BRIEF COMMUNICATION

TIME DEPENDENCE OF THE CALCIUM-ACTIVATED POTASSIUM CURRENT

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ABSTRACT We investigated the dependence of the kinetics of the Ca^{2+}-activated K^+ current of the molluscan neuron soma upon membrane potential. The K^+ current was activated by intracellular Ca^{2+} ion injection in neurons with blocked inward Na^+ and Ca^{2+} currents. The difference between currents was measured with brief pulses (<100 ms) before and immediately after Ca^{2+} injection and was used as the Ca^{2+} activated K^+ current at difference membrane potentials. The results in normal (10 mM) and in high (200 mM) external K^+ show that the time-course of the Ca^{2+}-activated K^+ current depends upon membrane voltage and that the current activates more rapidly with membrane depolarization.

INTRODUCTION

In many cells an increase in the intracellular Ca^{2+} concentration causes a change in the membrane permeability to potassium ions (Lew and Ferreira, 1978; Meech, 1978). Although the mechanism by which Ca^{2+} increases K^+ permeability is not fully understood, several important facts are known. An increase in the free, intracellular Ca^{2+} concentration, produced either by direct internal Ca^{2+} ion injection or by Ca^{2+} influx through voltage-dependent Ca^{2+} channels, causes an outward K^+ current. Various evidence suggests that it is the rise in Ca^{2+} concentration at the inner membrane surface that activates this current (Meech and Standen, 1975; Clusin and Bennett, 1976; Gorman and Thomas, 1980; Eckert and Tillotson, 1978). It has been shown that the affinity of the activation site for Ca^{2+} ions is much greater than its affinity for other divalent cations (Gorman and Hermann, 1979). It has also been found (Gorman and Thomas, 1980) that the K^+ conductance activated by Ca^{2+} influx or by internal injection depends on membrane potential (it increases *e*-fold for about 25-mV depolarization). More recently, it was shown (Woolum and Gorman, 1980) that the ionic selectivity of the pathway for K^+ current flow is similar to that for other voltage-dependent K^+ channels, i.e., $P_K > P_{Rb} > P_{NH4} > P_{Cs} > P_{Li}, P_{Na}$. Finally, voltage-dependent K^+ currents, but not the Ca^{2+}-activated K^+ current, are blocked by the pharmacological agent 4-aminopyridine (Thompson, 1977; Hermann and Gorman, 1978). Taken together, these data show that the Ca^{2+}-activated K^+ current is not a leakage current and that it differs from other K^+ currents. It is not clear, however, whether Ca^{2+} opens separate K^+ channels or has its effect on voltage-dependent K^+ channels. In excitable cells, the identification of a separate ionic pathway or channel is based upon differences in the time-course or kinetics of the current as

well as upon its selectivity for various ions and its independent modification by pharmacological and chemical agents (Hille, 1978). The marked dependence of the Ca^{2+}-activated K^+ conductance upon membrane potential raises the possibility that the time-course of activation of the K^+ current by Ca^{2+} may also depend upon membrane voltage. We have examined the kinetics of the K^+ current, activated by direct internal Ca^{2+} ion injection, at different membrane voltages and we report here the results of these experiments.

METHODS

Neurons R-15 and L-2 to L-6 in the abdominal ganglion of *Aplysia californica* were used, and experiments were carried out in a modified artificial sea water (ASW) medium that contained (in mM/liter) 500 NaCl, 10 KCl, 60 $MgCl_2$, and 10 mM Tris at pH 7.7 and at a temperature of 15°C. Ca^{2+} free ASW was used to eliminate the Ca^{2+} current and, therefore, any Ca^{2+} influx during depolarization. Tetrodotoxin (5×10^{-5} M) or complete replacement of external Na^+ with tetramethylammonium was used to eliminate the early Na^+ current. The voltage and current electrodes contained 3 m KCl and had resistances in ASW of ~1–2 MΩ and 0.4–0.8 MΩ, respectively. The procedures for differential recording and voltage clamping the soma membrane were similar to those described (Gorman and Hermann, 1979; Gorman and Thomas, 1980) except that the current electrode was shielded to within ~100 μM of the tip and the series resistance was compensated electronically. The Ca^{2+} injection electrode contained 0.2M $CaCl_2$ and was positioned close to the inner membrane surface (Gorman and Hermann, 1979). Ca^{2+} ions were injected iontophoretically in the voltage-clamp mode so that there was no net flow of current across the membrane caused directly by the injection. In the experiments described below, the membrane was depolarized (or hyperpolarized) briefly to various potentials before and 1–5 s after a 20 or 30-s Ca^{2+} injection. The current responses were digitized and the difference between currents before and after Ca^{2+} injection was obtained using a Tracor Northern Signal Analyzer (TN-1500; Tracor Northern, Middleton, Wis.) and used as the Ca^{2+}-activated K^+ current measured at the indicated membrane potential.

RESULTS

Fig. 1 *A* shows a series of representative records of the Ca^{2+}-activated K^+ current measured at the indicated membrane potentials in Ca^{2+}-free ASW containing normal (10 mM) K^+. The current measured at the end of the pulse is plotted vs. membrane potential in Fig. 1 *B*. Its increase at positive membrane potentials is similar to the increase in the Ca^{2+}-activated K^+ current measured under steady-state conditions (Gorman and Thomas, 1980). Inspection of the records in Fig. 1 *A* shows that the current begins without a delay because of the channels that are open at the holding potentials. Fig. 1 *B* also shows that the initial slope (dI/dt) after this initial current jump is not zero (as for the delay rectifier) and that its time-course changes as the membrane is depolarized to more positive potentials. The dependence of the current time-course on membrane voltage is shown more clearly in Fig. 1 *C* where the time to one half the estimated maximum current or the half time ($T_{1/2}$) is plotted vs. membrane potential. The half time increases as the membrane is depolarized. Fig. 1 *D* shows a semilogarithmic plot of the current response to potential jumps from −50 mV to +10 mV and to +110 mV. Although the current does not follow a simple exponential time-course at either potential, it is clear that the variation of current with time is slowed at the more positive potential. The Ca^{2+}-activated K^+ current of cell R-15 is blocked by external tetraethylammonium (Hermann and Gorman, 1979). Fig. 1 (*A* and *B*) shows that 100 mM TEA abolishes most of the outward Ca^{2+}-activated K^+ current, but that there is some relief of the block at positive membrane potentials (Fig. 1 *B*).

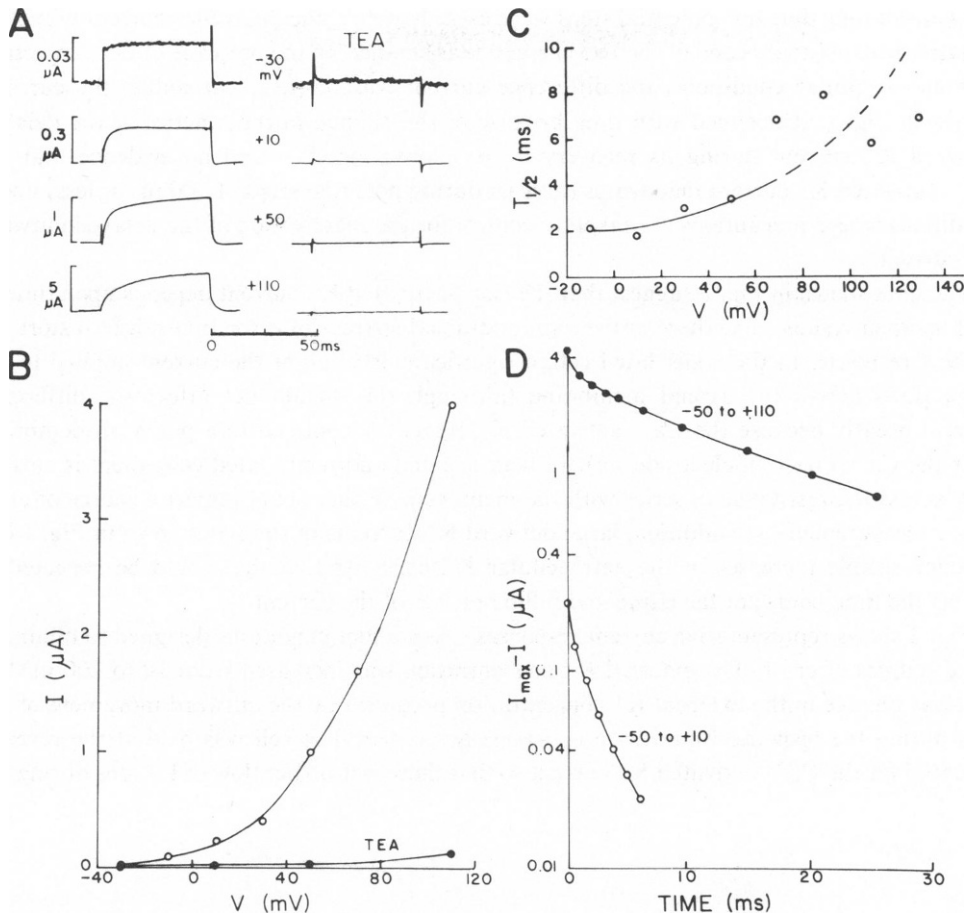

FIGURE 1 Effects of membrane potential on the Ca^{2+}-activated K^+ current. *A*, Digitized records of the difference between currents measured with 50-ms pulses from a holding potential of −50 mV to the indicated voltages before and 3 s after a 20-s intracellular Ca^{2+} injection (injection intensity 200 nA). The external bathing media contained Ca^{2+} free ASW and 50 μM TTX. The records in the right-hand side of *A* were recorded after the addition of 100 mM TEA to the bathing media. *B*, Plots of the peak outward difference current vs. pulse voltage under the two conditions shown in *A*. *C*, Plot of the time at which the difference current was half its estimated half-maximal value ($T_{1/2}$) vs. pulse potential. *D*, Logarithimic plot of two current responses measured with different clamp pulses vs. time.

The current difference method used to obtain a measure of the Ca^{2+}-activated K^+ current depends critically on the full subtraction of other membrane currents from successive records. Under the experimental conditions outlined above and in the absence of internal Ca^{2+} injection, the current across the neuronal membrane is carried primarily by K^+ ions flowing out of the cell through the delayed K^+ channel and through the leakage channels. The delayed outward K^+ current inactivates slowly with time in these cells and its recovery from inactivation is prolonged (Aldrich et al., 1979; Gorman and Thomas, 1980)—particularly when potential steps of long duration (>80 ms) are used. Typically, a stimulus interval (usually 1 min) was chosen so that, in the absence of internal Ca^{2+} injection, subtraction of current responses to successive pulses produced no net membrane current flow. When shorter

intervals or long duration potential steps were used, however, the difference current was often negative because the second of the two currents was smaller. In the presence of Ca^{2+} injection, and under similar conditions, the difference current was positive, but unlike the currents shown in Fig. 1, it declined with time because of the change in the kinetics of the delayed outward K^+ current during its recovery from inactivation. We find no evidence that the Ca^{2+}-activated K^+ current inactivates (at least during potential steps of 100 ms or less) under conditions where precautions are taken to control for the inactivation of the delayed outward K^+ current.

The data shown in Fig. 1 suggest that the Ca^{2+}-activated K^+ current depends upon time as well as upon voltage, but there are several additional sources of error that might distort the current response. In the nonisolated cell, a significant fraction of the current applied to the soma flows across the axonal membrane (although this should not affect the difference current greatly because the Ca^{2+}-activated K^+ current is confined to a patch of membrane near the Ca^{2+} injection electrode tip). In both isolated and nonisolated cells there is current flow across the resistance in series with the membrane. Either could impart a source of error in our measurements. In addition, large outward K^+ currents of the type shown in Fig. 1 can produce sizable increases in the extracellular K^+ concentration that could be expected to distort the time-course of the rising and falling phases of the current.

Fig. 2 shows representative current responses taken under conditions designed to minimize these sources of error. The external K^+ concentration was increased from 10 to 200 mM so that any change in the external K^+ concentration produced by the outward movement of K^+ ions during the response would be proportionally smaller. The cell was held at the reversal potential for the Ca^{2+}-activated K^+ current so that there was no net flow of K^+ ions during the

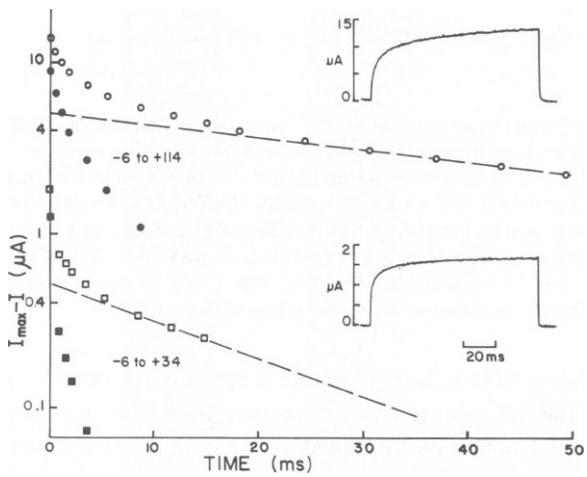

FIGURE 2 Effects of membrane potential on the kinetics of the Ca^{2+}-activated K^+ current. The inserts show digitized records of the difference between currents measured with 100-ms pulses from a holding potential of −6 to +34 mV (bottom) and to +114 mV (top) before and 3 s after a 20-s internal Ca^+ injection in an isolated neuron. The external media contained Ca^{2+} free ASW with 200 mM K^+ and 50 μM TTX. The current responses (open symbols) are plotted on a logarithimic scale vs. time. The closed symbols represent the residual currents at each potential after subtraction of single exponentials fitted to the later experimental points (open symbols) and indicated by the dashed lines.

period of Ca^{2+} ion injection. At this potential (~ -5 to -10 mV) the problem of obtaining zero-difference currents with no Ca^{2+} injection was eliminated; probably because of the more complete inactivation of voltage-dependent K^+ channels. The soma was isolated from the ganglion so that the proportion of current flow out of the axon was minimized. Finally two thirds of the normal series resistance of ~ 1.5 k Ω was compensated for electronically. The results (Fig. 2 *A*) are similar to those shown in Fig. 1. The time-course of the Ca^{2+}-activated K^+ current (both the rise and decay of the current) is slowed at positive membrane potentials. There is again a dependence of the time-course on membrane potential. The current does not follow a simple exponential time-course, or a time-course determined by two exponentials, or by a single exponential raised to a power >1. The kinetics of the Ca^{2+}-activated K^+ current are the same at different times after the Ca^{2+} injection (as long as there is a measurable change in intracellular Ca^{2+}).

DISCUSSION

Our results show that activation of the Ca^{2+}-activated K^+ current depends on time as well as upon membrane voltage and upon the intracellular Ca^{2+} concentration. Previous investigators (Eckert and Tillotson, 1978; Hofmeier and Lux, 1979; Lux and Heyer, 1979) have reported a slowing of the rate of activation of the K^+ current initiated by Ca^{2+} influx at positive membrane potentials. These experiments are difficult to interpret because of the intrinsic time and voltage dependence of the Ca^{2+} channel and, therefore, of Ca^{2+} influx. Another factor is the possible change in the kinetics of intracellular Ca^{2+} accumulation near the inner membrane under different conditions of Ca^{2+} influx. More recent measurements (Gorman and Thomas, 1980) of the Ca^{2+}-activated K^+ current measured near the Ca^{2+} equilibrium potential immediately after a sizable Ca^{2+} influx show a similar slow rate of activation and are consistent with our present findings.

A number of different voltage-dependent K^+ currents have been identified in nerve cells (Hodgkin and Huxley, 1952; Connor and Stevens, 1971; Neher, 1971; Brown and Adams, 1980) on the basis of their high selectivity for K^+ ions and their different kinetics. The kinetics of the Ca^{2+}-activated K^+ current differ from those of all previously described voltage-dependent K^+ currents that activated with a delay and become faster rather than slower at potentials where the membrane conductance to K^+ ions is increased. Our results suggest that the Ca^{2+}-activated K^+ current represents the movement of K^+ ions through channels that differ from other voltage-dependent K^+ channels.

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