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EFFECTS OF IMMOBILIZATION STRESS ON DISTRIBUTION OF NADPH-D REACTIVE NEURONS IN RAT'S PARAVENTRICULAR NUCLEUS

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Abstract — **Regulatory control of the hypothalamic–pituitary–adrenocortical axis (HPA) originates principally from the paraventricular nucleus (PVN). PVN contains a substantial population of nitric oxide synthesizing (NOS) neurons. In the present study, the effect of immobilization stress over these neurons was investigated by means of the NADPH–diaphorase histochemical technique. A considerable increase in the number of NADPH-diaphorase reactive neurons was observed following acute immobilization of rats. Our data showed that NO activity in rat's PVN was significantly affected by acute immobilization stress. This suggests an important role of this part of the brain and NO-ergic system in anti-stressor system which main role is to process strategies for coping with different types of stress and to restore the disrupted homeostasis.**

Key words: **Acute immobilization stress; Paraventricular nucleus; Nitric oxide; Histochemistry; Rat**

I. INTRODUCTION

Stress is associated with activation of the hypothalamic–pituitary–adrenocortical (HPA). Various stress related inputs converge upon the neurons located in the paraventricular nucleus (PVN) (Cullinan et al., 1996; Dzambazova et al., 2008; Dzambazova et al., 2009a). In fact, this nucleus has a pivotal role in the control of pituitary–adrenocortical activity in response to stress. The neurons located in this nucleus synthesize and release several secretagogues, corticotropin releasing factor and vasopressin, responsible of adenocorticotropin-induced release of corticosteroids from the adrenal cortex. Nowadays, it is well known that these neurons also coexpress the neuronal isoform of nitric oxide synthesizing (NOS) neurons (Sanchez et al., 1994; Siaud et al., 1994; Torres et al., 1993; Yamada et al., 1996). NOS has been demonstrated to be present in the PVN by means of the NO-histochemical method (Alonso et al., 1992a; Alonso et al, 1992b; Arevalo et al., 1993; Crespo et al., 1998; Sanchez et al., 1994; Sanchez et al., 1996a; Sanchez et al, 1996b; Sanchez et al., 1998a; Sanchez et al., 1998b), immunohistochemistry and in situ hybridization (Calza et al., 1993; Ceccatelli et al., 1993; Ceccatelli et al., 1996; Kishimoto et al, 1996). In addition, it is well known that immobilization stress activates neuronal NOS in the HPA (Calza et al., 1993; Kishimoto et al., 1996). The present study was undertaken to define whether or not immobilization stress promotes changes in the expression of the NO-activity in the hypothalamic PVN.

II. MATERIALS AND METHODS

Male Wistar rats (180-220 g) were divided into two groups. The first group represented intact controls. The second group was subjected to acute immobilization stress (IS) - rats were immobilized for 1 hour in a restrainer. Three animals of each group were anaesthetized with thiopental (40 mg/kg, b.w.) immediately after immobilization. After perfusion through the heart with fixative (4% paraformaldehyde in 0.1M phosphate buffer, pH 7,2) brains were removed and coronal sections were cut on a freezing microtom at 40 μm, and collected in Tris-HCl buffer 0.05M, pH 7.6. After a histochemical procedure for NADPH-d reactive neurons NO activity was estimated. Morphometric analysis was performed using a microanalysis system (primary magnification 20 x objective). Data of the entire drawings were entered in computer programme (Olympus CUE-2), recorded automatically, calculated and compared by Student's t-test. All procedures were approved by the Animal Care and Use Committee of the Medical University, Sofia

III. RESULTS AND DISCUSSION

A histochemical procedure for NADPH-d-reactive neurons in rat's PVN was used as marker of NO activity (Fig. 1). Increased NO stimulates guanylate cyclase and increases the levels of cyclic guanosine 3′5′-monophosphate in the cells. Thus the NADPH-d histochemical method allows the direct visualization of the neurons which use NO. The control animals that were not immobilized showed a cluster of intensely stained NADPH-d positive neurons with varicose fibers in the PVN (Fig. 2). The acute stressor – 1 hour immobilization showed statistically significant increase in the number of the NADPH-d positive neurons (Fig. 3) compared to the control group ($p < 0.01$) (Fig. 4).

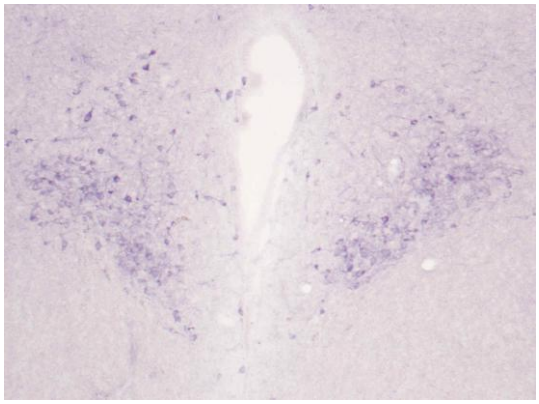

Fig. 1. NADPH-d-reactive neurons in PVN of hypothalamus in intact animal (x100).

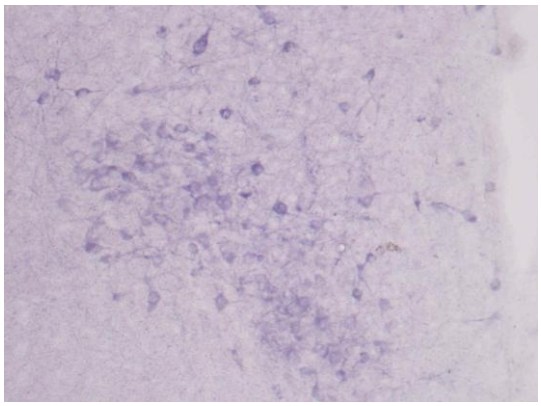

Fig. 2. NADPH-d-reactive neurons in PVN of hypothalamus in intact animal (x200).

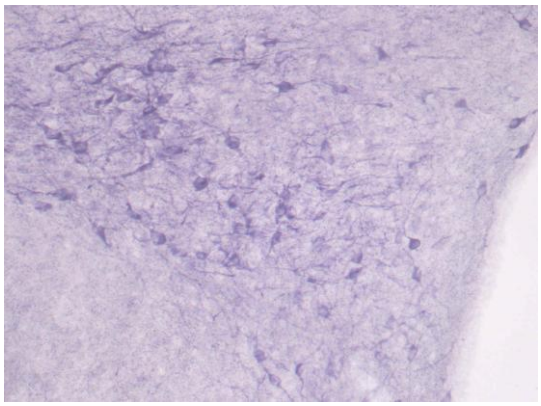

Fig. 3. NADPH-d-reactive neurons in PVN of hypothalamus in animal sacrificed immediately after 1h immobilization stress – increased number of neurons (x200).

These results support some author's data about stress-induced increasing of nitric oxide activity in PVN (Dzambazova et al., 2008, 2009a), PAG (Dzhambazova et al., 2011; Landzhov et al., 2012a,b), cerebral cortex (Dzambazova et al., 2011), caudate putamen (Landzhov et al., 2011a), striatum (Landzhov et al., 2011b), claustrum (Edelstein et al., 2012a, 2012b), in the thalamic reticular nucleus (Landzhov et al., 2003, 2004). There is also literature data about modulatory effects of some neuropeptides released during stress on NO activity (Dzambazova et al., 2009b, 2011b, 2015; Landzhov et al., 2011a) and involvement of different neurotransmitter systems (Bozhilova-Pastirova et al., 2006, 2012, 2013; Bocheva et al., 2008, 2009; Malinova et al., 2011; Pencheva et al., 2012; Landzhov et., 2012c, 2013a,b; Malinova et al., 2013a,b; Dzhambazova et al., 2014a,b). Some authors have studied newly synthesized analogues of neuropeptides which neuromodulatory effect on nitric oxideergic system was even stronger (Dzambazova et al., 2009; Hadjiolova et al., 2009). The work of Dzhambazova et al (2011) revealed that D-Kyotorphin (D-KTP) showed an increased number of NADPH-d reactive neurons in rat's periaqueductal gray after immobilization stress. Although D-KTP is incapable of crossing the blood-brain barrier the authors suggest that their modulatory effect is due to indirect mechanism. This mechanism involves strong stimulation of opioidergic system by D-KTP, which is structural and functional related with NO-ergic system in the brain.

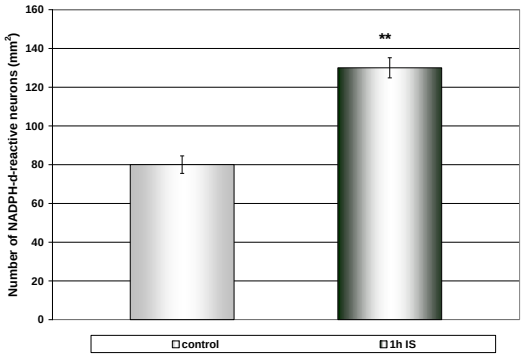

Fig. 4. Effect of 1 h immobilization stress (IS) on NADPH-d-reactive neurons in male Wistar rat's PVN. Mean values ± S.E.M. are presented **P < 0.01 vs. control.

IV. Conclusion

In conclusion our results showed that NO activity in rat's PVN was significantly affected by acute immobilization stress. This suggests an important role of this part of the brain and NO-ergic system in anti-stressor system which main role is to process strategies for coping with different types of stress and to restore the disrupted homeostasis.

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