

LOCALIZATION OF CB1 RECEPTORS IN RAT'S PERIAQUEDUCTAL GRAY AFTER IMMOBILIZATION STRESS AND EFFECTS OF PEPTIDE TYR-W-MIF- 1. IMMUNOCYTOCHEMICAL STUDY

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ABSTRACT

Periaqueductal gray (PAG) is a midbrain structure closely involved in the stress-induced analgesia. It suppresses nociception by the descending efferent pathways to the dorsal horn of the spinal cord. Except stimulation of opioid receptors, the PAG is specialized to produce cannabinoid-mediated stress-induced analgesia. Attractive candidates for opiate modulators are neuropeptides from Tyr-MIF-1 family. These peptides also are involved in the development of stress. Based on behavioral and anatomical data about direct interactions of cannabinoid type 1 (CB1) receptors and μ -receptors in the PAG we decided to investigate the effects of the Tyr-W-MIF-1 neuropeptide on expression of CB1 immunoreactive neurons in rat's PAG after immobilization stress. Light microscopic study was used to determine the distribution of CB1 receptor immunoreactivity. The obtained results showed that stress itself increased the expression of CB1 immunoreactive neurons in the PAG compared with intact animals, while Tyr-W-MIF-1 decreased stress-induced CB1 expression mentioned above probably by opioid/cannabinoid interaction. Further studies are needed to understand the exact role of Tyr-W-MIF-1 on CB1 receptors in response to immobilization stress.

Key words: PAG, CB 1 receptors, neuropeptides, immobilization stress, Tyr-W-MIF-1

INTRODUCTION

The PAG is a mesencephalic structure that surrounds the cerebral aqueduct and can be divided along its rostrocaudal axis into dorsomedial, dorsolateral (dIPAG), lateral, and ventrolateral columns (3). The previous data have shown that PAG is a significant modulator involved in neuronal

circuits taking part in the stress-induced analgesia (4). Stress activates neural systems that inhibit pain sensation. This adaptive response depends on neuronal pathways that project from the cortical neurons to the midbrain periaqueductal gray (PAG) and descend to the brainstem rostro-ventro-medial medulla and dorsal horn of the spinal cord and suppresses nociception (8,20).

The CB1 receptor expression was detected in regions influencing a number of key functions, including mood, motor coordination, autonomic function, memory, sensation, cognition and stress. Electron microscopy studies demonstrated CB1 receptors predominantly on presynaptic terminals (11,15) but they were found also on postsynaptic structures and glia (3). They activate G-proteins that inhibit adenylate cyclase and calcium channels

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Received: February 01, 2013

Accepted: February 25, 2013

and enhance potassium currents, thereby reducing neural firing and neurotransmitter release (18).

Tyr-W-MIF-1 is a member of Tyr-MIF-1 family of neuropeptides which have been isolated from human parietal cortex and bovine hypothalamic tissue. Previous studies showed that Tyr-MIF-1 family members are attractive candidates for opiate modulators. It is known that these peptides participate in different types of physiological processes including pain and evolution of stress. Tyr-W-MIF-1 has high selectivity for μ -opioid receptors (12).

The aim of this study was to investigate the effects of the Tyr-W-MIF-1 neuropeptide on expression of CB1 immunoreactive neurons in rat's periaqueductal gray after immobilization stress using immunohistochemistry.

MATERIALS AND METHODS

Animals

The experiments were carried out on male Wistar rats kept under normal conditions at ambient room temperature. Each group included 3 rats.

Acute model of immobilization stress (IS)

The animals were placed for 1 hour in a plastic tube with adjustable plaster tape on the outside so that the animals were unable to move. There were holes for breathing.

After the completion of the stress model the animals were injected with Tyr-W-MIF-1 (1mg/kg) obtained from Sigma. The neuropeptide was dissolved in sterile saline (0.9% NaCl) solution and was injected intraperitoneally (i.p).

Immunohistochemistry

After stress procedure, 24 h later the animals were anaesthetized with Thiopental (40 mg/kg, i.p.) and perfused through the heart with fixative (4% paraformaldehyde in 0.1M phosphate buffer, pH 7,2). Brains were removed and sectioned by freezing microtome. The coronal sections we took between bregma +1.70 - +5.2. Free-floating sections were preincubated for 1 h in 5% normal goat serum in PBS. Afterwards, incubation of the sections was performed in a solution of the primary antibody for 48 h at room temperature. We used a polyclonal anti-CB1 antibody (Santa Cruz, USA), in a dilution of 1:1000. Then sections were incubated with biotinylated anti-mouse IgG (dilution, 1:500) for

2 h and in a solution of avidin-biotin-peroxidase complex (Vectastain Elite ABC reagent; Vector Labs., dilution 1:250) for 1 h. This step was followed by washing in PBS and then in 0.05 M Tris-HCl buffer, pH 7.6, which preceded incubation of sections in a solution of 0.05% 3,3'-diaminobenzidine (DAB, Sigma) containing 0.01% H₂O₂ for 10 min at room temperature for the visualization.

All procedures were approved by the Animal Care and Use Committee of the Medical University, Sofia.

RESULTS AND DISCUSSION

Immunohistochemistry labeling in PAG sections was viewed using a light microscope. Images from three groups of animals were analyzed to determine the distribution of CB1 receptor immunoreactivity. In control animals CB1 expression was diffuse and extensive (Fig. 1). We found it on some cell bodies as well as in axons and dendrites of the PAG. In the neurons the CB 1 receptors were mostly expressed on the cell membranes. Even there is also a great deal of CB1 receptor immunoreactivity outside of cell bodies. Our results showed increased expression of CB1 immunoreactive neurons in the PAG after 1 h immobilization stress compared with intact animals (Fig. 2). Injection of Tyr-W-MIF-1 (1 mg/kg, i.p.) immediately after stress procedure apparently decreased expression of CB1 immunoreactive neurons in the PAG (Fig. 3).

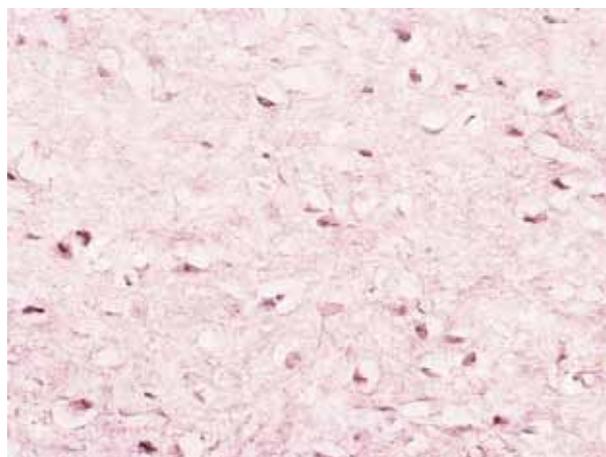


Fig. 1. Distribution of CB1 immunoreactive neurons in PAG, intact animals. The CB 1 receptors were mostly expressed on the cell bodies as well as in axons and dendrites (x400)

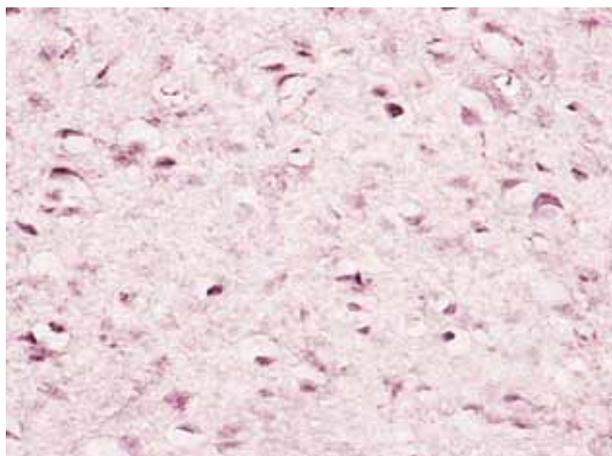


Fig. 2. Distribution of CB1 immunoreactive neurons in PAG after 1 h immobilization stress (x400)

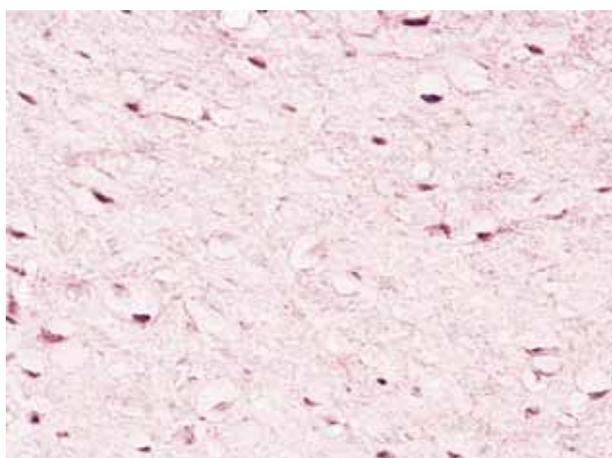


Fig. 3. Distributions of CB1 immunoreactive neurons in PAG after injection of Tyr-K-MIF-1 (x400)

The periaqueductal gray is part of a descending pain modulatory system that, when activated, produces widespread and profound antinociception. Furthermore, except opioid stimulation of opioid receptors, the dorsolateral PAG is specialized to produce cannabinoid-mediated stress-induced analgesia (10).

There are no data available about the effects of Tyr-MIF-1 peptides on expression of CB1 receptors in rat's PAG after any kind of stress. Our previous data demonstrated that Tyr-W-MIF-1 injected alone in naive rats had analgesic effect and it also decreased the effects of morphine, L-NAME and immobilization stress-induced analgesia, which corresponds to the hypothesis about anti-opioid peptides (5,6,7).

It's known that members of Tyr-MIF-1 family of peptides are particularly attractive candidates for opiate modulators. Unlike most other putative opiate-modulating peptides, they bind to the μ opiate receptor and to their own non-opiate sites (7). Immobilization stress-induced antinociception is also reduced in rats by Tyr-W-MIF-1, as measured by the paw-pressure test (6). This opioid effect is greater when the tetrapeptide is injected after exposure to the stress. Tyr-W-MIF-1 also decreases the analgesic effect of morphine in the tail-flick and paw-pressure tests (5). Now, the results in our experiments showed that Tyr-W-MIF-1 injected in animals immediately after immobilization stress procedure apparently decreased expression of CB1-immunoreactive neurons. Thus, the investigated neuropeptides counteract the effect of stress and we may confirm its anti-stressor effect.

There are scientific data that CB1 and μ -opioid receptors are each found in somatodendritic profiles in the PAG (14). In the dorsal horn of the spinal cord, both receptor types co-localize in dendrites (1).

Previous reports have shown that chronic drug administration can induce changes in the density of the CB1 and μ -opioid receptors in several sites in the central nervous system. In reward-related brain areas, there is a reciprocal up-regulation of CB1 and μ -receptors after chronic drug exposure. Similarly, chronic morphine administration increases the density of both CB1 and CB2 receptors in the dorsal horn of the spinal cord (9).

The behavioral and anatomical data cited above suggest that CB1 and μ -receptors have direct interactions in the PAG. Opioids and cannabinoids have been shown to have analgesic properties. Systemic co-administration of cannabinoid and opioid agonists results in synergistic antinociception (13), and systemic pre-treatment or co-administration of cannabinoids attenuates the development of morphine tolerance (17). It is possible that cannabinoid/opioid interactions occur between neurons where the receptors are not co-localized on the same cell, but interact synaptically. Endocannabinoids are retrograde messengers, with CB1 receptors located on presynaptic terminals (2, 19). Thus, CB1-receptive terminals contacting μ receptor-immunoreactive cells could be alternative sites of opioid/cannabinoid interaction.

CONCLUSION

In conclusion our results showed that expression of CB1 immunoreactive neurons in rat's PAG were increased by immobilization stress. To our knowledge, this is the first report showing that neuropeptide Tyr-W-MIF-1 decreased stress-induced CB1 expression mentioned above probably by opioid/cannabinoid interaction. Further studies are needed to understand the exact role of Tyr-W-MIF-1 on CB1 receptors in response to immobilization stress.

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