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SHORT COMMUNICATION

Acetaldehyde at Clinically Relevant Concentrations Inhibits Inward Rectifier Potassium Current I_{K1} in Rat Ventricular Myocytes

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Summary

Considering the effects of alcohol on cardiac electrical behavior as well as the important role of the inward rectifier potassium current I_{K1} in arrhythmogenesis, this study was aimed at the effect of acetaldehyde, the primary metabolite of ethanol, on I_{K1} in rat ventricular myocytes. Acetaldehyde induced a reversible inhibition of I_{K1} with IC_{50} = 53.7±7.7 μM at –110 mV; a significant inhibition was documented even at clinically-relevant concentrations (at 3 μM by 13.1±3.0 %). The inhibition was voltage-independent at physiological voltages above –90 mV. The I_{K1} changes under acetaldehyde may contribute to alcohol-induced alterations of cardiac electrophysiology, especially in individuals with a genetic defect of aldehyde dehydrogenase where the acetaldehyde level may be elevated.

Key words

Acetaldehyde • Arrhythmias • Inward rectifier • I_{K1} inhibition • Rat ventricular myocytes

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Alcohol intoxication may induce electrocardiographic changes (Aasebø *et al.* 2007, Cameli *et al.* 2009), arrhythmias (Haissaguerre *et al.* 1984, Kodama *et al.* 2011), and even the sudden cardiac death (Templeton *et al.* 2009). Effects of ethanol on the cardiac action potential (AP) configuration and most of the pivotal ionic membrane currents in cardiomyocytes are also known (Williams *et al.* 1980, Habuchi *et al.* 1995, O'Leary 2002, Bébarová *et al.* 2010). In our recent study, we described the effect of ethanol at clinically relevant concentrations on the inward rectifier potassium current I_{K1} in rat ventricular myocytes (Bébarová *et al.* 2014).

Acetaldehyde, the primary metabolite of ethanol, is of particular importance being several times more toxic than ethanol itself (Brien *et al.* 1983). Acetaldehyde was shown to cause AP prolongation in both canine Purkinje fibres (Williams *et al.* 1980) and guinea pig ventricular myocytes (Chen *et al.* 1999). In contrast, AP shortening was observed in bullfrog atrial cells (Chen *et al.* 2012). In addition, Chen *et al.* (1999, 2012) reported an increase of calcium current at high concentrations of acetaldehyde (above 100 μM) while potassium currents including I_{K1} were not affected. Since both genetic and pharmacological modifications of I_{K1} and I_{K1} heterogeneity are known to play an important role in the pathogenesis of arrhythmias (Piao *et al.* 2007, Sekar *et al.* 2009, Tristani-Firouzi and Etheridge 2010), we decided to examine I_{K1} changes at clinically relevant acetaldehyde concentrations in rat ventricular myocytes.

Experiments were performed on cardiomyocytes enzymatically isolated from right ventricles of adult male Wistar rats. The dissociation procedure, solutions, and electrophysiological measurements were as previously described (Bébarová *et al.* 2014). Acetaldehyde (Sigma-Aldrich) was added to the Tyrode solution to obtain a final concentration of 0.3-300 μM. The whole cell patch-clamp technique in current clamp and voltage clamp mode was employed to record APs and I_{K1}, respectively. Experiments were carried out at 23±1 °C. I_{K1} was evaluated as the current sensitive to 100 μM $BaCl_2$ at the end of a 500-ms

pulse to voltages between –130 and 0 mV (expressed as the current density in pA/pF). Potential contaminating currents were inhibited by 2 mM $CoCl_2$, 50 mM tetraethylammonium chloride, 1 μM atropine, and 10 μM glybenclamide (Sigma-Aldrich). The results are presented as means ± SEM from n cells; $P<0.05$ was considered statistically significant. The results were corrected for the junction potential by –10 mV. Experiments were carried out with respect to recommendations of the European Guidelines on Laboratory Animal Care; the experimental protocol (MSMT-29203/2012-30) was approved by the Local Committee for Animal Treatment at Masaryk University, Faculty of Medicine.

Figure 1 illustrates the significant inhibitory effect of acetaldehyde on I_{K1} at –110 mV. The effect was fully reversible during the subsequent wash-outs, even after exposure to the higher concentrations of 30 and 100 μM (Figs 1A and 1B; the experimental protocol: a 500-ms hyperpolarizing test pulse to –110 mV from the holding potential of –85 mV was preceded by a 15-ms prepulse to –50 mV to inactivate sodium current). I_{K1} was evaluated as the Ba^{2+} sensitive current. As shown in Figure 1C, a small Ba^{2+} insensitive component of the current was resistant even at 100 μM acetaldehyde (–0.23±0.08 pA/pF at acetaldehyde vs. –0.24±0.08 pA/pF in control, at –110 mV; n=5; $P>0.05$). The insensitivity of this component to acetaldehyde was verified in the voltage range between –130 and 0 mV (data not shown). The concentration dependence of the acetaldehyde effect on I_{K1} at –110 mV is shown in Figure 1D. The pooled data were approximated by the Hill equation; the resulting inhibitory concentration at 50 % inhibition (IC_{50}) was 53.7±7.7 μM (Hill coefficient n_H=0.8±0.1). A significant inhibition was documented even at clinically relevant concentrations (13.1±3.0 % inhibition at 3 μM; n=16).

Both the development and wash-out of the acetaldehyde effect on I_{K1} showed a single exponential time course. The resulting time constants were virtually identical at 3 and 100 μM acetaldehyde (the development of the effect: 24.5±3.5 s at 3 μM and 24.2±2.4 s at 100 μM; n=5 and 12, respectively; the wash-out: 41.5±3.6 s at 3 μM and 39.2±4.2 s at 100 μM; n=6 and 12, respectively; $P>0.05$ for both).

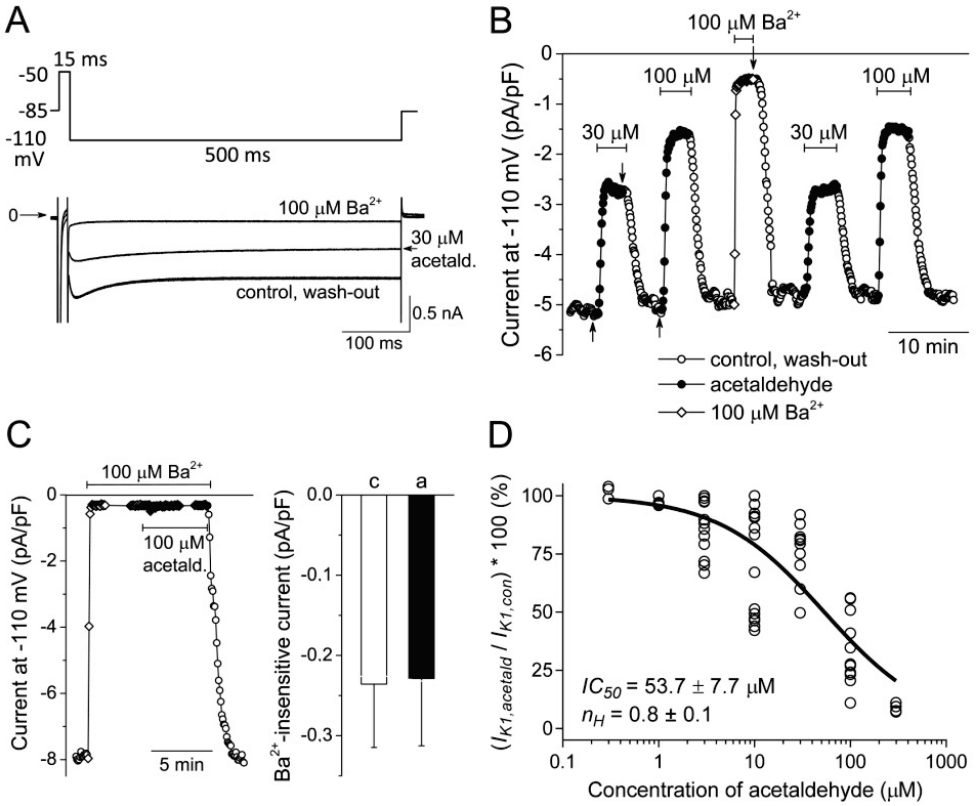

Fig. 1. Effect of acetaldehyde on inward rectifier potassium current I_{K1}. **A**: The experimental protocol (applied at 0.2 Hz) and representative current traces in control, under 30 μM acetaldehyde and after its wash-out, and under specific I_{K1}-inhibiton (100 μM Ba^{2+}). **B**: Time course of the representative experiment (partly shown in A). Arrows indicate the time of recording of traces shown in A. **C**: Effect of 100 μM acetaldehyde in the presence of 100 μM Ba^{2+}. A representative time course of the recorded current (left panel) and its evaluation (n=5, right panel) before (**c**) and after addition of acetaldehyde (**a**). **D**: Concentration dependence of acetaldehyde effect on I_{K1} evaluated as the Ba^{2+}-sensitive current (n=3-16).

Figure 2 illustrates the effect of 100 μM acetaldehyde on AP (A) and the average current-voltage relationship of I_{K1} in control conditions and during exposure to acetaldehyde (n=5; B). AP duration measured at 90 % repolarization (APD_{90}) was prolonged by 6 %. A significant acetaldehyde-induced reduction of I_{K1} was observed over the entire voltage range that was examined. At physiological voltages (above –90 mV), I_{K1} was reduced by ~20 and 60 % at 10 and 100 μM acetaldehyde, respectively (C).

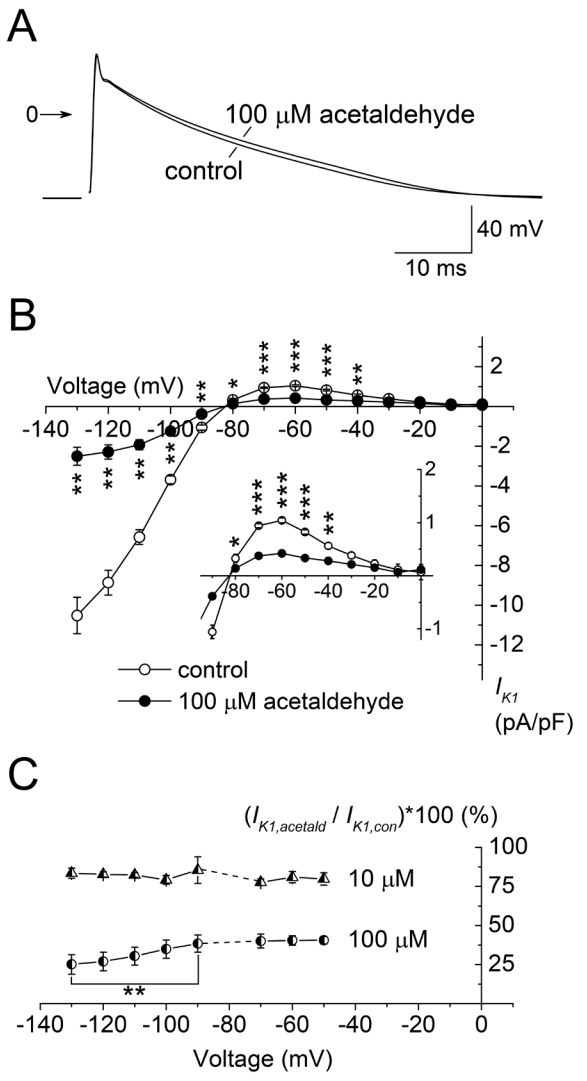

Fig. 2. Effect of acetaldehyde on action potential (AP) configuration and current-voltage relations of I_{K1}. **A**: AP recorded in control conditions and under 100 μM acetaldehyde (the experimental protocol: 0.5-ms suprathreshold current pulses of 4-10 nA at 1 Hz). **B**: Current-voltage relationship of I_{K1} (Ba^{2+}-sensitive current) in control and under 100 μM acetaldehyde (n=5). The experimental protocol was as in Figure 1A; the voltage level of 500-ms pulses varied in 10-mV steps between the −130 to 0 mV; *, **, *** – statistical significance at P<0.05, 0.01, 0.001, respectively. Inset: changes of the physiologically relevant portion of I_{K1} in detail. **C**: Voltage dependence of relative changes of I_{K1} induced by 10 and 100 μM acetaldehyde; ** – statistical significance at P<0.01.

In this study, we have documented a significant and reversible inhibitory effect of acetaldehyde, the primary metabolite of ethanol, on ventricular I_{K1} for the first time. In previous studies analyzing the effect of acetaldehyde on cardiac cells (Chen *et al.* 1999, 2012), much higher concentrations of acetaldehyde were used (500 and 1000 μM) when compared to the present study. In spite of that, the authors did not observe changes of I_{K1} in poikilothermic bullfrog atrial cells (Chen *et al.* 2012) and even in guinea pig ventricular cells (Chen *et al.* 1999). We do not have a simple explanation for this discrepancy, however, differences in species and tissue types must be considered. The cardiac I_{K1} channels are homo- or heteromeric tetramers composed of different *Kir2.1*, *Kir2.2*, and *Kir2.3* isoforms with various proportions. The varying subunit composition of I_{K1} channels may lead to differing drug sensitivity, even in atria and ventricles of the same heart (Dhamoon *et al.* 2004, Zhang *et al.* 2013, Gómez *et al.* 2014).

Clinically relevant blood concentrations of acetaldehyde after low alcohol consumption (the alcohol dose of ~0.3 g/kg) vary between ~1.5 and 6.5 μM in people with an adequate activity of aldehyde dehydrogenase (Di Padova *et al.* 1986, Jones *et al.* 1988). In our experiments, we observed a significant reduction of I_{K1} even at these clinically relevant concentrations of acetaldehyde (Fig. 1D, Fig. 2C). Importantly, in humans with genetic polymorphisms in aldehyde dehydrogenase (often present in the Asian population), the blood acetaldehyde levels may be much higher (30-100 μM and even more; Harada *et al.* 1983, Yoshida 1992). Hence, the observed acetaldehyde-induced changes of I_{K1}, if present to a similar extent in human cardiomyocytes, may indeed contribute to the reported alterations of cardiac electrophysiology related to alcohol intoxication.

Conflict of Interest

There is no conflict of interest.

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