Effect of the Perinatal Alcohol Abuse on the Development of Neuronal Population in the Hippocampus

Milotová M., Riljak V., Langmeier M., Marešová D., Jandová K., Pokorný J., Trojan S.
Institute of Physiology of the First Faculty of Medicine, Charles University in Prague, Czech Republic

Received January 25, 2005, Accepted February 11, 2005

Abstract: The study deals with neurotoxic effects of alcohol on the CNS of laboratory rats in the prenatal period. The aim of the experiment is to analyse structure of the hippocampus after the prenatal exposure to alcohol and to identify the most vulnerable hippocampal regions. Pregnant Wistar rats of our own breed received alcohol (2g per100g of body i.p.) each day since the first to the last day of pregnancy. Since the birth till the age of 34 days offsprings were kept together with their mother and were not exposed to alcohol. At the age of 35 days animals were perfused under the deep thiopental anaesthesia with buffered solution of paraformaldehyde. In the CA1 area of the hippocampus groups of degenerating cells were observed. In the CA3 area degenerating cells were also found. Some cells with fine granulated karyons were identified, which were accompanied with high number of glial cells. Our results demonstrate the neurotoxic effects of alcohol and the high vulnerability of the developing CNS. Remarkable is the observation of the high number of dying cells 35 days after the last exposition to alcohol. It suggests a long-term process of neuronal circuit remodelling in the juvenile tissue, probably triggered by apoptosis. The identification of cells with fine granulated karyons indicates the role of apoptotic mechanism in the cell death.

Key words: Alcohol – Hippocampus – Rat – Degeneration of neuronal populations

This study was supported by grants: GAČR 305/03/H 148, GAČR 309/05/2015 and GAUK 45/2004.

Mailing address: Mgr. Martina Milotová, Institute of Physiology of the First Faculty of Medicine, Charles University, Albertov 5, 120 28 Prague 2, Czech Republic, Phone +420 224 968 429, e-mail: martina.milotova@lf1.cuni.cz

Introduction

It is known that alcohol is a neurotoxic and teratogenic substance, which can alter several molecular cell processes. The most vulnerabile CNS region are neocortex, cerebellum and hippocampus. Hippocampus lies deep in the temporal lobe of the brain and is involved in memory. Although the precise role of the hippocampus in specific aspects of memory is controversial, it probably plays a role in the consolidation of memory traces [1]. It is well known that the hippocampus is one of the target sites for neurotoxic effect of ethanol during brain development [2, 3, 4, 5, 6, 7, 8]. Hippocampal pyramidal neurons are generated during late gestation from the ventricular zone [9, 10, 11, 12] and after the exposure to ethanol during the early life they can remain vulnerable [13]. The long-lasting hippocampal postnatal development allows studying changes, which are caused by intervention in the development during prenatal life. In this study we focused for specific defects in the CA1, CA3 area of the hippocampus and in the dorsal and ventral blades of the dentate gyrus after the long-term prenatal exposure to ethanol.

Methods

Female Wistar rats of our own breed were used for the experiments. Two animal groups were used in the experiment:

- animals exposed to alcohol (experimental group)
- animals exposed to saline solution (control group)

24 animals was included in each group. Pregnant Wistar rats received 2 g per 100g of body weight of 20% alcohol i.p. each day since the first to the last day of the gravidity (21 days). Control group of pregnant Wistar rats was exposed to the same amount of normal saline solution. Since the birth (the day 1) till the age of 34 days the young animals were kept together with their mother and they were not exposed to alcohol. At the age of 35 days males of young animals were perfused under the deep thiopental anaesthesia with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were removed, postfixed for one hour in 4% buffered paraformaldehyde and then submerged for 1 hour into 20% sucrose for cryoprotection. Brains were sliced in the frontal plane into 40 μm thin sections with a cryostat and the free-floating sections were placed in 0.1 phosphate buffer. Tissue sections were mounted onto gelatinized slides and allowed to dry at room temperature. Slides were then placed in staining racks (one slide/slot for even staining) and immersed in 100% ethanol solution for 3 minutes, in 70% ethanol solution for 1 minute, in distilled water for 1 minute, in 0.01 % potassium permanganate ($KMnO_4$) for 15 minutes with gentle shaking. Slides were washed in distilled water three times. Staining proceeded in dim place by immersing slides into 0.001% Fluoro-Jade B solution for 30 minutes with occasional gentle shaking [14]. After that slides were rinsed in the distilled water three times for 1 minute.

Slides were then immersed in 0.01% Hoechst staining solution for 10 minutes and dehydrated (in alcohol series), cover-slipped using DPX. Neutral Mounting Medium and allowed to dry. Fluro-Jade B positive neurons were observed in four regions of the hippocampal formation: i) in CA1 area of the hippocampus, ii) in CA3 area of the hippocampus, iii) in the dorsal blade of the dentate gyrus, iv) in the ventral blade of the dentate gyrus. Material was examined and Fluoro-Jade B positive neurons quantified under the light microscope Olympus Provis AX-70 with epifluorescence.

Results

In the CA1 (Colour fig. 1, 2) area of the hippocampus DNA staining Hoechst and Fluoro-Jade B revealed positive groups of degenerating cells. In the CA3 (Colour fig. 3, 4) area degenerating cells were also found. Some cells with fine granulated nuclei were identified. These cells were accompanied with high number of glial cells. In both the dorsal (Colour fig. 5, 6) and ventral blade (Colour fig. 5) of the dentate gyrus many Fluoro-Jade B positive cells and neurons with fine granulated nuclei in the DNA staining were identified.

Discussion

Several experimental studies have shown that the hippocampus is one of regions, which are particularly vulnerable to the effects of ethanol exposure during the early life [2, 15].

Our results these statements confirm and demonstrate the neurotoxic effects of alcohol and the high vulnerability of the developing CNS. Remarkable is the observation of the high number of dying cells in the CA1-CA3 region of the hippocampus and in the ventral and dorsal blades of dentate gyrus 35 days after the last exposition to alcohol. It suggests a long-term process of neuronal circuit remodelling in the juvenile tissue, probably triggered by apoptosis. Apoptosis is described as an orchestrated collapse of a cell characterised by membrane leaking, cell shrinkage, condensation of chromatin, and fragmentation of DNA followed by rapid engulfment of the fragments by neighbouring cells [16].

Our identification of cells with fine granulated nucleus indicates the role of apoptotic mechanism in the cell death but further research is required to confirm or deny this hypothesis.

References

1. BERMAN F. R., HANNIGAN J. H.: Effect of prenatal alcohol exposure on the hippocampus: Spatial behaviour, electrophysiology, and neuroanatomy. *Hippocampus* 10: 94–110, 2000.
2. BARNES D. E., WALKER D. W.: Prenatal ethanol exposure permanently reduces the number of pyramidal neurons in rat hippocampus. *Brain Res.* 227: 333–340, 1981.
3. BOTHIUS D. J., WEST J. R.: Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. *Alcohol Clin. Exp. Res.* 14: 107–118, 1990.

4. MIKI T., HARRIS S. J., WILCE P., TAKEUCHI Y., BEDI K. S.: Neurons in the hilus region of the rat hippocampus are depleted in number by exposure to alcohol during early postnatal life. *Hippocampus* 10: 284–295, 2000.
5. MIKI T., HARRIS S. J., WILCE P., TAKEUCHI Y., BEDI K. S.: A stereological analysis of the effect of early postnatal ethanol exposure on neuronal numbers in rat dentate gyrus. *Image Anal. Stereol.* 19: 99–104, 2000.
6. MIKI T., HARRIS S. J., WILCE P., TAKEUCHI Y., BEDI K. S.: The effect of alcohol exposure during early life on neuron numbers in the rat hippocampus. I. Hilus neurons and granule cells. *Hippocampus* 13: 388–398, 2000.
7. MILLER M. V.: Generation of neurons in the rat dentate gyrus and hippocampus: effects of prenatal and postnatal treatment with ethanol. *Alcohol. Clin. Exp. Res.* 19: 1500–1509, 1995.
8. PIERCE D. R., GOODLETT C. R., WES J. R.: Diferential neuronal loss following early postnatal alcohol exposure. *Teratogy* 40: 113–126, 1989.
9. SCHLESSINGER A. R., COWAN W. M., GOTTLIEB D. I.: An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *J. Comp. Neurol.* 159: 149–175, 1975.
10. SCHLESSINGER A. R., COWAN W. M., SWANSON L. W.: The time of origin of neurons in Ammon's horn and the associated retrohippocampal fields. *Anat. Embryol.* 154: 153–173, 1978.
11. BYER S. A.: Development of the hippocampal region in the rat I.. Neurogenesis examined with H-thymidine autoradiography. *J. Comp. Neurol.* 190: 87–114, 1980.
12. BYER S. A.: Development of the hippocampal region in the rat II.. Morphogenesis during embryonic and early postnatal life. *J. Comp. Neurol.* 190: 115–134, 1980.
13. MIK T.,TAKEUCHI Y., HARRIS S. J., BEDI K. S., WILCE P. A.: Effect of age and alcohol exposure during early life on pyramidal cell numbers in the CA1-CA3 region of the rat hippocampus. *Hippocampus* 14: 124–134, 2004.
14. SCHMUED L. C., HOPKINS K. J.: Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Res.* 874: 123–130, 2000.
15. WEST J. R., HAMRE K. M., CASSEL M. D.: Effect of ethanol exposure during the third trimester equivalent on neuron number in rat hippocampus and dentate gyrus. *Alcohol. Clin. Exp. Res.* 10: 190–197, 1986.
16. KERR J. F., WYLLIE A. H., CURRIE A. R.: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer.* 26: 239–257, 1972.