Evaluation of Toll-like receptors expression in rat brain under alcoholization and ethanol withdrawal

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In experiments on rodents and in the study of postmortem brain samples of people suffering from alcoholism, it was shown that ethanol increases the expression of Toll-like receptors in the brain, which persists for a long time. Toll-like receptors are known to be also involved in the development of autoimmune reactions. In the present study the expression of Toll-like receptors in hippocampus, amygdala, and medial entorhinal cortex of rats during prolonged alcoholization and alcohol withdrawal was assessed. In the group of prolonged alcoholization with 20% ethanol for 1 month there was no change in Toll-like receptors mRNA levels in the studied rat brain structures, except for a slight decrease in Toll-like receptor 3 mRNA levels in the hippocampus of alcoholized rats and its slight increase in medial entorhinal cortex. However, Toll-like receptors gene expression undergoes changes in all rat brain structures studied during alcohol withdrawal.

Keywords: rat, brain, alcoholism, withdrawal syndrome, TLRs.

Introduction

The chronic alcoholism alters all organs and systems, imposing, probably, deepest impact on the nervous and immune system functions and neuroimmune interactions. Recently, more and more attention of researchers is attracted by changes in the mechanisms of neuroimmune brain signaling during prolonged alcoholism [1–2]. In experiments on rodents and in the study of postmortem brain samples of people suffering from alcoholism, it was shown that ethanol increases the expression of TLRs (Toll-like receptors) in the brain, which persists for a long time, however, the level of expression of Toll-like receptors has not been studied previously in various brain structures of rats in association with the development of different forms of psychoactive substance addiction, including alcoholism [3]. The study of neuroimmune signaling mechanisms activation by Toll-like receptors in different brain structures of rats in conditions of long-term alcoholism is relevant in the light of data on the impact of the Toll-like receptor-dependent processes on autoimmunity and the increased risk of several autoimmune diseases, for example — orchitis — in alcoholism [4]. It can open up new targets for drug exposure.
Materials and methods

In the experiment 42 male Wistar rats were used. To simulate long-term alcoholization, rats were subjected to semi-forced alcohol consumption of 20% ethanol solution for 1 month. The control group of rats received water. After a month, the rats were decapitated. The study included control group, alcoholization group (1 month), and alcohol withdrawal groups: on day 1, day 7 and day 14. Samples of brain structures studied [hippocampus, amygdala (AMG), and medial entorhinal cortex (mEC)] were extracted. Total RNA was isolated using trizol reagent (“Ambion”, USA). The cDNA synthesis was performed by reverse transcription method using m-MuLV reverse transcriptase (Promega, USA). PCR with real-time detection (“Mx3005P”, “Stratagene”, USA) was performed in a mixture containing SYBR Green (“Eurogen”, Russia), a mixture of specific forward and reverse primers (“Beagle”, Russia). The obtained data were normalized to the gene expression levels of GAPDH gene (encoding glyceraldehyde-3-phosphate-dehydrogenase) and calculated in relative units in relation to the value of the expression of the studied gene by 2-ΔΔCT method. The program Graph Pad Prizm V.6 was used for statistical processing of the obtained data.

Results

In the long-term alcoholization group, Toll-like receptor 3 mRNA levels decrease in the hippocampus, increase in mEC, and remain unchanged in amygdala compared to the control group. Ethanol withdrawal leads to increased levels of Toll-like receptor 3 mRNA in the hippocampus at all studied withdrawal periods. In medial entorhinal cortex the mRNA level was lowered by day 1, but on the 7th and 14th days it increases, exceeding the level of control on the 14th day. In amygdala mRNA level increases on the 1st day, but on day 7 it returns to the level of control, and on the 14th day gets down below the level of control values. The Toll-like receptor 4 mRNA level did not change significantly in any of the studied brain structures in the long-term alcoholization group. In the hippocampus, there was an increase in the level of Toll-like receptor 4 mRNA on days 7 and 14 of alcohol withdrawal. In mEC and amygdala mRNA level increased on day 1, then decreases both in amygdala and medial entorhinal cortex, reaching the level of control values on day 7. In amygdala, mRNA level decreases, acquiring a value below the controls on the 14th day. The level of Toll-like receptor 7 mRNA did not change significantly in any of the studied brain structures in conditions of prolonged alcoholization.

In the hippocampus, the level of Toll-like receptor 7 mRNA decreases on day 1 of alcohol withdrawal, then increases by days 7 and 14 of alcohol withdrawal. In medial entorhinal cortex the levels of Toll-like receptor 7 mRNA are unchanged on all withdrawal days. In amygdala the Toll-like receptor 7 mRNA level does not change on days 1 and 7, however, decreases on the 14th day.

Conclusion

In the group of prolonged alcoholization with 20% ethanol for 1 month there was no change in Toll-like receptors’ mRNA levels in the studied rat brain structures, except for a slight decrease in Toll-like receptor 3 mRNA levels in the hippocampus of alcohol-
ized rats and its slight increase in medial entorhinal cortex. However, Toll-like receptor gene expression undergoes changes in all rat brain structures studied during alcohol withdrawal. Toll-like receptors may be of crucial importance in control of interactions between immune system, pathogens and self organs, including brain, determining its outcome. Thus, their role in pathogenesis of multiple sclerosis has been recently appreciated — both in fighting infection and in activating autoimmunity [5]. The practical significance of the data presented in this article is related to clinical pathophysiology of various cerebral autoimmunopathies.

References

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