

IMMUNOHISTOCHEMICAL STUDY ON DISTRIBUTION OF CANNABINOID CB1 RECEPTORS IN THE RAT'S PREFRONTAL CORTEX AFTER COLD STRESS PROCEDURE

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ABSTRACT

The prefrontal cortex (PFC), which mediates the emotional coping response to different stressful paradigms, is composed of distinct parts depends on stimulus involved physical or psychological stress. It also plays a role in a number of neurological conditions. It's known that neuroendocrine control of homeostatic and reproductive functions including stress response and energy metabolism is fulfilled by important signaling molecules as endogenous cannabinoids. The aim of the present study was to examine the effects of cold stress on distribution of CB1-receptors in PFC of rats. Immunohistochemical procedure for CB1-receptors was performed in adult male Wistar rats. The data were entered in the computer program, recorded automatically, calculated and compared by Student's t-test. We found CB1-immunoreaction in axons and dendrites as well as in cell bodies where they presented as puncta on somata. The cell bodies were comprised of several distinct shapes: pyramidal, oval, fusiform and multipolar. Numerous fine-beaded fibers and puncta were seen on a handful of pyramidal large-sized neurons and many puncta were observed around the oval-shaped small- and medium-sized neurons. The PFC in cold stress rats demonstrated around 18% higher density of CB1-receptors compared with controls. In conclusion our results showed that cold stress exposure increased distribution of CB1-receptors in PFC of rats. These experimental data suggest that endocannabinoid system in this brain area may play an important role in the continuity of homeostasis in cold stress.

Key words: CB1-immunoreactive neurons, cold stress, prefrontal cortex, rats

INTRODUCTION

Stress can be described as an organism's response to any influence exerted by environmental or endogenous factors that disrupt homeostatic mechanisms within the organism (14). Stressors can be physical (cold or hot exposure), psychological (emotional) or mixed (immobilization) in nature, and have wide-ranging effects on neuroendocrine, autonomic, immune, and hormonal function (4,17). A sharp decrease in temperature using either cold water or freezer has been used frequently to induce acute stress (4).

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During the last decade, numerous investigations have evidenced that the endocannabinoid (eCB) system is able to crucially control stress coping. Its central component, the cannabinoid type 1 receptor (CB1 receptor), is located at the presynapse, where it is able to attenuate neurotransmitter release after its activation by postsynaptically produced and released eCBs. CB1 is the dominant receptor in the CNS. Immunohistochemical studies revealed that CB1 is present in many brain regions, including prefrontal cortex (6,9). The prefrontal cortex (PFC) is one of the brain parts that reacts with distinct emotional coping strategies to different types of stress. The acute stages of immobilization increase the density of CB1-immunopositive neurons in PFC (5,15) parallel with increasing on the density of nitric oxide immunoreactivity neurons (7). It is well established that PFC damage alters dramatically the capacity of higher mammals to cope emotionally with stressful situations (2,3,18). The PFC area also consists of several subregions containing diverse glutamatergic projection neurons and GABAergic interneurons. It is mainly divided into two regions, the orbital and medial PFC, which gives rise to two distinct networks. Both regions are connected with different brain structures, but also send projections toward each other (13,19). Of special interest regarding the contribution of the PFC to the stress response is the medial PFC, which represents its main output region. Glutamatergic neurons from the subregions are known to innervate other limbic regions as well as the hypothalamus and brainstem areas (1,16).

Taking into account all this background, the aim of the present study was to examine the effects of cold stress on distribution of CB1-receptors in prefrontal cortex of rats using immunohistochemistry.

MATERIAL AND METHODS

Twelve adult eight-week old male Wistar rats were divided in two groups – control and experimental (animals subjected to cold stress). All animals were carried for in compliance with the “Principles of Laboratory Animal Care” of the Medical University, Sofia.

Cold stress procedure

Animals were placed in a refrigerating chamber at 4°C for 2 hours.

Immunohistochemistry

After stress termination animals of each group were anaesthetized immediately 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 coronal sections were cut on a freezing microtome at 40 µm, and collected in Tris-HCl buffer 0.05M, pH 7.6. 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 hs at room temperature. We used a polyclonal rabbit anti-CB1 antibody (Santa Cruz, USA), in a dilution of 1:1000. Then sections were incubated with biotinylated anti-rabbit IgG (dilution, 1:500) for 2 hs 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 0.05 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.

Statistical Analysis

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

RESULTS AND DISCUSSION

Immediately following cold stress, rats from experimental and control groups were killed, the brains were excised for immunohistochemical procedure. In PFC we found CB1-immunoreaction in axons and dendrites as well as in cell bodies where they presented as puncta on somata. The cell bodies were comprised of several distinct shapes: pyramidal, oval, fusiform and multipolar. Numerous fine-beaded fibers and puncta were seen on a handful of pyramidal large-sized neurons and many puncta were observed around the oval-shaped small- and medium-sized neurons (Fig. 1A,B). The PFC in rats subjected to cold stress demonstrated more than 18% higher density of CB1-receptors compared with controls. These results confirm that temperature fluctuation induces stress and eCB system is involved.

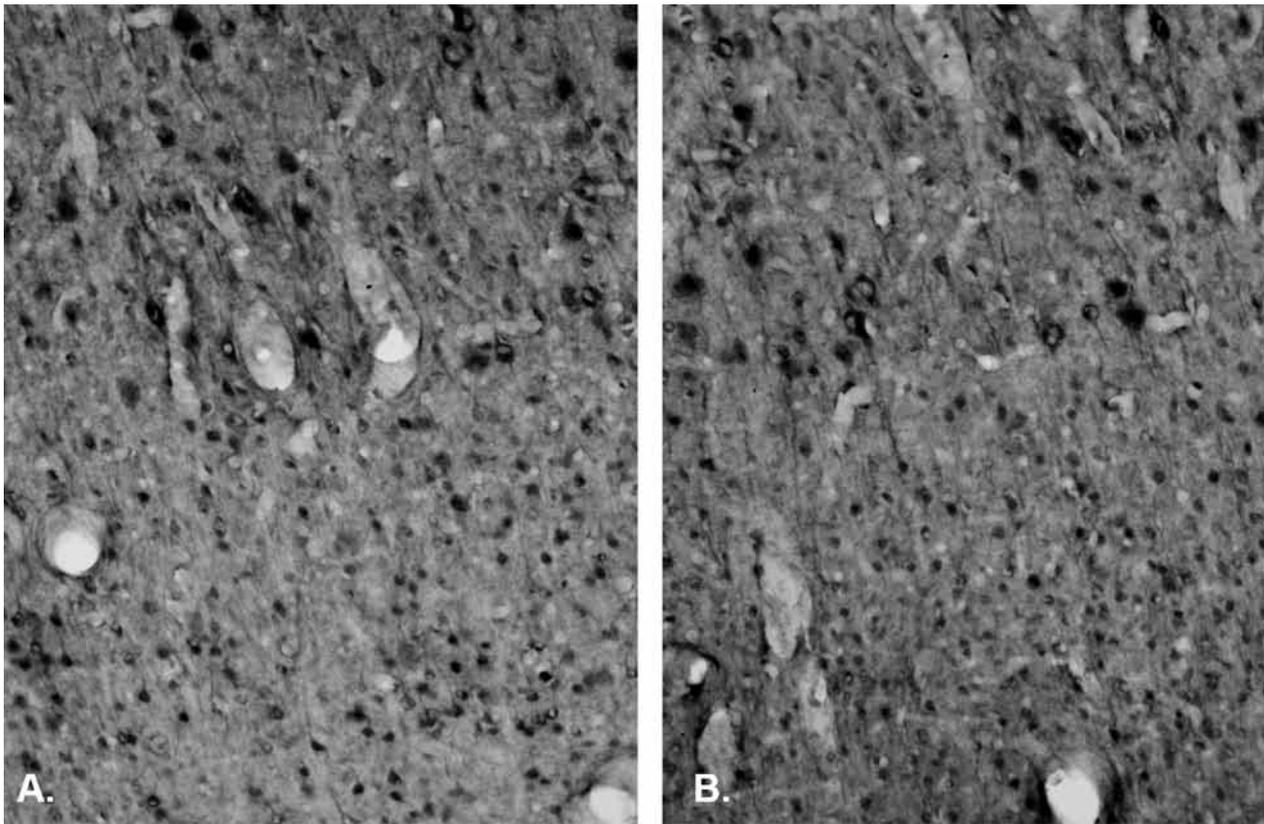


Fig. 1. A. Distribution of CB1-receptors is presented as puncta on somata. The cells bodies were comprised of several distinct shapes: pyramidal, oval, fusiform and multipolar (x200). B. Numerous fine-beaded fibers and puncta were seen on a handful of pyramidal large-sized neurons and many puncta were observed around the oval-shaped small- and medium-sized neurons (x 200)

One of the earliest steps in the stress response is the brain's perception that an event is threatening, which determines how an organism responds physiologically, emotionally, and behaviorally to the stressor. All animals, including humans, react with distinct emotional coping strategies to different types of stress. Active coping strategies (e.g. confrontation, fight, escape) are evoked if the stressor is controllable or escapable. Passive coping strategies (e.g. quiescence, immobility, decreased responsiveness to the environment) are usually elicited if the stressor is inescapable and help to facilitate recovery and healing. According to classification mention above, applied from us cold stress is physical, inescapable stressor with emotional component (11). Acute change in temperature leads to stressful conditions by activation of temperature regulatory centre in the hypothalamus and subsequently hypothalamic-pituitary-adrenal (HPA) axis. It leads to acute release

of adrenocortical hormones in the blood stream responsible for acute stress (4).

Recent evidence indicates that the eCB system may modulate HPA axis function both directly and more centrally, via regulation of limbic brain systems that control HPA-axis activity (9). Altered regulation of the hypothalamic-pituitary-adrenal axis is associated with stress-induced changes in cognitive, emotional, and physical health.

Because of its vast abundance of the CB1 receptor in the mammalian CNS, it is not surprising that the receptor is also found in the major brain structures involved in the stress response. The most prominent expression of the CB1 receptor can be seen in the hippocampal formation, in the amygdala, claustrum (8) and in the PFC (5,12,15). Medial PFC represents the main output region to the stress response. Glutamatergic neurons from the subregions are known to innervate other limbic

regions as well as the hypothalamus and brainstem areas (1,16).

Our data are in accordance with literature data that excitatory input from the FC inhibits HPA axis activity by activating inhibitory projections to PVN neurons (10).

CONCLUSION

In conclusion our results showed that cold stress exposure increased distribution of CB1- receptors in PFC of rats. Located at the presynapse, CB1 receptor, is able to attenuate neurotransmitter release after its activation by postsynaptically produced and released eCBs. To date, the eCB system has been found to control the neurotransmitter release from several neuron populations (e.g. GABA, glutamate, catecholamines and monoamines), suggesting a general mechanism for tuning neuronal activity, and thereby regulating emotion and stress responses. These experimental data suggest that eCB system in PFC may play an important role in the continuity of homeostasis in cold stress.

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