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Alteration in nitric oxide activity in the ventrolateral periaqueductal gray after immobilization stress in rats

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Nitric oxide (NO) synthesized by the enzyme nitric oxide synthase (NOS) affects the secretion of stress hormones and NO system is a stress-limiting system. Also, NO is involved in NO-molecular ways, which affect through auto regulation different signaling molecules – like opioids, endocannabinoids and others. Many stress models have been reported to affect the levels of nitric oxide and stimulate the expression of NOS engaged in their synthesis. Stimulation of opioid receptors within the periaqueductal gray (PAG) activates descending opioid and noradrenaline inhibitory pathways and suppresses nociception.

Because PAG has been identified as region that mediates the response to different stressful paradigms and contains distinct, longitudinally organised neural substrates, our goal was to investigate the effect of 3 hours immobilization stress (IS) on NO activity in rat ventrolateral PAG (vlPAG) by a histochemical procedure for nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d). According to obtained data NADPH-d reactive neurons in rat's vlPAG and NO activity respectively was increased by stress model mentioned above.

Key words: Nitric oxide, immobilization stress, vlPAG

All animals, including humans, react with distinct emotional coping strategies to different types of stress. According literature data, immobilization stress (IS) is physical, inescapable stressor which usually evoke passive emotional coping. On the other hand, the midbrain periaqueductal gray (PAG) has been identified as region that mediates the response to different stressful paradigms and the main structure involved in pain modulation and perception. One of the mechanisms involved in PAG's stress response modulation is activation of opioidergic and nitric oxideergic pathways. PAG region also contains distinct, longitudinally organised neural substrates which mediate either active or passive emotional coping strategies. It's known that active emotional coping of stress is evoked by activation of either the dorsolateral (dl) or lateral (l) PAG columns, whereas passive coping is triggered by activation of the vlPAG column.

The aim of our study was to investigate the efect of 3h IS on NO activity in rat vlPAG by a histochemical procedure for nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d).

Material and Methods

Nine male Wistar rats (180-220 g) were divided in three groups. Each group included 4 rats: Animals from first group (control group) were not submitted to stress procedure. Animals from second group were submitted for acute model of IS for 3 h.

Acute immobilization stress: The animals were placed in a plastic tube with adjustable plaster tape on the outside so that the animals were unable to move. There were holes for breathing. The control group was not submitted to restraint. The immobilization procedure was carried out for 3 hours.

Histochemical procedure: The animals of each group were anaesthetized with thiopental (40 mg/kg, i.p.). 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 mm, and collected in Tris-HCl buffer 0.05M, pH 7.6. The NADPH-d histochemical procedure was used as a marker of NO activity in the neurons. All animals were cared for in compliance with the Principles of Laboratory Animal Care of the Medical University, Sofia.

Statistical analysis: The data were entered in the computer program (Olympus CUE-2), recorded automatically and calculated. The values from controls and rats undergoing immobilization stress were compared by Student's t-test.

Results and discussion

Nitric oxide (NO) is a unique neurotransmitter, which participates in many physiological and pathological processes in the organism. It was synthesized by the family of enzymes called nitric oxide synthase (NOS), which belong to three subtypes – neuronal (nNOS), inducible (iNOS) and endothelial (eNOS)) and had widespread distribution in the brain [6, 9, 10, 11].

Our previous results revealed that some neuropeptides have modulating effects on acute immobilization stress-induced analgesia in rats maybe by opiod and non-opioid systems [2, 3, 4,]. Recent study have shown that the vlPAG can be activated by acute immobilization stress. 3 h immobilization stress significantly increased the number of NADPH-d neurons in the vlPAG compared to the control group (Fig. 1 and 2). This correlate with literature data that nNOS is increased in response to stress [1, 5, 7, 8, 12,]. There are no data available about the effects of IS in different columns of PAG. Our study have shown that acute immobilization stress activate mainly vlPAG.

Some investigators demonstrated that both nNOS and corticotrophin-releasing factor (CRF) are increased in response to stress [1, 7]. In our experiments the number of NADPH-d reactive neurons in vlPAG were significantly potentiated by investigated immobilization stress model. The obtained result correlate with literature data that nNOS is increased in response to stress.

During stress opioidergic system is strongly stimulated and also NO-ergic system because they have structural and functional relations within the PAG.

Conclusion

In conclusion our results showed that acute IS rises NADPH-d reactive neurons in rat's vlPAG and NO activity respectively. These data suggest that NO activity in rat's vlPAG may play an important role in the continuity of homeostasis disrupted by stress.

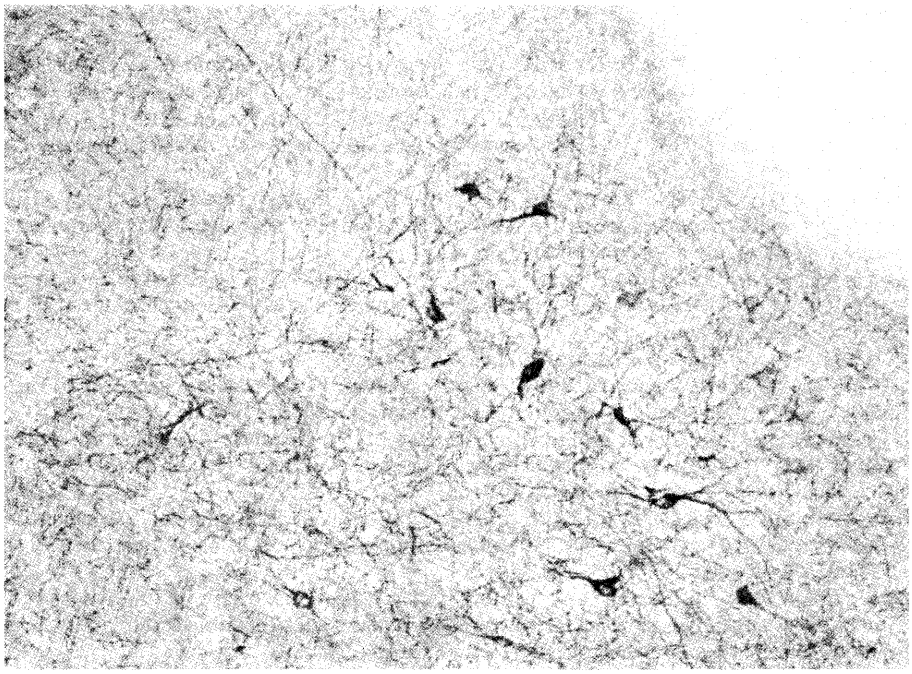

Fig. 1: NADPH-d reactive neurons in the vlPAG after 3 h immobilization stress. (x200).

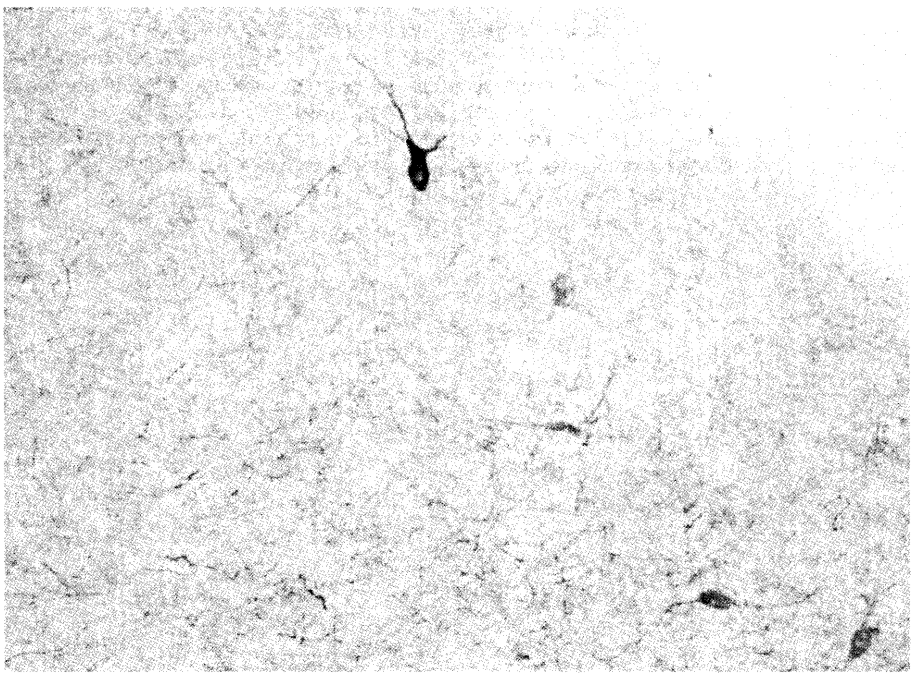

Fig. 2: NADPH-d reactive neurons in the vlPAG. Control group (x200).

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