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Blockade of the Hyperpolarization-activated Inward Current by Ethanol in Cerebellar Purkinje Cells

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ABSTRACT

This study was performed to examine the acute effect of ethanol (EtOH) on the hyperpolarization-activated inward current (I_h), one of the currents that is considered to control the excitability and firing patterns of Purkinje cells (PCs) using an intracellular recording method in slice preparation of rat cerebellum. The result showed that the amplitude of depolarizing sag and rebound depolarization, which have been known to be mediated by I_h, were reduced in 68.8% and 66.7% of PCs tested, respectively. In a voltage clamp study, a slowly activating inward relaxation was also decreased with EtOH application in 57.1% of PCs tested.

Key words: Ethanol, hyperpolarization-activated inward current (Ih), cerebellar purkinje cells, rebound depolarization

INTRODUCTION

The cerebellar Purkinje cell (PC) is one of the most sensitive targets of ethanol (EtOH) action (Urrutia and Groul, 1992; Lin et al., 1994; Netzeband and Groul, 1995; Groul et al., 1997). Although most studies show a depressant effect of EtOH on excitability, in some instances EtOH enhanced or produced a biphasic response consisting of a transient increase followed by a stable depression in PCs (Siggins et al., 1987; Urrutia and Groul, 1992; Lin et al., 1994; Netzeband and Groul, 1995; Groul et al., 1997; Freund and Palmer, 1997). With regard to the EtOH effect on ionic currents, it has been demonstrated that EtOH inhibits the voltage-activated calcium current and calcium-activated potassium current (Moore et al., 1990; Urrutia and Groul, 1992; Widmer et al., 1998). However, other voltage-clamp studies show an increment or no effect of EtOH on the calcium-activated potassium current (Siggins et al., 1987). It has been suggested that such considerable variability in the responses may be due to differences in EtOH doses, administration methods, and animal species (Basil et al., 1983).

PCs display distinct firing activity with a variety of intrinsic conductance, and these properties generate various patterns of firing activity (Llinas and Sugimori, 1980a; Llinas and Sugimori, 1980b; Chang et al., 1993; Raman and Bean, 1997). It has been considered that hyperpolarization-activated inward current (I_h) controls the excitability and firing pattern in PCs (Chang et al., 1993; Kapoor et al., 1988).

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We demonstrated that EtOH altered the spontaneous firing patterns of PCs significantly in a previous study (Seo and Suh, 2001). Even though EtOH was consistently demonstrated to alter spontaneous firing activities by influencing calcium and calcium-activated potassium currents, there were few attempts to examine the possible effect of EtOH on I_h in PCs. Therefore, this study was performed to examine the effect of EtOH on I_h in PCs.

MATERIALS AND METHODS

Experiments were performed in cerebellar slices (350μm) of male rats (*Sprague-Dawley*, 80～110 g) as described previously (Chang et al., 1993; Seo et al., 1999; Seo and Suh, 2001). The cerebellar vermis was sliced using vibroslice (Electron Microscopy Science, OTS-3000-04). Slices were constantly superfused with artificial cerebrospinal fluid (ACSF) and gassed with 95% O_2/5% CO_2. The ACSF consisted of (in mM) NaCl 124, KCl 5, $MgSO_4$ 1.15, KH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.5, and glucose 10 (pH 7.4). For the intracellular recording, micropipettes were prepared using micropipette pullers (P-80, Sutter Instrument) and their resistance was 60-100 MΩwhen filled with 3 M KCl. The PCs were current-or voltage-clamped with a bridge or switching clamp circuit (AxoClamp 2B, Axon Instrument).

For all experiments, TTX was applied onto the slice at the concentration of 0.3μM to block synaptic transmission and the Na^+-dependent action potential (Moore et al., 1990; Aubry et al., 1991; Chang et al., 1993). EtOH was applied by a bath superfusion as the percentage of solution by volume (0.5%) in the present study. In our previous study, various concentrations of EtOH (0.05, 0.1, and 0.5%) were used to evaluate the concentration effect of EtOH on firing patterns (Seo and Suh, 2001). The result of the previous study showed that the firing patterns were not affected by EtOH concentration. In addition, the PCs superfused with 1, 2, and 4% of EtOH were easily ruptured when injected current and voltage pulses during the current- and voltage-clamp study. In the present study, therefore, 0.5% of EtOH was used (Seo and Suh, 2001). Only one dose of EtOH was tested on each neuron.

For data analysis, pClamp and Axotape program (Axon Instruments) were used. I_h was determined by the depolarizing sag and rebound depolarization in the membrane voltage responses during hyperpolarizing pulses (-0.2, -0.4, -0.6, -0.8, -1.0 nA) as shown in Fig. 1A. In the voltage clamp study, the amplitude of I_h was measured by the difference between steady-state and instantaneous currents which were activated during hyperpolarizing voltage steps (-30, -60, -90, -120, -150, -180 mV) from a holding potential for 1 second (Fig. 2A). The amplitudes of I_h before and after EtOH application were compared with the difference I-V curve obtained by subtracting steady-state and instantaneous I-V relations.

RESULTS

PCs, which exhibited the typical electrophysiological properties, were recorded in the present study (Llinas and Sugimori, 1980a; Llinas and Sugimori, 1980b; Chang et al, 1993). I_h was measured using current- and voltage-clamp methods before and after EtOH application. It has been previously presented that the voltage- and time-dependent depo-

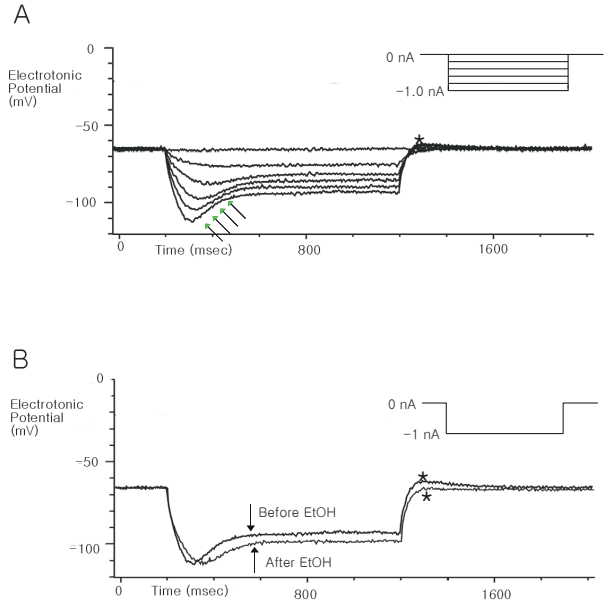

Fig. 1. A: Hyperpolarizing current pulses evoked voltage- and time-dependent depolarizing 'sags' (arrows) in the current clamp. B: Application of EtOH (0.5%) resulted in decreasing the amplitude of sag (bottom trace). Also note the decreased rebound depolarization (*) after EtOH application.

larizing sag during hyperpolarizing current injection was governed by I_h, and completely abolished by Cs^+ (Chang et al., 1993). Other investigators reported that the rebound depolarization was also mediated by I_h (Spain et al., 1987; Foehring and Water, 1991). Therefore, we measured the depolarizing sag and rebound depolarization as indicators for the presence and amplitude of I_h in the current clamp mode.

Fig. 1A shows prominent sags (indicated by arrows) in the voltage response of the membrane during hyperpolarizing current injection. This time- and voltage-dependent sag, which corresponded to I_h, were reduced by EtOH (0.5%) application in 11 out of 16 PCs (68.8%) in the present study (Fig. 1). In the remaining 5 PCs, the depolarizing sag was either increased (n=2) or not changed (n=3) after EtOH application. Such an effect of EtOH was observed regardless of the firing patterns of PCs.

In addition, EtOH decreased the rebound depolarization in 6 out of 9 PCs (66.7%, Fig. 1B). The rebound depolarization (indicated by *) was induced by hyperpolarizing current pulse injection and appeared to be voltage-dependent. On the other hand, the rebound depolarization was increased in 1 PC and not changed in 2 PCs after EtOH application.

In the voltage clamp experiment, the amplitude of a slowly activating inward current relaxation (represented as I_h) that was induced by hyperpolarizing voltage steps was calculated as the difference between the instantaneous and steady state currents (Fig. 2A). Therefore, the difference I-V curves were obtained by subtracting the instantaneous I-V relation from steady state I-V relation (Fig. 2B). Among 14 PCs tested, 8 PCs (57.1%) showed

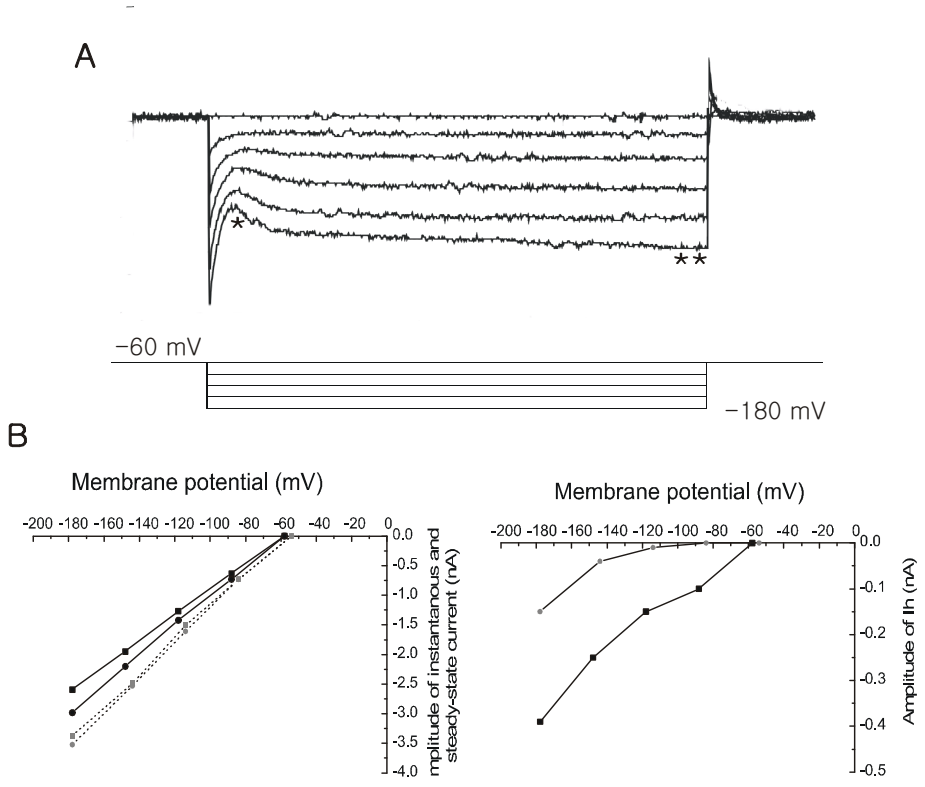

Fig. 2. A: Hyperpolarizing voltage steps elicited slow inward currents which could be measured by the difference between the instantaneous (*) and steady-state currents (**) (top trace). The duration of each hyperpolarization pulse was 900 msec. B: The voltage clamp I-V relationships derived from a PC shown in the Fig. 3B. The holding potential of this PC was -60 mV. Left: the instantaneous (■) and steady-state (●) current before (solid line) and after (dot line) EtOH (the same voltage steps and time scales as shown in Fig. 2A were used). Right: the difference curve (represents the amplitude of I_h) of the instantaneous and steady-state currents (I_{diff}= I_{ss}-I_{inst}) before (■) and after (●) EtOH.

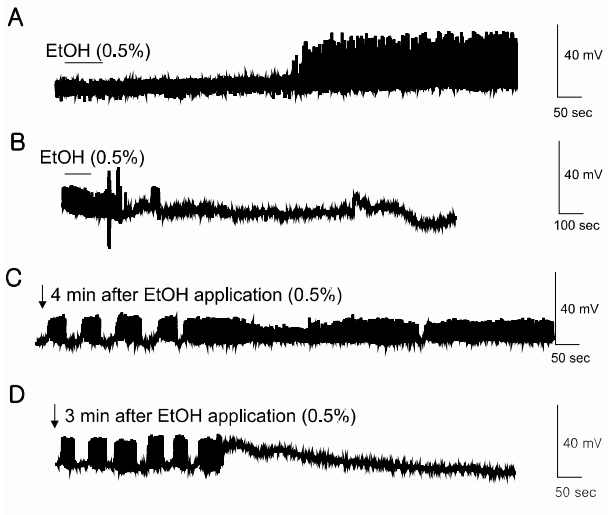

Fig. 3. A: Application of EtOH (0.5%) on a quiescent PC resulted in initiation of spontaneous activity. B: Application of EtOH (0.5%) on a PC displayed simple spike activity resulted in decreasing firing activity and finally terminating all spontaneous activity. C & D: After EtOH application (0.5%), cyclic manners of oscillating activities were converted to simple spiking activities (C) or completely stopped to fire (D).

decrease in the amplitudes of I_h after EtOH application (0.5%) regardless of the firing patterns of PCs (Fig. 2). Of the remaining 6 PCs, I_h was either increased (n=2) or not changed (n=4) by EtOH application.

DISCUSSION

Our previous studies showed that EtOH application altered the firing patterns of PCs: initiated firing activities in quiescent PCs, suppressed firing activities in randomly spiking PCs, and terminated cyclic manners in oscillating PCs (Seo and Suh, 2001). In addition, the firing patterns after EtOH application were not significantly affected by EtOH concentrations (Seo and Suh, 2001). Such an alteration in firing patterns after EtOH application was observed in the present study, too (Fig. 3). The effect of EtOH on firing patterns seemed to be generated by modulating intrinsic properties of PCs since TTX, a synaptic blocker, was applied to all PCs tested in the present and previous studies (Moore et al., 1990; Aubry et al., 1991; Chang et al., 1993). One of the currents, which have been considered to determine excitability and firing patterns in PCs, is the I_h (Kapoor et al., 1988; Chang et al., 1993; Raman and Bean, 1997). The presence of I_h has been proposed to underlie, in part, the low membrane potential and their ability to fire spontaneously. This time- and voltage-dependent inward rectifier, I_h, is reported to be a major determinant for firing patterns in thalamic relay neurons as well as in PCs (McCormick and Pape, 1990; Chang et al., 1993). The result of our previous study that EtOH altered the firing patterns raises a question whether EtOH exerts an effect on I_h. Therefore, the amplitudes of I_h before and after EtOH application were measured and compared in the present study.

The prominent voltage- and time-dependent sag and rebound depolarization mediated by I_h were clearly reduced after EtOH application in the present study (Spain et al., 1987; Foehring and Water, 1991; Chang et al., 1993). The voltage clamp study also showed that the amplitude of I_h was decreased after EtOH application. We have observed that the spontaneous firing activity, either simple or oscillatory firing activity, was suppressed and the membrane potential was hyperpolarized after EtOH application in the present and previous studies (Seo and Suh, 2001). Such an effect might be due to blockade of I_h by EtOH application. In thalamocortical relay cells, reducing I_h also resulted in abolishment of pacing activity and hyperpolarized the cells (Harris et al., 1994). Similarly, a point to be considered was that T-type Ca^{2+} channels also trigger the rebound depolarization (Aizenman and Linden, 1999), therefore, further study is necessary to clarify the effect of EtOH on rebound depolarization via I_h.

The non-inactivating, voltage-dependent K^+ current, called the M-current, which also creates an inward relaxation, was of some concern because of its similarity to I_h (Moore et al., 1990). However, we considered that the suppressive effect of EtOH on inward relaxation was not mediated by M-current since inward relaxation recorded in this study was best observed at membrane potentials more negative than resting membrane potential, clearly outside the M-current activation range (Moore et al., 1990). It has been shown that the calcium-activated potassium current ($I_{K(Ca)}$) and voltage-dependent Ca^{2+} current are very sensitive to EtOH and are reduced by EtOH in various neurons (Urrutia and

Groul, 1992; Widmer et al., 1998). This might also affect the spontaneous firing activities of PCs.

Taken together with our previous study results, the differential EtOH sensitivity persisted in the endogenously generated spontaneous activity in PCs, and this effect appeared to be generated by modulating intrinsic properties of PCs since it persisted in the presence of TTX. We demonstrated that, in addition to its effects on the voltage-dependent calcium and calcium-activated potassium current shown in other studies, EtOH also decreased I_h, and this might contribute, in part, to the alteration of the firing patterns of PCs.

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