

Investigation of changes in somatosensory evoked potentials in rats in which chronic alcoholism was induced

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Abstract

The average of alcohol consumption per year in Korea is 14.80 liter per person which is ranked eleventh in countries that consume the most alcohol. Alcoholism causes worse quality of life as well as financial problems. In addition, liver disease, gastritis, pancreatitis, hypertension, esophagitis, diabetes and alcoholic amnesic disorder can be caused by alcohol, and both motor nerve and sensory nerve in the peripheral nervous system and the central nervous system are damaged. In our study, Group I was a control group(n=6) and Group II was an alcohol intake group. Alcoholism was induced in rats by supplying 30% alcohol for 13 months. Then, somatosensory evoked potentials were measured by electromyography, and latency and waveform of responses in the nervous system activated by somatosensory pathway were determined through a series of standardization formulas. In the result, the weight of rats in the long-term alcohol intake group was reduced compared to that of the control group. Latency and waveform of somatosensory evoked potentials measured by electromyography were extended compared to that of the normal group. Therefore, long-term and chronic alcohol intake can cause hypoxia by reduction of a metabolic rate and it suggests damage to the nerve pathway from the posterior column pathway(Group IA) of afferent nerve and the posterior of spinal cord of Group II cutaneous afferent nerve(medial lemniscus) via the thalamus to the cerebral cortex or the somatosensory region

Keywords: Somatosensory Evoked Potential, Alcohol intake, Brainstem, Latency

1. Introduction

The average of alcohol consumption per year in Korea is 14.80 liter per person which is ranked eleventh in countries that consume the most alcohol. Alcoholism can

cause financial problems, loss of the aim of life and worse quality of life. In addition, the risk of alcoholic liver disease[5,6,7], gastritis[11,13], pancreatitis[14,15], hypertension[16, 17], esophagitis[11, 13], diabetes[17] and alcoholic amnesic disorder[8, 9, 10, 12] due to alcoholism increases. In particular, among the causes of dementia, alcoholic amnesia is ranked second, following Alzheimer's disease in Korea and therefore it is definitely one of social issues. That alcohol causes many diseases is being commonly studied and its magnitude has been widely known. In the study, we used rats in which chronic alcoholism was induced, to investigate changes in somatosensory evoked potentials of electromyography. A somatic evoked potential provisionally arises due to stimulation of peripheral nerve and is recorded according to various sensory nerve pathways. It is measured by using electric impulse. Although this signal is weak, responses in the nervous system activated by somatosensory pathway are expressed as a series of waveforms. Electrical stimulation is supplied to peripheral nerve fiber to record responses. If changes in signaling of stimulation from skin surface and peripheral nerve to the central nervous system, abnormal signaling in the nervous system can be checked by objective and noninvasive method. The somatosensory evoked potential is delivered from the posterior column pathway(Group IA) of afferent nerve and the posterior of spinal cord of Group II cutaneous afferent nerve(medial lemniscus) via the thalamus to the cerebral cortex or the somatosensory region [3,4]. In general, median nerve in the human wrist or posterior tibial nerve in the human ankle is electrically stimulated, and electrodes are placed on the scalp, spine and peripheral nerve in the upper region stimulated for recording. The posterior column lemniscal pathway is anatomically a main pathway in the central nervous system. Therefore, it is meaningful to evaluate functions of the sensory nervous system objectively. Furthermore, functions of the spinal cord or brainstem can be properly evaluated, so damage to the

nervous system caused by harmful substances, such as alcohol, can be traced and changes in pathological lesions in the nervous system be identified. Stable anesthesia for rats is required when noninvasive SEP is being analyzed. Various anesthetic drugs can activation and inhibition of the nervous system and therefore we used avertin(2,2,2 tribromoethanol)[18,19] which is not classified into nicotinic drugs. We maintained rapid and stable anesthesia during a short test and minimized the effect of anesthesia on the nervous system. Then, a somatosensory evoked potential test was performed to objectively latent time in the posterior column lemniscal pathway affected by long-term alcohol intake.

2. Material and method

2.1 Animals and housing conditions

We purchased 4-week-old male Sprague Dawley rats (♂) Gyeonggi-do, Korea) between 100 and 120 g from the animal facility without pathogens. The environmental temperature was 22 ± 2 °C and the humidity was 55-60%. The night and day cycle was adjusted to 12 hours. Water and feed were provided without limitation. We tried to use the minimum number of animals and conducted tests according to the regulation of International Association for the study of Pain (IASP) to minimize pain even though we used vertebrates.

2.2 Anesthesia

The rats were anesthetized with avertin. The standard dose was 0.2 ml of working avertin solution(rat/kg), and the working solution was made by diluting stock solution(100 g 2,2,2 tribrometnanol), dissolved in 100 ml tertiary amyl alcohol) 40-fold in 0.9% NaCl. 2,2,2 tribromoethanol (97%) was purchased from Junsei(Sigma-Aldrich, Germany). Rat was measured after 5 minutes of intraperitoneal injection.

2.3 Classification of animal groups

Laboratory animal groups were classified as follows: Group I was the water intake group (control group, n=6). Group II was the alcohol intake group(30 ml/kg(rat weight), n=6). Rats were kept in a separate cage for five

days a week from February 1, 2014 to March 1, 2015. 30 mL/day of 30% alcohol, instead of drinking water, was supplied.

2.4 MEG measurement and data analysis

A rat was weighed and then completely anesthetized. An acupuncture needle(0.20 ×15 mm, DongWon Acupuncture, Sungnam, Korea) was inserted to cranial C4 and Fz of the anesthetized rat in the prone position. A stimulus of 0.8~1.0 %(by active stimulator, part no 31E15) was given to the skin surface on the front right leg at a rate of one per second a total of 200 times and standardization of waveform was recorded (natus neurology Dantec Keypoint, In., USA) [20]. Analysis equipment was Ver 5.13, S/N 5252, License jkq7 ivhc x71f (prone position, Figure 1).

Waveforms were analyzed by LabChart 7 software(AD instruments, Bella Vista, australia). In SEP analysis, the frequency was 20 uV/D and the cycle was 5 ms/D. Those were measured by high-pass filter setting of 3 Hz at 0.1 ms with a stimulus of 1 mA or lower. All data was expressed as mean±standard error and the significance of a difference between the test group and the control group was proved by ANOVA. SPSS (version 18.0) was used for statistical analysis and the significance level was $P \leq 0.05$.

3. Result

3.1 Rat weight average

The weight of the control group and the test group was compared as shown in Table 1. The weight of the test group was statistically reduced compared to the control group in the study period ($P \leq 0.05$).

3.2 Somatosensory evoked potentials (SEP)

The result of SEP is shown in Figure 2. The latency of somatosensory was extended up to about 5ms/D.

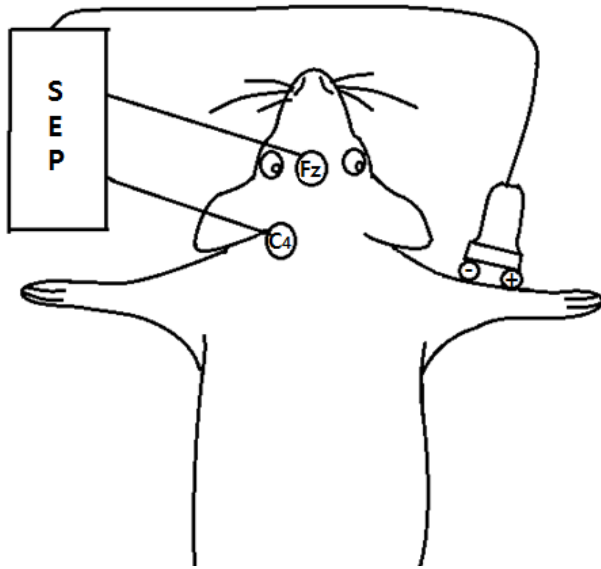


Figure. 1 Acupuncture needle electrodes are inserted subcutaneously according the the C4, Fz Somatosensory Evoked Potential scheme and stimulator are right foreleg.

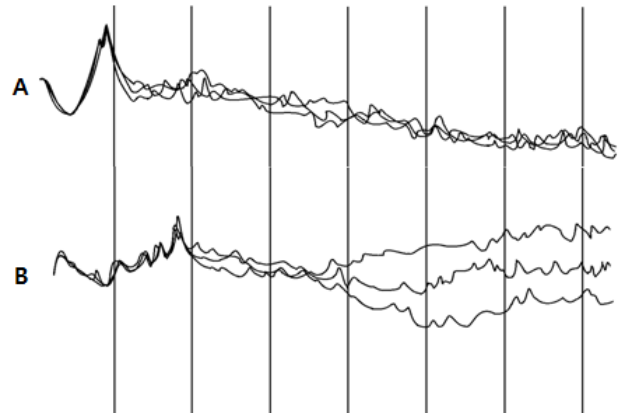


Figure. 3. Designations of the foreleg median nerve evoked somatosensory evoked potential (SEP). A: Control SEP. B: Alcohol intake SEP

Table 1 : Comparison of Body weight in the Rat

	N	mean±S.D.	S.E.
Con	3	382.9±30.78	17.77
Alcohol	3	503.3±11.47*	6.62

Data are expressed ± SD. *, $P < 0.05$ compared with control. Abbreviation: Con, control

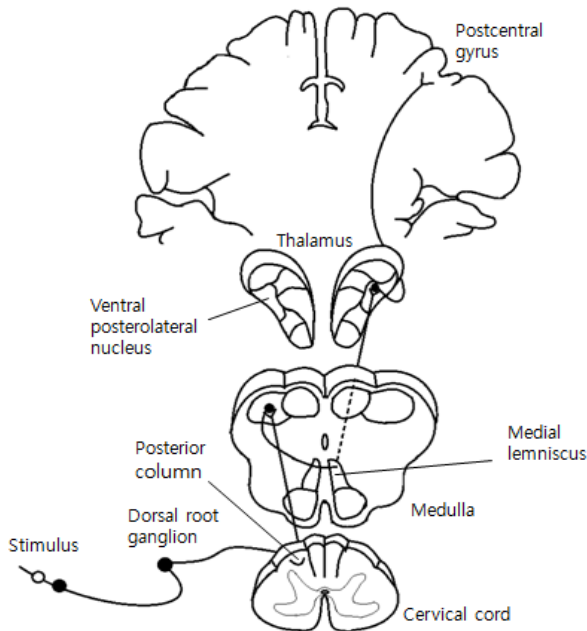


Figure. 2. Pathway for the somatosensory evoked potential(SEP). The SEP is mediated peripherally by large IA sensory fibers and centrally by the dorsal column-medial lemniscal system.

4. Conclusions

Today, people in their life are under stress. Some depend on alcohol to relieve stress, so it causes a social problem [1]. Long-term and chronic alcohol intake has been known to cause chronic metabolic disease[16,17] and alcoholic amnesia[8,9,10,12] (2). To investigate whether long-term alcohol intake induces damage to the nervous system, somatosensory evoked potentials in electromyography were analyzed. The SEP is largely mediated by large-diameter IA sensory fibers in the peripheral nerve and the dorsal column-medial lemniscal system in the Central nervous system (Figure 2) [3,4,21]. Long-term alcohol intake definitely caused weight loss (Table 1). The result accords with the precedent studies [22,23,24]. In other studies, it was confirmed that chronic alcohol intake caused damage to vital organs and, in particular, liver [5,6, 7,16,17] or renal injuries [25,26]. In the liver and kidney, the production of hemopoietin is interrupted and the inappropriate regulation of the hematopoietic system may

cause hypoxia to impair nerve cells and even fatal damage. In the somatosensory evoked potential test of electromyography, the latency in the alcohol intake group was extended. It suggests that there is a problem in nerve conduction of the nerve pathway between the IA sensory nerve pathway of the peripheral nervous system and the signaling pathway in the cerebral cortex. In the intermediate neuronal signaling pathway, signals are crossed in the spinal cord and medulla and neuronal signals delivered to the thalamus are sent to the cerebral cortex. Long-term alcohol intake induces active oxygen and inflammatory cytokines [27]. In particular, IL-6[28], IL-1 β [29] and TNF α [30] induces activation of microglia to produce insoluble protein. Eventually, deposition will cause damage to nerve cells. Thus, problems will arise in the neuronal signaling pathway. The frontal lobe which controls learning, humanity, morality and conscience has been known as an important regulating region to form personality required for human life [31,32]. FZ analysis can predict damage to the frontal lobe which causes problems in personality, such as humanity and morality. In addition, the parietal lobe in the brain is a critical region to control and activate the somatic motor control system and the comprehensive somatic motor control system. Thus, damage to the parietal lobe can cause problems in general somatic motor control due to disharmony of motility control [33]. In conclusion, long-term and chronic alcohol intake may cause problems in erythropoietic-stimulating protein regulation in the kidney and the liver to damage the hematopoietic system. Thus, it is expected that general hypoxia is caused to damage the central nervous system and peripheral nerve cells to extend the latency in the neuronal signaling pathway from nerve fibers in the peripheral nervous system to the cerebral cortex. Afterward, localization of the cerebral cortex in the peripheral nerve pathway will be necessary to investigate the region where the latency is extended.

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