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Alcohol induced depressive-like behavior is associated with a reduction in hippocampal BDNF☆

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ABSTRACT

Strong positive correlation between depression and alcoholism is evident in epidemiological reports. However, a causal relationship for this co-morbidity has not been established. We have observed that chronic daily exposure to a relatively high dose of alcohol can induce depressive-like behavior in rats and that pretreatment with nomifensine or imipramine can block the "depressogenic" effects of alcohol. Since brain derived neurotrophic factor (BDNF) is considered to play an important role in depressive-like behaviors and its elevation, particularly in the hippocampus, appears to be critical for the action of many antidepressants, we hypothesized that: 1. WKY rats, a putative animal model of depression, will show a lower hippocampal BDNF compared to their control Wistar rats, 2. Alcohol-induced depressive like behavior will be associated with a significant decrease in hippocampal BDNF and 3. Treatments with antidepressants will normalize hippocampal BDNF. These postulates were verified by measuring hippocampal BDNF in Wistar and WKY rats at baseline, following chronic (10 day) treatment with alcohol and combination of alcohol with nomifensine or imipramine. Alcohol was administered via inhalation chamber (3 h/day) such that a blood alcohol level of approximately 150 mg% was achieved. Nomifensine (10 mg/kg) or imipramine (10 mg/kg) was administered i.p. daily immediately after alcohol exposure. BDNF was measured by standard ELISA kit. The results support a role for central BDNF in depressogenic effects of alcohol and antidepressant effects of nomifensine and imipramine. Moreover, depression per se as manifested in WKY rats may be associated with a reduction in hippocampal BDNF.

1. Introduction

Epidemiological studies have consistently shown that alcoholism and depression commonly occur together (Regier et al., 1990; Grant and Harford, 1995; Sullivan et al., 2005). Depressed patients have higher rates of current and lifetime alcohol problems than the general population (Regier et al., 1990; Grant and Harford, 1995; Kessler et al., 1996; Sullivan et al., 2005) and similarly alcohol dependent individuals have a high prevalence for depression (Kessler et al., 1996).

Alcoholism and depression seem to share similar behavioral, neurochemical and pathophysiological changes. Prolonged exposure and withdrawal from alcohol has been shown to induce depression-like symptoms that disappear after period of abstinence (Davidson, 1995; Schuckit et al., 1997). A dysregulation in serotonergic system has been implicated in the development of depression (Hariri and Holmes, 2006; Davis, 2008) and subpopulations of alcoholic patients (Nevo and Hamon, 1995; Ressler and Nemeroff, 2000; Davis, 2008). Selective serotonin reuptake inhibitor (SSRI) treatments can help reduce depression and it has been suggested that understanding the modulation of the 5-HT system may lead to viable pharmacological therapies for alcoholism in a sub-set of patients (Nevo and Hamon, 1995; Johnson, 2004; Wrase et al., 2006; Davis, 2008). Disruptions in the hypothalamic–pituitary–adrenal (HPA) axis have also been implicated in both depression and alcoholism, dampening the ability to cope with stress in both populations (Nemeroff et al., 1984; Lovallo et al., 2000; O'Malley et al., 2002; Kiefer and Wiedemann, 2004; Adinoff et al., 2005; Nemeroff and Vale, 2005; Pariante and Lightman, 2008). Imaging studies reveal reductions in hippocampal volume and in the frontal lobes of both alcoholics and depressed patients (Coffey et al., 1993; Sullivan et al., 1995; Sheline et al., 1996; Agartz et al., 1999; Kril and Halliday, 1999; Bremner et al., 2000; Miguel-Hidalgo and Rajkowska, 2003; Gerritsen et al., 2011). However, the causal link between these two disorders is still unclear.

Brain derived neurotrophic factor (BDNF) is a molecule of interest thought to be involved in a number of psychiatric disorders such as depression, stress, anxiety, and drug addictions (Horger et al., 1999; Hall et al., 2003; Murakami et al., 2005; Pandey et al., 2006; Davis, 2008). BDNF, like most neurotrophins is responsible for neuronal survival, development and plasticity. It also acts as a modulator of some neurotransmitters and plays an important role in use-dependent plasticity such as long-term potentiation and learning and memory

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(Hyman et al., 1991; Thoenen, 1995; Li et al., 1998; Lyons et al., 1999; Hall et al., 2000; Huang and Reichardt, 2001; Guillin et al., 2001; Chao, 2003). BDNF supports survival of cholinergic (Alderson et al., 1990), nigral dopaminergic (Hyman et al., 1991) and serotonergic neurons (Altar, 1999; Madhav et al., 2001; Davis, 2008).

Human studies have shown significant reduction in peripheral levels of BDNF in several psychiatric disorders including major depression (Karege et al., 2002; Shimizu et al., 2003; Gonul et al., 2005; Karege et al., 2005; Aydemir et al., 2005, 2006; Cunha et al., 2006; Lee et al., 2007), suicidal depression (Kim et al., 2007) and alcohol dependent patients (Joe et al., 2007). Similar findings in central nervous system (CNS) of postmortem suicide patients have also been observed. Thus, significant decreases of BDNF protein (Dwivedi et al., 2003; Karege et al., 2005) and BDNF mRNA (Dwivedi et al., 2003) in the hippocampus and frontal cortex have been reported in this population. Finally, antidepressant treatments can increase peripheral and central BDNF levels (Chen et al., 2001; Shimizu et al., 2003; Aydemir et al., 2005; Gervasoni et al., 2005; Gonul et al., 2005; Bocchio-Chiavetto et al., 2006).

Experimental studies have shown that different stress paradigms (i.e. forced swim test, learned helplessness, restraint stress) that induce depressive-like symptoms in rodents can reduce BDNF protein and mRNA levels in the hippocampus and/or frontal cortex (Itoh et al., 2004; Russo-Neustadt et al., 2001; Murakami et al., 2005; Song et al., 2006; Takeda et al., 2006). In addition, hippocampal BDNF mRNA expression is suppressed after chronic ethanol (EtOH) exposure (MacLennan et al., 1995). In vitro, studies provide further evidence that chronic EtOH can reduce BDNF secretion, which suggests that BDNF might be linked to EtOH-induced cell damage (McGough et al., 2004; Sakai et al., 2005).

Wistar–Kyoto (WKY) rats are considered a putative animal model of depression. These animals exhibit a number of depressive-like symptoms such as psychomotor retardation, behavioral despair, abnormalities in monoamines and hyperactivity in the HPA axis reflected in high circulating corticosterone levels (Paré, 1992a,b; Paré and Redei, 1993a,b; Redei et al., 1994; Tejani-Butt et al., 1994; Paré and Kluczynski, 1997; De La Garza and Mahoney, 2004; Getachew et al., 2010). Moreover, WKY rats may also be considered a model for treatment resistant depression as they do not respond to selective serotonin reuptake inhibitors (SSRIs) (Tejani-Butt et al., 2003; Lopez-Rubalcava and Lucki, 2000; Griebel et al., 1999). However, these rats do respond to tricyclic antidepressants such as imipramine and nomifensine (Tejani-Butt et al., 2003; Paré et al., 2001, 1999; Getachew et al., 2008, 2010). Interestingly, similar to what is seen in human population (Kessler et al., 1993, 1996), higher prevalence of these behaviors is manifested in the female compared to male WKY rats (Paré and Redei, 1993a). However, the relationship between BDNF and the depressive-like characteristics observed in this animal model has not been explored.

Previous findings from our laboratory indicated that exposure to a relatively high ethanol level (150 mg%) via inhalation induced depressive like behavior in female Wistar rats and exacerbated that of WKY rats (Getachew et al., 2008; 2010). In addition, treatment with the clinically effective antidepressants nomifensine a NE/DA uptake inhibitor and imipramine, a NE/5HT uptake inhibitor, reduced EtOH-induced changes in both strains (Getachew et al., 2010). In the current study we sought to test the hypotheses that: 1) there are baseline differences in BDNF levels in discrete brain regions (hippocampus, and frontal cortex) between female WKY and WIS rats, 2) chronic EtOH will reduce BDNF and 3) treatments with nomifensine or imipramine will normalize the BDNF levels.

2. Materials and method

2.1. Animals and drugs

Age matched adult female WKY and Wistar rats (Harlan, Indianapolis, IN) were used throughout the study. The animals were housed four per cage and kept on a 12:12 hour reversed light/dark cycle (lights on at 7:00 A.M.) in a temperature-controlled room (24–26 °C). The animals had ad libitum access to food and water. USP 200 proof ethyl alcohol (VWR Scientific Products, USA) was diluted down (95% ethanol v/v) with distilled water to be used in the vapor inhalation chamber. Nomifensine and imipramine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and dissolved in saline and injected intraperitoneally (i.p.) (10 mg/kg).

2.2. Vapor EtOH exposure

2.2.1. Apparatus

Air-tight and dynamic EtOH inhalation chambers (La Jolla Alcohol Research Inc., La Jolla, CA) for rats (dimensions: 21.6 cm H × 26 cm W × 47 cm L) were utilized. Briefly, in this set up 95% EtOH is pumped, at regulated rate, from 5 gallon reservoir via a peristaltic pump to be delivered to 5000 ml Erlenmeyer vacuum flask that is kept on a warming tray (52 °C). EtOH is then volatilized and mixed with pressurized air. The flow of this mixture is controlled by a pressure gauge and delivered to the individual chambers. The variability in the EtOH concentration between similarly controlled chambers is minimal (Lee et al., 2000). EtOH vapor then leaves the chamber through an outlet flow tube connected to a vacuum. The control group received only air via exactly similar system. The advantages of this system over liquid diet consumption include: a) precise temporal control of duration and termination of exposure and b) achieving the targeted blood alcohol level (BAL) (Kliethermes et al., 2004).

2.3. Procedure

EtOH-naive adult (4 month old) female WKY and Wistar rats were randomly placed in either EtOH inhalation chambers (treatment group, 4–5/chamber, n = 10/strain) or air chambers (control, 4/chamber, n = 8/strain). EtOH vapor was administered for 3 h daily for 10 days. We used the same procedure as in previous study where the behavioral effects of alcohol as well as pretreatments with nomifensine and imipramine were evaluated (Getachew et al., 2008).

2.4. Blood alcohol determination apparatus and procedure

Two WKY and two Wistar rats were placed in the EtOH inhalation vapor chambers along with experimental animals for BAL determination. Blood was sampled by tail bleed technique every 3 days immediately after the end of daily EtOH exposure. Briefly, tail blood (0.5 ml) was collected in tubes coated with 0.2 M EDTA (Sigma-Aldrich CO., St. Louis, MO) and centrifuged for 5 min at 3200 rpm at 4 °C. The plasma was extracted and BALs were assayed by injecting 5 µl plasma into GM7 Micro-Stat Analyzer (Analox Instruments Ltd., Lunenburg, MA). For the antidepressant study, exactly the same EtOH exposure protocol as above was used, but the daily EtOH exposure was followed either with i.p. injection of nomifensine (10 mg/kg), imipramine (10 mg/kg) or saline (control).

2.5. Brain dissection and BDNF analysis

Animals were sacrificed by decapitation 18–20 h after the last injection. Brains were quickly removed, frozen on dry ice and stored at −80 °C. For sample collection, frozen brains were thawed on ice and frontal cortex and hippocampus (bilateral) were dissected alternating between strains and treatment groups as described previously (Tizabi et al., 1999, 2000; Getachew et al., 2010). The discrete brain regions were placed in 1.0 ml of ice cold lysis buffer (pH 8.0) containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% Igepal, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF),10 µg/ml aprotinin, 1 µg/ml leupeptin and 0.5 mM sodium vanadate. After adding buffer to the tissue, samples were homogenized and then centrifuged for 10 min at 10,000 rpm (4 °C). Promega BDNF Emax® ImmunoAssay

System (ELISA kit) was used to determine the levels of BDNF which were expressed per protein content of the areas analyzed. Protein analysis was performed by Peirce protein assay using BCA reagent.

2.6. Statistical analysis

All data were analyzed using two-way analysis of variance (ANOVA), followed by Student–Newman–Keuls post hoc test when significant main effects were indicated. All analyses were two-tailed and $P<0.05$ was considered significant.

3. Results

Fig. 1 depicts the basal BDNF level and the effect of chronic alcohol in the hippocampus of WKY and Wistar (WIS) rats. A two-way ANOVA showed significant main effects for strain where WKY rats had significantly lower baseline BDNF levels (approx 19%) in the hippocampus [$F(1, 28) = 12.65$, $p = 0.001$] compared to WIS rats. There was significant main effect of treatment where EtOH resulted in significant reduction in BDNF levels in both WKY (approx 12%) and WIS (approx 29%) rats [$F(1, 28) = 70.70$, $p = 0.001$]. The two-way ANOVA also revealed significant interaction between strain and treatment for basal hippocampus levels [$F(2, 28) = 25.34$, $p = 0.001$]. The WIS controls had the highest BDNF levels compared to all other groups [$p<0.05$]. There were no significant differences between WIS ALC and WKY ALC groups.

Fig. 2 depicts the basal BDNF level and the effect of chronic alcohol in the frontal cortex of WKY and Wistar rats. There were no significant differences in basal BDNF levels between the WKY or WIS rats [$F(1, 28) = 0.026$, $p = 0.86$]. EtOH did not affect the FCX BDNF levels in either strain [$F(1, 28) = 1.96$, $p = 0.17$].

Fig. 3 depicts the hippocampal BDNF level after chronic alcohol and following treatment with nomifensine and imipramine in WKY and Wistar rats. A two-way ANOVA showed significant main effects for both strain [$F(1, 30) = 38.70$, $p = 0.001$] and treatment [$F(2, 30) = 92.51$, $p = 0.001$]. There was also significant interaction between strain and treatment [$F(2, 30) = 14.66$, $p = 0.001$]. Both nomifensine and imipramine resulted in significant increases in hippocampus of WKY (Nom approx 12%, Imip approx 22%) and WIS (Nom approx 25%, Imip approx 50%) rats compared to ethanol only [$p = 0.001$]. WIS rats had the highest increase in BDNF levels after antidepressant treatment compared to WKY rats [$p<0.05$]. In addition, imipramine increased BDNF levels more than nomifensine in hippocampus [$p<0.05$].

Fig. 4 depicts the frontal cortex BDNF level after chronic alcohol and following treatment with nomifensine and imipramine in WKY and Wistar rats. A two-way ANOVA showed significant main effects for both strain [$F(1, 30) = 27.44$, $p = 0.001$] and treatment [$F(2, 30) = 100.29$, $p = 0.001$]. There was also significant interaction between strain and treatment [$F(2, 30) = 6.62$, $p = 0.004$]. Similar to the hippocampus, both nomifensine and imipramine resulted in significant increases in frontal cortex of WKY (Nom approx 72%, Imip approx 88%) and WIS (Nom approx 100%, Imip approx 135%) rats compared to ethanol only [$p = 0.001$]. The WIS rats had the highest increases of BDNF levels after antidepressant treatment compared to WKY rats [$p<0.05$]. Similar to hippocampus, imipramine increased BDNF levels more than nomifensine in frontal cortex [$p<0.05$].

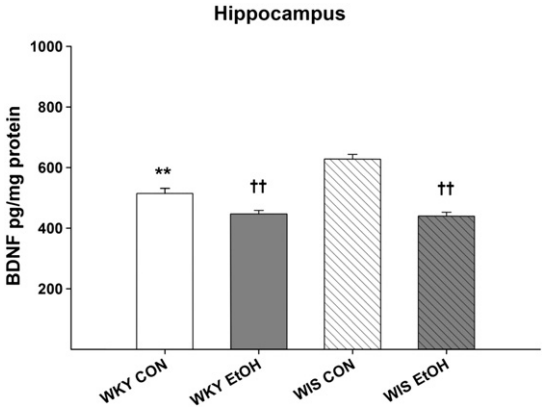

Fig. 1. Effects of 10 daily EtOH vapor exposures on BDNF levels in the hippocampus of WKY and WIS rats. Values are mean BDNF pg/mg protein ± SEM. $^{**}P<0.001$ compared to WIS control, $^{††}P<0.001$ compared to respective control. n = 8/group.

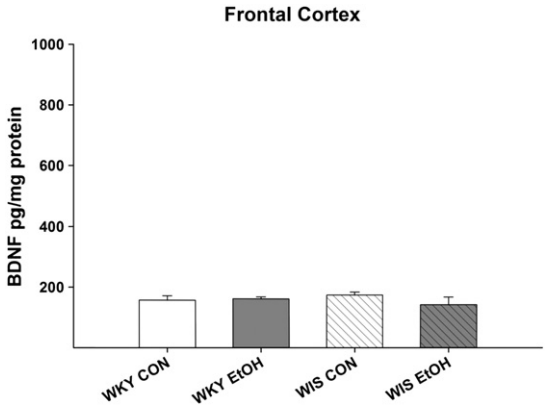

Fig. 2. Effects of 10 daily EtOH vapor exposures on BDNF levels in the frontal cortex of WKY and WIS rats. Values are mean BDNF pg/mg protein ± SEM. n = 8/group.

4. Discussion

Understanding the neural mechanisms that may be involved in mediating the co-morbidity of depression and alcoholism is a crucial step in developing novel pharmacological treatments for this condition. Previously we had observed that chronic alcohol exposure via inhalation chambers induced depressive-like behavior in Wistar rats and exacerbated the existing depressive characteristic in WKY rats (Getachew et al., 2008, 2010). In addition, treatments with nomifensine and imipramine ameliorated alcohol-induced effects (Getachew et al., 2010). The findings from the current study indicate that the hippocampal BDNF levels were significantly lower in WKY compared to Wistar rats and that alcohol exposure reduced hippocampal BDNF levels in both strains. Moreover, treatments with either nomifensine or imipramine reversed alcohol effects on BDNF levels, similar to the reversal of alcohol's behavioral effects. Thus, the results suggest

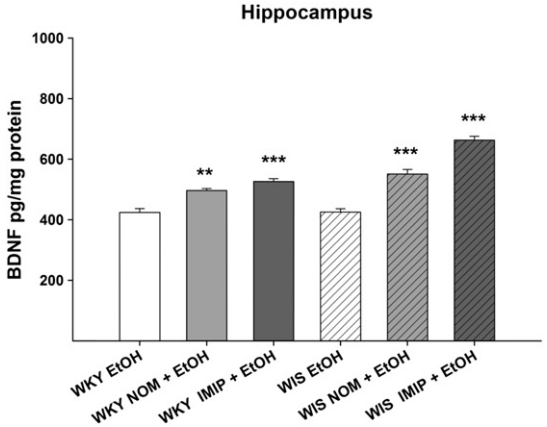

Fig. 3. Effects of 10 daily EtOH vapor exposures and followed by treatments with nomifensine (10 mg/kg/day) and imipramine (10 mg/kg/day) on BDNF levels in the hippocampus of WKY and WIS rats. Values are mean BDNF pg/mg protein ± SEM. $^{**}P<0.01$, $^{***}P<0.001$ compared to respective ethanol group. n = 8/group.

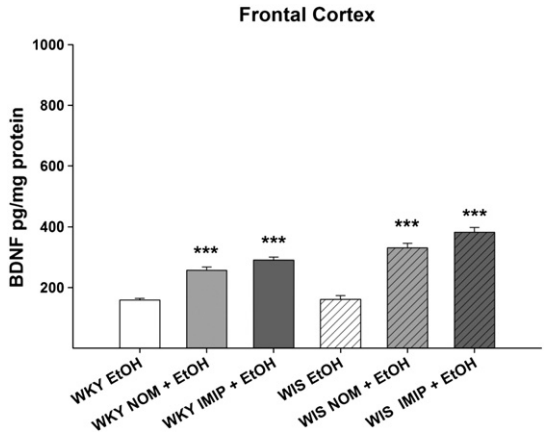

Fig. 4. Effects of 10 daily EtOH vapor exposures and followed by treatments with nomifensine (10 mg/kg/day) and imipramine (10 mg/kg/day) on BDNF levels in the frontal cortex of WKY and WIS rats. Values are mean BDNF pg/mg protein ± SEM. ***$P<0.001$ compared to respective ethanol group. n = 8/group.

a role for hippocampal BDNF in depressogenic effects of alcohol and the effects of antidepressants.

Other studies have also provided evidence that alcohol exposure can reduce BDNF expression in the hippocampus (Caldwell et al., 2008; Tapia-Arancibia et al., 2001; MacLennan et al., 1995). In addition, Caldwell et al. (2008) have reported that adult mice that were prenatally exposed to alcohol showed increased learned helplessness and immobility in the FST. They also demonstrated that alcohol-induced depressive-like behavior was associated with decreased total BDNF mRNA and BDNF transcripts in both hippocampal as well as in the frontal cortex of these mice (Caldwell et al., 2008). In our study, however, we did not observe any effect of alcohol on cortical BDNF. This might be due to species differences between the studies as well as the specific paradigms used (pre vs. postnatal). Nonetheless, both studies support a role for central BDNF in depressogenic effects of alcohol.

Our findings of increased central BDNF following treatments with nomifensine and imipramine are compatible with previous reports showing increased central BDNF following treatment with various antidepressants (Chen et al., 2001; Shimizu et al., 2003; Aydemir et al., 2005; Gervasoni et al., 2005; Gonul et al., 2005; Bocchio-Chiavetto et al., 2006). Hence, variations in BDNF may be an important mechanism associated with depression and possibly other adverse effects of alcoholism. Therefore, manipulation of this molecule may offer novel interventions in alcoholism–depression co-incidence. Since antidepressants are capable of reversing both the neurochemical and the behavioral effects of alcohol, the current findings provide further support for the suggestion that pretreatment with antidepressants could be a suitable intervention even prior to detoxification (Getachew et al., 2008, 2010). Interestingly, it has been reported that imipramine can reduce alcohol intake in animals (Katkov et al., 1994) as well as in depressed alcoholics where an improvement in mood is also obtained (McGrath et al., 1996; Nunes et al., 1993).

The hippocampus is one of the few areas of the CNS that undergoes neurogenesis which is postulated as a requirement for the effectiveness of antidepressants (Santarelli et al., 2003; Schmidt and Duman, 2007). BDNF and other neurotrophins appear to be crucial for neurogenesis in the hippocampus and other neuronal plasticity systems (D'Sa and Duman, 2002; Schmidt and Duman, 2007). Imaging studies have shown that the hippocampus volume is reduced in depressed patients and that antidepressant treatment results in volume recovery (Sheline et al., 2003; Czeh and Lucassen, 2007; Sheline, 2011). Interestingly, the WKY rats also have reduced hippocampal volume compared to their control the WIS rats (Tizabi et al., 2010). A recent study in adolescent male WKY rats has also reported a decrease in dentate gyrus BDNF compared to WIS rats (Malkesman et al., 2009). It is important to note, however, that a reduction in central BDNF may not necessarily be a requisite for manifestation of depression although the therapeutic effects of many antidepressants are associated with an elevation of hippocampal BDNF (Saarelainen et al., 2003; Monteggia et al., 2004; Adachi et al., 2008). Thus, the Flinders Sensitive Line rat (FSL), another established animal model of depression does not show reduction in hippocampal BDNF levels (Angelucci et al., 2000). Interestingly, gender based differences in BDNF and BDNF signaling have recently been reported (Autry et al., 2009; Elfving et al., 2010; Hill and van den Buuse, 2011).

We did not find any basal differences in the frontal cortex between WKY and WIS rats. Our findings are in line with previous studies that did not find any basal changes in cortical BDNF of FSL rats (Elfving et al., 2010; Angelucci et al., 2000). It is important to note, however, that involvement of BDNF in the cortex or other brain regions in effectiveness of antidepressants cannot be ruled out. This point is further highlighted by our results where both nomifensine and imipramine caused even higher elevation in frontal cortex BDNF compared to that of hippocampus. Other studies also have shown an increase in cortical BDNF following antidepressant treatments (Cooke et al., 2009; Balu et al., 2008). The lower increases of BDNF in WKY rats in response to antidepressants in comparison to Wistar rats are likely due to genetic differences between the two strains. Similar strain differences in response to antidepressants have been observed by others. For example, Lahmame et al. (1997) observed a differential antidepressant response to imipramine between various strains including WKY rats.

We have previously observed that frontal cortex noradrenergic system could be involved in depressogenic effects of alcohol and its reversal by nomifensine and imipramine (Getachew et al., 2010). The noradrenergic system may also influence BDNF expression (Haenisch et al., 2009; Chen and Russo-Neustadt, 2007; Chen et al., 2007; Juric et al., 2006; Garcia et al., 2003). It has been reported that norepinephrine (NE) locally applied to hippocampal neurons can enhance the expression of BDNF in a time- and dose-dependent manner (Chen and Russo-Neustadt, 2007; Chen et al., 2007). NE may also transiently increase BDNF in cultured cortical and cerebellar astrocytes (Juric et al., 2006). In a study involving knockout mice it was observed that norepinephrine transporter knockout mice are resistant to stress induced depressive like behaviors as well as stress induced reduction of BDNF levels (Haenisch et al., 2009). Interestingly, similar to our findings antidepressant treatment reduced the depressive like behavior and increased BDNF levels in stressed wild-type mice (Haenisch et al., 2009). Thus, it would be of significant interest to further investigate possible interactions between central noradrenergic system and BDNF in WKY rats, particularly in regulation of mood as it might offer novel therapeutic intervention for depression and/or alcoholism.

In summary, the results indicate a lower hippocampal but not cortical BDNF level in WKY rat model of depression, reduction of hippocampal BDNF by alcohol in both WKY and Wistar rats and an increase in both cortical and hippocampal BDNF by imipramine and nomifensine. The findings suggest usefulness of antidepressants in reversing alcohol-induced neurochemical changes.

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