Quantitative Analysis of Brain Synaptophysin (p38) in Offspring of Male Rats with Chronic Morphine Intoxication

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Cerebral synapses in offspring of male rats treated with morphine for a month before mating were studied using synaptophysin (p38) as a synaptic marker. The content of p38 in the nucleus accumbens, hippocampus, and layers III and V of the somatosensory cortex was below the control, while no significant changes were found in the motor cortex, caudate nucleus, and ventrolateral thalamic nuclei.

Key Words: *brain; morphine; offspring; synaptophysin*

The health of offspring of opium addicts becomes an important problem. The offspring of male rats receiving morphine before coupling shows disturbances in exploratory activity, passive avoidance learning, and responses to opiates [6]. Physiological tests revealed impaired learning and concentration and poor memory in offspring of opium addicts [7,3]. However, the mechanisms of these disturbances remain unclear. We assume that they are associated with modulation of synapses in different cerebral structures.

Synapses are highly sensitive to different factors, in particular various drugs. Electron microscopy of the cerebral cortex after morphine administration showed the presence of damaged, activated, and newly formed synapses [1]. However, there are no data on the state of neuronal contacts in the offspring of opiate addicts.

This study was aimed at investigation of neuronal contacts in the offspring of males with chronic morphine intoxication.

Synaptophysin (p38), a synaptic vesicle membrane protein was used both as a synaptic marker [11] and as a participant of neurotransmitter release during synaptic transmission [8].

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MATERIALS AND METHODS

Male Wistar rats (200 g) received intraperitoneal morphine twice a day for one month: the dose increased from 10 to 60 mg/kg (by 10 mg/kg daily) for the first week and then remained at this level. These males were coupled with intact females to produce offspring which comprised an experimental group (n=4). The offspring of males receiving intraperitoneal saline for 30 days served as the control (n=4).

The animals under deep nembutal anesthesia (60 mg/kg, intraperitoneally) were transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, the brains were removed and fixed in the same solution for 2 weeks at 4°C. Frontal serial sections (100 μ) prepared with a vibratome were washed with 0.1 M phosphate buffer for 1 day and incubated with rabbit antisynaptophysin antibodies (1:2000) at 4°C for indirect immunofluorescence assay (the antibodies were kindly provided by P. Greengard). p38 immunofluorescence was measured in the somatosensory and motor cortex, hippocampus (CA1 area), nucleus accumbens, nucleus caudatus, and ventrolateral nuclei of the thalamus [13] in both cerebral halves using a BioRad MRC 600 CLSM confocal scanning microscope connected with a Nikon FXA fluorescent microscope with filters for FITC (488 DF10). Some sections were not incubated with primary antibodies to control the non-

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specific binding of secondary antibodies. They showed no fluorescence.

Slice preparation for immunofluorescence, scanning, and quantitative analysis of synaptophysin with Pixel Anatomy software were performed as described earlier [2].

The data were analyzed statistically with StatView software using nonparametric ANOVA and Fisher's tests ($p<0.05$).

RESULTS

In the experimental group, the content of p38 in the nucleus accumbens, hippocampus, and somatosensory cortex was significantly lower than in the control group (Table 1), while in the motor cortex, ventrolateral thalamic nuclei, and caudate nucleus it did not differ from the control (Table 1).

TABLE 1. The Content of p38 in Offspring of Morphinized Rats and Controls (% of immunopositive cells, $M\pm m$)

Brain Structure	Control	Experiment
Motor cortex		
Layer III	35.2±1.1	31.3±1.7
Layer V	32.5±2.9	30.8±2.2
Somatosensory cortex		
Layer III	46.3±0.8	34.1±0.9*
Layer V	39.3±1.5	3.2±1.7*
Nucleus accumbens	41.3±0.6	30.1±1.7*
Hippocampus	35.8±1.8	22.1±2.9*
Caudate nucleus	43.2±2.5	42.8±1.6
Ventrolateral nuclei of the thalamus	27.8±3.1	27.4±1.6

Note. *$p<0.05$ in comparison with the control.

The reduced content of p38 can be associated with reduced density of synapses and/or inhibition of synaptic transmission in these brain regions.

p38 is an integral protein of synaptic vesicle membranes [9], which is thought to participate in neurotransmitter release [8] and is used as a specific marker of synapses [14]. Quantitative changes in p38 correlate with the density of nerve terminals [11].

It is remarkable that changes in p38 content in experimental rats were revealed in the nucleus accumbens and the hippocampus. The nucleus accumbens is the projection area of mesencephalic ventral tegmentum [10]. The latter contains clusters of catecholaminergic neurons (group A10 [4]), which originate pathways of the mesolymbic and mesocortical dopaminergic systems projecting on the nucleus accumbens and frontal cortex [5]. This system plays an important role in forming opiate abuse and realization of opiate reward [12]. The experimental rats were not given morphine, but they were bred from males receiving morphine for 1 month and showed quantitative changes in p38 in structures related to the brain reward system. It can be assumed that the decreased content of p38 in the nucleus accumbens, which plays a leading role in the realization of acute and chronic effects of opiates, reflects modifications in its functional state. The offspring of males exposed to chronic morphinization exhibits disturbed habituation to novel environment [3]. This can be explained by modification of cortical and hippocampal neuronal contacts revealed in the present study.

Of particular interest is the decreased content of p38 in the somatosensory cortex. This cortical area integrates information from proprio- and exteroreceptors. The reduced synaptic density in this region can indicate alterations in the afferent synthesis of somatosensory information. The content of p38 was reduced in layers III and V, enriched with projection and associative afferent terminals. No significant quantitative changes in p38 were found in the motor cortex responsible the formation of the motor response. Hence, it can be assumed that behavioral disorders in the offspring of morphinized parents [7] are due to changes in afferent processes.

Thus, we first used p38 to study modifications of neuronal contacts in the offspring of males with chronic morphine intoxication. We suggest that the reduced content of p38 in the somatosensory cortex, hippocampus, and nucleus accumbens of the experimental offspring results from of morphine intoxication of parents. It indicates impaired in interactions between neurons, which, in turn, can represent a morphological substrate for functional changes in these structures and in the whole brain. The low content of p38 in the nucleus accumbens, the central structure of the brain reward system suggests that the offspring of opiate addicts is at high risk of opiate abuse.

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