ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2008, 68: 484–493

Early postnatal ethanol exposure induces fluctuation in the expression of BDNF mRNA in the developing rat hippocampus

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Effects of early postnatal ethanol exposure on brain-derived neurotrophic factor (BDNF) mRNA expression in the rat hippocampus were investigated. Wistar rats were assigned to either ethanol treatment (ET), separation control (SC) or mother-reared control (MRC) groups. Ethanol exposure was achieved by a vapor inhalation method for 3 hours a day between postnatal days (PND) 10–15. On PND 16, 20, 30, and 60, the expression of BDNF mRNA in the hippocampus was determined using real-time RT-PCR analysis. There was a significant age-related increase in the BDNF mRNA expression between PND 30–60 in MRC animals. The BDNF mRNA expression in ET rats was increased at both PND 16 and 20 and thereafter decreased at PND 60 compared to SC animals. Such age-related fluctuation in the expression of BDNF mRNA differed from that of MRC animals. The exact functional implications, if any, of these ethanol-induced changes in BDNF mRNA expression remain unknown although it can be speculated that they may have an effect on the behaviors known to be influenced by the hippocampal formation.

Key words: BDNF, development, fetal alcohol syndrome, hippocampus, neurotoxicity, real-time RT-PCR

INTRODUCTION

It has been well established that maternal ingestion of alcoholic beverage during pregnancy can induce harmful effects on the developing fetus. These effects manifest themselves in a condition now known as fetal alcohol syndrome (FAS) (Clarren and Smith 1978). Three major signs are considered necessary for the diagnosis of FAS: (1) growth retardation, (2) specific facial features and (3) central nervous system (CNS) dysfunction (Streissguth and Martin 1983). The term fetal alcohol effect (FAE) is applied to alcohol-associated pathologies of somewhat lesser severity than FAS. Dysfunctions of CNS can occur in the absence of other gross morphological defects often associated with FAS (Streissguth et al. 1990). These children lack certain of the FAS morphological features (e.g. facial dysmorphia), but are nonetheless characterized by numerous cognitive and behavioral difficulties (Rosett 1980). Among FAS symptoms, CNS dysfunctions are perhaps the most serious consequences. A number of laboratory studies have shown that the hippocampus is one of the regions of the brain that appears to be particularly vulnerable to the effects of ethanol exposure during early life (Barnes and Walker 1981, West et al. 1986). The hippocampus is known to be involved in the control of several learning behaviors, including spatial learning. This has led to speculation that etha-

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Received 29 February 2008, accepted 02 September 2008

nol-induced hippocampal damage may be one of the causes of alterations in learning ability commonly seen in human FAS.

The timing of ethanol exposure is known as one of the important factors for human FAS (Miki et al. 2008) with many of the deleterious effects induced by exposure during the last trimester of the gestation period. Numerous published studies examining the effects of ethanol exposure during brain development have used rats or mice in a rodent model. Any animal model has to ensure that a similar stage of development is assessed as to that which exists in the developing human brain during the last trimester of gestation. Although the brain growth spurt, which is a period of rapid brain growth, occurs largely during the third trimester of human fetal development, it occurs between about 4 and 15 post-natal days in rats (Dobbing and Sands 1973, 1979, Bayer et al. 1991). It is known that the developing brain is particularly vulnerable to various extrinsic factors, including exposure to ethanol during the brain growth spurt period (Dobbing and Sands 1979, West et al. 1987).

We have previously shown that ethanol exposure of rat pups during PND 10–15 can lead to a deficit in the total number of pyramidal and hilar neuron in the hippocampus (Miki et al. 2000, 2003, 2004). We hypothesized that this loss of cells may lead to an accompanying change in the level of brain-derived neurotrophic factor (BDNF) as this is known to be closely involved in cell survival, differentiation, and neurite outgrowth in various brain regions, including the hippocampus (Maisonpierre et al. 1990, Das et al. 2001). BDNF is a member of a family of neurotrophic factors (NTFs) which includes nerve growth factor, neurotrophin-3, neurotrophin-4/5, neurotrophin-6 and neurotrophin-7 (Lewin and Barde 1996, Nilsson et al. 1998, Aid et al. 2007). BDNF acts mainly through its high-affinity tyrosine kinase receptor, TrkB (Squinto et al. 1991, Timmusk et al. 1993, Barbacid 1994, Lindvall et al. 1994).

We have now extended our previous studies in order to determine the effects of ethanol exposure of rats between PND 10–15 (i.e., in the middle of the postnatal brain growth spurt period) on the expression levels of BDNF mRNA using real time PCR techniques. We believe that this information may provide an important insight into the possible mechanisms involved that leads to a loss of certain hippocampal cells in rats exposed to ethanol.

METHODS

Animals

Pregnant Wistar rats were obtained from CLEA Japan (Tokyo, Japan) and housed in individual cages in a temperature controlled room maintained on a 12/12-h light/dark cycle. They were checked at 9:00 AM each day in order to determine whether they had given birth. The day of birth was designated as PND 0. On PND 2 all pups were removed from their mothers and placed together in a temporary holding cage. The pups from this pool were then randomly assigned back to the lactating mothers so that each mother received 12 pups. These litters were then randomly assigned to either ethanol treatment (ET), separation control (SC) or mother-reared control (MRC) groups. This study was carried out in compliance with the guidelines for experimental use and care of laboratory animals set forth by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Kagawa University Animal Ethics Committee.

Ethanol administration

The exposure to ethanol was achieved by a vapor inhalation method similar to that described by Karanian and coauthors (1986). Specifically, on each day during PND 10–15 all of the pups from a given litter assigned to the ET group were removed from their mother between the hours of 1:00 PM to 4:00 PM and placed in an enclosed saucer-shaped Perspex plastic chamber (600 mm in diameter and 300 mm in height). Ethanol vapor mixed with medical grade air was allowed to flow into this chamber at a rate of 6 l/min. After the three hour-treatment period each day the pups were weighed and returned to their mother. The pups within the SC litters were also removed from their mother for a period of three hours per day between PND 10–15. During this period of separation these pups were placed in under similar conditions as the ET group except that ethanol vapor was not added to the air. The pups within the MRC groups were allowed to remain with their mother for the duration of the experiment. Blood alcohol concentrations in some of the ET rats were measured immediately after the period of ethanol exposure on PND 10, 12, or 15. The enzymatic method described by

Lundquist (1959) was used for these determinations. The pups used for this purpose were not those used in the main part of the experiment.

Hippocampal slice preparation for BDNF mRNA extraction

At 16, 20, 30, and 60 days of age groups of ET, SC and MRC pups were weighed, deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused intracardially with a 70–300 ml of medical grade physiological saline (Otsuka Pharmaceutical Co., Ltd., Japan). Five male rats from 3 dams (1–2 male rats from each dam) were used in each of the 16-, 20-, and 30-day old ET group, and the other (i.e., SC and MRC) groups.

After perfusion the brains were removed from the skull and sectioned in the horizontal plane to yield 1 mm-thick slices using a Leica vibratome (VT 100S). The hippocampus region was identified in these slices, and carefully removed in chilled physiological saline with the aid of a dissection microscope (WILD, M650). Total RNA was extracted from these hippocampal slices by homogenizing the tissue in TRIzol reagent (Invitrogen, USA). During this procedure, RNase AWAY (Molecular BioProducts, USA) was used to remove RNase. The concentration and purity of the extracted RNA were evaluated by optical density measurements at 260 nm and 280 nm using a spectrophotometer (Hitachi, U-3300). These RNA samples were stored at −80°C until required.

Real-time RT-PCR procedure

TrueScript II reverse transcriptase (Sawady, Japan) was used to perform reverse transcription. For a 20 µl reaction mixture, the following reagents were used; 4 µl of 5× RT buffer, 2 µl of 10 mM dNTPs, 0.5 µl of RNase inhibitor (40 unit/µl), 1 µl oligo dT primer (10 µM), 10.5 µl of RNase-free water and 1 µl of sample RNA. Reverse transcription was carried out at 42°C for 60 min, followed by 99°C for 5 min and stored at 4°C.

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) analysis was performed as described previously (Kuma et al. 2004, Okamoto et al. 2006). Briefly, our pilot study revealed that GAPDH (Willoughby and Rosene 2001) was a suitable gene to use as an internal standard (housekeeping gene). We used the following forward (F) and reverse (R) BDNF primers for amplification of BDNF mRNA in the hippocampus (Timmusk et al. 1993, Aid et al. 2007). For BDNF (gene accession number; X67108), F: GAT GAG GAC CAG AAG GTT CG, R: GAT TGG GTA GTT CGG CAT TG, for GAPDH (gene accession number: AB017801), F: GTA TTG GGC GCC TGG TCA CC, R: CGC TCC TGG AAG ATG GTG ATG G.

Real-time PCR was performed using a LightCycler rapid thermal cycler system (Roche Diagnostics Ltd, Lewes, UK) according to the manufacturer's instructions. Reactions were performed in a 20 µl volume with 2 µl of the cDNA diluted 10 times, 0.5 µM primers and reagents included in the LightCycler-FastStart DNA Master SYBR Green I mix (Roche Diagnostics Ltd, Lewes, UK). The amplification protocol consisted of one cycle at 95°C for 10 min followed by 30 cycles at 95°C for 10 s, 65°C for 10 s, 72°C for 20 s, and 87°C for 2 s. Additionally, to assess an appropriate internal control, co-amplification of a GAPDH mRNA was performed in each sample. Detection of the fluorescent products was carried out at the end of the 87°C extension period.

To confirm amplification specificity, the PCR products from each primer pair were subjected to melting curve analysis and subsequent sequence analysis. To exclude DNA genomic contamination (Bustin 2002, Stankovic and Corfas 2003), electrophoresis of the PCR products amplified from cDNA of BDNF and GAPDH primers was carried out on 2% agarose gel (SeaKem GTG agarose, BMA, USA) and stained with ethidium bromide. A similar electrophoresis of the amplification product without reverse transcription (RT) was also performed for each sample as a negative control. The amounts of target gene BDNF mRNA were normalized against housekeeping gene GAPDH mRNA in the corresponding samples.

Statistical analysis

Group means and standard errors were calculated for the three groups of animals. Data on body weight and BDNF mRNA were analyzed by two-way analysis of variance (ANOVA) procedures. *Post-hoc* tests were carried out where appropriate using Tukey-Kramer's test (Sokal and Rohlf 1981). All statistical analyses were carried out using SigmaStat (Systat Software, version 3.1) statistical software.

RESULTS

Blood ethanol concentration

The mean blood alcohol concentration (BAC) of rats exposed to ethanol vapor during PND 10–15 was 336.51 ± 25.72 mg/dl (mean ± SEM; n=13).

Body weights

The mean ± SEM body weights of ET, SC, and MRC rats at PND 16, 20, 30, and 60 are shown in Table I. Two-way ANOVA revealed a significant main effect of group ($F_{2,48}$=9.170, P<0.01), age ($F_{3,48}$=1 029.532, P<0.01) and group × age interaction ($F_{6,48}$=5.662, P<0.01). *Post-hoc* analysis with Tukey-Kramer's test revealed that the body weights of ET and SC rats was significantly greater than those of MRC animals, with no significant difference between SC and ET rats at PND 60. However, there was no significant difference between ET rats and their age-matched controls at PND 16, 20, or 30.

Real-time RT-PCR

As we showed in our previous reports (Kuma et al. 2004, Okamoto et al. 2006), we performed the following series of experimental procedures. These profoundly verified that specific amplification had been accomplished in the present study. Melting curve analyses of the PCR products amplified from BDNF and GAPDH had a single and sharp transition. It was confirmed that a single PCR product was present, and primer-dimer formation was a rare occurrence within the number of cycles required for quantification. Agarose gel electrophoresis of these PCR products showed a single band. When omitting RT, no amplification products were observed (Fig. 1).

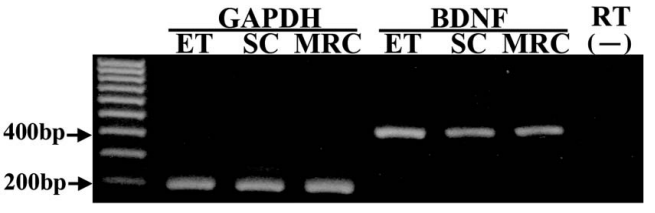

Fig. 1. A representative agarose gel electrophoresis of PCR products of ET, SC and MRC rats amplified from cDNA with GAPDH and BDNF primers showing a single band. An identical amplification procedure without RT resulted in no band [RT(−)]. A similar electrophoresis of the PCR products was carried out for each sample to exclude genomic contamination. Molecular weight markers are in the left lane.

Figure 2 shows the mean ± SEM ratio between the expressions of mRNA for BDNF and the housekeeping gene GAPDH in the different groups of rats at the various ages studied. This revealed significant main effects of group ($F_{2,48}$=17.330, P<0.01), age ($F_{3,48}$=19.199, P<0.01) as well as a significant group × age interaction ($F_{6,48}$=15.336, P<0.01). *Post-hoc* analysis with Tukey-Kramer's test was carried out to determine differences between ages within a given group as well as between groups within a given age.

There was a significant increase in the expression of BDNF mRNA between PND 30 and 60 in the MRC group but no other significant changes between any pair of ages (Fig. 2D). In SC animals, there was no significant change in the expression of BDNF mRNA between 16 and 20 days of age but thereafter the levels increased significantly at both 30 and 60 days of age (Fig. 2D). In ET animals there was a significant increase between 16 and 20 days of age but no further significant changes in the expression levels of BDNF

Table I

Mean ± SEM body weights (g) of ET, SC, and MRC rats at PND 16, 20, 30, and 60

Group	PND 16	PND 20	PND 30	PND 60
ET	31.35 ± 0.84 (5)	41.36 ± 0.51 (5)	85.14 ± 0.19 (5)	297.90 ± 9.42 (5)
SC	25.22 ± 0.90 (5)	34.49 ± 1.10 (5)	72.44 ± 4.42 (5)	279.34 ± 8.29 (5)
MRC	26.79 ± 1.76 (5)	37.34 ± 1.77 (5)	79.19 ± 5.79 (5)	238.36 ± 13.61 (5)

The number in parenthesis shows a number of animals examined. Abbreviations: (PND) postnatal day; (ET) ethanol treated; (SC) separation control; (MRC) mother-reared control.

mRNA (Fig. 2D). Comparisons between groups within an age group revealed that at 16 days of age BDNF mRNA expression of ET rats was significantly elevated compared to that of SC (Fig. 2A). At PND 20 the expression level in ET rats was significantly greater than both MRC and SC animals (Fig. 2A, B). At PND 30 the expression levels were greater in both ET and SC animals compared to MRC rats (Fig. 2B, C). At PND 60 the SC rats had a significantly greater expression level than both MRC and ET animals (Fig. 2A, C).

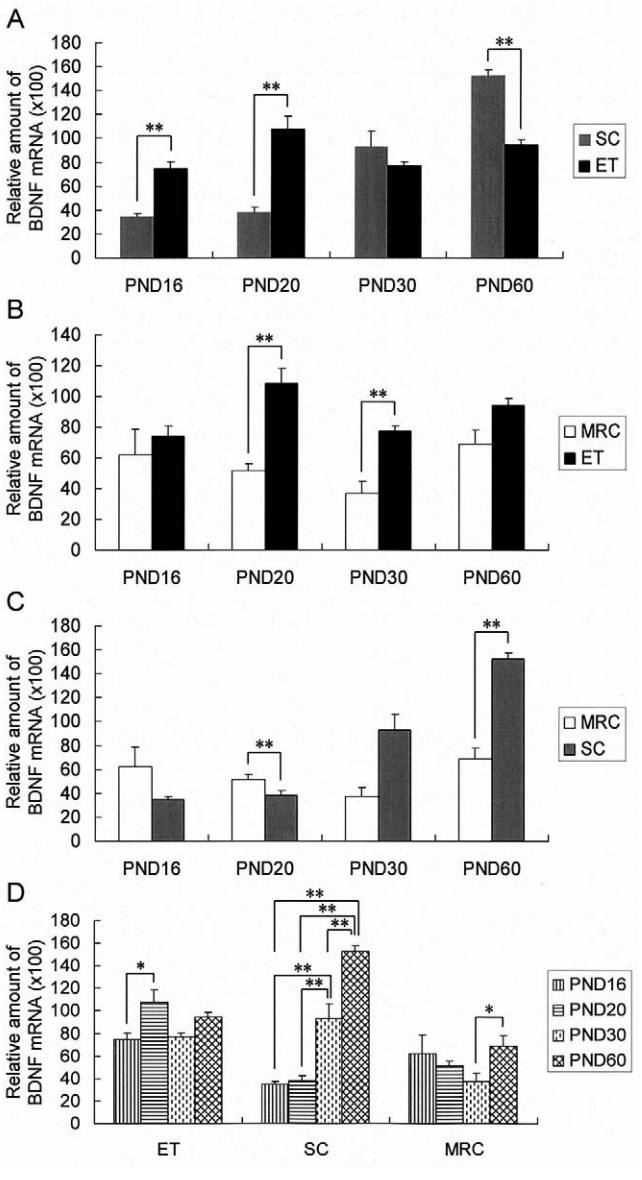

Fig. 2. The expressions of BDNF/GAPDH mRNA (mean ± SEM, n=5 each group) in the rat hippocampus across groups and ages. Comparisons of BDNF/GAPDH mRNA levels between various groups and ages, i.e., (A) SC and ET, (B) MRC and ET, (C) MRC and SC, and (D) age-dependent changes in ET, SC and MRC groups. Abbreviations: (PND) postnatal day; (ET) ethanol treated; (SC) separation control; (MRC) mother-reared control. *P<0.05, **P<0.01 (two-way ANOVA followed by Tukey-Kramer's *post-hoc* test).

DISCUSSION

We have shown that exposure to alcohol for a short period of time during early postnatal life can lead to change in the expression of BDNF mRNA in the rat hippocampus. The changes are complex, with an increase in the expression level of BDNF mRNA of ET rats that can last during PND 16–20 and PND 20–30 compared to SC and MRC animals, respectively. This increase in BDNF mRNA level is not permanent and in fact 60-day-old ET rats appear to have no such difference showing in turn a decrease in BDNF mRNA level compared to SC animals. In deed, the BDNF mRNA expression level in ET rats was increased at both PND 16 and 20 and thereafter decreased at PND 60 compared to SC animals. It appears that ethanol exposure reversed the decrease in BDNF mRNA levels on both PND 16 and PND 20 produced by maternal separation. Interestingly, a similar paradoxical phenomenon is reported by Bellinger and others (2006). Their group showed a decrease in NMDA receptor protein in the rat hippocampus resulting from periodic maternal deprivation. This effect is either prevented or reversed by treatment with ethanol exposure during separation. Further studies are needed to clarify these observations and investigate the mechanisms that may be involved in ethanol reversing some of the effects of maternal separation.

The interaction effect between ethanol exposure and maternal separation on BDNF expression is of some interest. The vapor chamber used to expose rat pups to ethanol also causes some level of separation of the pups from maternal care. This separation period is controlled for in the SC rats but not the MRC animals. It is therefore important to carry out comparisons between the ET and SC animals rather than the ET and MRC rats. Comparisons of all three data sets reveal the interaction between ethanol exposure and maternal separation. The maternal separation paradigm used in the present study was identical to that of our previous study reported by Kuma and coworkers (2004). Therefore, the data from the study aimed at determining the effect of maternal separation on hippocampal BDNF mRNA have been previously analyzed and discussed (Kuma et al. 2004).

As we have mentioned above, it is worthy to determine the effect of ethanol exposure by comparing the ET and SC groups. It is reported that early postnatal ethanol exposure (PND 4–10) can cause an elevation of BDNF protein level on PND 10, and then return to control level by PND 21 (Heaton et al. 2000). Despite the difference in experimental design between Heaton's and our study, it seemed to conclude that hippocampal BDNF is elevated earlier following early postnatal ethanol exposure. BDNF is known to play key roles in cellular survival, differentiation, plasticity and maintenance of function in the developing brain. Therefore, it is possible to speculate that the increased expression of BDNF mRNA as a result of ethanol exposure may be an attempt to protect the central nervous system (CNS) from the neurotoxic effects of the ethanol exposure (Beck et al. 1994, Cheng and Mattson 1994, Acheson et al. 1995). In contrast to the neuroprotective or neurorestorative effects of BDNF, it has also been paradoxically reported that elevated BDNF levels can harmfully influence synaptic plasticity by changing the dendritic structure of granule cell arbors (Tolwani et al. 2002). BDNF may have both beneficial and harmful effects on the developing brain depending on its level and spatio-temporal expression. In deed, Croll and colleagues. (1999) have reported that over-expression of BDNF in the brain can interfere with normal brain function by causing learning impairment and increased excitability.

The regulation and timing of neurite outgrowth processes in various brain regions are strictly controlled by several tissue-specific trophic growth factors and/or proteins (Hannigan et al. 1999). Inappropriate temporal and spatial expression of these important factors and/or proteins (including BDNF), during brain development can influence the modeling and remodeling of axonal and dendritic arborization. This in turn may lead to modifications of neural connectivity (Sapolsky et al. 1990, Lapchak and Hefti 1992).

In the present study we found that in MRC rats there was a significant age-dependent increase in BDNF mRNA expression between 30 and 60 day-old-rats but that there were no significant age-related differences among other ages of animals. Das and coauthors (2001) reported that BDNF mRNA expression increased in an age-dependent manner reaching a plateau at PND 14. The developmental fluctuation of BDNF mRNA expression in the hippocampus of MRC rats during PND 16–30, coincides with the findings by Das's group (2001). However, our findings showing a significant increase at PND 60, were different from results presented by Das and others (2001). This exact reason for this discrepancy are unknown at present, but may be partially due to the differences in animal strain used or to the methodology employed. In contrast to the MRC animals, the ET rats showed a different pattern of age-related fluctuations in the expression of BDNF mRNA. The BDNF mRNA expression in ET rats was increased firstly and thereafter decreased compared to SC animals. It can be speculated that such an abnormal alteration in the expression of BDNF mRNA in the hippocampus may lead to structural and functional abnormalities. These may in turn lead to learning disabilities or behavioral abnormalities similar to those seen in human FAS. However, the exact mechanisms involved in ethanol-induced changes to the expression of BDNF mRNA are currently unknown. Further studies are required to clarify these mechanisms.

We have previously reported that ethanol exposure during PND 10–15 at a BAC of about 430 mg/dl resulted in a significant deficit in body weight compared to age-matched MRC and SC animals at PND 30 but not PND 16 (Miki et al. 2004). In the present experiments, although a similar manipulation was used to expose neonatal rats to ethanol vapor, the BAC achieved was only about 340 mg/dl. This lower BAC level may explain our current finding, which is in conflict with our previous study (Miki et al. 2004), that such ethanol exposure did not result in any significant deficits in body weight at PND 16 and 30. It is, however, of interest to note that the ethanol exposure achieved in the present study resulted in a significant deficit in body weight compared to that of MRC rats, but not SC animals, although this did not manifest itself until the treated rats reached 60 days of age. Given that the BAC level is one of the key factors involved in the onset of FAS (Bonthius and West 1988, West et al. 1990), it is not to surprising that different levels of BAC could lead to some differences in the extent of the deficits observed in various studies. Ethanol exposure which results in a higher BAC level is likely to have a more severe effect. We believe that the discrepancy in the outcome on body weight observed in the present study compared to our previous experiments (Miki et al. 2000, 2003, 2004) may be explained by differing levels of BAC achieved in the two studies.

Data accumulated in recent years suggest that neuronal activity regulates the transcription of BDNF gene, the transport of BDNF mRNA and protein into dendrites, and the secretion of BDNF protein. The biological effects of BDNF are mediated through its binding to the high-affinity tyrosine kinase receptor TrkB. This receptor exists in two alternative spliced forms. One is a full-length tyrosine kinase-containing isoform (TrkB-fl) and the other is a truncated tyrosine-kinase lacking isoform (TrkB-T) (Middlemas et al. 1991). There is also evidence for activity-dependent regulation of the trafficking of TrkB, including its cell surface expression and ligand-induced endocytosis. Therefore, it seems appropriate to discuss the relationship between BDNF and TrkB. Many studies have examined the effects of ethanol exposure during the development of the hippocampus on BDNF and TrkB expression. Feng and colleagues (2005) reported a decreased BDNF expression in hippocampus with gestational ethanol exposure, but no change in the TrkB. Similarly, Moore and others (2004a, b) reported a decrease in hippocampal TrkB at PND1 with gestational exposure. In contrast, Heaton and coworkers (2000) found a transient increase in hippocampal BDNF at PND 10 with neonatal ethanol exposure. It may be that the discrepancy between these reports may be due to the timing of period of ethanol exposure and/or age of the animals examined. It has been suggested that changes in the levels of BDNF due to ethanol exposure may result in neuronal damage, impaired synaptogenesis and accerelated apoptosis (Davis 2008). Further experiments examining the effects on signaling through BDNF/TrkB system during and after ethanol exposure are required (Light et al. 2002, Ge et al. 2004).

Recent evidence indicates that BDNF and its receptor TrkB are also involved in food intake, body weight control and olfactory development (Deckner et al. 1993, Fritzsch et al. 1997, Zhang et al. 1997). The first report suggesting a role for BDNF in food intake regulation was made more than 15 years ago, when Lapchak and Hefti (1992) described that central administration of BDNF attenuated weight gain in rats. Another study showed that administration of BDNF to the lateral ventricle of rats induced severe, dose-dependent appetite suppression and weight loss (Pelleymounter et al. 1995). In addition, it has been reported that BDNF may also be involved in energy balance through melanocortin receptors (Xu et al. 2003). Disturbances in the levels of BDNF and/or its TrkB receptor may therefore contribute to abnormal feeding behavior in animals (Lebrun et al. 2006, Davis 2008). Although we have not examined the effects of ethanol on food intake/feeding behavior in the present study, the fact that BDNF expression levels are affected could help to explain the changes observed in body weights of the rats in our study.

The fluctuations in BDNF mRNA expression observed in our present study are similar to previous reports in the literature that have addressed the effects of ethanol exposure on neurotrophic factor (NTF) proteins in the developing brain. For instance, nerve growth factor protein was increased in the hippocampus of 15-day-old rats following ethanol exposure during development (Angelucci et al. 1997). The functional implications of such a fluctuation in the hippocampus are not fully understood at present. However, it is known that an increased level of expression of NTF may modulate the neurotoxic effects of ethanol and may, as a consequence, have long-term effects on the development of the hippocampus. As neurotrophins have a key role in brain development including its growth, differentiation, and plasticity, it is possible that even transitory changes in NTF could have serious potential effects on brain development, ultimately leading to functional and morphological abnormalities in the hippocampus.

Maternal separation during pre-weaning period is thought to markedly alter the stress status in young rats (Uno et al. 1989, Liu et al. 2000). In such circumstances normal development of rat neonates may be seriously compromised and make their development more vulnerable to minor changes in various other factors such as hormones, cytokines, and neurotrophins (Gould et al. 1991a, b, Hall et al. 1999). It is possible that this may be a factor in any experiments which involve short periods of separation of pups from maternal care (Cirulli et al. 1998, Roceri et al. 2002, Kuma et al. 2004). It is generally accepted that maternally separated animals showed changes in levels of various neurotrophins, including BDNF in the hippocampus, increased corticosterone levels and lower serum antioxidant potential. Moreover, theses animals exhibit depressive-like behavior during adulthood. It is suggested that maternal separation could cause down-regulation of neurotrophins in the hippocampus, possibly as an effect of high corticosterone levels. However, compensatory mechanisms to counteract against the

deleterious effects of these changes may also exist which may cause an elevation of neurotrophin levels in the hippocampus (Kuma et al. 2004, Marais et al. 2008). The balance between these factors at any given time could cause fluctuations in the levels of neurotrophins which could disrupt normal brain development.

In contrast to maternal separation, ethanol administration is considered as an exposure to extrinsic insult. Therefore, the extent of BDNF mRNA elevation should be greater in ethanol exposure than in maternal separation paradigm. Such difference appears to be reflected to inter-groups and time-dependent fluctuations of BDNF mRNA expression seen in the present study. Taken together, the changes in BDNF mRNA expression as a result of maternal separation and/or ethanol exposure may lead to the brain dysfunctions seen in animals exposed to ethanol during early life.

CONCLUSIONS

Our experiments show that exposing rat pups to ethanol during PND 10–15 can cause an abnormal developmental fluctuation in the expression of the hippocampal BDNF mRNA, first an increase and then a decrease. The long-term morphological and functional implications of the changes in the expression of BDNF mRNA for are uncertain at present. However, it is possible to speculate that the disruption in the normal pattern of expression level of BDNF mRNA in the hippocampus of animals exposed to ethanol during early postnatal life can ultimately lead to learning and/or behavioral abnormalities.

ACKNOWLEDGEMENT

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (C-18591220 and C-18591292).

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