the N-terminally extended form somatostatin-28 (SRIH-28) (Lewin and LeRomancer, 1996). The diverse physiological effects are mediated by a family of G-protein-coupled cell surface receptors, the somatostatin receptors, named sstr1 to sstr5 (Ösapay, and Ösapay, 1998). However clinical application of either SRIH-14 or SRIH-28 has been limited because of their short half-life in plasma (a few-minutes) (Sheppard et al., 1979).

The first synthetic somatostatin analogue, known as Octreotide or Sandostatin, synthesized by Bauer in February 1980 and consisting of 8 amino acid residues (Bauer et al., 1982), was introduced for the treatment of GH-secreting adenomas because of its longer half life (around 110 min) (Wass 1990) in comparison with SRIH-14 and SRIH-28. At present, Octreotide is in clinical use for cancer therapy and gastrointestinal disorders and it was shown by Scarpignato (1996) to interact primarily with the sstr2 receptor and was more stable than native somatostatin.

In this report, the effects of intracerebroventricular (i.c.v.) Octreotide administration on adrenocortical (ACTH) cells in adult female rats are demonstrated by analysis of stereological parameters and immunocytochemical properties.

MATERIAL AND METHODS

Animals

Adult Wistar female rats (200 - 220 g), maintained at the Institute for Biological Research, Belgrade were used. They were kept under a 12:12 h light-dark cycle, at 22 ± 2 ^{0}C. Feed (rat chow, a product of D.D.Veterinarski zavod, Subotica,Yugoslavia) and tap water were available ad *libitum*.

Animal preparation

Surgical procedures were performed under ether anesthesia (aether ad narcosis Ph. lug. III. produced by "Lek" Pharmaceutical Works, Ljubljana, Slovenia). A headset implanted into the rats was used for i.c.v. injections. A minimum recovery time of 5 days was permitted before initiation of the experiments. The headset consisted of a silastic-sealed 20-gauge cannula (Starčević et al., 1988), implanted into a lateral cerebral ventricle, 1 mm posterior and 1.5 mm lateral to the bregma, and 3 mm below the cortical surface. A small stainless steel anchor screw was placed at a remote site on the skull. The cannula and screw were cemented to the skull with dental acrylic (Simgal; ICN Galenika, Belgrade, Yugoslavia).

Treatment of animals

When recovered from surgical trauma the rats were divided into 2 experimental groups each consisting of five animals. Those from the first group were given i.c.v.three 1 μg doses of Octreotide (Sandoz, Switzerland) dissolved in 10 μl saline with 48-h-intervals between the treatments. The second group, serving as a control, was treated in the same way by the same time schedule with physiological saline. All animals were sacrificed in deep anaesthesia by decapitation 5 days after the last injection.

Light microscopy and immunocytochemistry

The pituitary glands were excised, fixed in Bouin-s solution for 48 h and embedded in paraffin. Serial 5-μm thick tissue sections were deparaffinized in xylol and serial alcohol. Pituitary hormones were localized by the peroxidase -antiperoxidase complex (PAP) method of Stanberger et al. (1986). The endogenous peroxidase activity was blocked by incubation in 9 mmol hydrogen peroxide solution in methanol for 30 min at ambient temperature. Before the application of the specific primary antiserum, nonspecific background staining was achieved by incubating the sections with nonimmune, *i.e.* normal porcine

serum diluted with phosphate buffered saline (PBS) pH 7.4, for 60 min. The sections were then overlaid with the appropriate dilutions of the specific primary antibodies (hACTH antiserum, Dako A/S, Glostrup, Denmark) for 24 h at 4 °C. After washing in PBS, the sections were incubated for another 60 min with the second antibody (swine-anti-rabbit IgG) for 45 min, rinsed again with PBS for 10 min and incubated with rabbit PAP serum for 45 min. Antibody localization was visualized by incubating the sections in Tris-HCl-buffered saline (0.5 mol/l, pH 7.4) supplemented with 3,3-diaminobenzidine tetrachloride (DAB, Serva, Heidelberg, Germany) and 9 mmol/l hydrogen peroxide. Slides were thoroughly washed under running tap water, counterstained with hematoxylin and mounted in Canada balsam (Alkaloid, Skopje, Former Yug. Rep. of Macedonia). Control sections were incubated without primary antisera or by substituting nonimmune rabbit serum for the primary antiserum.

Morphometry

Volume densities (Vv) of both the nuclei and the cytoplasm of ACTH-immuno-reactive cells, as well as numerical density (Na) of their nuclei *per* μm^3 were measured using 50 test areas of the pituitary gland at a magnification of X1000, using the multipurpose test system M_{42} (Weibel, 1979).

The number of nuclei of immunoreactive ACTH-cells *per* mm^3 was estimated applying the formula of Weibel and Gomez (1962) according to Weibel (1979). Since rat ACTH cells are mononucleated, the numerical density of the nuclei (Nv) corresponds to the number of cells *per* mm^3 :

$$Nv = (k/\beta) \times (Na^{3/2}/Vv^{1/2})$$

On the basis of earlier karyometric studies of Malendowicz (1974), the shape coefficient was estimated to be 1.382, for the pituitary cells. It relates Nv (number of cells counted *per* volume unit) to Na (number of cells counted *per* mm^3) and Vv (volume density) and depends on the axial ratio of the nuclei.

Volume densities of the ACTH-positive cells were expressed as percentages, taking total pituitary cells in μm^3 as 100%.

Statistical analyses

Biochemical and morphometric data obtained from individual rats were averaged *per* experimental group and the standard deviation of the mean was calculated. A one-way analysis of variance (ANOVA), followed by the multiple range test of Duncan (Pharmacological Calculation System, 1986) was used for statistical comparisons between the groups. A probability value of 5% or less was considered statistically significant.

RESULTS

Data on body weight, absolute and relative weight of the pituitary in the Octreotide-treated group and in the corresponding control are summarized in Table 1. Octreotide treatment did not lead to a significant change ($p>0.05$) of these values in comparison with the controls.

The characteristic features of immunohistochemically labelled ACTH cells in intact females are their localization between the capillaries and their stellate shape with the cytoplasmic processes among neighbouring cells. The nucleus follows the shape of the cell body. Small, specific secretory granules are distributed mainly at the periphery of the cytoplasm as shown in Fig. 1a. The shape, and localization of ACTH immunoreactive cells in octreotide-treated female rats was not significantly changed in comparison with the controls (Fig. 1b).

Table 1. The effects of Octreotide on body weight, absolute and relative pituitary weight in adult female rats

Experimental group	Body weight (g)	Absolute pituitary weight (mg)	Relative pituitary weight (mg%)
Control	234 ± 15.1	14.4 ± 2.3	6.1 ± 0.7
Octreotide	223 ± 8.3 (-5%)	12.5 ± 0.6 (-13%)	5.6 ± 0.2 (-8%)

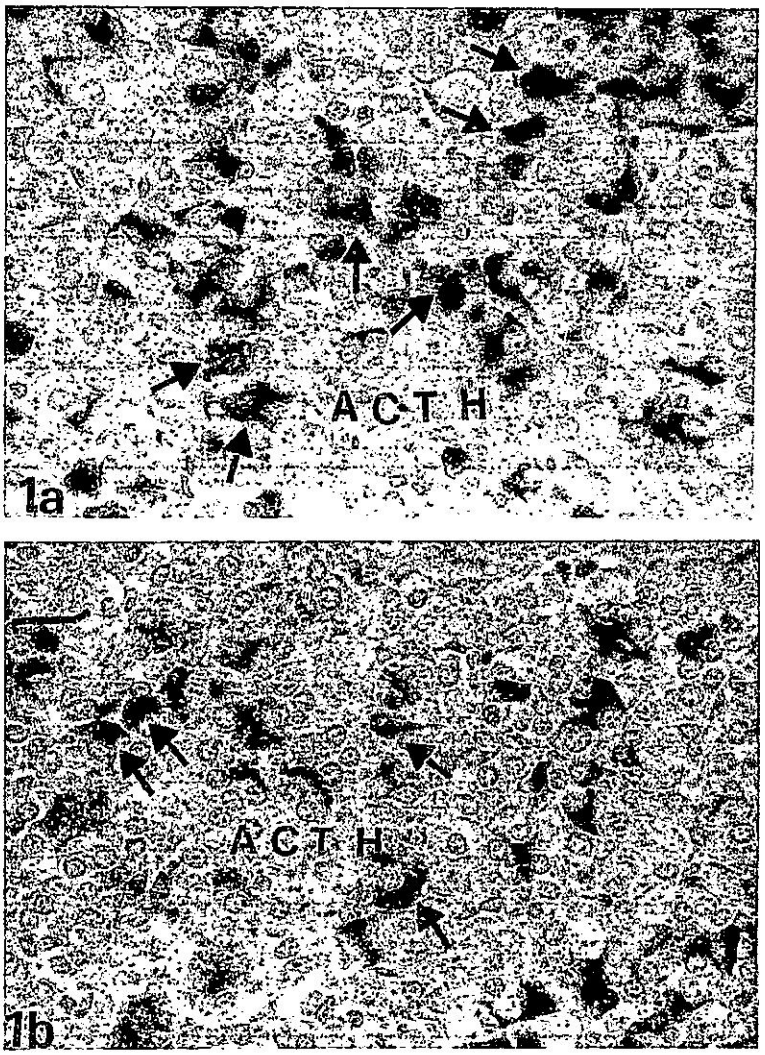

Fig. 1. Immunohistochemically labelled ACTH cells in : a) intact rats, b) Octreotide treated rats (PAP, 1256 X).

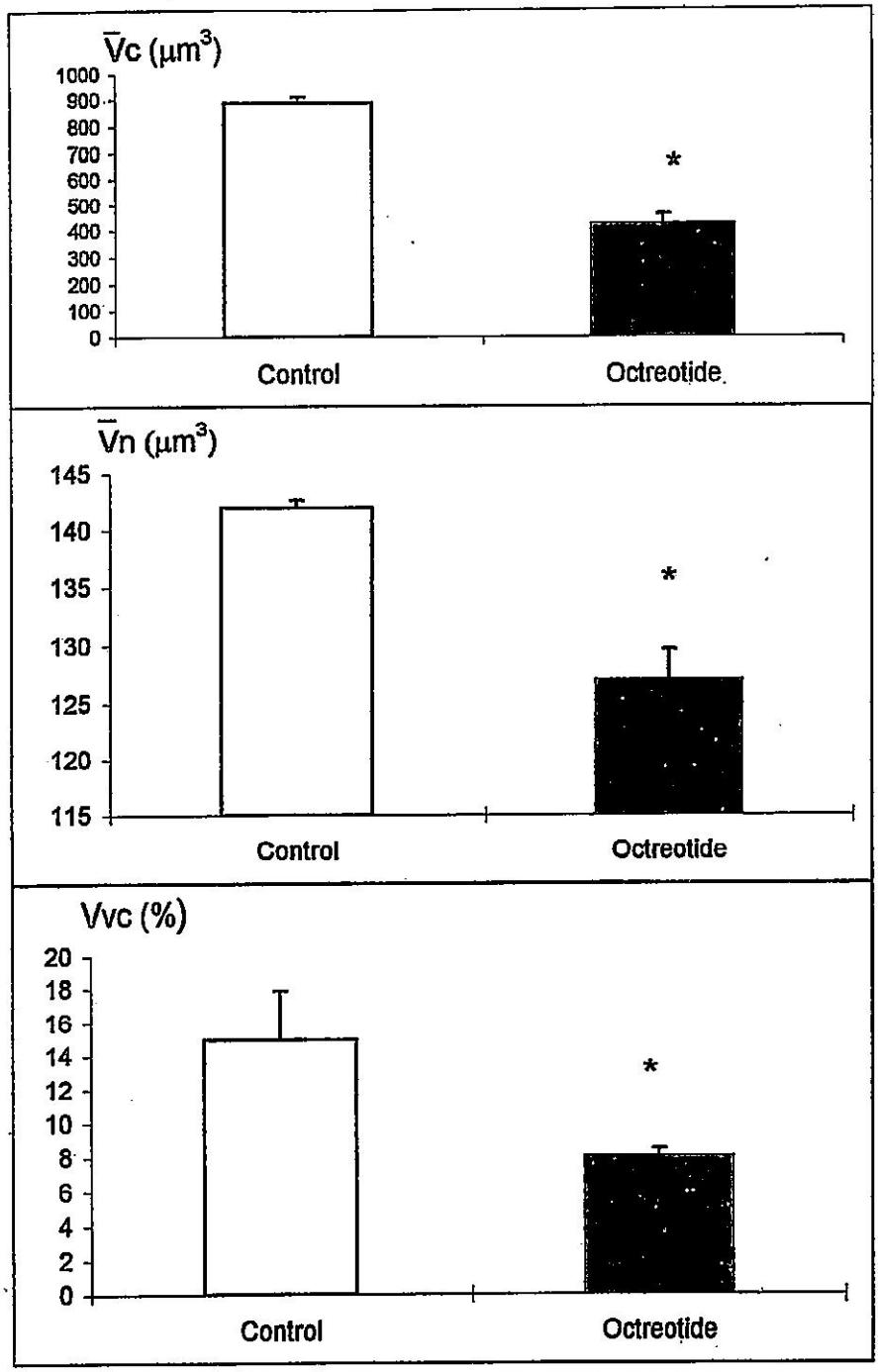

Fig. 2. A) Cellular volume (V_c; μm³) of the immunoreactive ACTH cells B) Nuclear volume (V_n ;μm³) of ACTH cells; C) Relative volume density (V_v; %) of cells expressed as a percentage of total gland tissue. All values are means ± SD. (n=5/group), [a]$p<0.05$ *vs.* control.

Morphometric parameters measured in the present study, *i.e.* volume of ACTH cells, their nuclei and volume density of these cells are presented in Fig. 2a, b, c. The mean volumes of ACTH cells and their nuclei were decreased in Octreotide-treated females by 52% and 11%, respectively, in comparison with the controls and the differences were statistically significant ($p<0.05$). The volume density of ACTH in Octeotride-treated rat females was also significantly decreased (by 47%; $p<0.05$) in comparison with control animals.

DISCUSSION

The results presented in this paper demonstrate, that repeated i.c.v. bolus injections of Octreotide significantly decreased all morphometric and immunocytochemical parameters of ACTH cells in adult female rats. Previously reported data on the effects of somatostatin on ACTH-secreting cells are somewhat conflicting (Richardson, 1981). Our earlier results (Starčević et al. 2000) indicated that in contrast to SRIH-14, i.c.v. administration of SRIH-28 to rat females exerted significant inhibitory effects on the function and morphometric charateristics of ACTH cells. We have also shown previously that i.c.v. treatment of adult male rats with either SRIH-14 or SRIH-28 did not result in significant changes of morphometric parameters of ACTH cells, or the blood concentration of ACTH, when compared to the corresponding controls (Milošević et al., 1994; Milošević, 1999). These data could be connected with the report of Browen et al. (1984) who demonstrated that SRIH-28 and ODT8-SS (an oligosomatostatin analogue) given i.c.v. acted by inhibiting stress-induced pituitary ACTH secretion in rats.

Octreotide primarily interacts with sstr2 and sstr5 somatostatin receptor subtypes and binds with a moderate affinity to sstr3 (Ösapay and Ösapay, 1998). However, it expressed no affinity for binding to the sstr1 and sstr4 subtypes of this receptor (Reisine and Ball, 1995). O·Carroll and Krempels (1995) found the mRNA of all five somatostatin receptor subtypes (sstr1-5) in somatotrophs, thyreotrophs, mammotrophs, corticotrophs and gonadotrophs. Octreotide was shown to inhibit the adenylate cyclase system *via* type 2 somatostatin receptors (Patel and Srikant 1994).

The mechanism of inhibition of ACTH secretion was hypothesized by several authors (Richardson, 1983; Browen et al.,1984; Shibasaki et al., 1988) to proceed through the inhibition of CRF release from the hypothalamus. Also, Litvin et al. (1986) observed that SRIH inhibits CRF-induced ACTH secretion from At20 cells *in vitro*. The action of SRIH was shown to be dose-dependent with a half-maximal effect at 1×10^{-9} M 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 tumor 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 female rats. These data, together with the reports of other authors, demonstrate that pharmacological manipulations of the central somatostatin receptors employing compounds exerting binding affinity for these receptors may provide a potent tool for altering ACTH physiology.

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EFEKTI OKTREOTIDA NA ACTH ĆELIJE HIPOFIZE ŽENKI PACOVA

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SADRŽAJ

Ispitivani su efekti intracerebroventrikularno (i.c.v.) ubrizganog analoga somatostatina, Oktreotida, na rast adrenokortikalnih (ACTH) ćelija adenohipofize ženki Wistar pacova. Sve eksperimentalne životinje su primile tri 1 μg doze Oktreotida rastvorenog u 10 μL fiziološkog rastvora, svaki drugi dan. Kontrole su tretirane na isti način fiziološkim rastvorom. Pacovi su žrtvovani petog dana po poslednjoj primljenoj dozi Oktreotida. ACTH ćelije su imunocitohemijski bojene PAP metodom. Dobijeni rezultati pokazuju da su statistički značajno smanjeni svi morfometrijski parametri tretiranih ženki pacova u odnosu na odgovarajuću kontrolu. Zapremina ćelija je smanjena za 52%, jedara za 11%, a volumenska gustina za 47% ($p<0.05$) u poredjenju sa odgovarajućom kontrolom. Na osnovu dobijenih rezultata može se zaključiti za Oktreotid deluje inhibitorno na rast ACTH ćelija adenohipofize ženki pacova.