

CBI RECEPTORS IN THE THALAMIC RETICULAR NUCLEUS DURING ACUTE IMMOBILIZATION STRESS OF THE RAT: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Cannabinoids and opioids interact in a number of ways that could be therapeutically beneficial. The CB1 receptors are implicated with the endocannabinoid-mediated modulation of stress, pain, visceral sensation, synaptic plasticity in the thalamus via GABAergic signaling. Thalamic reticular nucleus (TRN) is a thin sheet of GABAergic neurons surrounding anterolateral surface of the thalamus. In our immunohistochemical study we demonstrated expression of CB1 immunoreactive neurons in a light microscope during a normal condition and after the acute stress in the rats. We found higher expression of CB 1 immunoreactivity in stressed animals compared with control group.

Opioids and cannabinoids have been shown to have analgesic properties and they are considered as drug targets for the treatment of numerous neurological disorders, pain and stress.

Key words: *Thalamic reticular nucleus, CB 1 receptors, immobilization stress, rat*

INTRODUCTION

The endocannabinoid system is composed of endogenous ligands, their enzymes for biosynthesis and degradation and cannabinoid receptors (13). The CB1 receptor is one of the most abundant G protein-coupled receptors expressed in the central nervous system (5). The regional distribution of CB1 receptors has been described in the rat brain (10, 15). During aging the number of CB1 receptors in the

rodent and human brains decreased (16). In many forebrain and midbrain structures CB1 receptors are expressed on GABAergic neurons, although there is functional evidence for CB1 receptor expression on some glutamatergic neurons in the forebrain (11). Thalamic reticular nucleus (TRN) is a slender sheet of neurons surrounding anterolateral surface of the thalamus. The main neuronal population in the TRN is the GABAergic cells (2,6). The nucleus is a key place for thalamocortical and corticothalamic fibers - between the external medullary lamina of the thalamus and the internal capsule (2,4,6). The CB1 receptors are involved with the endocannabinoid-mediated modulation of stress, pain, visceral sensation, synaptic plasticity in the thalamus via GABAergic signaling pathways (9). TRN neurons have been found to have receptors for opioid peptides, particularly type μ (1). This work additionally demonstrates that the response to

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μ -opioids is heterogeneous, and raises the question of the role of these different cell types (tonic and burst mode) in thalamocortical processing. CB 1 receptors are considered as drug targets for the treatment of numerous neurologic disorders, including convulsive epileptic seizures (17).

MATERIAL AND METHODS

We used twelve eleven-week male Wistar rats shared out into two groups: six animals were individually housed in an empty cage for one hour (control group), and six animals were exposed to one hour of immobilization. The animals were placed in a plastic tube with adjustable plaster tape on the outside so that the animals were unable to move. Stress group there were holes for breathing. All animals were cared for in compliance with the "Principles of Laboratory Animal Care". After stress procedure, 24 h latter the animals were anaesthetized with thiopental (40 mg/kg b.w.). Transcardial perfusion with 4% paraformaldehyde in 0,1 phosphate buffer, pH 7,2 was done. Brains were removed and coronal sections were cut on a freezing microtome (Reichert-Jung) at 40 μ m. 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-rabbit 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% H_2O_2 for 10 min at room temperature for the visualization and then were studied on the light microscope.

RESULTS AND DISCUSSION

We found CB1-like immunoreactivity in axons, cell bodies and dendrites, where it appeared as puncta in somata and branches in the all parts of the TRN (Fig. 1).

Our results demonstrated that CB 1 receptor-positive neurons were in all parts of the TRN.

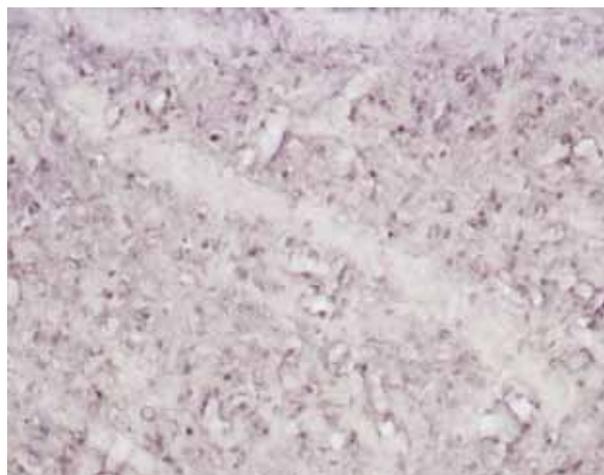


Fig. 1. Distribution of CB1 immunoreactive neurons in TRN, intact animals under the normal condition. The CB 1 receptors were expressed on the cell bodies as well as in cell membranes and neuronal branches. (x200)

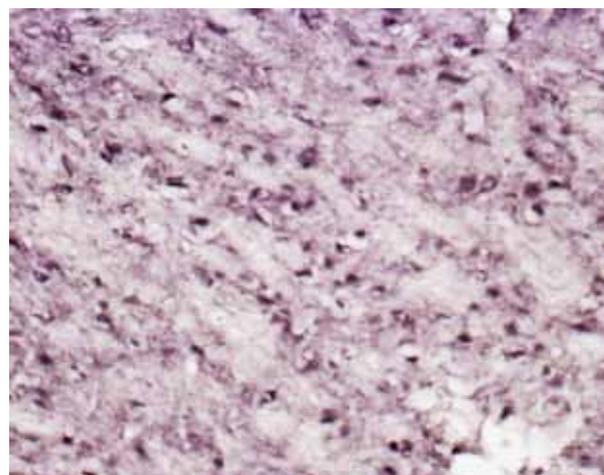


Fig. 2. Distribution of CB1 immunoreactive neurons in TRN after 1 h immobilization stress in the rostral part (x200)

In the rostral part of TRN the neurons were typically oval in form (Fig. 2). CB 1 receptors were visualized as distinct puncta on the cytoplasm and cell membrane. In the middle part we found CB 1 positive neurons typically arranged in clusters of three-to-five. Between these clusters there are a lot of thalamo-cortical and cortico-thalamic fibers. In the caudal part the CB 1 immunopositive neurons were fusiform in shape (Fig. 3). CB 1-immunostainig was associated with dense network of fibers in the middle and caudal parts.

During the normal condition the expression of the CB 1 receptors is relatively low (Fig. 1), while

undergoing the immobilization stress the animals showed more intensive immunostaining for CB 1 and more numerous immunopositive puncta in the all parts of the TRN compared with intact animals (Fig. 2, 3).

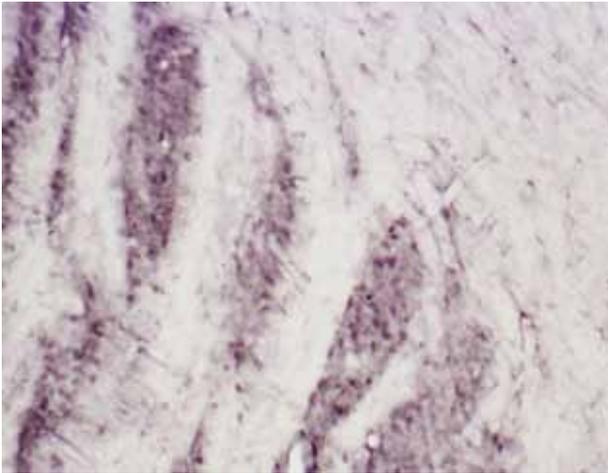


Fig. 3. Distributions of CB1 immunoreactive neurons in TRN after 1 h immobilization stress in the middle and caudal parts (x200)

TRN neurons are interrelated by chemical (7,17) and electrical (3,8) synapses. A number of studies have shown that intra-TRN inhibition mediated by chemical synapses is essential in keeping thalamic circuits from entering a hypersynchronous state (3).

Here, we have shown that changes in the expression of CB1 receptors are associated with immobilization stress. These new data support the view that the cannabinoids play an important role in different neuronal functions as neuromodulators or neurotransmitters. The previous study has shown that depolarization of TRN neurons led to prominent suppression of inhibitory synaptic strength, mediated by the calcium-dependent release by blocking from TRN neurons acting on presynaptic CB 1 receptors, while this suppression there was not at TRN synapses contacting thalamic relay neurons (14). These findings suggested that presynaptic specializations alone could explain target-dependent endocannabinoid signaling at TRN synapses. According to the morphology and location a lot of CB1-like immunoreactive neurons are GABAergic (11). Therefore, cannabinoids and cannabinoid receptors may play a role in modulating GABAergic

neurons. CB1 receptors are considered as drug targets for the treatment of numerous neurological disorders, including convulsive and non-convulsive epileptic seizures, stress and pain (12).

In conclusion our results demonstrated that during the normal condition expression of CB 1 immunoreactivity in the TRN was low. The rats undergoing acute immobilization stress showed higher CB 1 immunoreactive expression.

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