

Acute Effect of Ethanol on Firing Patterns of Purkinje Cells in the Rat Cerebellar Slice Preparation

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This study examined the acute effects of ethanol (EtOH) on the firing patterns of Purkinje cells (PCs) using an intracellular recording in slice preparation of rat cerebellum. The experiments were performed in sagittal cerebellar slices (400 μ m) of adult Sprague-Dawley rats (80-100g). Ethanol was applied by a bath superfusion with a known concentration expressed as the percentage of solution by volume (v/v) at 0.1, 0.5, 1, 2, and 4%. The result of the Chi-square test illustrated that the firing patterns were altered significantly after EtOH ($p=0.007$). However, the firing patterns that were altered by EtOH application were not affected by EtOH concentration ($p=0.1296$). Among the 54 PCs tested, 30 PCs did not display any spontaneous firing activity and 24 PCs displayed spontaneous spike activity, either spiking in the simple manner ($n=14$) or cyclicly oscillating ($n=10$). In the presence of EtOH, 31 PCs were quiet, 22 PCs exhibited simple spiking activity and 1 PC continued to oscillate. Most PCs that displayed spontaneous activity before EtOH application progressively slowed their spike activity after EtOH superfusion. Especially, it was evident that 9 out of 10 oscillating PCs stopped their regular cyclic activity. In addition, 9 out of 14 PCs that displayed simple spike activity ceased to fire after EtOH application. Eleven out of 30 quiet PCs began to fire irregularly after EtOH application and this phenomenon usually occurred with membrane depolarization. EtOH induced spontaneous activity in 36.7% (11/30) of the quiescent PCs. In conclusion, there was differential EtOH sensitivity in the vitro slice preparation. EtOH depressed the endogenously generated spontaneous activity, especially the oscillatory firing activity. In contrast, the silent PCs were excited after EtOH application. Since this differential sensitivity persists in the presence of tetrodotoxin (TTX), it is suggested that this differential sensitivity is

peculiar to the PCs.

Key Words: Ethanol (EtOH), cerebellar Purkinje cells, spontaneous firing patterns

INTRODUCTION

Understanding ethanol (EtOH) actions in the brain has been complicated by the possibility that EtOH may alter neuronal activity not only through direct action on a given neuron, but also through indirect action, EtOH-induced alteration of the synaptic input to the neuron.¹⁻³ It has been reported that the cerebellum is a sensitive target for EtOH action when EtOH is administered systemically or locally.³⁻⁹ Although most studies described the depressant effect that EtOH has on the neuronal excitability of cerebellar PCs, in some instances EtOH either enhances or produces a biphasic response consisting of a transient increase followed by a stable depression.^{1,3-9}

Intracellular studies showed that PCs exhibit a variety of intrinsic conductances. These properties generate various patterns of firing activity such as a simple spike and a repetitive firing activity (oscillation).¹⁰⁻¹³ Kaczmarek and Levitan¹⁴ suggested that the electrical characteristics of a given neurons is closely related to its particular role in controlling specific physiological functions. Therefore, changes in the intrinsic properties of the firing pattern or the rate may alter the most basic mechanism of neurons such as encoding and processing sensory information or generating motor input.¹⁴ EtOH has been reported to alter the neuronal excitability and firing patterns of hippocampal pyramidal cells,⁵ molluscan neurons¹⁵ and Aplysia pacemaker neurons.¹⁶ In the

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present study, the effects of EtOH on the neuronal excitability in the cerebellar PCs were investigated using an intracellular recording in the slice preparation of a rat cerebellum.

MATERIALS AND METHODS

Experiments were performed in the sagittal cerebellar slices (350 μ m) of adult Sprague-Dawley rats (80-100g) as described previously.¹²⁻¹⁷ The slices were prepared using a vibroslice (Electron Microscopy Science, OTS-3000-04) and held at room temperature submerged in an oxygenated bath containing an artificial cerebrospinal fluid (ACSF). The ACSF consisted of (in mM) NaCl 124, KCl 5, MgSO₄ 1.15, KH₂PO₄ 1.25, NaHCO₃ 26, CaCl₂ 2.5, and glucose 10 (pH 7.4). The slices were placed for recording in an interface type chamber that was constantly superfused with ACSF and gassed with 95% O₂/5% CO₂ at room temperature.

Intracellular recordings of the PCs were performed using micropipettes formed on a micropipette puller (Flaming-Brown P-80, Sutter Instrument). The micropipette had a resistance of 60-100 M Ω when filled with 3M KCl. The neuron was current clamped with a AxoClamp 2B amplifier (Axon Instrument). Current commands, data acquisition and analyses were performed with the aid of a computer running pClamp and Axotape software (Axon Instrument). For all experiments, tetrodotoxin (TTX) was applied by microdrop onto the slice at a concentration of 0.3 μ M to block synaptic transmission and the Na⁺-dependent action potential.^{12,18,19} EtOH was applied by a bath superfusion with various concentrations (as the percentage of solution by volume of 0.1, 0.5, 1.0, 2.0, and 4.0%) to define concentration effect.^{16,20}

Only one EtOH dose was tested on each neuron.

For data analysis, the effect of EtOH on the membrane potential was classified into 4 occasions, depolarization, hyperpolarization, depolarization after hyperpolarization, and hyperpolarization after depolarization. To examine whether EtOH application altered the firing patterns significantly and the firing patterns before and after EtOH application was related, a Chi-square test was applied. In addition, whether or not the EtOH concentration affected the firing patterns was examined using ANOVA.

RESULTS

Previous results have shown that the PCs exhibit 3 distinct activities at the resting membrane potential as intrinsic properties: quiescent, regular or irregular simple spike activity and oscillatory firing activity.¹²⁻¹⁷ In this study, 54 PCs were recorded. Among them, 30 (55.6%) were quiescent, 14 (25.9%) showed irregular simple spike activity and 10 (18.5%) displayed the regular cyclic manner of oscillation (Table 1). Eleven out of 30 quiescent PCs, 9 out of 14 randomly firing PCs, and 9 out of 10 oscillating PCs exhibited an alteration in their firing patterns after EtOH application (Table 1). To determine whether such changes in firing patterns by EtOH application were statistically significant, Chi-square test was applied. The result showed that EtOH altered the PCs' firing pattern significantly ($\chi^2=9.823$, $p=0.007$).

The average resting membrane potential for the PCs that exhibited quiescent and simple spike activity was -59.4 (\pm 8.0) mV (quiescent PCs: -60.1 \pm 8.0 mV, simple spiking PCs: -57.3 \pm 7.8 mV). The membrane potentials in the oscillating PCs were in continuous fluctuation, and were re-

Table 1. The Number of PCs in Accordance with Firing Patterns before and after EtOH Application

Firing patterns before EtOH application	Firing patterns after EtOH application			
	Total # of PCs	Quiet	Simple spike activity	Oscillatory firing activity
Quiet	30	19	11	0
Spike activity	14	9	5	0
Oscillatory activity	10	3	6	1
Total # of PCs	54	31	22	1

corded in the range from -45 to -70 mV between bursting. There were no significant differences in the membrane potentials between the quiescent and simple spiking PCs ($F=0.46$, $p=0.6334$).

Effect of EtOH on quiescent PCs

The most common type of firing pattern in PCs is characterized by being quiescent, although PCs of this type can generate action potentials by injecting depolarizing pulses. EtOH superfusion with various concentrations was performed in 30 quiescent PCs (Table 1). EtOH application on 30 quiescent PCs resulted in the initiation of spontaneous activity in 11 cells (36.7%), which was usually accompanied by depolarization of the membrane potential (Fig. 1A). However, the induced spike activity was usually depressed within a few minutes of EtOH application. The rest of the PCs ($n=19$) did not initiate spike activity at all

(Fig. 1B).

EtOH application altered the membrane potential in the quiescent PCs. The average resting membrane potential of 30 quiescent PCs was $-60.1 (\pm 8.0)$ mV. After EtOH application, the membrane potentials were depolarized in 14 PCs (46.7%), hyperpolarized in 3 (10.0%), biphasically hyperpolarized and then depolarized or vice versa in 12 (40.0%) and unchanged in 1 (Table 2). Depolarization to the membrane potentials by more than 20 mV was frequently observed after EtOH application. However, the hyperpolarization was not greater than 1 to 5 mV. The effect of EtOH on the membrane potentials was produced regardless of ability of EtOH to initiate spiking activity on the quiet PCs.

Effect of EtOH on simple spiking PCs

In this study, 14 out of 54 PCs fired action

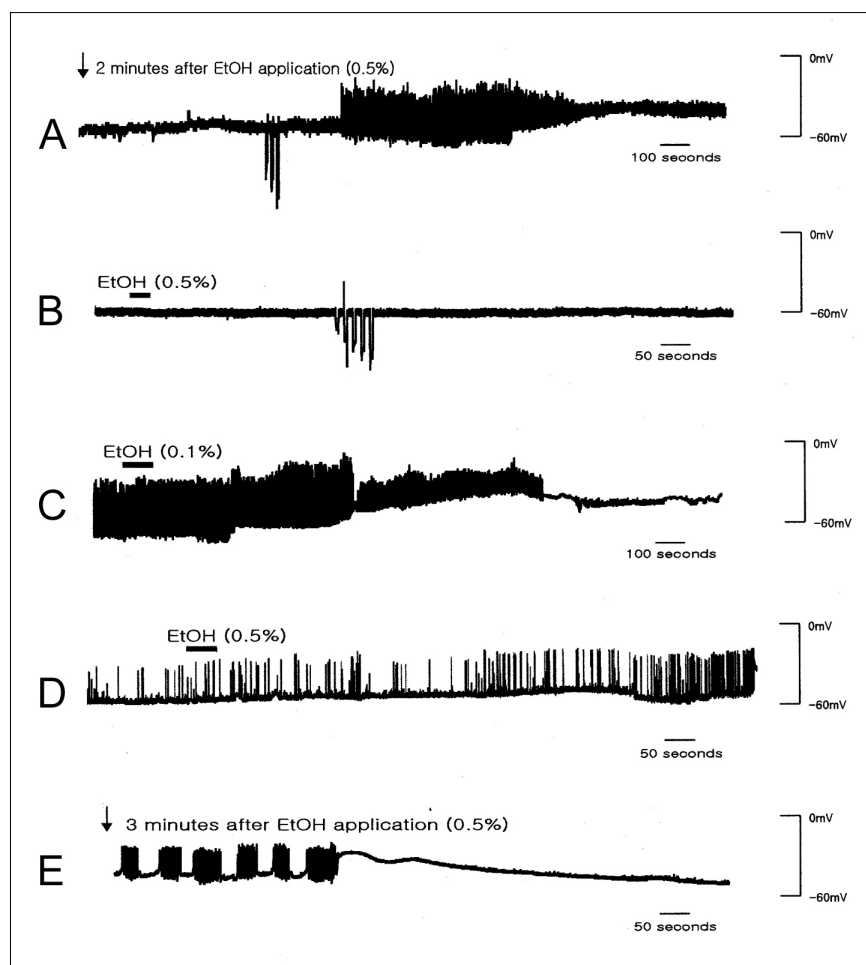


Fig. 1. Firing patterns of PCs before and after EtOH superfusion. A. Application of EtOH (0.5%) on a quiescent PC resulted in initiation of spontaneous activity and this phenomenon was accompanied with depolarization of membrane potential. Downward deflections were hyperpolarizing current test pulses. B. A PC did not initiate spike activity after EtOH application (0.5%). Downward deflections were hyperpolarizing current test pulses. C. Application of EtOH (0.5%) on a PC displayed simple spike activity resulted in decreasing firing activity and finally terminating all spontaneous activity. D. A spontaneous active PC continued to fire its activity after EtOH application (0.5%). E. After EtOH application (0.5%), cyclic manners of oscillating activities were completely stopped.

Table 2. The Number of Cells in Accordance with the Alteration of the Membrane Potentials after EtOH Application

M.P. after EtOH Firing patterns (# of PCs)	↑	↓	Biphasically (↑ ↓ or ↓ ↑)	No change	Can not determined
Quiescent (30)	14	3	12	1	0
Simple spike activity (14)	9	1	1	2	1
Oscillatory activity (10)	7	1	1	0	1
Total (54)	30	5	14	3	2

M.P.: Membrane potential.

↑: Depolarized.

↓: Hyperpolarized.

potentials regularly or irregularly (Table 1). Applying EtOH to 14 PCs resulted in a reduction in firing activity and finally terminating all spontaneous activity in 9 PCs (64.3%, Fig. 1C) although 5 PCs continued to fire spontaneously (Fig. 1D). The average resting membrane potential of the simple spiking PCs prior to EtOH application was $-57.3 (\pm 7.8)$ mV. After EtOH application, the membrane potential was depolarized in 9 PCs (64.3%), hyperpolarized in 1 (7.1%), biphasically hyperpolarized and then depolarized in 1 (7.1%) and was unchanged in 2 (14.3%) (Table 2). Usually, the membrane depolarization occurred at greater than 20 mV.

Effects of EtOH on oscillating PCs

After EtOH application, the cyclic manners of the oscillating activity was stopped completely (9 out of 10 PCs) (Fig. 1E, Table 1). This effect was obvious even at the membrane potential, which was still in the range of the oscillatory firing activity. The membrane potential was temporarily altered after EtOH application: depolarized in 7 PCs (70%), hyperpolarized in 1 (10%) and biphasically hyperpolarized and then depolarized in 1 (10%) (Table 2). The oscillating PCs predominantly depolarized after EtOH application in a similar way to the quiescent and simply spiking PCs. Likewise, the degree of depolarization was frequently over than 20 mV.

Whether the effect of EtOH on the PC firing pattern depended on the concentration of EtOH was examined. The ANOVA results showed that the firing patterns after EtOH application were not affected by EtOH concentration ($F=2.83$, $p=$

0.1296). Table 1 shows the firing patterns before and after EtOH application as discussed above in detail. Using this data, we examined whether there was a relationship between the firing patterns before and after EtOH application. However, the result of the Chi-square test showed that there was no relationship between the firing patterns before and after EtOH application ($\chi^2=2.990$, $p=0.224$).

DISCUSSION

The firing pattern of a neuron is determined by the mechanism that is intrinsic to its own membrane and by the synaptic inputs received from other.¹⁴ Such changes in the pattern and the firing rate may be brought either by altering the amount of transmitters released presynaptically or by modulating specific ion conductances postsynaptically. On the other hand, changes in the firing pattern may produce alterations in the neuronal response such as encoding and processing sensory information or generating motor input.¹⁴ It has been suggested that different patterns of electroresponsiveness in thalamocortical neurons reflect different functional states of the sleep-wake cycle.²¹ Many studies have examined the effects of EtOH on neurons in various regions of the brain. The results of these studies show a considerable degree of variability in the observed response.⁴ This has been due in part to differences in the EtOH dose, the routes of administration and animal species.⁴ Other studies on Purkinje neurons *in vivo* and *in vitro* have reported that the excitatory effect was generally linked to low EtOH

doses and the inhibitory effect was linked to high EtOH doses but both effects were not observed consistently.⁵ The main interest of this study was in the acute effects of EtOH on different basal firing patterns of PCs. However, it is not possible to identify the specific conductances involved with the data currently available.

Among the 54 PCs tested, 30 did not display spontaneous firing activity and 24 displayed spontaneous spike activity, either randomly spiking ($n=14$) or cyclicly oscillating ($n=10$). In the presence of EtOH, 31 PCs were quiet, 22 showed randomly spiking activity and 1 continued to oscillate. Most PCs that displayed spontaneous activity before EtOH application progressively slowed their spike activity after EtOH superfusion. In particular, it was quite obvious that 9 out of 10 oscillating PCs stopped their regular cyclic activity. They either continued to fire spike activity irregularly or terminated all spontaneous activity. In addition, 9 out of 14 PCs that displayed irregular spike activity ceased to fire completely after EtOH application and the remaining 5 cells also slowed their firing activity. All these alterations in the PCs firing patterns induced by EtOH were not correlated with EtOH concentration, and there was no relationship between the firing patterns before and after EtOH application.

The endogenously generated spontaneous activity was mediated by a voltage sensitive mechanism intrinsic to PCs, including Na^+ , Ca^{2+} and K^+ conductance.¹⁰⁻¹² However, the mechanism underlying the generation of spontaneous activity, especially the oscillatory firing activity in the presence of TTX was Ca^{2+} - and K^+ -dependent.^{5,12} Several investigators have suggested that EtOH inhibits the voltage-activated calcium current directly^{5,22} and this might explain the depressive effects on spontaneously active cells, especially on the oscillatory firing PCs. It has been reported that $I_{\text{K}(\text{Ca})}$ is very sensitive to EtOH. However, voltage clamp studies demonstrated that EtOH increases $I_{\text{K}(\text{Ca})}$ in one study, decreases or does not affect $I_{\text{K}(\text{Ca})}$ in others.^{1,5,15,18} The reason for this discrepancy is unknown. In this study, the main focus was on the firing pattern alteration by EtOH. The sensitivity of the conductances (I_{Ca} , $I_{\text{K}(\text{Ca})}$, I_{A}) to EtOH was not assessed directly.

On the other hand, 11 out of 30 quiet PCs began

to fire irregularly after EtOH application, which usually occurred with membrane depolarization. EtOH induced spontaneous activity in 36.7% of quiescent PCs. However, statistical analysis revealed that this effect was not related to concentration of EtOH superfused. It has been proposed that EtOH increases the neuronal firing rate in other neurons such as ventral tegmental area²³ and substantia nigra.²⁴

EtOH caused changes in the membrane potential. Although depolarization was the main response, hyperpolarization, biphasic hyperpolarization followed by depolarization or vice versa and unresponsiveness were also observed after EtOH application. Depolarization to the membrane potentials more than 20 mV was frequently observed after EtOH application and this might be produced by the more dominant effect of EtOH on the I_{K} .¹⁵ Hyperpolarization occurred in some cells, but usually not greater than 1 to 5 mV. However, it has been also reported that EtOH concentrations as large as 150-200 mM had no detectable effect on the resting membrane potential of the pyramidal neurons in the hippocampal slice preparation.¹

In conclusion, we have demonstrated that the differential EtOH sensitivity persists in the *in vitro* slice preparation of rat brain. EtOH depressed the endogenously generated spontaneous activity, especially the oscillatory firing activity. On the contrary, the silent PCs were excited by EtOH application. It is unlikely that the diverse effects of EtOH were determined by the concentration or the resting membrane potentials. Since this differential sensitivity persists in the presence of TTX, it is suggested that this differential sensitivity is intrinsic to the Purkinje cells.

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