Ethanol reduces hyperpolarization-activated cation currents (I_h) recorded in hippocampal CA3 pyramidal neurons: implication on the inhibitory action on neuronal excitability

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It is widely accepted that ethanol (EtOH) may produce a broad spectrum of pharmacological and behavioral effects through its interaction with a number of membrane proteins as well as by interfering with many signal transduction mechanisms. While a large plethora of experimental data indicates the inhibitory GABAergic system as one of the most sensitive target for EtOH's central actions, other ligand- and voltage-dependent ion channels may also be involved in the action for this drug. The hyperpolarization-activated cation current (I_h), often referred as pacemaker current, has been suggested as being important for generating specific neuronal activities in different brain regions as well as in specific sub-regions of the hippocampal formation, contributing to the neuronal resting membrane potential and action potential (AP) discharge (Robinson and Siegelbaum, 2003). These I_h currents are present in both hippocampal GABAergic interneurons as well as pyramidal neurons of CA1 and CA3 sub-regions. It has been recently reported that EtOH, in a concentration-dependent manner, increases the firing rate of hippocampal GABAergic interneurons through the positive modulation of I_h currents (Yan et al., 2009), an effect that may lead to an increase of GABA release from presynaptic terminals in both CA1 and CA3 sub-regions (Sanna et al., 2004, Galindo et al., 2005). Since robust I_h currents are also present in CA3 glutamatergic neurons (Cobb et al., 2003), we here investigated the action of EtOH on I_h in order to evaluate the sensitivity of these cation channels to this drug and its implication on the excitability of these cells. Whole-cell patch-clamp experiments were conducted in acute hippocampal coronal slices obtained from young (PND 15-30) male Sprague-Dawley rats. Current-clamp recordings revealed an I_h-induced sag when hyperpolarizing currents were injected. Bath application of CsCl (5 mM) completely antagonized the I_h-currents evoked in CA3 pyramidal neurons. Perfusion of EtOH (20 – 80 mM) inhibited in a concentration-dependent and reversible manner the amplitude I_h-currents. Moreover, the inhibition of I_h-currents by EtOH was independent by the membrane potential. Furthermore, EtOH, but not CsCl, perfusion, markedly decreased the frequency of APs in response to injection of depolarizing currents, suggesting that this effect is independent on I_h-current inhibition. Given that EtOH can act through a modulation of the GABAergic transmission, we evaluated whether the effect of EtOH on both I_h-currents and AP firing could involve the interaction with $GABA_A$ receptors. Bath application of the GABA antagonist bicuculline (20 μM) completely abolished the inhibitory effect of EtOH on evoked AP firing, but did not alters the inhibitory effect on I_h currents, suggesting that this latter effect is independent on the GABAergic system. In summary, these data demonstrate, for the first time, that EtOH reduces the excitability of hippocampal CA3 pyramidal neurons not only facilitating the function of GABAergic synapses, but also by an inhibitory action on I_h currents. Because the effect of EtOH on I_h-currents occurs at concentrations of the drug similar to those acting at other membrane receptor and ion channels, it is conceivable that such effect may contribute to the overall central effects of EtOH.

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Cobb et al. (2003). Neuropharm. 44, 293-303.
Galindo et al. (2005). J Neurochem. 94, 1500-1511.
Robinson and Siegelbaum. (2003). Annu. Rev. Physiol. 65, 453-80.
Sanna et al. (2004). J Neurosci. 24, 6521-6530.
Yan et al. (2009). J Neurophysiol. 101, 67-83.