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Research paper

Does retigabine affect the development of alcohol dependence?—A pharmaco-EEG study

Ewa Zwierzyńska, Dariusz Andrzejczak, Bogusława Pietrzak*

Department of Pharmacodynamics, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland

HIGHLIGHTS

- We examine the effect of retigabine on the development of alcohol dependence.
- Drug decreased ethanol-induced EEG changes during alcohol administration period.
- Retigabine mitigated ethanol abstinence-induces changes in the EEG recordings.
- This may be a significant element of its mechanism of action in AUD therapy.

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ABSTRACT

New antiepileptic drugs have been investigated for their potential role in the treatment of alcohol dependence. One of these drugs is retigabine and this study examines the effect of retigabine co-administered with ethanol on the development of alcohol dependence and the course of acute withdrawal syndrome. A pharmaco-EEG method was used to examine this impact in selected brain structures of rabbits (midbrain reticular formation, hippocampus and frontal cortex). Retigabine was administered *p.o.* at a dose of 5 mg/kg/day with ethanol ad libitum for 6 weeks and then alone for 2 weeks during an abstinence period. Changes in bioelectric activity, which demonstrated the inhibitory effect of alcohol on the brain structures, were already visible after 2 weeks of ethanol administration. In the abstinence period, changes were of a different nature and significant neuronal hyperactivity was observed, particularly in the midbrain reticular formation and the hippocampus. This findings reveal that retigabine decreased ethanol-induced changes during both alcohol administration and abstinence periods. In particular, the modulatory effect of retigabine on the hippocampus may be a significant element of its mechanism of action in alcohol dependence therapy.

1. Introduction

Alcohol Use Disorder (AUD) and its therapy still represent challenges for both research and clinical practice. The effect of previous experimental research of drugs were beneficial for animal models of addiction but the results of clinical trials were not satisfactory. Although a few drugs are currently recommended for AUD therapy, their efficacy is low and dependent on additional factors. One of the reasons for the limited effectiveness of these drugs appears to be that their mechanism of action is associated with a particular neurotransmitter system [1]. It is now known that many closely interdependent neurotransmitter systems are involved with the pathogenesis of AUD, which itself is complex. Furthermore, the role of memory processes in the development of addiction has been emphasized in recent years. Addiction and memory are very similar types of neuronal plasticity and they involve most of the same brain regions [2].

Recently, attention has been drawn to the new generation of antiepileptic drugs (AEDs) whose mechanism of action is associated with various neurotransmission systems also involved in the pathogenesis of alcohol dependence [1]. Therefore, current research is focused on their possible use in this addiction therapy and their results are promising [3,4]. Individual studies in this research area are focused on new AEDs, such as retigabine [5,6].

Retigabine (ezogabine, DC23129) is characterized by a complex mechanism of action. The drug activates the Kv 7.2–7.5 voltage-gated potassium channels, particularly Kv 7.2 and Kv 7.3 [7], and increases GABAergic transmission. Furthermore, although retigabine affects glutamatergic transmission, it only exerts a direct effect at high concentrations [8]. Hansen et al. [9] suggest that the drug

* Corresponding author. Fax: +48 42 678 83 98.
E-mail address: boguslawa.pietrzak@umed.lodz.pl (B. Pietrzak).

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also reduces dopaminergic activity through potassium channel Kv activation and neuronal excitability inhibition.

A review of available literature reveals little data about the effect of retigabine on ethanol-induced changes in the central nervous system, particularly in the hippocampus. It has been demonstrated retigabine decreases ethanol consumption in rats [5,6]. The recent research also shows that Kv7 channels are modified by chronic ethanol administration [6]. Our previous pharmaco-EEG study reports that retigabine in multiple doses reduces the changes induced by an acute dose of ethanol in the rabbit hippocampus [10].

These findings prompted us to investigate whether retigabine co-administered with ethanol affects the development of alcohol dependence and the course of acute withdrawal syndrome. To this end, a pharmaco-EEG investigation was performed and quantitative changes in the spectrum of EEG recordings from selected brain structures were analyzed.

2. Materials and methods

2.1. Animals and treatment

Twenty-one rabbits (Medical University of Lodz, Poland), males ($n = 12$) and females ($n = 9$), were used in the study. Animals weighed 4.5–5.5 kg and their weights did not change significantly during the experiment. Eleven animals were used in the experimental group and ten animals in the ethanol group. Rabbits were housed in individual cages under normal laboratory conditions (20–22 °C, 12 h light/12 h dark cycle) with free access to commercial chow. They were provided free access only to a solution of ethanol which was given in increasing concentrations (ethanol:sucrose: 1st week 10%:10%, 2nd week 15%:7%, 3rd–4th weeks 17%:5%, 4–6th weeks: 19%:0%). Following this, a two-week period of abstinence took place in which the animals received water.

The experimental group received 0.2 ml/kg retigabine (Glaxo Group Ltd.®) *p.o.* repeatedly once a day at a dose of 5 mg/kg/day as a suspension in 1% methylcellulose solution during the entire experiment. The ethanol group received 1% methylcellulose solution *p.o.* All experiments were performed during the light cycle.

The experiments were carried out in strict accordance with Polish governmental regulations concerning experiments on animals (Dz.U.05.33.289). All the experimental protocols have been approved by the Local Ethical Committee for Experimentation on Animals (resolution no. 77/LB 587/2011).

2.2. EEG procedure

Monopolar electrodes were implanted into the rabbit brain structures: the midbrain reticular formation – MRF (P 8 mm, L 3 mm, H 15 mm); the dorsal hippocampus—Hp (P 3 mm, L 5 mm, H 5 mm); and the frontal cortex – FC (A 3 mm, L 2 mm), according to Sawyer et al. [11]. The implantation was performed under butorphanol (0.1 mg/kg.), ketamine (10 mg/kg) and xylazine (0.5 mg/kg) anesthesia. The cortical electrodes were made of 0.15 mm diameter silver wire with a ball at the tip. The subcortical electrodes were made of 0.11 mm diameter Teflon-covered steel wire (Leico Industries, New York). First pharmaco-EEG recording were performed 4 weeks after surgery but before alcohol administration period (initial value). Then EEG were recorded after each week of the study.

EEG recordings were taken with an 8-channel electroencephalograph (Medicor-EEG 8S, Hungary) with the time constant set at 0.3 s and the high filter set at 60 Hz. During the recordings, the animals remained in a cage with a transparent roof and front. The cage was located in a quiet room, and camera recorded their behavior.

One-minute artifact-free EEG recordings selected by the experimenter were taken for computer analysis. EEG samples were digitized at the rate of 128 samples/s, and the Fourier transform of consecutive 4 s epochs for each channel was calculated. Each spectrum consisted of 512 terms for a frequency range between 0 and 45 Hz, with each term having a width of 0.25 Hz. For further statistical analysis, the transformed data was compressed into the six frequency bands: 0.5–4 Hz (delta rhythm), 4–7 Hz (theta rhythm), 7–10 Hz (slow-alpha rhythm), 10–13 Hz (fast-alpha rhythm), 13–30 Hz (slow-beta rhythm), 30–45 Hz (fast-beta rhythm).

2.3. Analysis of results

The results are presented as a percentage change of the initial value. The normality of the distribution was checked by the Kolmogorov–Smirnov test, with Lilliefors correction. Statistical analysis was performed with the Kruskal–Wallis (ANOVA) test and the Mann–Whitney *U*-test (comparison between groups), or the Wilcoxon matched pair test (comparison in a group), using the Statistics 10. A *p*-value of 0.05 or less is considered to indicate a statistically significant difference for all statistical tests.

3. Results

Due to the fact that the forced ethanol model was used, there was no significant differences between groups in alcohol drinking. Ethanol consumption rate was 2.12 ± 0.23 g/kg/day in the ethanol group and 2.07 ± 0.37 g/kg/day in experimental group.

Changes were noted in the bioelectric activity of all selected brain structures after 2 weeks administration of ethanol (Fig. 1A). Changes were most pronounced in the recording from the MRF, where a significant increase in the 4–13 Hz range was observed. An increase in the 13–30 Hz band was observed in the hippocampus, and also in the 4–7 Hz range in the FC recordings.

While ethanol-induced changes were indicated in all studied brain structures after 4 weeks of alcohol administration, these were less pronounced in the FC recordings (Fig. 1B). A higher significant increase was observed in the 4–13 Hz range in the MRF compared to the previous week. A significant increase in low frequencies (0.5–4 Hz) and decreases in the 10–13 Hz and 30–45 Hz bands were observed in the Hp, while a decrease of low frequencies (0.5–4 Hz) was only noted in the FC.

After 6 weeks of ethanol administration, significant increases remained in the 4–10 Hz bands and decrease in the 30–45 Hz band were observed in the EEG recordings from the MRF (Fig. 1C). An increase in low frequencies (0.5–4 Hz) was still noted in the Hp recordings. However, the 4–7 Hz band increased while the 10–45 Hz bands decreased in the FC.

After one week of abstinence, the changes in the EEG recordings from rabbits which had previously received ethanol varied depending on the studied brain structure (Fig. 2A). In the MRF, a significant increases were still present in the 4–10 Hz bands, while the low frequency band (0.5–4 Hz) remained decreased. In the Hp, the 10–13 Hz band decreased while the low (0.5–4 Hz) and high frequency band (30–45 Hz) increased. Finally, a significant decreases in the 0.5–4 Hz and 10–13 Hz bands and an increase in the 4–7 Hz band were still observed in the FC.

In the EEG recordings from rabbits treated previously with ethanol, the most pronounced changes, compared to the 6th week of ethanol administration, were observed in the hippocampus (Fig. 2B). After the first week, a significant increases were seen in high bands (13–45 Hz), whereas, changes were visible across the entire EEG spectrum after 2 weeks. In the MRF and FC, increases in the higher frequency bands were only demonstrated after 2 weeks of abstinence.