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Voluntary alcohol consumption and plasma beta-endorphin levels in alcohol preferring rats chronically treated with lamotrigine

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HIGHLIGHTS

- Low level of beta-endorphin may be responsible for alcohol dependence.
- Alcohol intake increases the level of beta-endorphin both in human and animals.
- Drugs that increase of beta-endorphin levels may be effective in alcoholism treatment.
- Lamotrigine decreases alcohol intake but not influences beta-endorphin.
- Lamotrigine may have therapeutic potential for alcohol addiction.

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ABSTRACT

Several recent studies have indicated that lamotrigine, similarly to other antiepileptic drugs, may be useful in the therapy of alcohol dependence. The rationale for using lamotrigine in the treatment of alcohol addiction is based on its multiple mechanisms of action which include inhibition of voltage-sensitive sodium channels, modulation voltage-gated calcium currents and transient potassium outward current. However, the known mechanism of lamotrigine does not fully explain its efficacy in alcohol addiction therapy. For this reason we have decided to examine the effect of lamotrigine on the opioid system. Our previous studies showed that topiramate and levetiracetam (antiepileptic drugs) as well as the most effective drugs in alcohol addiction therapy *i.e.* naltrexone and acamprosate, when given repeatedly, all increased plasma beta endorphin (an endogenous opioid peptide) level, despite operating through different pharmacological mechanisms. It is known that low beta-endorphin level is often associated with alcohol addiction and also that alcohol consumption elevates the level of this peptide. This study aims to assess the effect of repeated treatment with lamotrigine on voluntary alcohol intake and beta-endorphin plasma level in alcohol preferring rats (Warsaw high preferring (WHP) rats). We observed a decrease in alcohol consumption in rats treated with lamotrigine. However we didn't observe significant changes in beta-endorphin level during withdrawal of alcohol, which may indicate that the drug does not affect the opioid system. We suppose that lamotrigine may be useful in alcohol dependence therapy and presents a potential area for further study.

1. Introduction

Numerous studies in recent years have increased the body of evidence indicating that antiepileptic drugs, such as topiramate, gabapentin, pregabalin, tiagabine and lamotrigine may have new clinical applications in the treatment of drug and alcohol dependence [1–6]. Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine) is a novel drug of phenyltriazine class, chemically unrelated to existing anticonvulsants. Preliminary data from case reports and open clinical trials suggest that lamotrigine may be effective in the therapy of addiction. Effects of lamotrigine in reducing craving have been reported for cocaine [7–12] alcohol [13,14] and abused inhalants [15]. At present, there are only a few published controlled clinical trials which support usefulness of lamotrigine in reducing alcohol abuse. An open-label clinical study found evidence that lamotrigine is safe and effective in the treatment of alcohol withdrawal syndrome [13]. That research also presented preliminary data suggesting that lamotrigine may have beneficial effects on reduction of craving and consumption of alcohol in dual-diagnosis patients presenting bipolar disorder and alcohol dependence. Decrease in craving for alcohol was also observed in alcohol dependent patients with comorbid schizophrenia [16]. Moreover, it was

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observed that lamotrigine inhibits somatic signs of alcohol withdrawal [17]. In prospective a clinical study including 240 patients with ICD-10 criteria for delirium tremens, lamotrigine proved efficient in the treatment of this condition [18].

Lamotrigine, similarly to other second generation antiepileptic drugs, presents several mechanisms of biochemical action. One of the proposed mechanisms of its action is connected with voltage-sensitive sodium channels. *In vitro* studies suggest that lamotrigine inhibits voltage-sensitive sodium channels and stabilizes the neuronal membrane and consequently inhibits release of excitatory neurotransmitters such as glutamate and aspartate [19,20]. Lamotrigine also modulates voltage-gated calcium currents and the transient potassium outward current [21]. These mechanisms may be effective to inhibit pathological excitation during withdrawal syndrome. Since lamotrigine prevents the release of glutamate, it may reduce drug reward and reinforcement as well as somatic symptoms of withdrawal and relapse-like behavior [22]. However, the precise mechanism of lamotrigine is unknown and those listed above do not fully explain the efficacy of lamotrigine in substance abuse treatment. For this reason, we decided to test another, possible mechanism of action of lamotrigine, namely its effect on endogenous opioid system, as this system is one of neuropharmacological targets in treating alcohol dependence. Many studies have demonstrated that beta-endorphin, an endogenous opioid, plays a role in alcohol dependence [23–25]. Low level of beta-endorphin seems to be one of a number of factors responsible for drug craving and withdrawal syndrome, as this peptide is known to play one of the key roles in the mesolimbic reward system [25,26]. The relationship between the level of this peptide and alcohol dependence was observed in people with high family risk of alcoholism [27]. Moreover, a correlation between predisposition to alcohol consumption and low level of beta-endorphin was observed in alcohol preferring rats [28,29]. Previously we found that the most effective drugs in the treatment of alcoholism, acamprosate or naltrexone, applied repeatedly, increased the levels of endogenous beta-endorphin [28,30]. Also two of the second generation anticonvulsant drugs, levetiracetam and topiramate, were observed to lead to an increase of beta-endorphin level [31,32]. On the other hand, fluoxetine which has only modest efficacy in alcoholism treatment, showed no effect on the level of this peptide [33].

The aim of this study was to examine the effect of repeated treatment with lamotrigine on voluntary alcohol intake in WHP rats as well as to investigate the effect of lamotrigine on beta-endorphin concentration in rats with a free access to ethanol and during withdrawal phase.

2. Materials and methods

2.1. Animals

The experiments were carried out on 42 female adult rats weighing 210–250 g from the F_{47} generation of the WHP animal line. The animal facility was 12:12 h light:dark cycle with light onset at 7.00 a.m., at temperature 22 ± 2 °C and humidity of approximately 55 ± 5%.

The rats were individually housed in stainless steel cages equipped with two graduated plastic drinking tubes, fitted with a steel sipper tube, with tip valves (steel balls) to prevent leakage, containing tap water or 10% v/v alcohol. The alcohol solution was prepared from water and a stock solution of 95% reagent grade ethanol.

2.2. Baseline period

Following the procedure established by Dyr and Kostowski [34], the rats had free access to a solution of 10% (v/v) ethanol available in the two drinking tubes over the first week, as a sole source of fluid. Food was available *ad libitum*. This procedure allowed them to become accustomed to drinking from the tubes and to experience the taste and pharmacological properties of alcohol. After the initial period the rats were given 24 h of free-choice access to 10% ethanol and water during three consecutive weeks. Alcohol and water intake were recorded and the tubes were refilled daily. Consumption of ethanol and water was measured daily at the same time, before lamotrigine or 1% methylcellulose (vehicle) administration. The position of the alcohol and water tubes was alternated daily to avoid the development of a position preference. The animals were weighed every 3 days. The data are expressed as the daily amount of ethanol ingested (g/kg), the total volume of fluid ingested daily (ml/kg) and preference. Alcohol consumption was determined by calculating grams of alcohol consumed per kilogram of body weight. The ethanol preference was calculated by the following formula:

$$\text{Ethanol preference}\ (\%) = \text{intake of ethanol}\ (\text{ml/kg/day})/\text{total fluid intake}\ (\text{ml/kg/day}) \times 100.$$

2.3. Treatment period

After the baseline period, the rats were assigned to one of six groups (each n = 6) and received intragastrically (by a gavage) lamotrigine in doses 5 or 15 mg/kg body weight; 2 ml/kg b.w., daily or 1% methylcellulose (1% MTC) as vehicle over the course of 14 days according to the schedule (1) Vehicle-Ethanol — group treated with 1% methylcellulose, with free access to ethanol and water; (2) Vehicle-Water — alcohol withdrawal group treated with 1% MTC; (3, 4) LTG5-Ethanol and LTG15-Ethanol — groups treated with lamotrigine in doses 5 or 15 mg/kg, respectively with free access to ethanol during experiment; (5, 6) LTG5-Water and LTG15-Water — groups treated with lamotrigine for 14 days without access to ethanol. Drugs were administered in light cycle. (7) Control group was offered only water as its sole source of fluid both in three week initial procedure and period of 14 days treatment with vehicle (see Table 1).

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Research Committee.

2.4. Materials

Sep-pak C 18 cartridges were obtained from Waters M.A., USA (Cat. No. WAT 020515); acetone (HPLC grade) and trifluoroacetic acid were from Baker. Aprotinin was from Finepharm, Sp. z o.o., Poland; lamotrigine (Lamitrin) was from GlaxoSmithKline, Great Britain. Ether was purchased from POCh, Poland. The plasma beta-endorphin radioimmunoassay kit was obtained from Phoenix Pharmaceuticals, Inc., USA.

2.5. Blood sample procedure

The rats were anesthetized with ether on the next day after the last administration of lamotrigine during the light cycle. The blood samples were collected by heart puncture and after blood sampling the animals were sacrificed by decapitation. The samples were transferred to centrifuge tubes containing aprotinin (500 KIU/ml) and gently rocked several times to inhibit proteinase activity. The samples were then cooled in an ice bath. The plasma was separated by centrifugation at 1600 ×*g* for 15 min at 4 °C. The plasma was frozen and stored at −20 °C until assessment.

2.6. Solid phase extraction of beta-endorphin from plasma

Beta-endorphin extraction was performed as reported previously [29]. The procedure for beta-endorphin extraction was based on the use of Sep-pak C 18 cartridges.