Acetaldehyde at clinically relevant concentrations inhibits inward rectifier potassium current I_{K1} in rat ventricular myocytes

Markéta Bébarová, Peter Matejovič, Milena Šimurdová, Jiří Šimurda

Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Short title: Acetaldehyde inhibits rat ventricular I_{K1}

Short communication

Corresponding author: Assoc. Prof. MUDr. Markéta Bébarová, Ph.D., Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; tel. +420-549493147, bebarova.lfmu@centrum.cz

Summary

Considering the effects of alcohol on cardiac electrical behaviour 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

Main body of the text

Alcohol intoxication may induce electrocardiographic changes (*e.g.* Aasebø *et al.* 2007, Cameli *et al.* 2009), arrhythmias (*e.g.* Haissaguerre *et al.* 1984, Kodama *et al.* 2011), and even sudden cardiac death (*e.g.* 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 (*e.g.* 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 (*e.g.* 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 ± S.E.M. 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 (No. 4-11-06-2012) 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 $n_H = 0.8 \pm 0.1$). 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 analysing 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 (*e.g.* 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 (*e.g.* 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; *e.g.* 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.

Acknowledgments. Authors thank to Prof. P. Bravený and Dr. D.M. Johnson for reading the manuscript and valuable comments, and to Mrs. Vyoralová for excellent technical assistance. This work was supported by the grant project NT14301-3/2013 from the Departmental Program for Research and Development of the Ministry of Health of the Czech Republic.

References

AASEBØ W, ERIKSSEN J, JONSBU J, STAVEM K: ECG changes in patients with acute ethanol intoxication. *Scand Cardiovasc J* **41**: 79-84, 2007.

BÉBAROVÁ M, MATEJOVIČ P, PÁSEK M, OHLÍDALOVÁ D, JANSOVÁ D, ŠIMURDOVÁ M, ŠIMURDA J: Effect of ethanol on action potential and ionic membrane currents in rat ventricular myocytes. *Acta Physiol (Oxf)* **200**: 301-314, 2010.

BÉBAROVÁ M, MATEJOVIČ P, PÁSEK M, ŠIMURDOVÁ M, ŠIMURDA J: Dual effect of ethanol on inward rectifier potassium current I_{K1} in rat ventricular myocytes. *J Physiol Pharmacol* **65**: 497-509, 2014.

BRIEN JF, LOOMIS CW: Pharmacology of acetaldehyde. *Can J Physiol Pharmacol* **61**: 1-22, 1983.

CAMELI M, BALLO P, GARZIA A, LISI M, PALMERINI E, SPINELLI T, BOCELLI A, MONDILLO S: Acute effects of low doses of red wine on cardiac conduction and repolarization in young healthy subjects. *Alcohol Clin Exp Res* **33**: 2141-2146, 2009.

CHEN FS, MOMOSE Y, YAMAMURA S, OKABE F, HATAKEYAMA N, YAMAZAKI M, MASUDA A, ITO Y: Effects of acetaldehyde on membrane potentials and ionic currents in single cardiac myocytes. *Pharm Pharmacol Commun* **5**: 565-570, 1999.

CHEN FS, SATOH Y, IDE Y, SUGANO T, IIMURA M, MOMOSE Y, TSUCHIDA K, TAGAMI M: Effects of acetaldehyde on action potentials and Ca2+ currents in single atrial myocytes from the bullfrog. *Pharmacology* **90**: 216-222, 2012.

DHAMOON AS, PANDIT SV, SARMAST F, PARISIAN KR, GUHA P, LI Y, BAGWE S, TAFFET SM, ANUMONWOSM JMB: Unique Kir2.x properties determine regional and species differences in the cardiac inward rectifier K^+ current. *Circ res* **94**: 1332-1339, 2004.

DI PADOVA C, ALDERMAN J, LIEBER CS: Improved methods for the measurement of acetaldehyde concentrations in plasma and red blood cells. *Alcohol Clin Exp Res* **10**: 86-9, 1986.

GÓMEZ R, CABALLERO R, BARANA A, AMORÓS I, DE PALM SH, MATAMOROS M, NÚŇEZ M, PÉREZ-HERNÁNDEZ M, IRIEPA I, TAMARGO J, DELPÓN E: Structural basis of drugs that increase cardiac inward rectifier Kir2.1 currents. *Cardiovasc Res* **104:** 337-346, 2014.

HABUCHI Y, FURUKAWA T, TANAKA H, LU L, MORIKAWA J, YOSHIMURA M: Ethanol inhibition of Ca^{2+} and Na^{+} currents in the guinea-pig heart. *Eur J Pharmacol* **292**: 143-149, 1995.

HAISSAGUERRE M, FLEURY B, LE METAYER P, GABINSKI C, CONRI C, COUZIGOU P, WARIN JF, BLANCHOT P: Arrhythmia in relation to acute alcoholic intoxication in the absence of obvious cardiopathy. *Presse Med* **13**: 1723-1726, 1984.

HARADA S, AGARWAL DP, GOEDDE HW, TAKAGI S: Blood ethanol and acetaldehyde levels in Japanese alcoholics and controls. *Pharmacol Biochem Behav* **18**: 139-140, 1983.

JONES AW, NEIMAN J, HILLBOM M: Concentration-time profiles of ethanol and acetaldehyde in human volunteers treated with the alcohol-sensitizing drug, calcium carbimide. *Br J Clin Pharmacol* **25**: 213-221, 1988.

KODAMA S, SAITO K, TANAKA S, HORIKAWA C, SAITO A, HEIANZA Y, ANASAKO Y, NISHIGAKI Y, YACHI Y, IIDA KT, OHASHI Y, YAMADA N, SONE H: Alcohol consumption and risk of atrial fibrillation: a meta-analysis. *J Am Coll Cardiol* **57**: 427-436, 2011.

O'LEARY ME: Inhibition of HERG potassium channels by cocaethylene: a metabolite of cocaine and ethanol. *Cardiovasc Res* **53**: 59-67, 2002.

PIAO L, LI J, MCLERIE M, LOPATIN AN: Transgenic upregulation of IK1 in the mouse heart is proarrhythmic. *Basic Res Cardiol* **102**: 416-428, 2007.

SEKAR RB, KIZANA E, CHO HC, MOLITORIS JM, HESKETH GG, EATON BP, MARBÁN E, TUNG L: IK1 heterogeneity affects genesis and stability of spiral waves in cardiac myocyte monolayers. *Circ Res* **104**: 355-364, 2009.

TEMPLETON AH, CARTER KL, SHERON N, GALLAGHER PJ, VERRILL C: Sudden unexpected death in alcohol misuse--an unrecognized public health issue? *Int J Environ Res Public Health* **6**: 3070-3081, 2009.

TRISTANI-FIROUZI M, ETHERIDGE SP: Kir2.1 channelopathies: the Andersen-Tawil syndrome. *Pflugers Arch* **460**: 289-294, 2010.

WILLIAMS ES, MIRRO MJ, BAILEY JC: Electrophysiological effects of ethanol, acetaldehyde, and acetate on cardiac tissues from dog and guinea pig. *Circ Res* **47**: 473-478, 1980.

YOSHIDA A: Molecular genetics of human aldehyde dehydrogenase. *Pharmacogenetics* **2**: 139-147, 1992.

ZHANG L, LIU QH, LIU CF, ZHAI XW, FENG QL, XU RL, CUI XL, ZHAO ZQ, CAO JM, WU BW: Zacopride selectively activates the Kir2.1 channel via a PKA signaling pathway in rat cardiomyocytes. *Science China. Life Sciences*, **56**:788-796, 2013.

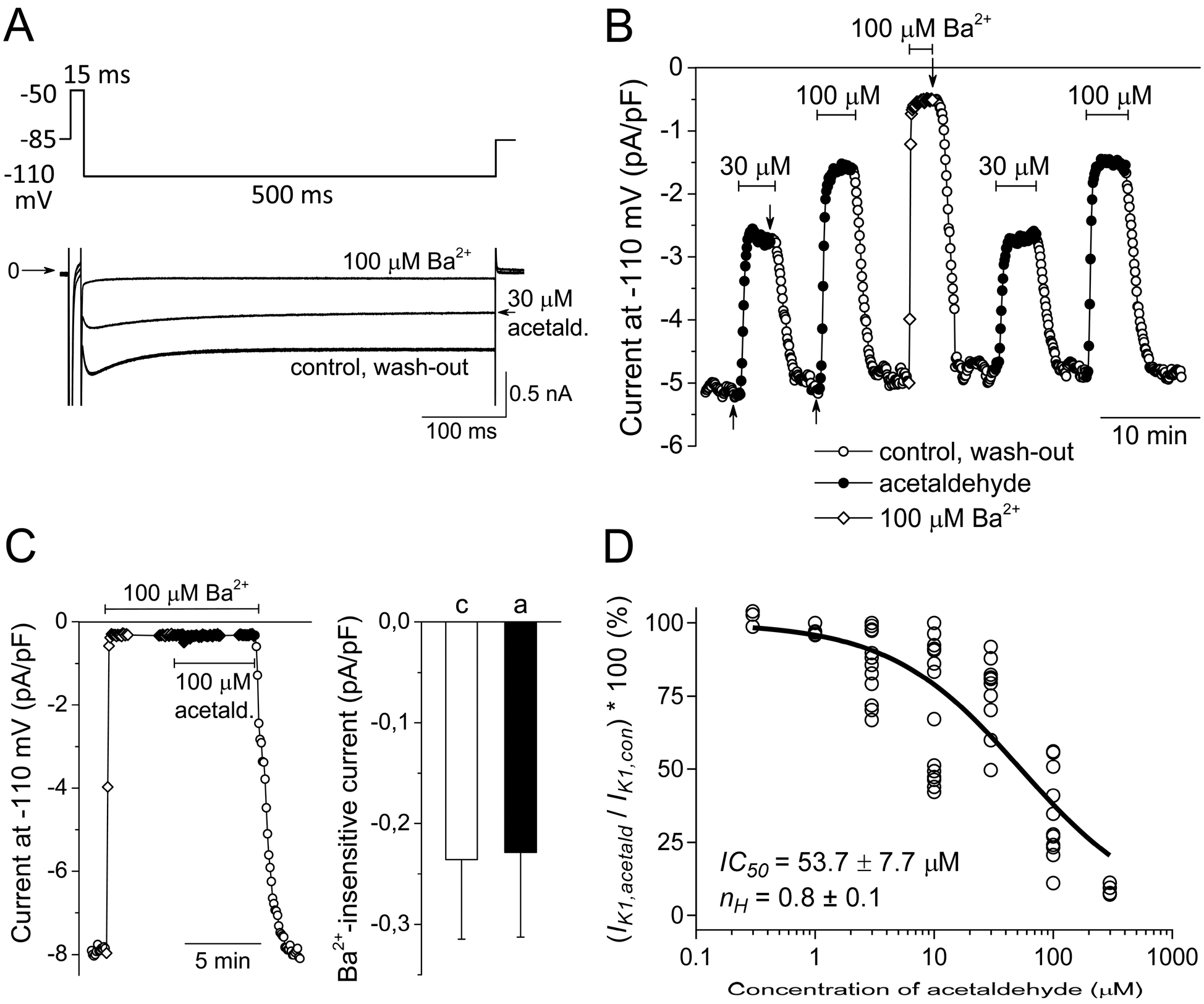

Fig. 1. Effect of acetaldehyde on inward rectifier potassium current I_{K1}. *A:* The experimental protocol (applied at 0.2 Hz) and representative current traces in control, under 30 μM acetaldehyde and after its wash-out, and under specific I_{K1}-inhibiton (100 μM Ba^{2+}). *B:* Time course of the 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).

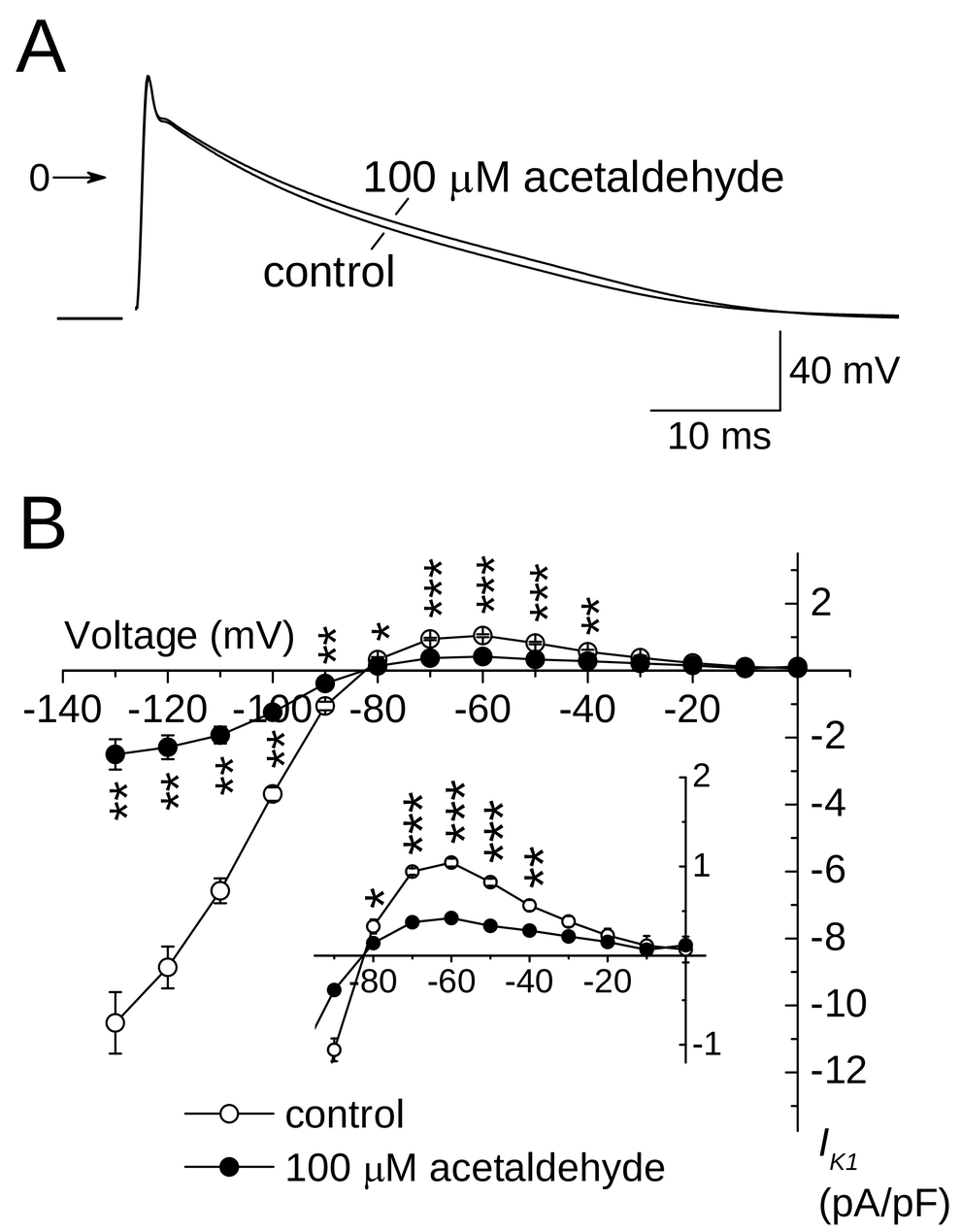

Fig. 2. Effect of acetaldehyde on action potential (AP) configuration and current-voltage relations of I_{K1}. *A:* AP recorded in control conditions and under 100 μM acetaldehyde (the experimental protocol: 0.5-ms suprathreshold current pulses of 4-10 nA at 1 Hz). *B:* Current-voltage relationship of 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.