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Research Article

Effects of Acetyl-L-Carnitine Administration on Auditory Evoked Potentials in Rats Exposed to Chronic Ethanol

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Abstract:

These days, one of the biggest issues facing public health is chronic alcohol consumption. Prolonged alcohol intake has been associated with a number of illnesses, including pancreatitis, cerebrovascular illnesses, and liver damage. It is known that the endogenous molecule acetyl-L-carnitine (ALCAR) has neuroprotective properties. The objective of this study was to examine the impact of ALCAR on auditory evoked potentials (AEP) in a model of chronic alcoholism in rats. Four groups (n = 10) were randomly formed from 40 three-month-old albino Wistar rats: Sham (Sh), chronic ethanol (CE), ALCAR administered (ALCAR), and chronic ethanol+ALCAR (CE+ALCAR). The rats were administered either distilled water, ethanol, ALCAR, or ethanol+ALCAR via gavage for a duration of 4 weeks. AEP recordings were acquired from each rat upon the conclusion of the experiment period. The amplitudes of P1N1 and N1P2, in addition to the latencies of the P1, N1, and P2 peaks, were analyzed. Our findings showed that P1, N1, and P2 latencies were considerably prolonged in the CE group compared to the Sh group. Furthermore, the amplitudes of P1N1 and N1P2 exhibited an increase in the CE group relative to the Sh group, a decline in the CE+ALCAR group in comparison to the CE group, and a subsequent return to normal levels. Finally, our results demonstrated that, at the dose and duration used here, ALCAR normalized the increased neuronal activity but had no effect on the conduction velocity of the hearing signal.

1. Introduction

Alcohol addiction is a significant issue that poses a threat to both individuals and society in the present day. With respect to the World Health Organization's 2014 Global Status Report on Alcohol and Health, roughly 3.3 million individuals die annually due to alcohol intake [1]. As per the report, the aforementioned figure surpasses the mortality rates attributed to AIDS, tuberculosis, or acts of violence. Furthermore, within the same report, it was documented that a notable proportion of global diseases and injuries, specifically 5.1%, can be attributed to the consumption of alcohol. Alcohol consumption has also been found to induce various behavioral, physiological, and cognitive

disorders in individuals [2]. Besides, chronic alcohol consumption has been shown to be associated with cardiomyopathy, cerebrovascular diseases, liver damage, hypertension, pancreatitis, and increased mortality [3]. Furthermore, alcohol consumption has been shown to cause lipid peroxidation and free radical formation in many vital organs [4]. Acetyl-L-carnitine (ALCAR) is an endogenous compound that is a pivotal component in energy metabolism, while also exhibiting neuroprotective and neurotrophic characteristics [5]. It plays crucial roles in the process of mitochondrial oxidative energy metabolism [6], making it easier for fatty acids to move from the cytosol to the mitochondria [7]. Furthermore, it facilitates the β-oxidation of fatty acids, a crucial process for ATP synthesis, and acts as a safeguard against toxicity caused by the accumulation of long-chain fatty acids [6, 7]. ALCAR exhibits antioxidant properties and serves to safeguard mitochondria against oxidative harm [8] by impeding the generation of oxidizing agents, scavenging free radicals, enhancing the defensive activities of mitochondrial antioxidants, and promoting the activation of antioxidant enzymes [7]. Furthermore, ALCAR prevents apoptosis induced by mitochondrial impairment [9]. ALCAR therapy has been reported to improve cognitive performance in Alzheimer's patients; however, no improvement was reported in some studies [10, 11]. The addition of ALCAR enhanced cognitive function and the transmission of signals between neurons in elderly rats [12, 13]. In another study, high doses of ALCAR were shown to lead to recovery after ischemia [14]. Auditory stimuli elicit morphological and functional alterations, including the proliferation of neurons that develop a sensitivity to these stimuli, enhancement of dendritic branching, facilitation of connections, and synchronization of synapses [15]. Neuroplasticity can be examined by studying the P1, N1, P2, and N2 components of Auditory Evoked Potentials (AEP) [16]. These components are produced by the electrical activity of thalamo-cortical regions in response to auditory stimulation. They have been demonstrated to be a useful tool for monitoring neurophysiological alterations in the auditory system. A study was conducted on a sample of 15 students with learning disorders and 15 healthy students to investigate the AEPs [17]. The findings indicated meaningful delays in the latencies of the N1, P2, and N2 peaks in the left ear, along with a notable decrease in the amplitude of the N2 component, among the students with learning disorders in comparison to the control group. In another study Dorman M.F. et al. [18] have reported that the latency of P1 can serve as a valuable clinical biomarker. In this study, a case report was conducted on a female individual who presented with congenital hearing loss. At the age of 5 months, the participant underwent the fitting of a hearing aid. Following a period of 7 months, cortical AEP was recorded, revealing the absence of the P1 peak. The patient, at the age of 19 months, satisfied the necessary requirements for cochlear implantation and subsequently underwent the surgical procedure to receive a cochlear implant. Cortical AEP recordings were acquired at four different time points following the implantation procedure: one week, one month, four months, and seven months. The recordings yielded a P1 peak, and the findings indicated that the latency of P1 returned to the standard range within a span of four months. The consumption of alcohol over an extended period of time has been widely recognized as a causative factor for various diseases affecting crucial organs, such as the brain. This indicates that the consumption of alcohol in large amounts may have detrimental impacts on brain functions and, consequently, on AEP. According to our literature review, no study on the effects of ALCAR on AEP in a chronic alcohol experimental model was found. Therefore, this study intended to examine the impacts of ALCAR administration on AEP in rats exposed to chronic alcohol.

2. Material and Methods

2.1 Experimental Groups

The studies conducted on rats adhered to the guidelines established by the "Local Ethics Committee for Animal Experiments" of Akdeniz University (protocol #: 1590/2023.05.006). The animals were housed under standard conditions at 23±1 °C and 50±5 % humidity, with a 12-hour circadian rhythm. Every experiment was conducted from 9:00 to 17:00. A total of forty male albino Wistar rats, aged three months and weighing between 250 and 300 grams, were housed in groups of four in stainless steel cages and given food and water ad libitum. Rats were separated equally into four groups (n=10): Sham (Sh), chronic ethanol (CE), ALCAR administered (ALCAR), and chronic ethanol+ALCAR (CE+ALCAR). Distilled water to the Sh group, 5 g/kg ethanol (25% w/v) to the CE group, 50 mg/kg ALCAR to the ALCAR group, and the same doses of ethanol+ALCAR to the CE+ALCAR group were given daily by oral gavage for four weeks.

2.2 AEP recording and analysis

Under urethane anesthesia (intraperitoneal injection, 1.2 g/kg, Sigma Aldrich, St Louis, MO, USA), AEP recordings were obtained from the

temporal region of the rat brains following the four-week experimental period. Every electrode had an impedance of under 10 kOhm. The EEG signal was converted to digital data at a sampling rate of 1000 Hz (Brainvision, Brain Products, Munich, Germany), amplified (Brainamp, Brain Products, Munich, Germany), and band-pass filtered (0.1–300 Hz). AEPs were recorded at 85 dB using 8000 Hz tones. The auditory stimulus was repeated once every hertz (Hz). The tones with an intensity of 85 dB were delivered via loudspeaker at an approximately 15 centimeters away from the rat's ear. The duration of the tones was 50 ms. 800 ms epochs (200 ms pre-stimulus/600 ms post-stimulus) were used to process the EEG data. A BrainVision Analyzer (Brain Products, Munich, Germany) was used to average the epochs. To obtain peaks in EEG waves, 100 waves were averaged. From the averaged waveforms, the latencies (in milliseconds) and peak-to-peak amplitudes (in microvolts) of the AEP components were measured for each rat. Peak latencies were measured in milliseconds from stimulus artifacts to the peaks. The latencies of P1, N1, and P2 peaks, as well as the peak-to-peak amplitudes of P1N1 and N1P2, were analyzed based on the measurements.

2.3. Statistical Analysis

The statistical analyses comparing the groups were performed with the SigmaStat® 3.5 software (Jandel Corp., San Rafael, CA). Utilizing the Shapiro-Wilk test, the normality of the quantitative data was ascertained. After conducting a Holm-Sidak post hoc test, a one-way ANOVA was applied to the data if they satisfied the normality test. If not, the Kruskal-Wallis test is utilized, and subsequently, Dunn's test is performed. A significance level of $p < 0.05$ was utilized to quantify statistical significance. Each data was expressed as a mean ± SEM.

3. Results and Discussions

The statistical evaluation of the acquired latency data revealed that the P1, N1, and P2 latencies exhibited a significant prolongation in the CE group when compared to the Sh group (Fig. 1). For these three latencies, however, no meaningful distinction existed between the CE and CE+ALCAR groups (Fig. 1). The statistical analysis of peak-to-peak amplitudes revealed that the CE group exhibited a significant increase in peak-to-peak amplitudes for P1N1 and N1P2 compared to the Sh group (Fig. 2). Furthermore, a substantial reduction in both amplitudes was determined in the CE+ALCAR group with respect to the CE group (Fig. 2).

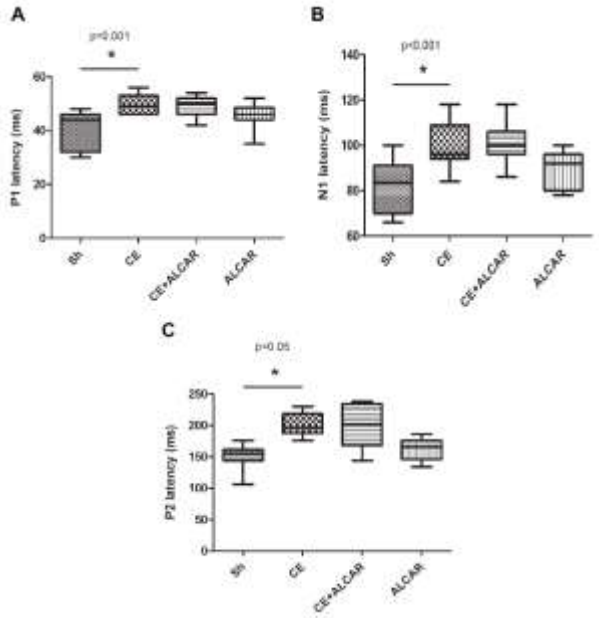

Figure 1. *Latencies of P1, N1, and P2 peaks of AEPs obtained from experimental groups. Sh: Sham, CE: Chronic ethanol, CE+ALCAR: Chronic ethanol+ALCAR, ALCAR: ALCAR administered.*

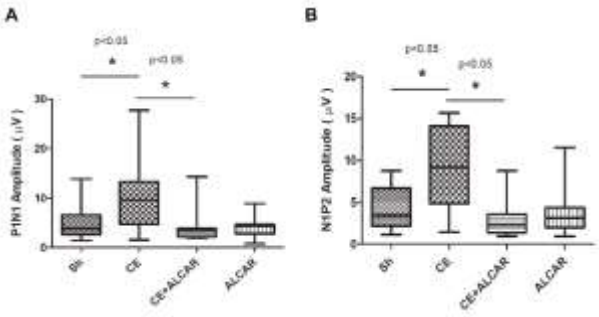

Figure 2. *P1N1 and N1P2 amplitudes of AEPs obtained from the experimental groups. Sh: Sham, CE: Chronic ethanol, CE+ALCAR: Chronic ethanol+ALCAR, ALCAR: ALCAR administered.*

The latency data of our P1, N1, and P2 peaks indicate that chronic alcoholism exerts a decelerating influence on signal transduction within the auditory pathway. Nevertheless, the data obtained from our analysis of P1N1 and N1P2 peak-to-peak amplitude indicate that chronic alcoholism is associated with increased neuronal activity in comparison to levels observed in individuals without alcohol use disorder. There is a lack of research in the literature pertaining to this particular topic. A study conducted by Cadaveira F. et al. (1992) examined the auditory evoked potentials of 32 individuals with chronic alcoholism (at least 8 years) [19]. These patients were observed after a period of 25 days of abstaining from alcohol. The study found that there was a significant increase in N2 latency and N1P2 peak-to-peak amplitude in these individuals. In a separate investigation, male rats were given 10% alcohol

mixed with drinking water over a period of three months. Subsequently, the mid-latency auditory evoked potentials were assessed. The study conducted by Floyd EA. et al. (1997) demonstrated that the latencies and amplitudes of the Na and Pa peaks (mid-latency AEP components) exhibited an increase in the group exposed to ethanol for a duration of three months, compared to the control group [20]. The findings of our investigation align with the existing body of research on the effects of long-term alcohol consumption.

The normalization of increased neuronal activity by ALCAR may be due to the antioxidant properties of ALCAR, as stated in Traina G. [7]. However, additional studies are needed to understand whether it has this effect by inhibiting the formation of oxidizing agents, scavenging free radicals, or promoting the activation of antioxidant enzymes. On the other hand, the inability of ALCAR to normalize the slowdown in signal transduction may be related to the dose administered and may be related to the interaction of ALCAR with neurotransmitters, which have important roles in signal transduction, and with membrane channel kinetics, which are very important in action potential generation.

The following can be said as limitations of our study: ALCAR can be administered at a higher dose and for a longer period of time in another study in order to observe the neuroprotective effects of ALCAR also in signal transduction. In addition, the interactions of ALCAR with neurotransmitters and membrane channels that have important roles in signal transduction can also be investigated.

4. Conclusions

In summary, the administration of ALCAR at the precise dosage and duration specified in this study resulted in the normalization of increased neuronal activity associated with chronic alcoholism. However, it did not exhibit any impact on signal conduction velocity. Additional research is required to examine the neuroprotective properties of ALCAR in the chronic alcoholism model, specifically focusing on various doses and durations.

Author Statements:

- **Ethical approval:** All the experimental animal protocols for this study were approved by the Local Ethics Committee for Animal Experiments of Akdeniz University (protocol #: 1590/2023.05.006)
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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