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Interaction of Tyr-W-MIF-1 and Tyr-K-MIF-1 with in PAG with CB1-receptors during cold stress

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During an acute stress response, physiological processes are important to redirect energy utilization among various organs and selectively inhibit or stimulate various organ systems, or their components to mobilize energy reserves and to be prepared for exposure to additional, unpredictable challenges. Under stress conditions the hypothalamic-pituitary-adrenal (HPA) axis is stimulated. Exitation of opioid receptors within the PAG activates descending opioid inhibitory pathways and suppresses nociception. Tyr-W-MIF-1 and Tyr-K-MIF-1 are neuropeptides, neuromodulators, which are able to inhibit the expression of some forms of stress.

The aim of our study was to investigate the effects of Tyr-W-MIF-1 and Tyr-K-MIF-1 on CB 1 expression in PAG after cold stres in rats.

Introduction

The midbrain periaqueductal gray (PAG) is the cell group surrounding the midbrain aqueduct. Recent evidence suggests that the important functions which are associated with the PAG autonomic regulation and analgesia are integrated by longitudinal arranged columns of neurons (1, 3). The Tyr-MIF-1 family of peptides includes Tyr-W-MIF-1 and Tyr-K-MIF-1 are neuropeptides. Endogenous opioid peptides are substances which are produced in the body and its take part in the various functions as hormones or neuromodulators. These peptides can modulate pain and stress by antiopiate and mu-specific processes (4). The endogenous opioid peptides Tyr-W-MIF-1 and Tyr-K-MIF-1 belong to the Tyr-MIF-1 family of peptides and have been shown to be involved in a wide spectrum of physiological processes, including the development of stress (4). Literature data showed that members of Tyr-MIF-1 family are particularly attractive candidates for opiate modulators. Unlike the most other putative opiate-modulating peptides, they bind to the opiate receptors and to their own non-opiate sites. It is known that Tyr-K-MIF-1 showed little activity on opiate binding, while Tyr-W-MIF-1 acted as a mixed μ2-opioid receptor agonist and μ1-opioid receptor antagonist (2, 4, 5, 6, 7).

Material and methods

Acute model of cold stress: The animals were placed in a refrigerating chamber at 4^0 C for 1 h. The control group was not submitted to 1 h cold stress procedure.

Drugs and treatment: Tyr-W-MIF-1 and Tyr-K-MIF-1 (both in dose 1 mg/kg) were obtained from Sigma. The neuropeptides were dissolved in sterile saline (0.9% NaCl) solution and were injected intraperitoneally (i.p). After completion of the cold stress model the animals were injected with Tyr-W-MIF-1 or Tyr-K-MIF-1. 24 h later the animals were anaesthetized with Thiopental (40 mg/kg, i.p.) and perfused through the heart with fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2).Brains were removed and sectioned by a freezing microtome. 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 antibody anti-CB1 antibody (Santa Cruz, USA) in dilution of 1:1000. Then the 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, Burlingame CA, USA; dilution 1:250) for 1 h. This step was followed by washing in PBS and then in 0.05M Tris-HCl buffer, pH 7.6, which preceded incubation of sections in a solution of 0.05% 3,30-diaminobenzidine (DAB, Sigma) containing 0.01% H_2O_2 for 10 min at room temperature for visualization. All procedures were approved by the Animal Care and Use Committee of the Medical University, Sofia.

Results and discussion

Our results showed that one-hour cold stress increases the expression of CB1-immunoreactive neurons in the periaqueductal grey compared with expression in intact animals. The results showed that after intraperitoneal treatment with Tyr-K-MIF-1 and Tyr-W-MIF-1 decreased the expression of CB1-immunoreactive neurons in the periaqueductal grey. In this investigation we have shown that changes in the expression of CB1 receptors are associated with cold stress. These findings correspond with several previous that the Tyr-W-MIF-1 and Tyr-K-MIF-1 exerted antiopioide effects under the different types of stresses (2, 5).

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