DAMAGING EFFECT OF ACETALDEHYDE ON THE MEMBRANE OF STRIATAL SYNAPTOSOMES AS EVALUATED BY ATOMIC-FORCE MICROSCOPY

O. V. Chumakova[1], A. V. Liopo[1], O. Yu. Komkov[2], S. A. Chizhik[2]

[1] Institute of Biochemistry of National Academy of Science of Belarus, Grodno, Belarus.
[2] Metal-Polymer Research Institute of National Academy of Science of Belarus, 32A Kirov St., 246050 Gomel, Belarus.

Introduction

Acetaldehyde (AA) is metabolite of ethanol, may be responsible for some of the behavioral and neurotoxic effects of ethanol [1]. AA is formed in brain by catalase from ethanol [2, 3]. The results of investigations on the role of AA in the ethanol neurotoxicity are contradictory [4]. Any structural changes of neuronal membrane may play an important role in acetaldehyde- and ethanol-induced brain toxicity [2, 5] because they could affect the nerve conductance. It is possible that even low doses of AA may bring about the structural changes of neuronal membrane.

We used the technique of visualization of isolated nerves endings (synaptosomes) of rat striatum by means of AFM for investigation of the neuronal membrane surface under normal conditions and following effect of AA . The aim of our study was to reveal the neuronal membrane changes by AFM. To achieve this aim, we developed the method of native synaptosomal fixation and visualization.

Biological material. The fraction enriched with synaptosomes was isolated according to Weiler et al. (1981) from the striatum of male albino rats of the Wistar line as described previously [6]. The fraction of washed synaptosomal membranes was obtained by a modification of the method [7].

Control of obtained synaptosomal fraction was realized by electronic microscopy. The synaptosomal fraction was biochemically controlled by measuring of high-affinity choline uptake, the most reliable presynaptic cholinergic marker[8].

Incubation with acetaldehyde. The samples containing synaptosomal suspension were incubated for 20 min at 37°C with AA at concentrations of 2, 20, 50, 400, 2000, 4000 μM in 20 mM Na-phosphate buffer (pH 7.4) before applied on a coverslip and AFM-investigations.

AFM of synaptosomes and synaptic membranes. The synaptosomal suspension (4 μl) was applied on a coverslip. The samples were dried at a room temperature and under a hot air stream within different temperature and time ranges.

Results and discussion

In our study, AA at different concentrations (2, 20, 50, 400, 2000 and 4000 μM) brought about changes in the synaptosomal surface which was detected by means of the AFM technique. The AFM image was no differences as compared to the control sample(Fig. 1*a*) at 2 μM AA. The dose-dependent changes of the synaptosomal surface were observed in the presence of AA beginning with 50 μM. The irregularities on the synaptosomal surface were already detected at AA concentration of 20 μM, but they were marginal (Fig. 1*b*). The clear alterations were seen at 50 μM AA when enlarged separate synaptosomes stuck together (Fig. 1*c*). With the growth of AA concentration up to 400 μM, the damages were manifested by substantial degradation of synaptosomes (Fig. 1*d*). Further increasing of the AA concentration up to 2000 and 4000 μM led to further degradation of synaptosomal membranes.

The interpretation of the obtained phase image is a fairly difficult task . However, as it was experimentally established for the investigated samples, the dark areas at the phase images correspond to a 'soft' contact between the probe and the sample surface while the bright areas to a 'rigid' contact. Figure 2*b* shows the phase image of synaptosomes treated with 50 μM of AA. The appearance of a distinct soft region

(dark spiral band) was noted as compared to the control sample, which can be seen as a completely bright spheres (Fig. 2*a*). We also studied the effect of this concentration on the washed synaptosome membrane. The control fraction of washed neuronal membranes formed a microregions of a continual film due to the aggregation occurring in the preparation process. The height of the cross-sectional profile was approximately 2225 nm for a dried membrane film. In our experiments, 50 μM AA caused clear flattening of the membrane film up to 911 nm. We also observed a diminution of some film part and almost complete absence of a contrast which characterized membrane structure.

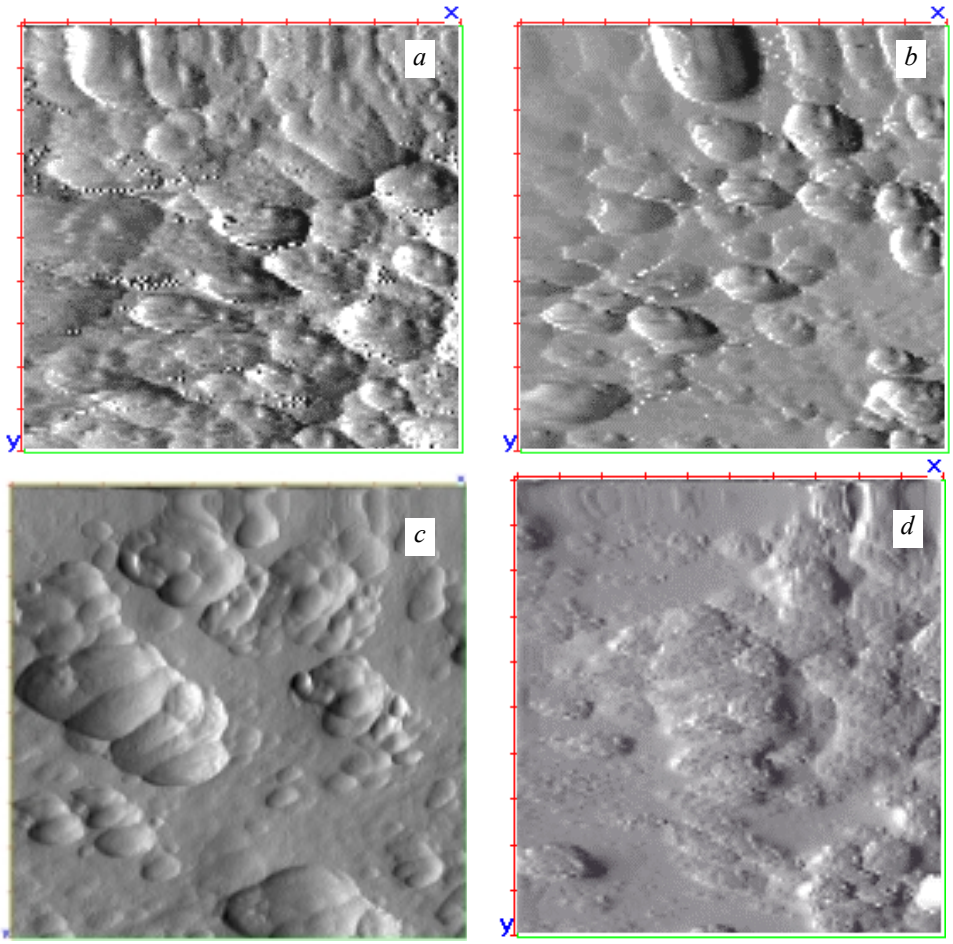

Fig. 1. AFM- imaging of striatal synaptosomes after 20 min incubation with acetaldehyde (AA) at concentrations: *a* – 2 μM (no differences as compared to the control samples); *b* – 20 μM; *c* – 50 μM; *d* – 400 μM. Scan size is 25×25 μm

Data available in literature suggested that clear response to AA application has been shown in different tissues at the AA concentrations of 5–10 μM and higher. In our study, the dose-dependent structural changes of the neuronal membrane surface were revealed in the presence of AA beginning with 50 μM. Brain tissue produces AA from ethanol, and the concentration of AA can rise to 50 μM during acute ethanol intoxication, which was established previously [3, 9]. The level of 50 μM AA in interstitial liquid is a threshold value because AA is detected in brain tissue starting from this level [10].

AA is a strong neurotoxic compound which is related to its capacity to covalently bind to various tissue proteins at relatively low concentrations. The structure and function of the membrane may thus be changed by AA. Such an effect of AA has been previously shown for the liver membrane proteins [11]. In our experiments at 50 μM of AA the nearly total absence of the contrast characterizing the membranous structure and flattening of the membrane film was observed as compared to control.

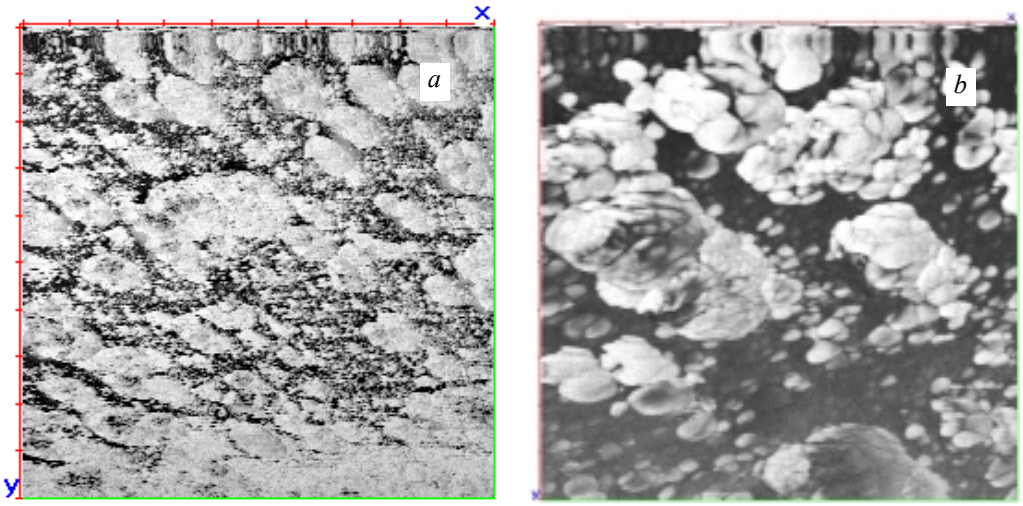

Fig. 2. The phase image of synaptosomes: *a* – native; *b* – after incubation with 50 μM of AA. Scan size 26.5 μm

These facts can be explained by the formation of a membrane film with a higher density resulting from the acetaldehyde action. A similar effect of forming a total junction for soft tissues due to surface forces is known as the effect of a precision contact [12, 13].

From our results we can conclude that the atomic-force microscopy technique allows to obtain the representations of both the native synaptosomes and washed synaptosomal membranes. The dose-dependent structural changes in the surface of both the native synaptosomes and washed synaptosomal membranes were observed in the presence of AA beginning with 50 μM.

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