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Distinct vasopressin content in the hypothalamic supraoptic and paraventricular nucleus of rats exposed to low and high ambient temperature

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ABSTRACT

Both high and low ambient temperature represent thermal stressors that, among other physiological responses, induce activation of the hypothalamic–pituitary–adrenal (HPA) axis and secretion of arginine-vasopressin (AVP). The exposure to heat also leads to disturbance of osmotic homeostasis. Since AVP, in addition to its well-known peripheral effects, has long been recognized as a hormone involved in the modulation of HPA axis activity, the aim of the present study was to elucidate the hypothalamic AVP amount in the acutely heat/cold exposed rats. Rats were exposed to high (+38 °C) or low (+4 °C) ambient temperature for 60 min. Western blot was employed for determining hypothalamic AVP levels, and the difference in its content between supraoptic (SON) and paraventricular nucleus (PVN) was detected using immunohistochemical analysis. The results showed that exposure to both high and low ambient temperature increased hypothalamic AVP levels, although the increment was higher under heat conditions. On the other hand, patterns of AVP level changes in PVN and SON were stressor-specific, given that exposure to cold increased the AVP level in both nuclei, while heat exposure affected the PVN AVP content alone. In conclusion, our results revealed that cold and heat stress influence hypothalamic AVP amount with different intensity. Moreover, different pattern of AVP amount changes in the PVN and SON indicates a role of this hormone not only in response to heat as an osmotic/physical threat, but to the non-osmotic stressors as well.

1. Introduction

Animals have developed various thermoregulatory strategies in order to successfully overcome frequently occurring variations in environmental temperature. Given that rats have evolved to cope well with extreme environmental conditions (Donald and Wittert, 1994), they are often used as an experimental model for studying these strategies. Although thermal stressor of altered ambient temperature belongs to a group of physical stressors (Kvetnansky et al., 2009), heat and cold differ in the manner they affect endothermic organisms. Exposure to heat, unlike cold, leads to disturbance of osmotic homeostasis. Generally, stressful events pose a threat to homeostasis and affect numerous physiological processes throughout the central nervous and endocrine system. Not surprisingly, there are many data showing different patterns of response to these stressors, judging by the blood catecholamine levels and altered hypothalamic–pituitary–adrenal (HPA) axis activity (Djordjevic et al., 2003; Pacak and Palkovits, 2001).

The activation of the HPA axis results in the rapid increase in circulating adrenocorticotropic hormone (ACTH), with the subsequent rise in glucocorticoids, which is critical for successful acclimatization (Dallman et al., 1992). Corticotropin releasing hormone (CRH) is long-known to regulate the corticotroph activity (Antoni, 1986). It was also demonstrated that the next step in triggering the HPA axis response is not only the CRH, but arginine vasopressin (AVP) secretion as well. The two hormones then synergistically stimulate ACTH release from the anterior pituitary (Yates et al., 1971; Leong, 1988). However, the patterns of CRH, AVP and ACTH secretion vary with the type and duration of the stressor (Donald and Wittert, 1994).

In the mammalian brain, AVP is mainly synthesized in the

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magnocellular neurons of the hypothalamic supraoptic nucleus (SON) and the paraventricular nucleus (PVN) but also within the parvocellular neurons of the PVN (pPVN) (Hawthorn et al., 1980). According to Aguilera and Rabadan-Diehl (2000), AVP expression and secretion from the pPVN may be independent of the osmotic status, being increased during stress and involved in the regulation of HPA axis activity. Explanation of this phenomenon is in the fact that these neurons project into the *zona externa* of the median eminence and secrete both CRH and AVP into the long portal vessels. This pathway constitutes the central neuro-endocrine part of the HPA axis, being active under both resting and stressful conditions.

In contrast, data regarding AVP of magnocellular origins are still controversial. Based mostly on data regarding neuronal projections of these neurons, some authors consider that the magnocellular AVP is responsible exclusively for water conservation and/or blood pressure regulation (Stricker and Sved, 2002). Namely, magnocellular AVP-producing neurons of the PVN project through the *zona interna* of the median eminence to the posterior pituitary (PP) where AVP is stored in the axon terminals and secreted into the systemic blood circulation upon stimulation. This indeed constitutes the main pathway of the AVP action on water retention under both resting and stressful conditions. However, the magnocellular originating AVP may also act as secretagogue for ACTH, reaching the anterior pituitary via at least two vascular pathways (Engelmann et al., 2004; Jasnic et al., 2013). Thus, during the stress conditions, AVP is released from axon terminals of the PP into short portal vessels, which connect the anterior and posterior pituitary. Furthermore, it is proposed that AVP secreted during stress (e.g. osmotic stress due to heat provoked dehydration) from the PP into the general blood circulation could also reach the corticotrophs (Engelmann et al., 2004; Jasnic et al., 2013), although the physiological relevancy of quantity released through suggested pathway is still under question (Plotsky, 1991).

Taken together the literature data on emerging roles of AVP and the fact that heat and cold differently challenge osmotic homeostasis impose the question whether there is a different pattern of the magnocellular and parvocellular AVP-secreting neurons response to respective thermal stressors. An answer to this question could provide a better insight into the AVP role as an ACTH secretagogue.

That said, and owing to the lack of data regarding the AVP amount in the particular hypothalamic areas during heat/cold stress, our goal was to examine the hypothalamic AVP content in acutely heat and cold exposed rats.

2. Materials and methods

Animal handling and treatments were carried out in compliance with the Serbian animal protection law and approved by the Ethical Committee of the University of Belgrade, Faculty of Biology. The work presented in the present article was also performed in accordance with the EU Directive 2010/63/EU for animal experiments, and the uniform requirements for manuscripts submitted to Biomedical journals.

2.1. Experimental design

The experiments were conducted on adult male Wistar rats, *Rattus norvegicus*, weighting 220 ± 20 g, bred in the vivarium of the Faculty of Biology, University of Belgrade. The animals were acclimated to 22 ± 1 °C, synchronized to a 12 h:12 h light:dark regime with lights on at 6:00 a.m. and off at 6:00 p.m., with free access to commercial rat food (Veterinary Institute, Subotica, Serbia) and tapwater. The rats were caged in groups of two for at least a week before they were randomly separated into three groups (control, the cold or heat exposed). While the control rats were left undisturbed during the course of the experiment, rats exposed to heat were transferred into a temperature chamber at +38 °C (Sutjeska, Belgrade, Serbia), and those exposed to cold into another temperature chamber at +4 °C. Immediately after one hour of high or low temperature treatment, rats were sacrificed and the brain tissue isolated according to the protocol as described below. In order to avoid day–night variations in the concentrations of the measured hormones, all experiments were performed between 8:00 a.m. and 10:00 a.m. Furthermore, a group of sacrificed animals always consisted of both treated and control animals, and this pattern was repeated until all experimental groups reached their full number ($n=5$ for immunohistochemistry and $n=6$ for western blotting).

2.2. Western blot analysis

The animals ($n=6$ per group) were decapitated with a guillotine (Harvard-Apparatus, Holliston, MA, USA) without anesthesia immediately after the stress exposure. The heads were immediately plunged into the iced water and hypothalami quickly dissected by the method of Glowinski and Iversen (1966), where the optic chiasm delimit the anterior part of the hypothalamus, the anterior commissure being the horizontal reference and the mammillary bodies delimit the posterior part of the hypothalamus.

The hypothalami were homogenized on ice with an Ultra-turrax homogenizer in protein isolating buffer which contained 1.5 mol l^{-1} NaCl, 10% Triton X-100, 0.5 mol l^{-1} Tris–HCl (pH 7.5), 10% SDS and a cocktail of protease inhibitors (Roche, Basel, Switzerland). After homogenization, the samples were sonicated (3×10 s) and centrifuged for 25 min at 14,000 r.p.m. The total protein concentration was determined using the method described by Lowry et al. (1951). The intrahypothalamic AVP level was determined by western blot analysis (Burnette, 1981). The samples containing 2 mg ml^{-1} of total protein were added to an equal volume of Laemmli buffer with 2-mercaptoethanol (200:5, v/v). After denaturation by boiling at 100 °C for 5 min, the samples were separated on a 20% polyacrilamide gel (120 V, Criterion Cell, Bio-Rad, Hercules, CA, USA) and electrotransferred (overnight, 20 mA per gel, Criterion blotter, Bio-Rad) onto 0.45 mm polyvinylidene difluoride (PVDF) membrane. Subsequent to the transfer of proteins, membranes were blocked for 3 h in 2,5% non-fat dry milk powder (Santa Cruz Biotechnology, Heidelberg, Germany) in Tris buffered saline containing 0.1% Tween 20 (TBST). After blocking step, the blots were incubated with primary antibody (AVP, rabbit polyclonal, 1:3000, ab39363 Abcam, Cambridge, UK) overnight at +4 °C, and thoroughly washed in TBST before a 1 h incubation with horseradish peroxidase-conjugated secondary goat anti-rabbit polyclonal antibody (1:5000, ab6721 Abcam, Cambridge, UK). After washing in TBST, the membranes were incubated with the enhanced chemiluminescence (ECL) plus detection system (Amersham, Bucks, UK) for 5 min. Excess ECL plus solution was then removed, and the immunoreactive bands detected in the dark chamber. The intensity of signals was evaluated by the Image Quant program (Molecular Dynamics, Amersham Biosciences) and calculated as the number of arbitrary units (a.u.) mg^{-1} of total protein. Those results were then recalculated and the specific protein content of all groups was expressed relative to the control group value on the graphs.

2.3. Immunofluorescence

Rats ($n=5$ per group) were anesthetized by intramuscular injection of ketamine (100 mg/kg) and xylazine (8 mg/kg) mixture and transcardially perfused first with physiological saline and then