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Nicotine Blocks the Depressogenic Effects of Alcohol: Implications for Drinking-Smoking Co-Morbidity

Olubukola Kalejaiye, Babur H. Bhatti, Robert E. Taylor, and Yousef Tizabi

Department of Pharmacology, College of Medicine, Howard University, Washington, DC 20059, USA
Address correspondence to Yousef Tizabi, ytizabi@howard.edu

Abstract Alcohol and nicotine are two very commonly abused legal substances. Although various hypotheses for such co-dependence have been suggested, it is not known whether the effects of alcohol and nicotine on mood behavior may also contribute to such co-abuse. Chronic exposure to high alcohol levels may lead to various neurochemical changes and precipitate depressive-like behavior. Nicotine, on the other hand, may exert an antidepressant-like effect. Here, we sought to determine whether nicotine may also block or mitigate the "depressogenic" effects of alcohol in a rat model. Moreover, since hippocampal brain-derived neurotrophic factor (BDNF) has been strongly implicated in mood regulation and effectiveness of antidepressants, the level of this neurotrophic factor in the hippocampus was also evaluated. Adult male Wistar rats were injected (i.p.) with alcohol (1.0 g/kg), nicotine (0.3 mg/kg) or their combination once daily for 14 days. Controls received saline. The behavior of these rats in open field locomotor activity (LMA), the forced swim test (FST), a measure of helplessness, and sucrose intake, a measure of anhedonia were evaluated 16–18 h after the last injection. Chronic alcohol did not affect LMA, but increased immobility in FST and decreased sucrose consumption, suggesting a "depressogenic" effect. Nicotine by itself did not affect any of the measured behavior but blocked alcohol-induced changes in FST and sucrose intake. Parallel to the behavioral changes, chronic alcohol resulted in a significant decrease in hippocampal BDNF, which was normalized by nicotine. These findings suggest that the opposing effects of alcohol and nicotine on depressive-like behavior may contribute to their co-abuse.

Keywords alcohol; nicotine; depression; hippocampus; BDNF

1. Introduction

Despite the well-established adverse effects of drinking (alcohol consumption) and smoking (nicotine intake), simultaneous consumption of these two substances is widespread [2,5,9,12,16,28]. It is also known that alcohol may increase the craving for nicotine and vice versa [3, 17,29,30]. Indeed, alcohol may be a major risk factor for relapse during smoking cessation [4,6,21], and conversely smoking may be a major risk factor for alcoholism relapse [6,37]. Considerable research has provided various explanations for co-morbid condition of drinking and smoking. These include genetic predisposition, enhanced analgesic, and/or rewarding effects as well as other pharma-codynamic and pharmacokinetic interactions [22,31].

Mood effects of alcohol and nicotine are also well-recognized phenomena. The high co-morbid condition of depression and alcoholism is believed to be precipitated by detrimental effects of high alcohol doses on neuro-transmitters intimately involved in mood regulation [1, 14,23]. Nicotine, on the other hand, has been shown to exert antidepressant effects in preclinical as well as clinical studies [13,27,39,40,41]. Consequently, smoking cessation or deprivation of nicotine may exacerbate the mood dysregulation and hence contribute to relapse. Thus, it is postulated that the high incidence of smoking in depressed population may be a self-medication attempt [27,33].

However, it is not known whether pharmacological interactions between alcohol and nicotine vis-à-vis mood effects may also contribute to the high incidence of co-morbid drinking-smoking. A major goal of this study was to provide some answers to this question using an experimental paradigm. Moreover, since a functional role for hippocampal brain-derived neurotrophic factor (BDNF) in mood regulation in general, and effectiveness of antidepressants in particular is well recognized [10,20, 25,32], the expression of this protein in hippocampus was also determined. Based on previously published findings, we hypothesized that chronic alcohol administration will induce depressive-like behavior and a decrease in hippocampal BDNF levels in Wistar rats and that nicotine would block or mitigate these effects of alcohol.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Harlan Laboratories, Indianapolis, IN, USA) were utilized through the course of the study. Upon arrival, the animals (8–10 weeks old) underwent 1-week quarantine period during which time they were not handled. After this period, they were handled once a day for one week to accustom them to the experimental procedure.

Wistar rats were housed in groups of two in standard plastic cages on standard bedding in a temperature controlled (24–26 °C) room designated for male rats. Animals had access to food and water ad libitum. In order to allow for measurements of behaviors during their active phase, animals were kept on a 12-h reversed light/dark cycle (lights on 7:00 p.m.–7:00 a.m.). A red light source was used for illumination during injection and behavioral studies. Each experiment consisted of four groups (8–10 rats per group). All experiments were carried out in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee.

2.2. Drug treatment

Rats were administered ethanol (1 g/kg), nicotine (0.3 mg/kg base) or their combination daily for 14 days as previously described by Getachew et al. 2008, Hauser et al. 2011 and Tizabi et al. 2009 [14, 19, 39]. The injections (i.p.) were carried out between 10:00 a.m. and 11:00 a.m. Controls received saline. The dose of nicotine and alcohol were based on our previous studies and preliminary results. Furthermore, the administration of the 1 g/kg dose of ethanol resulted in a blood alcohol level (BAL) of approximately 90 mg/dL. Reports from the CDC and NIAAA found that human studies show alcoholics' BAL may range between 80 mg/dL to 150 mg/dL throughout a given day. Thus, chronic daily administration of this dose was resulting in equivalent BAL as seen in some alcoholics.

2.3. Behavioral evaluations

Eighteen to 20 h after the next to the last injection the rats were assessed for sucrose intake (described below). The rats were injected once again after the sucrose test and approximately 18 to 20 h following this last injection, open-field locomotor activity (LMA) followed by the forced swim test (FST) was conducted. This was to ensure that the sedative effects of alcohol were not present during the behavioral tests.

2.4. LMA monitoring

The behavior of the rats in the open-field locomotor activity test was assessed to distinguish the treatment effect on FST immobility from the effect on general locomotor behavior. Locomotor activity was measured first for each animal for 5 min in an open-field activity monitoring cage (27 × 27 × 20.3 cm, Med Associates, Inc., St. Albans, VT, USA), which automatically records the number of infrared beam interruptions. The activity of the animal during this entire 5-min period was recorded as a measure of LMA.

2.5. FST

Immobility in the FST was measured by a modification of the method introduced by Detke et al. [11] as described previously [14, 19, 40]. Briefly, the rats were individually placed into a Pyrex cylinder filled with room temperature water (25 ± 1 °C) at a height of 30 cm to ensure that animal could not touch the bottom of the container with its hind paws or its tail. A time-sampling scoring technique was used whereby the predominant behavior (i.e., immobility or swimming) in each 5-s period of the 300-s test was recorded blindly.

2.6. Sucrose preference test

In this study, in addition to FST, we also evaluated the effects of the treatments on sucrose intake, a test reflective of anhedonia, another hallmark characteristic of depression [42]. The animals were conditioned to sucrose solution for four days prior to initiation of drug treatments. During this conditioning phase, each rat was given free access to both water and a 1% sucrose solution for a period of 4 h. The bottles were pre-weighed prior to placing them on the cages and the position of the bottles was reversed 2 h into the conditioning period to avoid side preference. Sucrose preference test was performed using the Grippo et al. method [18] with some modification. On the day of testing, the animals were housed individually in a clean standard polypropylene shoebox cage (42 × 20.5 × 20 cm) on hardwood chip bedding (alpha-dry). Each rat was given free access to two bottles for 6 h: one bottle was filled with a 1% (w/v) sucrose solution, and the other bottle was filled with water. Each bottle was weighed before and after the 6-h testing period. The two bottles were swapped 3 h into the testing to prevent side preference. The total amount of sucrose consumed by each rat was recorded.

2.7. Tissue preparation

For neurochemical analysis, age-matched groups of rats (6–8/group) were treated exactly as described above, but in this case, the animals were sacrificed 18–20 h after the last injection by decapitation without any behavioral test. The brains were rapidly removed, frozen on dry ice, and stored at −80 °C. Each frozen brain was later thawed on ice, and hippocampus was dissected out [38] for western blot analysis.

2.8. Western blot

Homogenate of the dissected tissues were made in lysis buffer (10 mM Tris-buffer, 5 mM EDTA, 150 mM NaCl, 0.5% Triton X-100 (v/v) with protease inhibitors (Sigma-Aldrich, St. Louis, MO, USA). The protein concentration in each sample was determined using a BCA protein Assay Kit (Pierce Biotechnology Inc., IL, USA), and equal protein amount (as confirmed by β-actin) was loaded in each immunoblot. The proteins were separated using 12% SDS-PAGE gel and transferred onto a nitrocellulose membrane. The membranes were blocked with a blocking reagent (5% nonfat milk in TBS buffer) for 30 min and
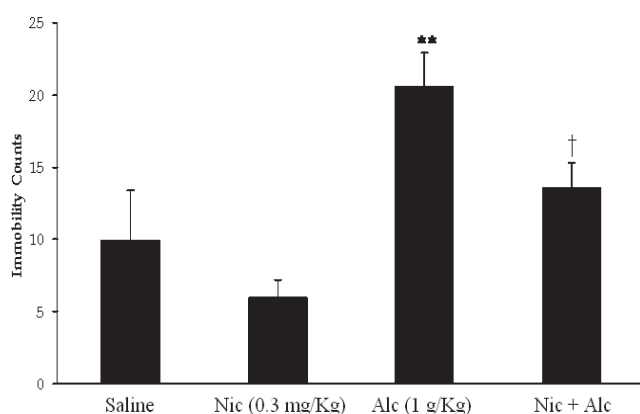
Figure 1: Effect of chronic alcohol, nicotine and their combination on immobility in the forced swim test (FST). Wistar rats were treated daily for 14 days and their behavior in FST was evaluated 18–20 h after the last injection. Values are ±SEM. **$P < .01$ compared to controls, †$P < .05$ compared to alcohol only. $N = 8$–10/group.

Figure 2: Effect of chronic alcohol, nicotine and their combination on open field locomotor activity (LMA). Wistar rats were treated daily for 14 days and their behavior in LMA was evaluated 18–20 h after the last injection. Values are ±SEM. $N = 8$–10/group.

incubated at 4 °C overnight with the primary antibody against BDNF (1:1,000, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The membranes were washed with TBST (TBS buffer with 1% Tween-20) and blocked with the blocking reagent. Membranes were then incubated for 1 h at room temperature in Goat Anti-Rabbit-HRP conjugated secondary antibody (1:3,000 in TBS, Bio-Rad Laboratories, CA, USA). The membranes were then washed in the TBST washing solution and then visualized using enhanced chemiluminescent kits (Bio-Rad Laboratories). The intensity of the protein bands on the gel was quantified using ChemiDoc XRS system (Bio-Rad Laboratories).

2.9. Statistical analysis

Statistical differences between treatment groups were determined by one-way ANOVA followed by post-hoc Newman-Keuls multiple comparison test to determine which groups differed. Significant difference was considered a priori at $P < .05$. Data were analyzed using Graphpad Prism 3 (Graphpad Software, Inc., San Diego, CA, USA).

3. Results

Figure 1 depicts the effects of chronic alcohol, nicotine and the combination on immobility counts in the FST. Alcohol alone resulted in doubling of the immobility counts in the FST ($P < .01$). Nicotine by itself did not affect immobility counts, but significantly reduced alcohol-induced increase in immobility counts in the FST. Thus, the combination effect was not significantly different from the control.

Figure 2 depicts the effect of chronic alcohol, nicotine and the combination on general locomotor activity (LMA). None of the treatments had any significant effect on LMA,
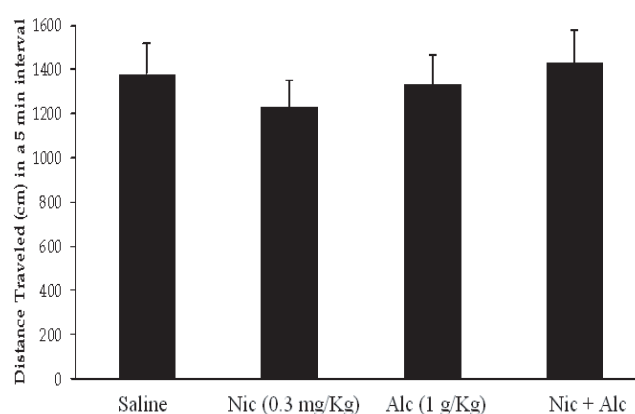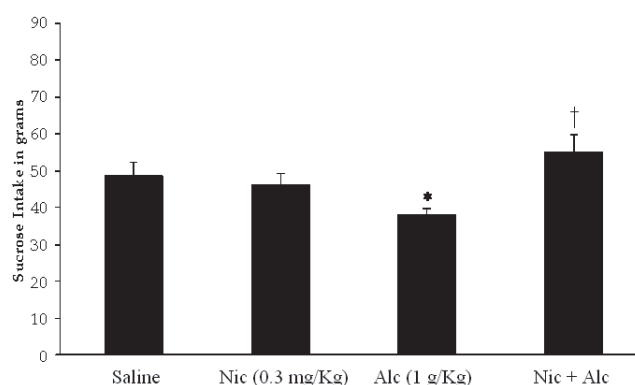

Figure 3: Effect of chronic alcohol, nicotine and their combination on sucrose intake. Wistar rats were treated daily for 14 days and their sucrose consumption over a 6-h period was measured 4 h after the last injection. Values ±SEM. *$P < .05$ compared to controls, †$P < .5$ compared to alcohol only. $N = 8$–10/group.

suggesting that the effects on immobility in the FST are independent of general locomotor activity.

Figure 3 depicts the effect of chronic alcohol, nicotine and the combination on sucrose intake. Alcohol resulted in a significant decrease in sucrose intake (approx. 25%, $P < .05$). Similar to what was observed in the FST, nicotine by itself did not affect the sucrose intake, but significantly reduced alcohol-induced decrease in sucrose intake. Thus, the combination effect was not significantly different from the control.

Figure 4 depicts the effect of chronic alcohol, nicotine and the combination on hippocampal BDNF levels. Parallel to behavioral observations, alcohol resulted in a significant decrease in BDNF levels (approx. 30%, $P < .05$). Nicotine
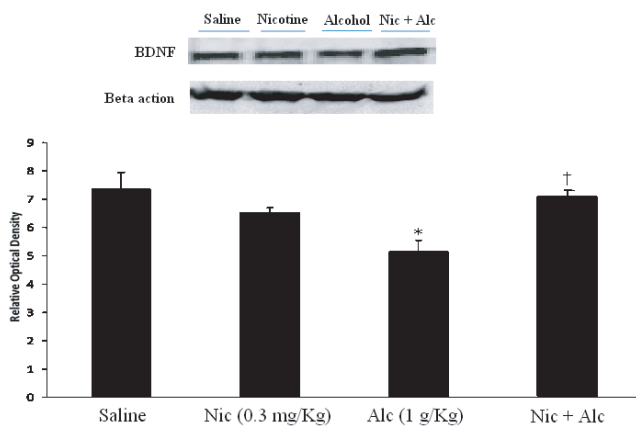
Figure 4: Effect of chronic alcohol, nicotine and their combination on hippocampal BDNF levels and beta actin protein. Wistar rats were treated daily for 14 days and the level of BDNF (relative optical density) in the hippocampus was evaluated 18–20 h after the last injection. The bands indicate equal loading of proteins (beta actin) and intensity of BDNF protein. Values ±SEM. $^*P < .05$ compared to controls, $^\dagger P < .5$ compared to alcohol only. $N = 6$–8/group.

by itself did not affect hippocampal BDNF, but significantly reduced alcohol-induced decreases in BDNF. Thus, the combination effect was not significantly different from the control.

4. Discussion

The results of the current study confirm and extend the previously observed depressogenic effects of chronic administration of a relatively high alcohol dosage. Thus, 2 weeks of daily injection of 1 g/kg of alcohol resulted in a significant increase in immobility in the FST, reflective of helplessness characteristic of depression. Moreover, the same regimen reduced sucrose intake, reflective of the anhedonic component of this mental disorder. In addition, the results support an involvement of hippocampal BDNF in this action of alcohol as chronic alcohol administration caused a decrease in BDNF level in the hippocampus. The more novel finding of this study is that nicotine co-administration blocked both the behavioral and neurochemical effects of alcohol suggesting that the antidepressant effects of nicotine in this paradigm may be a contributory factor to the drinking-smoking co-morbidity.

Previous studies have reported that alcohol-induced depressive-like behavior is associated with a reduction in hippocampal BDNF [7, 19, 34]. Interestingly, human alcoholics also manifest a decrease in serum BDNF levels [44]; however, the extent to which changes in peripheral BDNF level may be reflective of central BDNF changes is not yet established [24, 36, 43]. It is important to note that in addition to the hippocampus, other brain regions

may also contribute to mood regulation. In this regard, a role for frontal cortex is well recognized [15, 26, 35]. However, in regard to BDNF changes, much higher concentrations of alcohol might be needed to affect BDNF or its messenger RNA in this area [19, 34].

Our findings of nicotine's effect in normalizing alcohol-induced decreases in hippocampal BDNF is also in agreement with previous studies where antidepressant effect of nicotine was shown to be associated with increased hippocampal BDNF [8]. Interestingly, other therapeutically used antidepressants have also been shown to normalize alcohol-induced changes in hippocampal BDNF as well as its depressogenic effects [14, 19]. A major distinction and likely drawback between possible nicotinic-based interventions and other antidepressants in regard to alcohol-associated depression is the likelihood that nicotinic agonists may actually enhance alcohol craving [37]. In this regard it would be of significant interest and clinical relevance to elucidate the extent to which depression may precede alcohol and/or nicotine co-abuse, or vice versa. Preliminary studies indicate that a past history of depression may actually enhance nicotine's reinforcing effects more in alcoholics than in non-alcoholics [1, 31].

In summary, our findings indicate that nicotine counteracts alcohol-induced depression and the associated decreases in hippocampal BDNF. This interaction may also be a contributory factor to drinking-smoking co-morbidity.

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