Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 19
Sofia • 2012

Immunocytochemical Study of CB1 Receptors in Rat's Dorsal Striatum after Immobilization Stress

A. Bozhilova-Pastirova[1], K. Grancharska[2], N.Pencheva[2], B. Landzhov[1], Lina Malinova[1] and W. Ovtscharoff[1]

[1] *Department of Anatomy and Histology, Medical University, Sofia 1407, Sofia, Bulgaria Prof. Anastasia Bozhilova-Pastirova*
[2] *Department of Kinesitherapy, South-West University, Blagoevgrad*

The aim of this study was to examine the immunocytochemistry of the cannabinoid receptors CB1 in neuronal elements in the rat dorsal striatum after acute immobilization stress. First, CB1 immunoreactivity appeared as puncta and was found in neuronal cell bodies, axons and dendrites. Second, the morphometric analysis revealed that the density of CB1 receptors in neurons of the dorsal striatum increases in the acute immobilized rats comparing with control rats.

Key words: acute immobilization stress, CB1 receptors, dorsal striatum, rat

Introduction

Stress is defined as a state of threaten to homeostasis, evoking adaptive responses of the organism that can be specific or nonspecific to the stressor. The adaptive response are in response to the activation of specific circuits and is genetically programmed and permanently modulated by environmental factors [1, 2]. The adaptive responses in due to an acute stressor include the physiological and behavioral processes that are essential to reestablish homeostatic balance.

The immobilization stress causes variable physiological, behavioral and endocrine responses by activating motor, autonomic, and hypothalamic-pituitary-adrenal systems. The descending loop of the immobilization evoked stress pathway arises from cortical, limbic, hypothalamic, and some mesolimbic efferents, which activate motor and autonomic output system [2]. The basal ganglia are an important subcortical center for the integration and control of cognitive, motor and limbic processes. The striatum is the largest nucleus of the basal ganglia and receives associative, motor and limbic projections in territories segregated throughout its associative, sensorimotor and limbic extension [3]. The striatum is involved in the control of many aspects of stress and can influence motor response to stress [4].

One of the mechanisms known to play a part in the response of an organism to stress is activation of the endocannabinoid system [5, 6]. The endocannabinoid system

is a signalling system, comprising of the endogenous cannabis-like ligands anandamide and 2-arachidonoylglycerol, which bind to a family of G-protein-coupled receptors, called CB1 and CB2 [7]. On the other hand, the presence of CB1 receptors in stress-responsive neural circuits suggests that it may play a crucial role in regulating behavioral responses to stress [8].

Futhermore, in the light of the above data, the purpose of the present study was to examine the density of CB1 – immunoreactive neurons in the dorsal striatum of control and acute immuobilized rats.

Materials and methods

Animals: The experiments were carried out on male (n = 6) Wistar rats (180-200 g) kept under normal conditions at ambient room temperature (22°C). Each group (control and experimental) included three rats.

Acute model of 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 1 h. There were holes for breathing.

Immunocitochemistry: After the completion of the stress model, 24 hs later they were anaesthetized with Thiopental (40 mg/kg, i.p.). Transcardial perfusion was done with 4% parafomaldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were removed from skulls and postfixed for 1 hour (h) in the same fixative at 4°C. Coronal sections were cut on a freezing microtom (Reichert-Jung) at 40 μm. 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 anti-CB1 antibody (, raised against the N-terminal, Santa Cruz, USA), in a dilution of 1:1000. Then sections were incubated with biotinylated anti-mouse IgG (dilution, 1:500) for 1 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 0.05 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% H_2O_2 for 10 min at room temperature for the visualization.

Morphometric analysis was performed by capturing images of) through a 40 objective using a microanalysis system Nikon photomicroscope ECLIPSE 80i (digital camera DXM 1200C and the measured area of 0.360185 mm^2). Data the entire drawings were entered in the computer program, recorded automatically, calculated and compared by Student's t-test. All values are presented as means ± standard error of the mean (S. E. M.).

Results and discussion

The staining patterns on coronal sections throughout the whole extension of dorsal striatum at levels of +2.2 to + 3.2 mm from bregma [9] were analyzed. The dorsal striatum in rodents (caudate – putamen) is not divided clearly into caudate and putamen and have a medial-lateral gradient of connectivity (ventromedial and dorsolateral), which is similar, but not identical to the connectivity in primates [10].

The principal findings were as follows. First, in the dorsal striatum there were numerous moderately stained CB1 immunoreactive neurons with punctated cytoplasm,

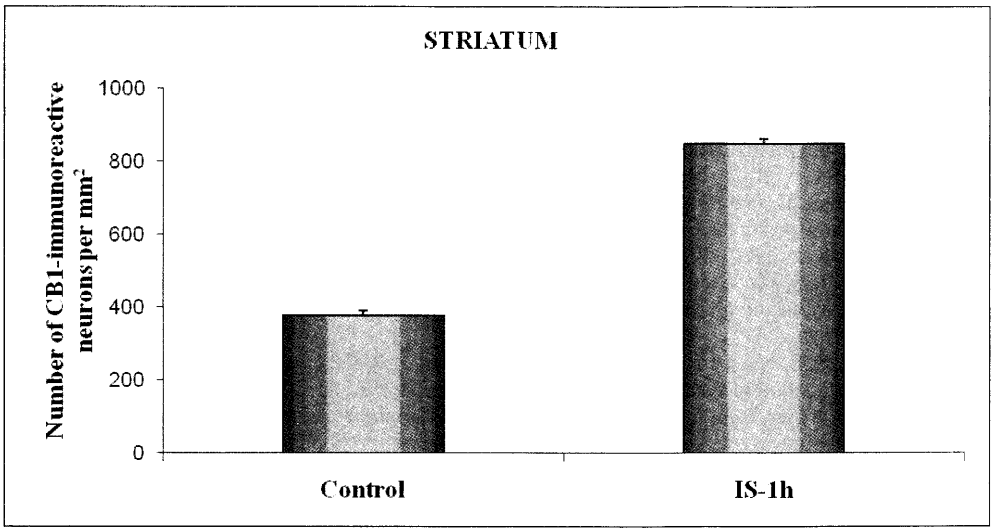

Fig. 1. The number of CB1-immunoreactive neurons in the dorsal striatum of control and acute immobilized rats (IS-1h), $P<0.01$. Values are presented as means ± S.E.M.

and unindented unstained nuclei. The distribution of the CB1-like immunoreactive neurons and neuronal elements generally coincided with that observed in previous studies that employed autoradiography. *in situ* hybridization and immunocytochemistry in CNS [11].

Second, in the dorsal striatum there was a lateral to medial density gradient and the lateral region being the more densely labeled. Third, our data provide the evidence about significant difference in the density of CB1 – immunoreactive neurons in the dorsal striatum between control and acute immobilized rats. Immubilized rats have greater density of CB1 – immunoreactive neurons than controls rats (Fig. 1). These results suggest that differences in the density of CB1-immunoreactive neurons in the immobilizes rat dorsal striatum can be related to the action of the stressers during the acute stages of immobilization [11].

Conclusion

In summary, our morphometric studies reveal differences in the number of CB1 – immunoreactive neurons in the rat dorsal striatum after acute immobilization. These new data may open the way to new studies in stress signalling in the dorsal striatum.

Acknowledgments: This work was supported by grant from Ministry of Education (*МОН НС"НИ" ДОО2-54/2008)*

Referenses

1. McEwen, B.S., L. Eiland, R G. Hunter, M.M. Miller. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. – Neuropharmacology, 62, 2012, 3-12.

2. Pacák, K., M. Palkovits. Endocr Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. – Rev., 22, 2001, 502-548.
3. McFarland, N.R., S. N Haber. Convergent inputs from thalamic motor nuclei and frontal cortical areas to the dorsal striatum in the primate. J. Neurosci. 20, 2000, 3798-3813.
4. Voorn, P., L. J. Vanderschuren, H. J. Groenewegen, T. W. Robbin, C. M. Pennartz. Putting a spin on the dorsal-ventral divide of the striatum. – Trends Neurosci., 27, 2004, 468-474.
5. McLaughlin, R. J., M. N. Hill, S S., Dang, S. R Wainwright, L. A. Galea, C. J. Hillard, B. B. Gorzalka. Upregulation of CB(1) receptor binding in the ventromedial prefrontal cortex promotes proactive stress-coping strategies following chronic stress exposure. -Behav Brain Res., 237C, 2012, 333-337.
6. Hill, M.N., B.S. McEwen. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. – Prog. Neuropsychopharmacol. Biol. Psychiatry, 34, 2010, 791-797.
7. Pazos, M.R., E. Núñez, S. Benito, R. M. Tolón, J. Romero. Functional neuroanatomy of the endocannabinoid system. – Pharmacol. Biochem. Behav., 81, 2005, 239-247.
8. Ganon-Elazar, E., I. Akirav. Cannabinoids prevent the development of behavioral and endocrine alterations in a rat model of intense stress. – Neuropsychopharmacology, 37, 2012,456-466.
9. Paxinos, G., C. Watson. – In: The Rat Brain in Stereotaxic Coordinates (II Ed.), San Diego CA, 1986.
10. Voorn, P., L. J.Vanderschuren, H. J. Groenewegen, T.W.Robbins, C. M. Pennartz. Putting a spin on the dorsal-ventral divide of the striatum. – Trends Neurosci. 27, 2004, 468-474.
11. Tsou, K., B.S. Rown, M.C. Sañudo-Peña, K. Mackie, J.M. Walker. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neurosci., 83, 1998, 393-411.