brief communication

TEA prevents inactivation while blocking open K^+ channels in human T lymphocytes

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ABSTRACT The whole-cell recording mode of the patch-clamp technique was used to study the effect of external tetraethylammonium ($[TEA^+]_o$) on the inactivating, voltage-dependent K^+ channels of human T lymphocytes. TEA^+ reduced the peak amplitude and slowed the time course of the K^+ current decay during a depolarizing pulse, resulting in a crossover of the current records in the presence and absence of TEA^+. In solutions with different $[TEA^+]_o$ both the peak K^+ current amplitude, $I_{K\,peak}$, and the time constant of the decay of the K^+ current, τ_d, were reduced in a dose-dependent manner, both with apparent binding constants, K_D, of 12 mM. The integral of K^+ current during a prolonged depolarizing pulse was unaltered in solutions with different $[TEA^+]_o$. The concentration dependence of $[TEA^+]_o$ on $I_{K\,peak}$, τ_d, and the unchanged current integral can be explained with a kinetic scheme in which open channels blocked by TEA^+ cannot inactivate.

INTRODUCTION

Voltage-gated K^+ channels in a variety of cell types are blocked by tetraethylammonium ions (TEA^+) either from the inside or outside of the membrane (Armstrong, 1975; Stanfield, 1983; Hille, 1984). Recent investigations have shown that the block of K^+ channels by external TEA^+ results in an apparent reduction in the single-channel conductance (Standen et al., 1985; Spruce et al., 1987; DeCoursey et al., 1987). This reduction of single-channel amplitude is consistent with the idea that blocking and unblocking by TEA^+ is fast compared with channel opening. In skeletal muscle the activation time course and mean open time of K^+ channels were unaltered by TEA^+, leading Stanfield and his colleagues to conclude that the TEA^+ block is independent of channel gating. In this paper we describe the action of external TEA^+ on the K^+ channels of human T lymphocytes and show that open K^+ channels blocked by TEA^+ cannot inactivate.

MATERIALS AND METHODS

The experiments were carried out on single cells of a human T-lymphoma cell line, Jurkat E6-1, using the whole-cell recording mode of the patch-clamp technique (Hamill et al., 1981). All experiments were done at room temperature (22°–26°C). The cells under investigation were bathed in normal Ringer solution (160 mM NaCl; 4.5 mM KCl; 2.0 mM $CaCl_2$; 1.0 mM $MgCl_2$; 5 mM Hepes); adjusted to pH 7.4 with NaOH; 290–320 mosmol. TEA^+ was purchased from Eastman Kodak Co. (Rochester, New York). TEA^+ concentrations were increased by replacing NaCl with TEA-chloride to keep the osmolarity constant. The bath solution could be changed during the recordings by bath perfusion. The patch-pipette usually contained 160 mM K-aspartate, 1.1 mM EGTA; 0.1 mM $CaCl_2$; 2.0 mM $MgCl_2$; 10 mM Hepes, adjusted to pH 7.2 with KOH; 290–320 mosmol.

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The holding potential was adjusted in all experiments to $E = -80$ mV. The patch-clamp amplifier (List L/M-EPC 7; Adam List Associates, Ltd., Great Neck, NY) was used in the voltage-clamp mode. Series resistance, R_s, values determined from the time course of capacity transients ranged from 7 to 14 MΩ. For the current amplitudes in these experiments, voltage errors due to R_s ranged between 3 and 15 mV and could, in principle, influence the time course of inactivation of the K^+ currents. At potentials more positive than +20 mV, however, the time course of inactivation is hardly voltage-dependent (Cahalan et al., 1985). Identical kinetic effects of TEA^+ were observed in cells with peak K^+ currents ranging from 200 pA to 2 nA at +40 mV. Furthermore, in two cells where R_s compensation was employed (>90%), there was no effect on the time course of inactivation, and the measured K^+ current amplitude was increased by <10% compared to uncompensated records. Electrodes were pulled from Accu-fill 90 Micropets (Becton, Dickinson & Co., Parsippany, NJ) in three stages, coated with Sylgard (Dow Corning Corp., Midland, MI), and fire-polished to resistances, measured in the bath, of 2–7 MΩ. K^+ channel inactivation was not different if hard pipette glass was used as pipette glass (compare Cota and Armstrong, 1988; Furman and Tanaka, 1988).

In all experiments, the command input of the patch-clamp amplifier was controlled by a computer (PDP 11/73; Scientific Micro Systems Inc., Mt. View, CA) via a digital-analog converter and membrane currents were recorded at a bandwidth of 2 kHz. Correction for linear leakage and capacitative currents was achieved by analog subtraction and by digital subtraction of an appropriately scaled mean current associated with 8 pulses delivered from a hyperpolarized potential. All potentials were corrected for the liquid junction potential that develops at the tip of the pipette if the pipette solution is different from that of the bath. The liquid junction potential between the normal internal (pipette) and external (bath) solution was −13 mV.

RESULTS

Fig. 1 demonstrates that TEA^+, as well as reducing peak K^+ current, alters the apparent inactivation rate of K^+

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FIGURE 1 K^+ currents in a Jurkat cell in normal Ringer solution (control) and during application of a Ringer solution containing 30 mM TEA^+. K^+ outward currents associated with 5-s depolarizing pulses to +40 mV from a holding potential of −80 mV. Cell 106.

FIGURE 2 Effect of $[TEA^+]_o$ on peak K^+ current amplitude, $I_{K\,peak}$, and current integral I_K of the K^+ current. The TEA^+ dose-response curve on $I_{K\,peak}$ was determined as

$$I_{K\,rel} = I_{K\,peak} \text{ in TEA}^+ \text{ Ringer}/I_{K\,peak} \text{ in normal Ringer.}$$

$I_{K\,peak}$ in normal Ringer solution is the average of $I_{K\,peak}$ before and after application of the TEA^+ Ringer solution. The curve through the squares was calculated assuming that $I_{K\,rel}$ is proportional to $K_D/([TEA^+]_o + K_D)$ with $K_D = 12$ mM. The calculation of the normalized current integral during long depolarizing pulses in the absence and presence of different $[TEA^+]_o$ is shown below:

$$I_K(t) = I_{Ko} \exp(-t/\tau_d)$$

$$\int_0^t I_K = I_{Ko}(-\tau_d)\exp(-t_t/\tau_d) - I_{Ko}(-\tau_d)\exp(-t_0/\tau_d)$$

$$\text{for } t \ggg \tau_d \text{ and } I_{Ko} = I_{K\,peak}$$

$$\int I_K = I_{K\,peak} \times \tau_{d\,norm} = I_{K\,rel} \times \tau_{d\,rel}.$$

The dotted line at 1.0 was drawn for clarity. The squares and triangles are averages of at least three experiments except the ones at $[TEA^+]_o$ = 1 and 40 mM (each one experiment). The bars on the points reflect the standard deviation.

channels in human T lymphocytes. The K^+ current in normal Ringer solution (control) activates very rapidly, reaches a peak within 12 ms, and then inactivates almost completely. 30 mM TEA^+ reduced the peak current amplitude, $I_{K\,peak}$, from 1,800 to 425 pA while slowing the apparent inactivation time constant, τ_d, obtained by fitting a single exponential to the decay of the K^+ current, from 275 to 1,065 ms. Thus, $I_{K\,peak}$ in Ringer with 30 mM TEA^+ is approximately one-quarter of $I_{K\,peak}$ under control conditions while τ_d is about 4 times slower. Note that the current records in the presence and absence of TEA^+ cross over. This was consistently observed in three other experiments with 30 mM TEA^+, and in experiments with lower and higher TEA^+ concentrations.

Fig. 2 illustrates dose-response relations for $[TEA^+]_o$ on the reduction of the peak K^+ current, $I_{K\,peak}$, and on the normalized current integral during long depolarizations. The fit of the dose-response curve for the reduction of $I_{K\,peak}$ yielded an apparent dissociation constant, K_D, for TEA^+ of 12 mM, in agreement with the findings of DeCoursey et al. (1984, 1987), who described a K_D for TEA^+ of 8 mM in human T-cell K^+ channels and 13 mM for "type *n*" K^+ channels of murine T lymphocytes. The relative integral of the current traces measured under control conditions and with different $[TEA^+]_o$ demonstrates that the current integral, calculated as the product of $I_{K\,rel} \times \tau_{d\,rel}$ (see legend of Fig. 2), is unaltered in solutions with different $[TEA^+]_o$ compared to normal Ringer solution. This indicates that the total time the channels spend in the open state is unchanged by TEA^+.

DISCUSSION

How is TEA^+ able to reduce $I_{K\,peak}$ and at the same time slow the time course of the decay of I_K? One possible explanation could be multiple populations of channels differing in their inactivation kinetics and sensitivity to TEA^+, with the more TEA^+-resistant channels having slower inactivation kinetics. However, in this case one would expect a reduction in I_K with no crossover of the current traces. The crossover of the current traces (Fig. 1), the constant current integral (Fig. 2), and the lack of obvious multi-exponential components during the time course of inactivation rule out this possibility. The fact that TEA^+ alters K^+ current kinetics in a single population of K^+ channels points out a potential hazard in using TEA^+ to pharmacologically dissect current components by subtraction of voltage clamp records.

To account for our results, we have considered the following kinetic scheme, which applies to the K^+ channel and its interaction with TEA^+, where only state O is conducting:

$$\begin{array}{ccccc} C & \underset{b_0}{\overset{a_0}{\rightleftharpoons}} & O & \underset{b_1}{\overset{a_1}{\rightleftharpoons}} & I \\ & & b_2 \Updownarrow a_2 & & \\ & & OB & & \end{array}$$

This is a simplified kinetic scheme that does not attempt to show all of the possible transitions; multiple closed

states would be needed to account for the time course of activation, and closed-blocked states are not ruled out. We modeled the experimental results with the assumptions that: (*a*) TEA^+ produces a fast block of the K^+ channel; (*b*) activation and unblocking are fast compared to inactivation ($b_2 \geqq a_0 >>> a_1$); and (*c*) TEA^+-blocked open channels cannot inactivate. Using this scheme, we show in Fig. 3 *A* that simulated traces can mimic the reduction in K^+ current amplitude, the decrease in apparent inactivation rate, and the crossover of the current traces. From the simulated current traces we calculated the effect of raising $[TEA^+]_o$ by raising the ratio of a_2/b_2 (see legend of Fig. 3) on $I_{K\,peak}$, τ_d, and on the current integral. Calculations illustrated in Fig. 3 *B* show the simulated dose-response curve of $[TEA^+]_o$ on the normalized $I_{K\,peak}$. The fit of this dose-response curve (*solid line*) yielded a dissociation constant K_D for TEA^+ of 12 mM, in agreement with the data shown in Fig. 2. In addition, Fig. 3 *B* shows the calculated relative current integral for different $[TEA^+]_o$ compared to the current integral under control conditions.

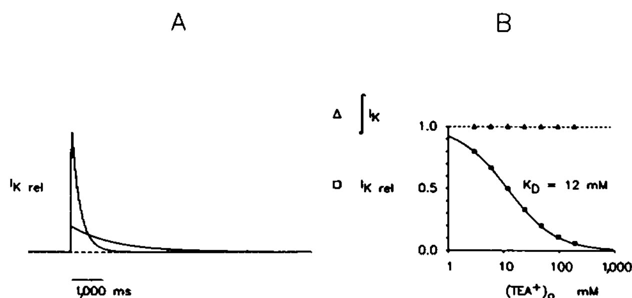

FIGURE 3 Calculated effect of TEA^+ on I_K. (*A*) Time course of I_K calculated under the assumption that an open channel blocked by TEA^+ cannot inactivate and that block by TEA^+ is fast compared to inactivation. For the calculations we used the kinetic scheme shown in the text with

$$a_0 = 200; b_0 = 0.1;$$

$$a_1 = 4; b_1 = 0.01;$$

and

$$b_2 = 200.$$

a_2 was 0 for the control sweep and 800 to simulate 48 mM TEA. We do not know the actual rates for the TEA^+ blocking and unblocking, but simulated current traces were the same as long as $b_2 \geqq a_0$, and the ratio of a_2 to b_2 remains constant. (*B*) Calculated effect of TEA^+ on $I_{K\,peak}$ and the current integral, I_K, under the assumption that a reduction of $I_{K\,peak}$ to 50% of the control value reflects a TEA^+ concentration of 12 mM, in this case the ratio of a_2 to b_2 is 1 ($a_2 = 200$). Changing this ratio simulates a change in TEA^+ concentration. The curve through the points was calculated assuming that $I_{K\,peak}$ is proportional to $K_D/([TEA^+]_o + K_D)$ with $K_D = 12$ mM. The normalized current integral during long depolarizing pulses in the absence and presence of different $[TEA^+]_o$ was calculated as shown in legend of Fig. 2.

Related kinetic schemes have been used to describe the action of blocking compounds on Na^+ channels (Yeh and Narahashi, 1977; Cahalan, 1978; Cahalan and Almers, 1979; Yamamoto and Yeh, 1984) and acetylcholine receptors (Beam, 1976; Neher and Steinbach, 1978). In these cases, the blocker appears to hinder gating transitions when occupying its binding site in the channel. Spruce et al. (1987) found that mean open times for skeletal muscle K^+ channels were unaffected by TEA^+, and suggested that channels blocked by TEA^+ are able to close normally. If this is also true for lymphocyte K^+ channels, the simple model above would predict an increase in the inactivation time constant in ensemble average single-channel records; mean open times would be expected to increase only if open → inactivated transitions predominate over an open → closed ones.

Our results on the lymphocyte K^+ channel indicate that TEA^+ can hinder K^+ channel inactivation gating; the crossover of the current records before and during TEA^+, the constant current integral, and the agreement of the data with the proposed model indicate that open K^+ channels that are blocked by TEA^+ cannot inactivate.

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