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Effect of the general anesthetic halothane on the activity of the transverse tubule Ca^{2+}-activated K^+ channel

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The effect of the general anesthetic halothane on the activity of the rat skeletal muscle Ca^{2+}-activated K^+ channel in planar lipid bilayers was investigated. Halothane concentrations in the clinical range (1.0–0.2 mM) alter the regulation of the channel by both Ca^{2+} and membrane potential. At Ca^{2+} concentrations between 10 and 250 μM and membrane potentials between 0 and −30 mV, halothane significantly decreases the open state probability without changing the channel conductance. The results demonstrate that halothane can act directly on the Ca^{2+}-activated K^+ channel or its lipid environment to alter the channel gating kinetics.

Ca^{2+}-activated K^+ channel; General anesthetic; Halothane; Transverse tubule

1. INTRODUCTION

The mechanism by which general anesthetics alter excitable membranes to cause loss of consciousness has not been established. Because the relative potency of general anesthetics is proportional to their ability to partition into oil or into lipid bilayers [1,2], it has been proposed that anesthetics alter signal transmission by excitable membranes by altering membrane fluidity or some other physical property of the membrane. Tas et al. [3] and Pancrazio et al. [4] demonstrated that halothane inhibits the Ca^{2+}-activated K^+ channel in rat glioma C6 cells and adrenal chromaffin cells respectively. Here we describe the effects of halothane on the rat skeletal muscle Ca^{2+}-activated K^+ channel inserted into a synthetic planar lipid bilayer.

2. MATERIALS AND METHODS

Transverse tubule vesicles were prepared from rat skeletal muscle by a modification of the procedure developed by Barchi and co-workers [5] as previously described [6]. The vesicles were stored in 0.4 M sucrose, 10 mM histidine (pH 7.0) at −70°C. Planar lipid bilayers were formed using the procedure and apparatus described by Meissner [7]. A 40 mg/ml phospholipid stock solution containing phosphatitylethanolamine, phosphatitylcholine, phosphatitylserine (purified from bovine brain by Avanti Polar-lipids Inc.) at a ratio of 1:0.75:0.25 (wt:wt:wt) in decane was used to form the lipid bilayers. Ca^{2+}-activated K^+ channels were incorporated into the planar lipid bilayer by fusion of transverse tubule vesicles (1–5 μg protein/ml) as described by Latorre and co-workers [8]. The *cis* chamber (chamber to which the vesicles are added) contained 200 mM KCl, 1.0 mM $CaCl_2$, 10 mM Tris-HEPES, pH 7.0, while the *trans* chamber contained 50 mM KCl, 10 mM Tris-HEPES, pH 7.0. The membrane potential of the bilayer was clamped to the desired voltage and the current measured with a L/M EPC7 Patch clamp (Medical Systems Corp., Greenvale, NY). The data was collected at a rate of 20,000/s. The current distribution was fitted to the Gaussian distribution function to determine the mean current, standard deviation, and the probability of being at each conductance level. In the analysis of the gating kinetics, the transfer from one conductance level to another was defined as a change in the current within one standard deviation of the new conductance level for at least 0.25 ms. The membrane potential refers to the *trans* chamber relative to the grounded *cis* chamber. Negative currents are in the same direction as positive charges moving from the *cis* to *trans* chamber. Halothane and Ca^{2+} were applied to the bilayer by perfusion of the *cis* chamber with solutions containing the desired halothane and Ca^{2+} concentrations. The chambers were covered with Parafilm to minimize halothane evaporation.

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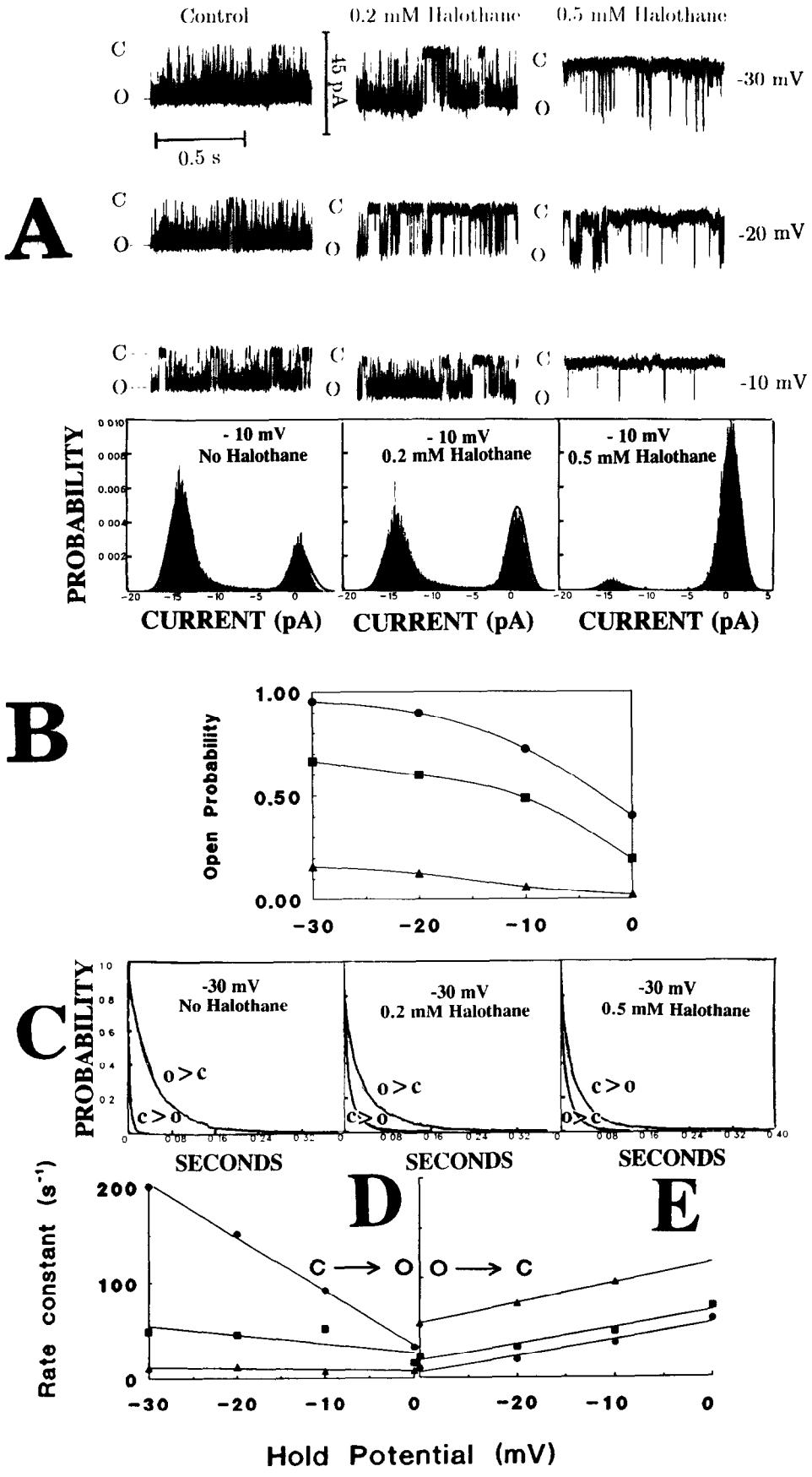
3. RESULTS

The skeletal muscle transverse tubule Ca^{2+}-activated K^+ channel was incorporated into an artificial planar lipid bilayer membrane by the fusion of transverse tubule vesicles using the method developed by Latorre et al. [8]. Because transverse tubule vesicles are cytoplasmic-side out, the Ca^{2+}-activated K^+ channel is expected to be orientated so that the cytoplasmic side of the channel faces the *cis* chamber.

Fig. 1A shows a typical current recording of a single Ca^{2+}-activated K^+ channel. The Ca^{2+} concentration in the *cis* chamber was 50 μM and the membrane potential was set at −30 mV, −20 mV, or −10 mV (*trans* side negative). The *cis* chamber contained 0.2 mM, 0.5 mM or no halothane. These halothane concentrations are within the clinical range [9,10]. Halothane at 0.2 mM and 0.5 mM corresponds to solutions equilibrated with about 0.4% and 1% atm. halothane respectively. The fluctuation in the current recording between two conductance levels (o=open, c=closed) is the result of the fluctuation of the Ca^{2+}-activated K^+ channel between


Control
0.2 mM Halothane
0.5 mM Halothane
C
O
45 pA
0.5 s
-30 mV
-20 mV
-10 mV
A
PROBABILITY
- 10 mV
No Halothane
- 10 mV
0.2 mM Halothane
- 10 mV
0.5 mM Halothane
CURRENT (pA)
B
Open Probability
1.00
0.50
0.00
-30
-20
-10
0
C
-30 mV
No Halothane
-30 mV
0.2 mM Halothane
-30 mV
0.5 mM Halothane
o > c
c > o
SECONDS
D
E
Rate constant (s⁻¹)
200
100
0
C → O
O → C
Hold Potential (mV)

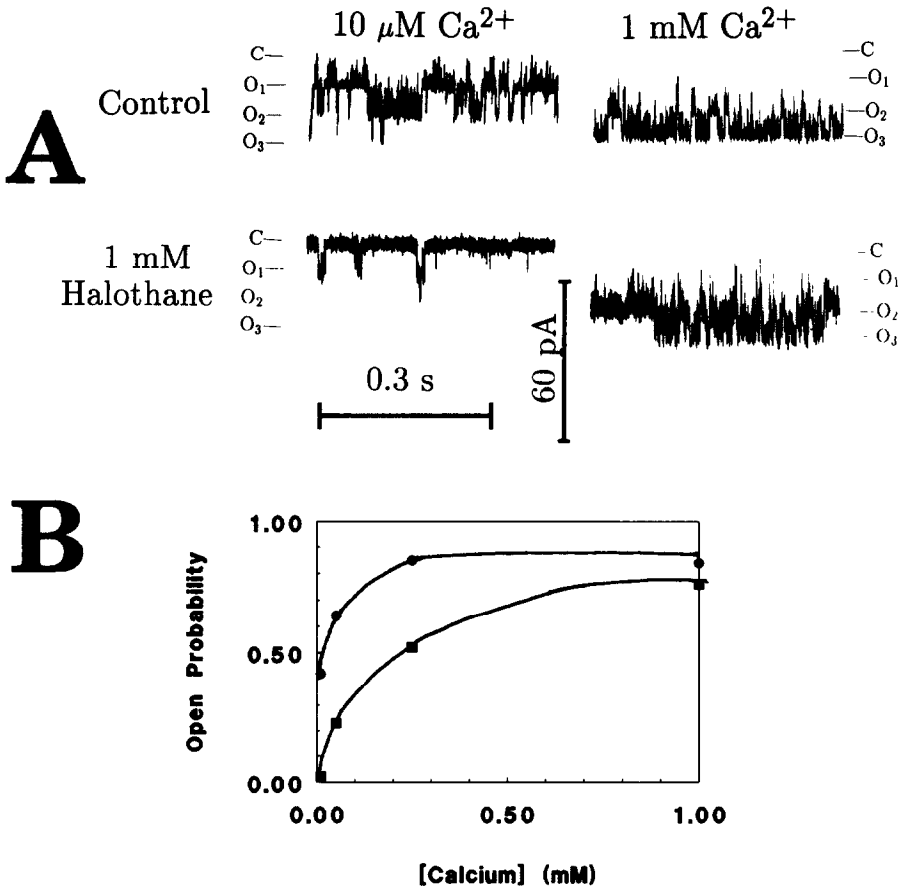


Fig. 2. Effect of the Ca^{2+} concentration on the activity of the Ca^{2+}-activated K^+ channel in the presence and absence of 1.0 mM halothane. (A) Current recording of three Ca^{2+}-activated K^+ channels at 0 mV in the presence of 0.01 mM Ca^{2+} (left column) or 1.0 mM Ca^{2+} (right column) with (bottom row) or without (top row) 1.0 mM halothane. Other experimental conditions were the same as Fig. 1. (B) The open probability as a function of the Ca^{2+} concentration in the absence (●) and in the presence (■) of 1.0 mM halothane.

the open and closed conformational states. As shown by Latorre and co-workers [8], the open state probability increases as the membrane becomes more polarized (*trans* side negative). Halothane reduces the time that the channel stays in the open state.

The relationship between the membrane potential and the open state probability at different halothane concentrations is shown in Fig. 1B. Halothane reduces the open probability at all potentials measured, but the percent decrease is greater at the lower polarizations. Similar results were obtained in two other experiments.

The channel conductance of the open state, 330 pS, (the slope of the line made by plotting the channel current vs. membrane potential) is not significantly altered by halothane.

The effect of 1 mM or less halothane on the gating kinetics was reversed by perfusion of the *cis* chamber with 10 volumes of a halothane-free solution, however irreversible alterations in the gating kinetics were observed after treating the membrane with halothane above 5 mM (data not shown).

The rate constants for the open to closed state transi-

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Fig. 1. Effect of halothane on the activity of the Ca^{2+}-activated K^+ channel. (A) Single channel current recordings of the Ca^{2+}-activated channel at −30 mV (top row), −20 mV (middle row), and −10 mV (bottom row) in the presence of 0 (left column), 0.2 mM (center column), or 0.5 mM (right column) halothane. The *cis* chamber contained 200 mM KCl, 10 mM Tris-HEPES (pH 7.2), and 50 μM Ca^{2+} and *trans* chamber contained 50 mM KCl, 10 mM Tris-HEPES (pH 7.2). Current histograms for the channel at −10 mV membrane potential are shown below the current traces along with the Gaussian distribution fit (bold line). (B) Open probability as a function of the membrane potential for the control without halothane (●), with 0.2 mM halothane (■), or 0.5 mM halothane (▲). (C) Probability distribution curves for the closed (no conductance) state (C > O) and the open state (O > C) of the channel at −30 mV membrane potential. The data is indicated by the bold line and the double exponential fit is shown by the thin line. The close state probability distribution curves were fitted to the the following equations: 'no halothane', $P(t) = 0.57e^{-641t}+0.43e^{-211t}$; '0.2 mM halothane', $P(t) = 0.57e^{-481t}+0.43e^{-211t}$; 0.2 mM halothane $P(t = 0.57e^{-481t}+0.43e^{-46t}$; 0.5 mM halothane $P(t) = 0.56e^{-151t}+0.44e^{-15t}$. The open state probability distribution curves were fit to the the following equations: 'no halothane', $P(t) = 0.52e^{-44t}+0.48e^{-15t}$; '0.2 mM halothane', $P(t)=0.53e^{-157t}+0.47e^{-21t}$; '0.5 mM halothane', $P(t)=0.55e^{-273t}+0.45e^{-57t}$. (D) The effect of membrane potential on the rate constant, $k_{2C\rightarrow O}$, for the closed to open transition without halothane (●), or with 0.2 mM halothane (■) or 0.5 mM halothane (▲). (E) The effect of membrane potential on the rate constant, $k_{2O\rightarrow C}$, for the open to closed transition without halothane (●), or with 0.2 mM halothane (■) or 0.5 mM halothane (▲).

tion ($k_{O\rightarrow C}$) and the closed to open state transition ($k_{C\rightarrow O}$) were obtained by analyzing the curve obtained by potting the probability (P) of the open or closed state lasting for time t against t. The probability distribution curves for both O→C and C→O transitions are described by a double exponential equation ($P(t) = A_1e^{-k_1t}+A_2e^{-k_2t}$) indicating the existence of at least 2 open states and 2 closed states with different transition rate constants ($k_{1O\rightarrow C}$ and $k_{2O\rightarrow C}$ for the O→C transition, $k_{1C\rightarrow O}$ and $k_{2C\rightarrow O}$ for the C→O transition) (Fig. 1C). A_1 is the fraction of transitions that occur with the rate constant k_1, and A_2 is the fraction of transitions that occur with the rate constant of k_2. A_1 and A_2 are both approximately 0.5 for both O→C and C→O transitions. The higher rate constants for the O→C ($k_{1O\rightarrow C}$) and C→O ($k_{1C\rightarrow O}$) transitions are above 200 s^{-1}; too rapid to determined accurately under these experimental conditions. The lower rate constants for the O→C ($k_{2O\rightarrow C}$) and the C→O ($k_{2C\rightarrow O}$) transitions in the presence of 50 μM Ca^{2+} ranged from 7 s^{-1} to 200 s^{-1} depending on the membrane potential and the halothane concentration (Fig. 1D and E). The rate constant, $k_{2C\rightarrow O}$, for the C→O transition is dependent on the membrane polarization in the absence of halothane, but following the addition of 0.5 mM halothane, the transition rate was not significantly affected by the membrane potential. Halothane increases the open to closed rate constant, $k_{2O\rightarrow C}$, but doesn't significantly alter the effect of membrane potential on the $k_{2O\rightarrow C}$.

The effect of halothane on the channel activity at different Ca^{2+} concentrations is shown in Fig. 2. In this experiment, the planar lipid bilayer contained three Ca^{2+}-activated K^+ channels. The membrane potential was held at 0 mV potential and the Ca^{2+} concentration in the *cis* chamber was varied between 0.01 and 1.0 mM. As shown by Latorre and co-workers [8], the open state probability increases as the Ca^{2+} concentration in the *cis* chamber is raised. At 1 mM Ca^{2+}, the probability of the open state with or without 1 mM halothane is about 0.8. However, at 10 μM Ca^{2+}, 1 mM halothane caused a 96% decrease in the open state probability. The Ca^{2+} concentration that gives a 50% open state probability was 10 μM in the absence of halothane and 130 μM in the presence of 1 mM halothane. Similar results were observed when the membrane potential was held at −10, −20 and −30 mV. These results were also obtained in three other experiments.

4. DISCUSSION

The main conclusion of this study is that halothane alters the gating kinetics of the skeletal muscle Ca^{2+}-activated K^+ at clinically relevant concentrations. Halothane increases the open to closed state transition rate and decreases the closed to open state transition rate by destabilizing the open state and stabilizing the closed state. Perhaps the closed state contains more exposed hydrophobic surfaces than the open state, and halothane interacts with these surfaces to stabilize the closed state conformation.

In the absence of halothane, the closed to open state transition rate is strongly dependent on the membrane potential, but in the presence of 0.5 mM halothane, this transition rate is not affected by the membrane potential. The interaction of the voltage sensor domain of the channel with halothane may block its voltage-sensing action. On the other hand, the sensitivity of the open to closed state transition rate constant to the membrane potential is not altered by halothane suggesting the closed to open state transition and the open to closed state transition may not share a common voltage sensor.

The role of halothane mediated inhibition of the Ca^{2+}-activated K^+ channel in the physiological response to halothane is not known. Inhibition of a K^+ channel would seem to increase the excitability of membranes by decreasing the hyperpolarization which accompanies the opening of the channel. However increasing the excitability of some cells may lead to the decrease activity of other cells that are linked through a negative feedback.

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