

Influence of Maternal Alcoholism on the Brain Benzodiazepine Receptor Development in Human Embryo and Fetus during Ontogeny

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Abstract

Graphical Abstract: Influence of maternal alcoholism on the brain benzodiazepine receptor development in human's embryo and fetus showed decreases their affinity and increases density as compensatory adaptation of the fetal nervous system to the effects of alcohol.

Abstract: Signaling mechanisms that are required for proper neuronal development and how these processes are impaired by ethanol resulting in harmful consequences to brain development are very important for our understanding. The aim of the present work was to study the development of synaptic Benzodiazepine Receptors (BzDR) (functionally associated with the brain GABAergic system) in the brains of embryos and fetuses aged 8-15 weeks obtained from alcoholic female patients.

Material and method: Abortive material (samples of brain tissue) was obtained from 33 women with grade II alcoholism (ICD-10 F10.201 and F10.202) and 30 control women were studied. The properties of BzDR were studied by radioreceptor binding with the selective ligand [3H]-flunitrazepam using a crude embryo brain synaptosomal fraction.

Results: In contrast to controls, brain cells developing in conditions of prenatal alcoholization showed formation of synaptic benzodiazepine receptors and alterations in their properties: decreases in their affinity and increases in their density. A decrease in the ability of receptors to bind agonist ligands impairs the ligand: receptor protein ratio, leading to decreased binding of the major neurotransmitter GABA and impairment to synaptic transmission.

Conclusion: These are interpreted as compensatory reactions promoting adaptation of the fetal nervous system to the effects of alcohol and functional deficiency of the GABAergic system.

Keywords: Ethanol; Alcoholism; Prenatal pathology; Brain synapse; Benzodiazepine receptors

Abbreviations: FAS: Fetal Alcohol Syndrome; GABA: Gamma-Aminobutyric Acid; GABA_AR: Receptor for Gamma-Aminobutyric Acid Type A; Cl⁻: Chlorine ion; CNS: Central Nervous System; BzD: Benzodiazepine; BzDR: Benzodiazepine Receptor (binding site for benzodiazepine); RRA: Radio Receptor Assay; Kd: Dissociation Constant of the ligand-receptor complex; Bmax: Density of Binding Sites for Selective Ligand; DBI: peptide that inhibits binding of diazepam on their receptor (Diazepam Binding Inhibitor); mAb: monoclonal Antibody; GABA_A/BzD receptor complex: Gamma-Aminobutyric Acid Type A receptor, coupled with allosteric binding site for benzodiazepine

Introduction

Influence of maternal alcoholism on brain development in human embryos and fetuses, the possible mechanisms of its effect on the formation of neural tissue and the development of synaptic structures and receptor systems in the brain have received insufficient study [1,2]. It has been shown, that ethanol triggers apoptotic neurodegeneration [3] in the developing brain, when administered to infant rodents during the period of synaptogenesis, also known as the brain growth spurt period [4]. These induce lifelong neurobehavioral disturbances associated with the human Fetal Alcohol Syndrome (FAS) [5,6]. Chronic alcohol exposure inhibits neurogenesis [7-9] and dendritic growth of newborn neurons [6]. Ethanol's harmful effects include neuronal cell death, impaired differentiation, reduction of neuronal numbers, and weakening of neuronal plasticity. These factors regulate development and differentiation of neurons by acting through various receptors and their signaling pathways [10]. Signaling mechanisms that

are required for proper neuronal development and how these processes are impaired by ethanol resulting in harmful consequences to brain development are very important for our understanding [11].

Developing networks follow common rules to shift from silent cells to coactive networks that operate via thousands of synapses. Some of these closely related to the neurotransmitter Gamma-Aminobutyric Acid (GABA), which operates primarily via chloride-permeable GABA_A receptor channels. Neurons have a higher intracellular chloride concentration at an early stage leading to an efflux of chloride and excitatory actions of GABA in immature neurons. GABA signaling is also established before glutamatergic transmission, suggesting that GABA is the principal excitatory transmitter during early development and can modulate the cell cycle, cells formation [12,13] and its migration [14].

Many investigations suppose significant role of GABA and GABA-

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ergic neurotransmission in mechanisms of ethanol's action and including GABA as a candidate of growth factor in Central Nervous System (CNS) [15,16]. An important point in the functioning of the GABA receptor complex is that this oligomeric protein complex contains various allosteric binding sites modulating the activity of the receptor. These allosteric binding sites are the targets for a variety of agents, including benzodiazepines and ethanol. Benzodiazepines, binding with specific sites – Benzodiazepine Receptors (BzDR) on GABA receptors – alter its conformation and affinity [17-20]. Substances acting on GABA type A receptors (GABA_AR) ethanol, benzodiazepines, and barbiturates) are able to influence adversely on development of CNS. Benzodiazepine and alcohol, reinforcing activity of GABA_AR also increase teratogenic effects in animals and human beings and give rise to defects of formation of neuronal tube. The early ontogeny of the benzodiazepine receptor was also evaluated in fluorographs (³H]-flunitrazepam) and immunoblots using the alpha 1-subunit-specific monoclonal antibody (mAb) bd-24. Specific radiolabeled proteins with molecular weights of 53K and 59K were visible in cortical membranes from gestational week 8, the earliest time investigated [21].

Because processes of brain development have close association with functioning of GABA_A – BzDR system, it is important to investigate the influence of GABA-active substances including ethanol on receptor's formation in developing CNS. Our investigations continue study of influence of alcohol on the BzDRs and in particular receptors of developing human brain.

The aim of this investigation was to study the dynamics of formation and development of BzDRs of brain synapses of embryos and fetuses aged 8-15 weeks of development, obtained from healthy women and alcoholic female patients.

Materials and Methods

The brains of human embryos and fetuses at 8-15 weeks of development were studied, obtained in compliance with the requirements of the Ethics Committee and with patients' consent during pregnancy termination procedures for medical indications.

A total of 33 embryos and fetuses were obtained from female alcoholic patients and constituted the study group. Alcoholic patients were aged 26-39 years and the duration of illness was 3-13 years. In all cases, alcoholism was diagnosed (ICD-10 F10.201, F10.202). Diagnoses of alcoholism were established at the Addictive States Department, Mental Health Research Institute of Siberian Branch of Russian Academy of Medical Sciences.

The control group consisted of embryos and fetuses from healthy women with no history of neurological or mental illnesses. Women of the control group were of comparable age as alcoholic patients. Significant information was obtained by using embryonic material only from cases in which there were no harmful influences with additional effects on embryo brain development (radiation, chemical substances, certain medical drugs, and maternal diseases during pregnancy, i.e., influenza, rubella, toxoplasmosis etc.).

The properties of BzDR were studied by radioreceptor binding with the selective ligand [³H]-flunitrazepam (85 Ci/mol; "Amersham") using a crude embryo brain synaptosomal fraction at a final concentration of 0.2-10 nM in 0.25 ml samples of the incubation volume at 0°C for 60 min. The final membrane protein concentration was 0.3 mg/ml in incubation volume. Nonspecific binding was performed in the presence of non-radioactive flunitrazepam at a concentration of 10 nM.

Bound ligand was separated by vacuum filtration through GF/B-filters ("Whatman"); the filters were washed with 15 ml Tris-HCL (50 mM, pH 7.4, 0°C), the filters were placed in glass vials containing 10 ml of scintillator. Radioactive analysis of the amount of bound ligands was carried out in scintillation β-counter - "Rack-beta" (LKB). Nonspecific binding (<10 %) was similar in control and test samples.

Radioanalysis of the quantity of bound ligand was performed in a Rack-beta (LKB) scintillation β-counter. The dissociation constant of the receptor-ligand complex (Kd) and maximum number of specific binding sites (Bmax) were determined by analysis of saturation curves in Scatchard coordinates and expressed in 10 nM and in mol/mg protein respectively. Distributions of parameters did not deviate from the normal, so statistical analysis of the data was performed by parametric variational statistics (Student's test) on Statistica 8.0; differences were regarded as significant at p<0.05.).

Experimental work was carried out in the Department of Clinical Neuroimmunology and Neurobiology of Mental Health Research Institute, SB RAMSci (Tomsk) and in the Laboratory of Clinical Biochemistry of Mental Health Research Center RAMSci (Moscow). All the studies were approved by the Ethics Committee of the Mental Health Research Institute SB RAMSci.

Results

Study the kinetic characteristics of [³H]-flunitrazepam binding (Kd and Bmax) to synaptosomal membranes prepared from brain tissue of human embryos and fetuses showed that absolute value of these parameters was increased during ontogeny in control and basic groups. These data support the general pattern consisting of an increase in receptor number in human brain during ontogeny. BDR affinity (inverse of the receptor dissociation constant - 1/Kd) also changed, reflecting a tendency to decrease with increasing developmental period, which was reflected in increasing values of Kd (Figure 1).

Studies of the properties of human brain BDR at 8-9 weeks of development showed that specific [³H]-flunitrazepam binding site density (Bmax) was greater in the study group than the control group. At the same time a decrease in receptor affinity for the selective ligand [³H]-flunitrazepam, in the study group has been found, reflected in an increase in the absolute value of Kd (Table 1). The value characterizing receptor affinity was the inverse of the ligand-receptor complex dissociation constant - 1/Kd. This provides evidence for a reduction

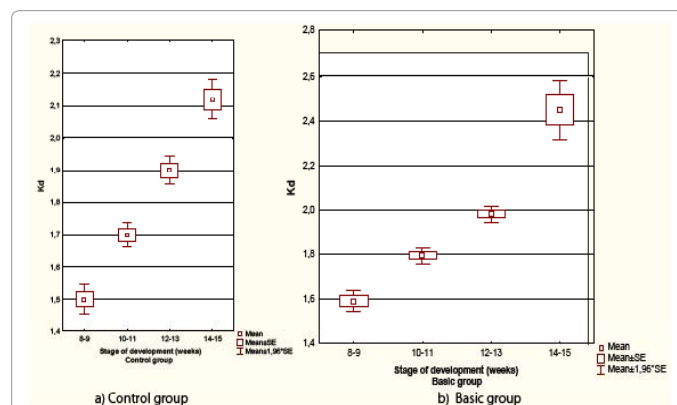
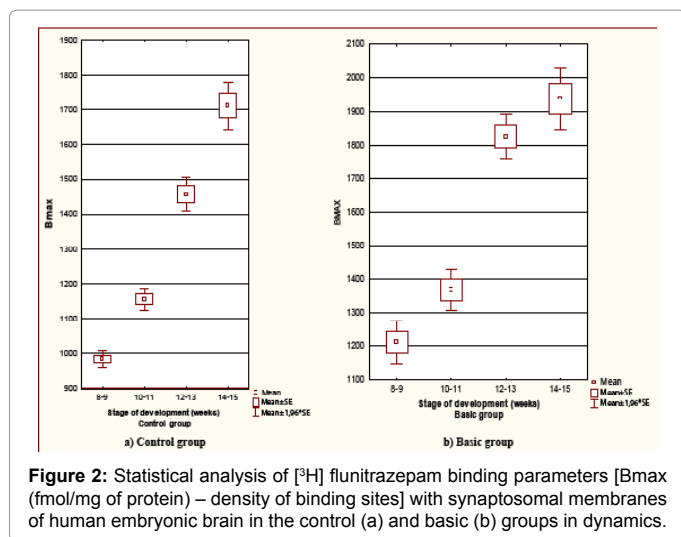


Figure 1: Statistical analysis of [³H] flunitrazepam binding parameters [Kd (nM) – constant of dissociation ligand-receptor complex] with synaptosomal membranes of human embryonic brain in the control (a) and basic groups (b) in dynamics.

Developmental period, weeks	Control group			Study group		
	B _{max} (fmol/mgprotein)	K _d (nM)	n	B _{max} (fmol/mgprotein)	K _d (nM)	n
8-9	984,22 ± 11,64	1,500 ± 0,024	9	1210,00 ± 32,79* p = 0,0001	1,591 ± 0,023* p = 0,014	9
10-11	1156,00 ± 15,22	1,700 ± 0,019	8	1367,40 ± 30,38* p = 0,0001	1,792 ± 0,019* p = 0,04	10
12-13	1456,29 ± 24,17	1,900 ± 0,023	7	1824,13 ± 33,51* p = 0,0001	1,982 ± 0,018* p = 0,014	8
14-15	1712,00 ± 35,24	2,120 ± 0,031	5	1938,17 ± 47,28* p = 0,005	2,450 ± 0,068* p = 0,0027	6

Note: B_{max} – [³H]-flunitrazepam binding density with synaptosomal BzDR; K_d – ligand-receptor complex dissociation constant ([³H]-flunitrazepam with synaptosomal BzDR); *statistically significant differences between study and control groups, p<0.01

Table1: [³H]-flunitrazepam binding properties with synaptosomal membranes from human embryo and fetus brains (8-15 weeks of development).



in receptor affinity, i.e., the affinity of BzDR, along with an increase in the number of BzDR in human embryo brains under the influence of maternal alcoholization.

At 10-11 weeks of development, benzodiazepine receptor density (Bmax) in the control group increased with the increase in embryo developmental period, while there was a reduction in the affinity of receptors for their ligand, apparent as an increase in the absolute value of Kd. These data provide evidence of changes in receptor affinity and density with growth and development of the nervous system in embryo brains (Figure 2).

The study group at this period showed the same tendency as in earlier periods of fetal development, which was apparent as an increase in receptor density and a decrease in receptor affinity as compared with controls. However, it should be noted that the dynamics of changes in receptor density was nonlinear. At 10 weeks of development, there were minor changes in [³H]-flunitrazepam binding characteristics in control and study groups. Receptor density increased slightly between the ninth and tenth weeks of fetal development; in the study group, where the developing brain was under the influence of alcohol, there were no significant differences in BzDR density in this period. There was some slowing in the increase in receptor density (Table 1), especially in the study group.

These results that indicate that changes in BzDR density between 8-9 weeks and 10-11 weeks were insignificant and it was only at 12-13 and 14-15 weeks of development that more marked growth in absolute values of Bmax was seen, supporting a significant increase in synaptic BzDR density in this period. The increase in receptor density in the control group from 8-9 weeks to 14-15 weeks of development reached almost 200%, with some delay at week 10.

Receptor density in the study group was greater than that in the control group in different development periods, the largest differences from controls being seen in later periods of development, i.e., 12-13 and 14-15 weeks. These data support the general pattern consisting of an increase in receptor number with increases in the developmental period of human embryos and fetuses during ontogeny.

BzDR affinity also changed, reflecting a tendency to decrease with increasing developmental period. Thus, the ligand affinity of receptors, which characterizes their sensitivity, was maximal in the earliest developmental periods, so that the early stages of human brain development are the most sensitive and vulnerable to the actions of alcohol.

The dynamics of changes in the affinity of BzDR for the selective ligand in the brains of human embryos and fetuses during ontogeny had developmental characteristics in the control group which were very close to the linear, apparent as decreases in receptor affinity with increases in embryonic and fetal developmental periods, approaching adult values. In the mature brain, the ligand affinity of receptors is somewhat lower than affinity at the earliest stages of development. In our study, receptor affinity in the brains of embryos and fetuses during development from 8-9 to 14-15 weeks decreased, i.e., Kd increased from 1.5 to 2.12 nM. In the study group, BzDR affinity in synaptosomal membranes isolated from the brains of human embryos and fetuses from alcoholic mothers was lower at all the developmental stages studied than in the control group, which was apparent as an increase in absolute Kd values from 8-9 to 14-15 weeks, i.e., from 1.59 to 2.45 nM. In addition, the dynamics of changes had a rather different nature, and Kd at the later period of development – 14-15 weeks – was greater than that in the control group: 2.12 and 2.45 nM, respectively. Fetal brain receptors in the study group in our experiments in developmental period 14-15 weeks had lower affinity than receptors in the normal mature human adult brain. A decrease in the ability of receptors to bind agonist ligands impairs the ligand: receptor protein ratio, leading to decreased binding of the major neurotransmitter GABA and impairment to synaptic transmission.

Discussion

Our investigations have shown that in contrast to controls, brain cells developing in conditions of prenatal alcoholization showed slowed formation of synaptic benzodiazepine receptors: decreases in their affinity and increases in their density. These are interpreted as compensatory reactions promoting adaptation of the fetal nervous system to the effects of alcohol and functional deficiency of the GABAergic system.

Fetal brain receptors in the study group in our experiments at developmental period 14-15 weeks had lower affinity than receptors in the normal mature human adult brain [22,23]. A decrease in the ability of receptors to bind agonist ligands impairs the ligand: receptor protein ratio, leading to decreased binding of the major neurotransmitter GABA and impairment to synaptic transmission. Simultaneous decrease in the affinity of synaptosomal BzDR, the tendency to an increase in

receptor density can be evaluated as a compensatory reaction directed at adapting the embryo and fetus nervous system to conditions of functional insufficiency of GABAergic neurotransmission.

The action of alcohol may lead to changes in BzDR conformation, with increases in affinity for the major agonist. The decrease in receptor affinity can be regarded as resulting from partial inhibition of BzDR due to formation of peptide DBI (diazepam-binding inhibitor) and its metabolites [20,24]. In addition, endogenous DBI peptide has been shown to have anxiogenic actions, i.e., to be an inverse agonist of BzDR [25]. Alcohol may stimulate the synthesis of an endogenous polypeptide interacting with BzDR and decreasing their binding affinity with the GABA agonist [³H]-flunitrazepam [25].

Against the background of the decrease the affinity of synaptosomal BzDR, the tendency to an increase in receptor density can be evaluated as a compensatory reaction directed at adapting the embryo and fetus nervous system to conditions of functional insufficiency of GABAergic neurotransmission [23,24].

Our results have shown that alcohol consumption by mothers influences on the properties of BzDRs linked with GABA_ARs in the developing brain of their offspring and may affect development of CNS of embryo and fetus through these receptors, that may explain reduction of efficacy of binding of BzD as a consequence of chronic alcohol consumption or as a possibility of alcohol dependence development. These results support the view that maternal alcohol consumption, which influences the processes of synaptosomal BzDR formation to modulate GABA receptor function, impairs the development of the embryo and fetus brain that can lead to various physical and mental disorders, including the development of FAS.

Prenatal exposure to alcohol on the developing brain could lead to disturbances in the trophic actions of GABA on some neurotransmitter systems in the embryonic brain and produce alterations in GABA_A-BzD receptor expression and function. These processes could lead to disturbances in postnatal functions of GABAergic system, possibly with behavioral consequences [1]. Because alcohol acts on GABA_A-BzD receptor complex as an agonist, one may suppose that alcohol consumption descendants of women abusing alcohol may be a compensatory mechanism under conditions of deficit of GABAergic function.

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