Protective Effect of Bupropion on Alcohol Abstinence-Induced Anxiety and Convulsions

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Chronic administration of ethanol (2g/kg po) on days 1-6 and its withdrawal produced anxiogenic reaction in mice as assessed in the mirror chamber test. Daily administration of bupropion (2 or 5 mg/kg, ip) prior to ethanol for 6 days prevented withdrawal-induced anxiety in mice. However, acute administration of a single dose of bupropion (5 mg/kg), to animals withdrawn from ethanol, i.e. on the 7th day, did not prevent withdrawal-induced anxiety. Ethanol withdrawal also induced a significant increase in the locomotor activity of mice indicating an anxiogenic response. Administration of bupropion (2 or 5 mg/kg) prior to ethanol for 6 days also prevented withdrawal induced increased locomotor activity. Ethanol withdrawal also sensitized the convulsogenic reaction to pentyleneetetrazole. A non convulsive dose (40-60 mg/kg) of pentyleneetetrazole produced full blown convulsions and increased mortality rate in ethanol withdrawn mice. Both acute and chronic administration of bupropion (2 and 5 mg/kg) exhibited a significant protection against ethanol withdrawal-induced reduction in pentyleneetetrazole threshold in mice.

Physical dependence on ethanol is defined by the presence of an ethanol withdrawal syndrome that becomes apparent following cessation of ethanol intake and elimination of ethanol from the system. Tolerance to the effects of ethanol can be demonstrated in both humans and experimental animals but the mechanism of this tolerance is not known for certain1. The withdrawal symptom is well characterized in humans and in animal models2,3, and it consisted of anxiety response, increased locomotion and convulsions (reduction in convulsive threshold) in animals. Alcoholism and withdrawal of its chronic intake are grave social and medical problems. There is no single drug therapy that helps patients in overcoming alcoholism4.

Bupropion HCl, an antidepressant of the aminoketone class, is chemically unrelated to classical or second generation selective serotonin re-uptake inhibitor, or other known antidepressant agents. Bupropion is a noradrenaline and dopamine re-uptake inhibitors (NDRI) which has both noradrenergic and dopaminergic activity5,6. Bupropion has been recently licensed for the treatment of smoking cessation in a number of countries7-11.

The present study was undertaken to evaluate the de-addiction potential of bupropion against alcohol withdrawal reactions in animals. The parameters used were alcohol withdrawal-induced anxiety, locomotion and reduction in convulsive threshold to PTZ in mice.

MATERIALS AND METHODS

Laka mice of either sex (20-25 g), bred in Central Animal House facility of the Panjab University, Chandigarh, were housed 5 per cage at room temperature and allowed to adapt to laboratory conditions for at least 2 days before the initiation of any experiment. The animals were housed under a natural light and dark cycle, and had free access to food and water. Each animal was used only once. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. The drugs used were bupropion (Zyban®, Glaxo-
SmithKline, UK), pentylentetrazol (PTZ, Sigma, USA) and ethanol (Bengal Chemicals & Pharmaceuticals Ltd, Kolkata).

PTZ and ethanol were prepared in distilled water. Bupropion powder was dissolved in distilled water and administered i.p. Ethanol (2 g/kg of 10% v/v in mice) was administered orally while PTZ (40–80 mg/kg) was injected i.p. PTZ was administered 24h after the last dose of ethanol (withdrawal animals) and bupropion was given 30 min prior to ethanol.

Treatment schedule:

In chronic studies, mice received (2g/kg of 10% v/v) ethanol, intragastrically, twice a day on 1st day and once daily on the successive days for a total of 6 days. On 7th day, i.e. 24h after the last dose of ethanol mice were tested for withdrawal reaction. The other treatment groups included (pretreatment:treatment) : (i) saline:saline  (ii) saline:bupropion (2 or 5 mg/kg; i.p.)  (iii) saline:ethanol, and (iv) bupropion (2 or 5 mg/kg; i.p):ethanol.

Control experiments were performed on day 7 to determine whether bupropion prevented the development of withdrawal syndrome or it simply altered the behavioral expression of the withdrawal symptoms. On this day, the treatments were reversed so that the animals that had received bupropion followed, 30 min. later, by ethanol on days 1 through 6 were challenged with saline. Similarly, the animals which received saline followed, 30 min later, by ethanol received only bupropion. The group chronically treated with saline received the same (saline only). The withdrawal reaction was assessed by studying the anxiogenic reaction in mirror-chamber and for decrease in threshold to pentylentetrazol (proconvulsive response to an otherwise non-convulsive dose in naïve-animals).

Measurement of anxiety and locomotor activity:

The mirror chamber used for mice consists of a wooden chamber having a mirror-chamber enclosed within it. During the 5 min test session the following parameters were noted: (i) transfer latency, (ii) the total time spent in mirror-chamber, (iii) number of entries the animal made in mirror-chamber. Animals were put individually at the distal corner of the mirror chamber facing towards the mirror-chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber26. The ethanol withdrawal-induced hyperlocomotor activity of mice was measured in actophotometer for a period of 5 min.

PTZ-threshold:

The onset of body jerks, clonic convulsions followed by tonic convulsions and death were recorded following PTZ challenge in naïve and ethanol withdrawn animals. Each animal was observed individually for 2 h for acute response. Reduction in the dose of PTZ to produce full blown convulsions in ethanol withdrawn animals was considered as withdrawal-induced reduction in the convulsive threshold21. The protective effect of bupropion following acute and chronic treatment was noted.

Statistical analysis:

The data expressed as means±SEM were analysed by Student’s t-test. Probability levels < 5% were considered significant.

RESULTS

Effect of bupropion on ethanol withdrawal-induced hyperlocomotor activity in mice:

Mice withdrawn from chronic ethanol treatment showed hyperlocomotor activity as compared with the vehicle-treated control group (fig. 1). Concomitant administration of bupropion (2 or 5 mg/kg, i.p.) along with ethanol showed reduced withdrawal-induced hyperlocomotor activity (fig.1).

![Graph](image)

Fig. 1: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the locomotor activity of mice.

Effect of co-administration of bupropion (BUP) on the long-term ethanol (2 g/kg) chronic treatment- and withdrawal-induced changes in the locomotor activity in mice. Values expressed means±SEM (5–8 mice). *P<0.05 as compared to control group. *P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Turkey’s test).
Fig. 2: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the transfer latency to the mirror chamber in mice.

Effect of co-administration of bupropion (BUP) on the long-term ethanol (2 g/kg) chronic treatment- and withdrawal-induced changes in the transfer latency to the mirror chamber in mice. Values expressed means±SEM (5-8 mice). *P<0.05 as compared to control group. **P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey’s test).

Mice treated repeatedly with saline followed by ethanol for 6 days and then challenged with bupropion on 7th day did not show a significant decrease in the ethanol withdrawal induced hyperlocomotor activity (fig. 1).

Effect of bupropion on ethanol withdrawal-induced anxiety:

Mice withdrawn from chronic ethanol treatment showed lower preference for mirror-chamber, increase in transfer latency to the mirror chamber and significant decrease in the duration of time spent and number of entries in mirror-chamber as compared with the vehicle-treated control group.

Concomitant administration of bupropion (2 or 5 mg/kg, ip) along with ethanol chronically for 6 days showed reduced withdrawal-induced anxiety. There was a significant decrease in the transfer latency to the mirror-chamber (fig. 2). The time spent (fig. 3) and the number of entries (fig. 4) in mirror-chamber increased significantly as compared with ethanol-withdrawn group.

Mice treated repeatedly with saline followed by ethanol for 6 days and then challenged with bupropion on 7th day
did not show reduced withdrawal-induced anxiety.

Effect of bupropion on PTZ-threshold in ethanol-withdrawn animals:

PTZ (80 mg/kg; i.p.) induced severe clonic-tonic seizures followed by 100% mortality in naïve mice. A lower dose (60 mg/kg; i.p.) induced mild clonic-tonic seizures with reduced mortality, whereas a still lower dose (40 mg/kg; i.p.) failed to cause any mortality or observable behavioral change in naïve mice. However, in ethanol-withdrawn mice PTZ (60 mg/kg; i.p.) produced severe clonic-tonic seizures and 100% mortality. Acute administration of bupropion (2 or 5 mg/kg; i.p.) to ethanol withdrawn mice showed protection against PTZ (60 mg/kg; i.p.) convulsions. Animal showed only mild clonic convulsions followed by recovery. Chronic administration of bupropion (2, 5 mg/kg; i.p.) followed by ethanol for 6 days exhibited only mild clonic seizures with delayed onset and 60% mortality following administration of PTZ (60 mg/kg; i.p.) on day 7 in mice (fig. 5).

DISCUSSION

Chronic ethanol administration is known to produce
Fig. 4: Effect of bupropion (BUP) on chronic ethanol (2 g/kg) treatment- and withdrawal-induced changes in the mean number of entries to the mirror chamber by mice. Effect of co-administration of bupropion (BUP) on the long-term ethanol (2g/kg) chronic treatment and withdrawal-induced changes in the mean number of entries in the mirrored chamber in mice. Values expressed mean±SEM (5-8 mice). *P<0.05 as compared to control group. *P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey’s test).

Fig. 5: Effect of bupropion (BUP) on mean onset time after PTZ challenge in ethanol withdrawn mice. Effect of co-administration of bupropion (BUP) on mean onset time after PTZ challenge (measurement convulsive threshold) in ethanol (ETOH)-withdrawn mice. Values expressed mean±SEM. *P<0.05 as compared to vehicle treated group. *P<0.05 as compared to control group. *P<0.05 as compared to ethanol (ETOH) treated group (for treatment schedule see text) (ANOVA followed by Tukey’s test).

bupropion followed by ethanol for 6 days displayed significant reversal of withdrawal-induced anxiety on 7th day. Acute administration of bupropion on 7th day to ethanol-withdrawn animals also displayed considerable decrease in 7th day. The anxiety reaction in ethanol-withdrawn animals may be attributed to the anxiogenic action of bupropion per se or to an acute interaction between bupropion and ethanol. Reversal of anxiogenic response and hyperlocomotor activity following substitution of bupropion with saline in ethanol-withdrawn animals suggests that bupropion need not be present during testing to observe anxiety in chronically bupropion treated animals.

PTZ, a chemoconvulsant, is reported to induce seizures by depressing chloride channel function by binding to picrotoxin site on the GABA receptor complex. In ethanol withdrawn animals lower doses of PTZ as compared to control animals was required so as to exhibit severe tonic seizures and mortality. Sensitization of inverse agonistic site on GABA-benzodiazepine receptor following ethanol withdrawal has been shown to be responsible for decrease in convulsive threshold to PTZ in ethanol-withdrawn animals. Bupropion significantly inhibited PTZ convulsions...
in these animals when given repeatedly for 6 days. Acute 
bupropion administration following ethanol withdrawal also 
produced significant attenuation of PTZ convulsions. The 
reversal of ethanol withdrawal sensitized convulsant 
response to PTZ suggests the effectiveness of this drug in 
suppressing ethanol withdrawal syndrome whether given 
acutely or chronically. The above data suggests that the 
anticonvulsant effect of bupropion alone or its acute 
interaction with ethanol is unlikely to account for its 
protective effect in addiction.

Bupropion is a safe drug having no apparent response 
per se on reward system. Concurrent administration of this 
drug may help in preventing the development of tolerance 
and dependence to ethanol and other psychotropic drugs. 
The drug is also effective in combating withdrawal reactions 
to chronic administration of ethanol.

Bupropion and its active metabolites compete for the 
neuronal transporters for dopamine (DA) and 
norepinephrine (NE), and thus, increase extracellular levels 
of these monoamines in synapses within the brain and in 
the interstitial spaces between the cells. Bupropion blocks 
reuptake of NE and DA, and also blocks nicotinic receptors 
in the low to intermediate micromolar range. The 
antidepressant effects of bupropion result from inhibition of 
dopamine and norepinephrine transporters (DAT and NET, 
respectively) however, its mechanism of action is not fully 
understood. Most drugs of dependence preferentially 
stimulate the release of DA in the nucleus accumbens, a 
principal terminal field of the mesolimbic system, and this 
property is fundamental to the addictive potential of all drugs 
of abuse. Bupropion-induced inhibition of DAT and NET 
function and associated increases in extracellular DA and 
NE concentrations, respectively, may substitute for drug-
evoked neurotransmitter release.

Bupropion is a selective re-uptake inhibitor of 
dopamine and noradrenaline which prevents or reduces 
 cravings and other features of nicotine withdrawal. The 
effects of bupropion on addiction may be through dual effects 
on dopaminergic and nicotinic systems. Its effects on DA, 
NE and indirectly 5-HT overflow in the brain may also 
contribute to attenuation of other symptoms associated with 
abstinence. Bupropion sustained release preparation 
provided the first non-nicotine pharmacological treatment 
approved for smoking cessation and was thought to be 
effective because of its dopaminergic activity on the 
pleasure and reward pathways in the mesolimbic system 
and nucleus accumbens. The underlying mechanism for 
bupropion in smoking cessation is thought to be mediated 
by increasing the concentration of dopamine in the nucleus 
accumbens. Thus, bupropion is believed to work by 
elimination of nicotine cravings and to decrease the 
physiologic and psychologic symptoms associated with 
nicotine withdrawal.

There are many evidences of interaction of 
bupropion with dopaminergic system. Certain in vivo 
 studies indicated that bupropion is a selective dopamine 
uptake inhibitor and that dopaminergic system plays an 
important role in its central nervous system pharmacology.

Bupropion is known to induce seizures in high 
doses per se. Recently, Tutka et al. (2004) demonstrated 
that bupropion hydrochloride a commonly used smoking 
cessation aid, dose-dependently caused clonic convulsions in 
mice, with the CD50 (convulsive dose 50, i.e., the dose 
producing convulsions in 50% of mice) at 119.7 mg/kg. An 
evaluation for anticonvulsant effects showed that bupropion 
in the doses of 15-30 mg/kg protected against convulsions 
induced by maximal electroshock with the ED50 (effective 
dose50, i.e., the dose protected 50% of mice against 
 convulsions) being 19.4 mg/kg. Bupropion had no effect on 
pentylenetetrazole- and kainic acid-induced convulsions. 
It was speculated that the anticonvulsant activity of 
bupropion may be exploited for use in the treatment of 
epilepsy but it requires further investigations.

Thus the present study provides evidence for the de-
addiction potential of acute as well as chronic 
administration of bupropion against ethanol. The observed 
effect may be due its interaction with dopamine in reward 
areas or any other neurotransmitter effecting dependence, 
but the exact mechanism remains to be explored.

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