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Effect of Ethanol on Tracheal Potassium Channels Reconstituted into Bilayer Lipid Membranes

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Summary

We examined the effect of ethanol on single potassium channels derived from plasma membranes of bovine tracheal smooth muscles. The observed potassium channels had a conductance of 296±31 pS (mean ± S.D.) in symmetrical 250 mmol/l KCl solutions, and exhibited a voltage- and Ca^{2+}-dependence similar to BK_{Ca} channels. Ethanol at 50, 100 and 200 mM concentrations increased the probability of open potassium channels to 112±5, 127±7 and 121±13% (mean ± S.E.M.), respectively. It is suggested that increased activity of the BK_{Ca} channels by ethanol hyperpolarizes the plasma membrane and thus may contribute to relaxation of tracheal smooth muscle.

Key words

Potassium channel • Trachea - Ethanol • Bilayer lipid membrane • Bronchodilation

Introduction

A bronchodilator ability of ethanol in constricted airways has been recognized for a long time (Ayres and Clark 1983a). Using electromechanical recording techniques, it was observed that ethanol induced hyperpolarization and relaxation in spastic airway smooth muscle preparation *in vitro* (Richards *et al.* 1989). Intravenous alcohol also induced bronchodilation in asthma (Ayres and Clark 1983b) and *in vitro* reduced the reactivity of isolated airway preparations, when they were subjected to electrical field stimulation (Schreiber and Slapke 1991). However, the mechanism of bronchodilation is not fully understood.

Therefore, we investigated here the effect of ethanol on potassium channels derived from plasma membranes of bovine tracheal smooth muscle. We have observed that ethanol increased activity of the potassium channels what may contribute to its bronchodilatory effects.

Methods

Chemicals were from Sigma and the lipids were supplied by Avanty Polar Lipids. Microsomes from bovine tracheal smooth muscle, prepared according to Gaburjakova *et al.* (1999), were provided by courtesy of Dr. J. Schlossmann (Technical University, Munich, Germany).

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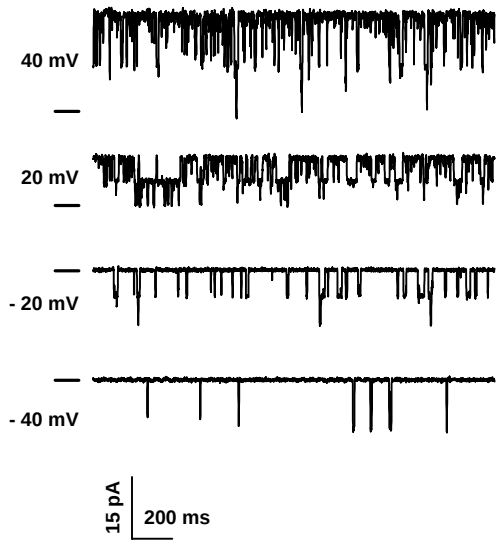

Fig. 1. *Single channel traces of two potassium channels in a bilayer lipid membrane measured at different voltage. The left lines mark closed state of the channels. The traces were measured in symmetric 250/250 mmol/l KCl solutions.*

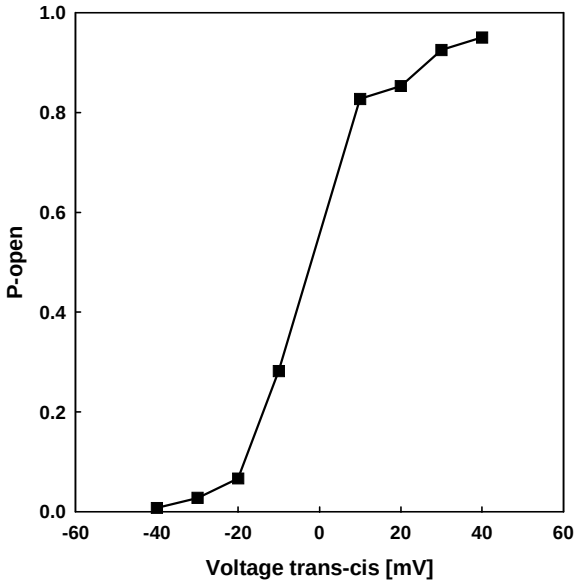

Fig. 2. *The voltage dependence of open probability (P-open) of the potassium channel in a bilayer lipid membrane.*

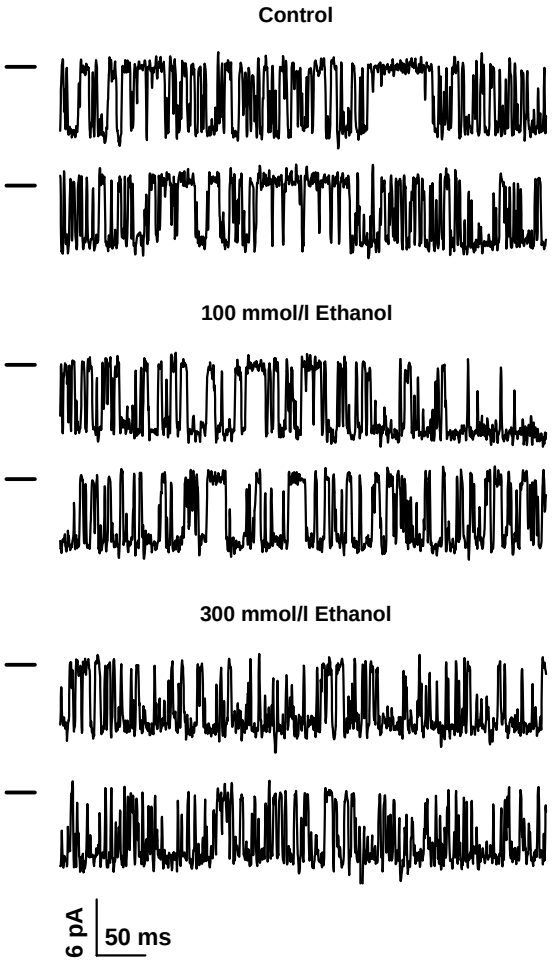

Fig. 3. *The effect of ethanol on the single potassium channel current. The left lines mark closed state of the channel. The current was measured at 0 mV in 250/50 mmol/l KCl asymmetric solutions.*

Bilayer lipid membranes (BLM) were formed over an apperture (diameter 0.1-0.2 mm) separating the *cis* and *trans* chambers, using a mixture of phosphatidylcholine and phosphoethanolamine at a molar ratio of 7:3 in n-decane (20 mg/ml), similarly to the method used in a previous study (Gaburjakova *et al.* 1999). The composition of solutions in *cis* and *trans* chambers (in mmol/l) was KCl 250, Hepes 10, Tris 5, EGTA 0.1, $CaCl_2$ 0.01, (350 nmol/l free Ca^{2+}; Schoenmakers *et al.* 1992).

The microsomes were fused into the BLM, using 250/50 mmol/l KCl gradient, from the *cis* chamber, which corresponds to the extracellular side and the *trans* chamber to the cytoplasmic side of the channel. The single channel current was measured using an amplifier (Axoclamp 1C, Axon Instruments, Foster City, USA), a converter (Labmaster) and acquisition software

(PClamp5, Axon Instruments). The single channel data were filtered at 1 kHz, digitized at 4 kHz and stored in an IBM-compatible computer. Data for figures were filtered at 200 Hz. Since the channel activity ran down in some cases, the open probability of the channels was identified for 1 min before and after ethanol addition by 50 % threshold analysis using program pStat (Axon Instruments, Foster City, USA). During the 50 % threshold analysis, channels were taken as open, when the channel current was higher than the 50 % of the single channel current. Ethanol effect (in percentage) was normalized using the formula

Effect [%] = 100*[(E-C)/(1-C)]

where C is open probability in control and E is the effect after addition of ethanol.

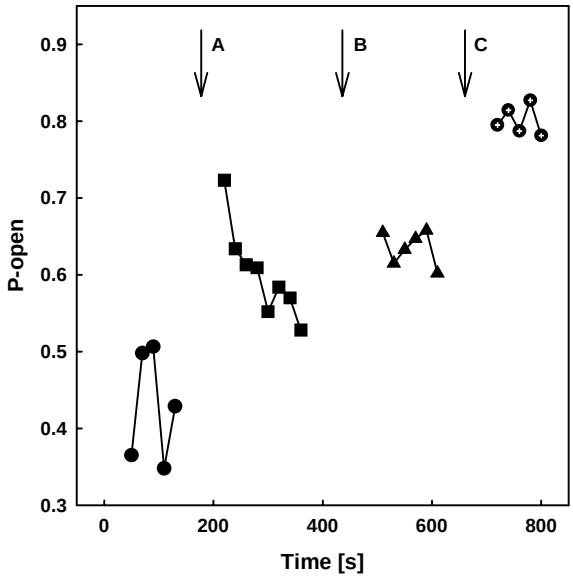

Fig. 4. *The time-dependent effect of ethanol on the open probability (P-open) of the potassium channel. P-open is the average of 20-sec intervals. Arrows indicates the addition of ethanol to the cis chamber of bilayer lipid membrane. Final concentration of ethanol: 100 mmol/l (A), 300 mmol/l (B) and 500 mmol/l (C).*

Results

Microsomes were incorporated into BLM using a 250/50 mmol/l KCl gradient. After the incorporation, the selectivity of channels was determined by applying membrane voltage ± 30 mV. In the case that channel was permeable only for K^+, the *trans* concentration of KCl was increased to 250 mmol/l to prevent further fusion.

Traces of single K^+-channels at different membrane voltages are shown in Figure 1. The BLM contained two K^+-channels. Their activity depended on the applied voltages. The open probability of the channels increased at positive voltages (Figs 1 and 2) and when Ca^{2+} concentration was increased in *trans*-cytoplasmic side (data not shown). The K^+-channel conductance at symmetric 250 mmol/l KCl was 296±31 pS (mean ± S.D., n=5). Effect of ethanol on potassium channel was studied using asymmetric 250/50 and symmetric 250/250 mmol/l KCl solutions in BLM chambers. Results were not dependent on the solutions. An example of the effect of ethanol on single K^+-channel activity is depicted in Figure 3. The K^+-channel activity increased in the presence of ethanol (Fig. 4). The increase of open probability at 50, 100 and 200 mM ethanol concentrations was to 112±5, 127±7 and 121±13% (mean ± S.E.M.), respectively (Fig. 5). Ethanol had no effect on the conductance of the channels (Fig. 6).

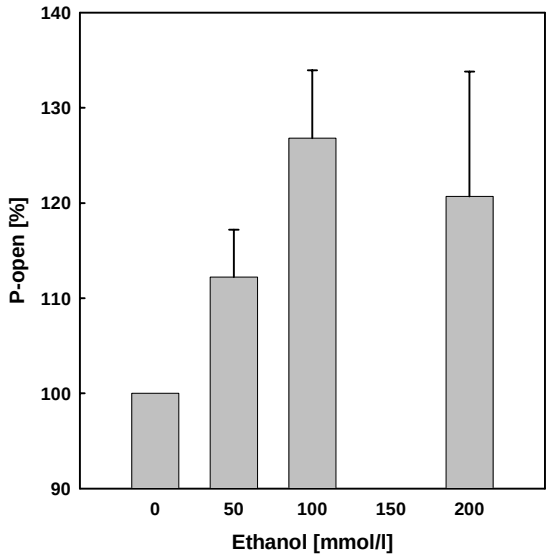

Fig. 5. *Concentration dependence of ethanol on open probability (P-open) in percentage of the potassium channel.*

Discussion

Conductance, voltage and Ca^{2+} dependence of the K^+-channels observed in our study are similar as reported for large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels. Garcia-Calvo *et al.* (1994) purified the BK_{Ca} channels from tracheal smooth muscle, which had a conductance of 235 pS at symmetric 150 mmol KCl and were voltage- and Ca^{2+}-dependent. Dumoulin *et al.* (1998) also reported 280 pS conductance of BK_{Ca} channels at symmetric 250 mmol KCl from bovine airway smooth muscles. Therefore, we suggest that the channels observed in our study are BK_{Ca} channels from plasma membrane of tracheal smooth muscle.

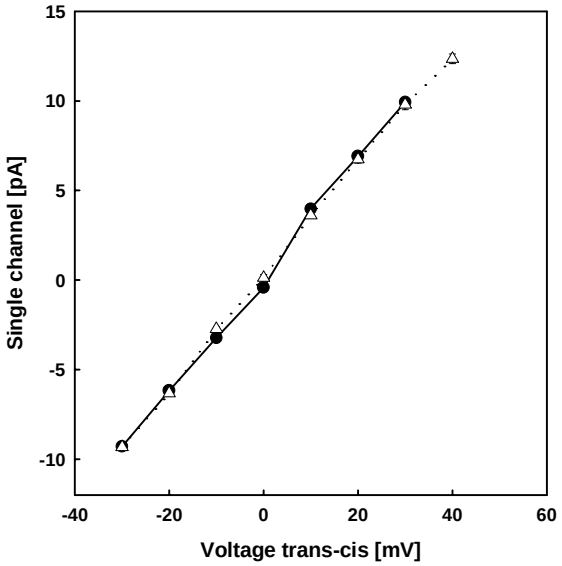

Fig. 6. *Unitary current-voltage relationship of the potassium channel at symmetric 250 mmol/l KCl solutions (filled circles) and in the presence of 100 mmol/l ethanol (open triangles).*

Ethanol influences multiple ion channels and there is growing evidence that it interacts directly with specific sites on ion channels (Harris 1999). Ethanol was reported to increase the activity, but not the conductance of BK_{Ca} channels isolated from neurohypophyseal terminals (Dopico *et al.* 1996, 1998) and from skeletal muscle T-tubule membranes (Chu *et al.* 1998). Recently Walters *et al.* (2000) observed an inhibitory effect of ethanol on the bovine aortic smooth muscle BK_{Ca} channels using 5-20 mM concentrations.

In the present study we found that ethanol increased activity of BK_{Ca} channels derived from tracheal smooth muscle membranes without affecting the channel conductance. The increased channel activity may cause repolarization or hyperpolarization of tracheal cells and this may contribute to the ethanol-induced bronchodilation.

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Reprint requests

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