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Preliminary reports

EFFECTS OF CENTRALLY APPLIED VASOPRESSIN ON THE PITUITARY ACTH CELLS IN MALE RATS

Dejan M. NEŠIĆ[1], Darko M. STEVANOVIĆ[1], Vladimir Z. AJDŽANOVIĆ[2], Saško D. VELKOVSKI[1], Verica LJ. MILOŠEVIĆ[2] and Vesna P. STARČEVIĆ[1]

[1]Institute of Physiology, School of Medicine, University of Belgrade,
[2]Institute for Biological Research "Siniša Stanković",
Belgrade, Serbia and Montenegro

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Arginine vasopressin (AVP) is synthesized in specific brain regions including the magnocellular and parvocellular divisions of paraventricular nucleus (PVN). While magnocellular AVP responds to osmotic stimuli and functions mainly - although not exclusively - as an antidiuretic hormone, AVP produced in the parvocellular region controls the hypothalamus-pituitary-adrenal (HPA) axis, in conjunction with CRH (FERRINI et al., 1997). ACTH release from anterior pituitary gland is principally driven by two hypothalamic hormones, corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) (TSIGOS and CHROUSOS,

Corresponding author: Dejan Nešić, MD, M Sc., Institute of Physiology, School of Medicine, University of Belgrade, Višegradska 26/II, 11 000 Belgrade, Serbia and Montenegro, Tel: (+381) 11 3611 438, E-mail: drdejannesic@yahoo.com

2002). AVP is a potent synergistic factor with CRH in stimulating ACTH secretion (LAMBERTS *et al.* 1984). AVP stimulates pituitary ACTH secretion through interaction with receptors of the V1b subtype (V1bR), located in the plasma membrane of the pituitary corticotropin releasing hormone (VOLPI *et al.* 2004).

Adult male Wistar rats (210 - 230 g) were used. They were implanted with a headset later serving for i.c.v. injections. A minimum recovery period of 5 days was permitted before the onset of experiments. The animals were divided into three experimental groups each including five individuals. The first and the second group consisted of rats, which were given (i.c.v.) three single 1-mg doses of AVP (No. V-9879; Sigma, St. Louis, Mo., USA) dissolved in 5 mL saline, every second day. The control group, comprised of rats treated in the same manner with an equal volume of saline. All animals were sacrificed by decapitation during deep aether anaesthesia 5 days after the last i.c.v. injection. Pituitary glands were excised, weighed in air, fixed in Bouin's solution and embedded in paraffin. Serial 5 mm 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 using the M_{42} multipurpose test system after WEIBEL (1979). Morphometric data obtained from each group were averaged, and the standard deviation of the mean was calculated by Student t-test. A probability value of 5% or less was considered statistically significant.

Data on body weight, absolute and relative weight of the pituitary in AVP-treated groups and control are summarized in Table 1. The body weight and absolute and relative pituitary weights were significantly increased ($p < 0.05$) by 44%, 89% and 61%, respectively, in comparison with corresponding control males.

Table 1. - The effects of intracerebroventricular AVP-treatment on body weight, absolute and relative pituitary weight in adult male rats

Experimental group	Body weight (g)	Absolute volume pituitary (mg)	Relative volume pituitary (mg%)
Control	242.2 ± 9.8	6.7 ± 1.9	2.3 ± 0.2
AVP	350.0 ± 14.1* (+44%)	12.8 ± 1.1* (+89%)	3.6 ± 0.4* (+61%)

The values are the means ± S.D. for five animals.
* $p < 0.05$ v.s. control

Immunohistochemically labelled ACTH cells in control rats were localized between the capillaries, stellate in shape, with the cytoplasmic processes between neighbouring, mostly somatotrophic cells. The nuclei followed the shape of the cell bodies. Small, specific secretory granules were distributed mainly in the peripheral cytoplasm. These cells were intensely stained (Fig 1a). In the rats i.c.v. treated with AVP neither the shape nor the localization of ACTH immunoreactive

cells were significantly changed in comparison to the corresponding controls, but their staining properties were significantly changed (Fig 1b).

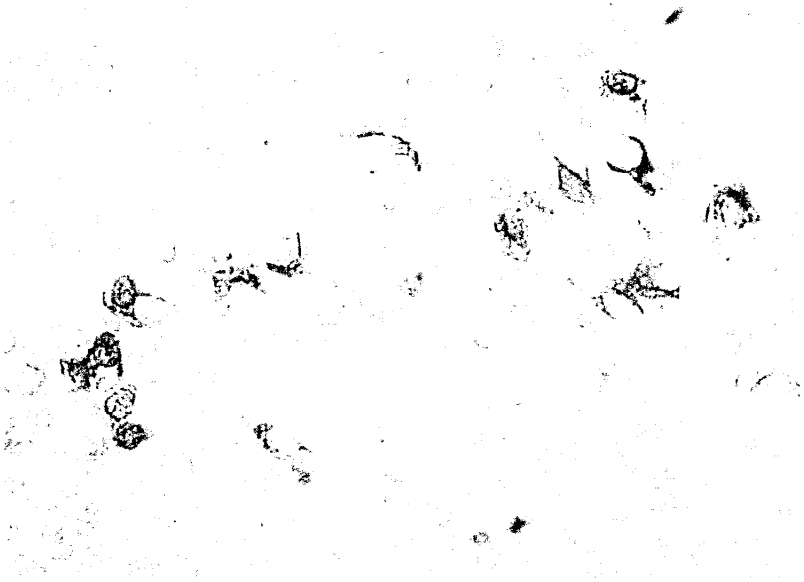

Fig 1a

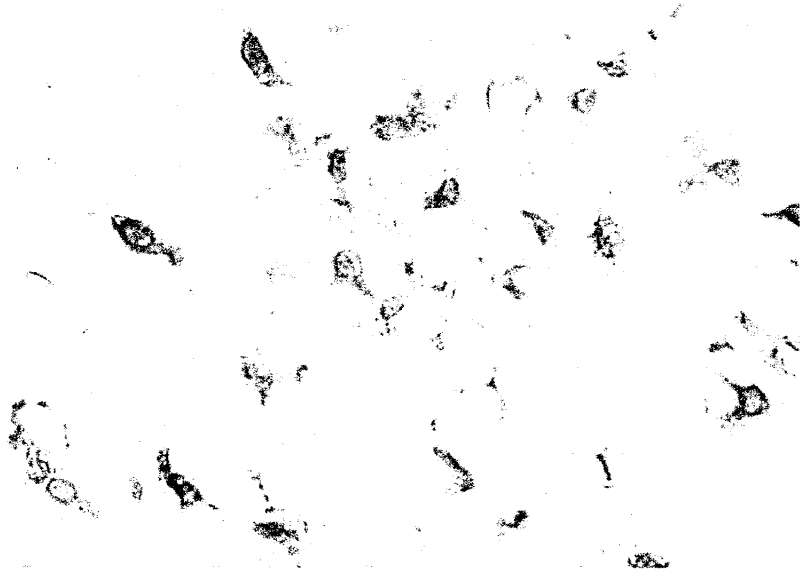

Fig 2b

Fig. 1 - Immunopositive ACTH cells in: a) control rats and b) AVP treated animals treated male rats. (PAP, magnification objektive 40x)

The volume (Vc) of ACTH cells and their nuclei (Vn) were significantly ($p < 0.05$) increased by 36% and 11%, respectively in AVP-treated animals in comparison with the controls. Volume density (Vv) of these cells treated groups was significantly increased by 33% compared to the controls.

The present results clearly demonstrate that i.c.v. administered AVP significantly increased body weight, absolute and relative pituitary weight compared to saline-treated controls. The results showed increase in body weight of treated rats by 44%. This increase is presumed to be a consequence of water accumulation in rat's body - antidiuretic effects of AVP (LOHMEIER, 2003).

Absolute and relative pituitary weights increased by 89% and by 61%, respectively, due to increased number of ACTH cells in hypophysis. Previous studies showed synergistic effects of CRH and AVP on pituitary ACTH cells (LAMBERTS *et al.* 1984; JIA *et al.* 1991).

Also, our results showed increase in all morphometric parameters. Similar results were reported by VOLPI *et al.* (2004) by studing the effects of vasopressinergic regulation of the hypothalamic pituitary adrenal axis and stress adaptation.

As a conclusion, our results indicate that i.c.v. applied AVP have significant stimulatory effect on growth of pituitary ACTH cells in adult male rats.

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REFERENCES:

FERRINI, M., GRILLO, C., PIROLI, G., DE KLOET, E., DE NICOLA, A. (1997): Sex difference in glucocorticoid regulation of vasopressin mRNA in the paraventricular hypothalamic nucleus. Cell. Mol. Neurobiol. 17: 671-686

JIA, L., CANNY, J. B., ORTH, N., D., LEONG, A., D. (1991): District classes of coticotropes mediate corticotropin-releasing hormone and arginine vasopressin-stimulated adrenokorticotropin release. Endocrinology. 128: 197-203.

LAMBERTS, S. W. J., VERLEUN, T., OOSTEROM, R., DEJONG, P., HACKENG, W. H. L. (1984): Corticotropin releasing factor and vasopressin exert a synergistic efect on adrenocorticotropin release in man. J. Clin. Endocrinol . Metabol. 58: 298-303.

LOHMEIER, E. T. (2003): Neurohypophysial hormones. Am J Physiol Regul Integr Comp Physiol. 285: 715-717.

STERNBERGER, L. A., HARDY P. H. J., CUCULIUS J. J., MEYER H. G. (1970): The unlabelled Antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18: 315-333.

TSIGOS, C., CHROUSOS, G. (2002): Hypothalamic-pituitary-adrenal axis, neuroendocrine actors and stress. J Psyhosomatic Res 53: 865-871.

VOLPI, S., RABADAN-DIEHL, C., AGUILERA, G. (2004): Vasopressinergic regulation of the hypothalamic pituitary adrenal axis and stress adaptation. Stress. 7(2): 75-83.

WEIBEL, E.R., (1979): Stereological methods: Practical Methods for Biological Morphometry, ol. 1, New York, Academic Press, pp. 1-415.

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