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Correlation between cold stress procedure and expression of CB1 receptors in the rat's basal nuclei. An immunohistochemical study

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The basal nuclei (BN) have been involved in stress response and nociceptive perception. They are complicated complex of internal anatomical and neurochemical organization that receive input from many brain areas includes the cortex and sends output to other components of the brain. They are designed to protect the individual through basic "drives" such as self preservation, bodily appetite and fear.

The endocannabinoid system is a recently identified neuromodulatory system involved in several physiological and pathopysiological processes. Endogenous cannabinoids are important signaling molecules in neuroendocrine control of homeostatic and reproductive functions including stress response and energy metabolism.

The aim of the present study was to examine the expression of endocannabinoid CB1 receptors after acute cold stress. We suggest that increased by cold stress CB1- immunoreactivity in rat's BN is possible protective role of CB1 receptors in stressful condition.

Key words: Basal nuclei, CB1 receptors, cold stress

When stress specifically activated one of these functions, the basal nuclei send a message to the limbic system – the part of the brain that initially processes emotions. Thus basal ganglia mediate the emotional coping of stress response to different stressful stimulus – physical or psychological. The cannabinoid CB1 receptor is the receptor that is expressed ubiquitously throughout most regions of the brain [5; 7; 9].

Literature data revealed that stress alters the levels of many biologically active substances including endocanabinoids. CB1 receptor is the major responsible for the behavioral effects of cannabinoids [3. 8]. Neuroanatomical studies have shown a very high density of cannabinoid CB1 receptors in neurons of the cerebellum, basal ganglia, limbic cortices.

Material and Methods

Ten adult eight-week old male Wistar rats were utilized for light microscopy. They were divided in two groups: control group – five animals were individually housed in an empty cage for three hours, cold stressed group – five animals were placed in a refrigerating chamber at 4°C for 1 h.

Immunohistochemistry: After stress termination three animals of each group were anaesthetized immediately 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 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.

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. All animals were caried for in compliance with the "Principles of Laboratory Animal Care" of the Medical University, Sofia.

Results and Discussion:

The BN are key region for the control of nociception containing higher levels of endocannabinoids in the brain [2].Our immunohistochemical study present CB1 immunoreactivity throughout the basal ganglia which was different in controls (Fig. 1, 3) and cold stressed rats (Fig. 2, 4). We found CB1-immunoreactivity (CB1-IR) in axons and dendrites as well as in cell bodies where they presented as puncta on somata. The cells bodies were comprised of several distinct shapes: pyramidal, oval, fusiform and multipolar. The CB1-like immunoreactive neurons were divided into three categories:

Fig. 1. CB1 immunoreactivity throughout the basal ganglia in controls (x100).

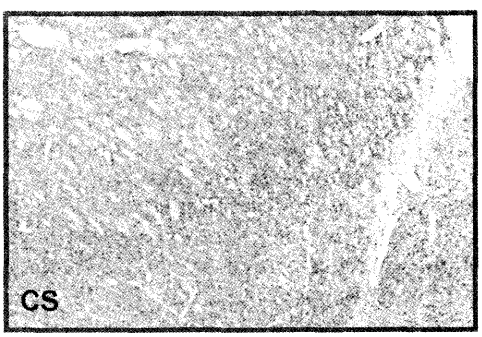
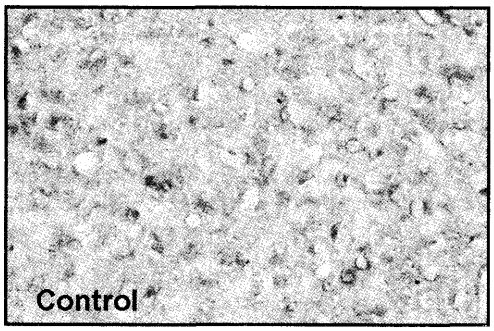
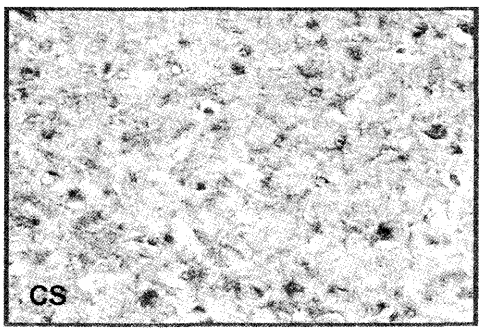


Fig. 2. CB1 immunoreactivity throughout the basal ganglia in cold stressed rats (x100).



Fug. 3. CB1 immunoreactivity throughout the basal ganglia in controls (x200).



Fug. 4. CB1 immunoreactivity throughout the basal ganglia in cold stressed rats (x200).

small-sized (10-15μm in diameter; 25%), medium-sized (16-20μm in diameter; 60%) and large-sized (21-30μm in diameter; 15%).

The BN contained higher levels of endocannabinoids, parallel with a high densitie of CB1 receptors [1, 4, 5, 6].The BN contained sparsely distributed, thin, varicose, labeled processes, as well as a diffuse immunoreactivity that was morphologically indistinct and contained a very dense meshwork of thin, smooth, CB1-IR processes that encircled large unstained fascicles, cell bodies, and wooly fibers.

Immunoreactivity was most often seen in medium-sized neurons, in the form of puncta. Numerous fine-beaded fibers and puncta were also seen on a handful of pyramidal large-sized neurons, while many puncta were observed around the oval-shaped small- and medium-sized neurons. As well, CB1-positive fusiform-shaped neurons were noted, evidenced by unstained nuclei and stained perikarya.

Conclusion:

CB1-IR in rat BN were increased by cold stress. We suggest possible protective role of CB1 receptors in stressful condition. Further experiments and specific markers are needed to understand the phenotype of the endocannabinoid producing neurons in the basal ganglia.

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