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 $IC_{50} = 53.7 \pm 7.7 \mu M$ at $-110 \text{ mV}$; a significant inhibition was documented even at clinically-relevant concentrations (at 3 $\mu M$ by $13.1 \pm 3.0 \%$). The inhibition was voltage-independent at physiological voltages above $-90 \text{ mV}$. 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 (Haisaguerre 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 $\mu M$) 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 $\mu M$. 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$\pm1^\circ\text{C}$. $I_{K1}$ was evaluated as the current sensitive to 100 $\mu M$ 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 CoCl₂, 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 Ba²⁺ sensitive current. As shown in Figure 1C, a small Ba²⁺ 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).
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).

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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