

Rapid Recovery from K Current Inactivation on Membrane Hyperpolarization in Molluscan Neurons

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ABSTRACT Recovery from K current inactivation was studied in molluscan neurons using two-microelectrode and internal perfusion voltage clamps. Experiments were designed to study the voltage-dependent delayed outward current (I_K) without contamination from other K currents. The amount of recovery from inactivation and the rate of recovery increase dramatically when the membrane potential is made more negative. The time course of recovery at the resting potential, -40 mV, is well fit by a single exponential with a time constant of 24.5 s ($n = 7$). At more negative voltages, the time course is best fit by the sum of two exponentials with time constants at -90 mV of 1.7 and 9.8 s ($n = 7$). In unclamped cells, a short hyperpolarization can cause rapid recovery from inactivation that results in a shortening of the action potential duration. We conclude that there are two inactivated states of the channel and that the time constants for recovery from both states are voltage dependent. The results are discussed in terms of the multistate model for K channel gating that was developed by R. N. Aldrich (1981, *Biophys. J.*, 36:519-532).

INTRODUCTION

The voltage-dependent delayed outward current, I_K , in molluscan neurons inactivates during long voltage-clamp depolarizations (Hagiwara et al., 1961; Connor and Stevens, 1971a; Lux and Eckert, 1974; Kostyuk et al., 1975; Aldrich et al., 1979a, b; Eckert and Ewald, 1982). Inactivation increases with depolarization and the kinetics of inactivation are characterized by the sum of two exponentials with time constants of ~ 0.5 and 2.0 s when measured at $+10$ mV (Aldrich et al., 1979a). Recovery from inactivation on return to the resting potential (approximately -40 mV) occurs much more slowly, requiring tens of seconds for half-recovery (Aldrich et al., 1979a; Kostyuk et al., 1975). Because of the rapid onset and slow recovery rates, inactivation can accumulate during repetitive voltage-clamp depolarizations or during repetitive action potentials. This results in a broadening of the action potential duration during low-frequency firing, especially in cells that possess a significant inward Ca current (Aldrich et al., 1979b). Inactivation of K current, therefore, can have a dramatic

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effect on the integrative functions and firing properties of these nerve cells. A similar cumulative inactivation of K current is seen in human T lymphocytes (Cahalan et al., 1984).

The present experiments show that recovery from inactivation is enhanced when the membrane is hyperpolarized beyond the resting potential. The steady state voltage dependence of recovery tends toward a saturating value with hyperpolarization, and half-recovery occurs at -40 to -45 mV, a voltage range that encompasses the resting potential. The time course of recovery at the resting potential could be fitted by an exponential with a time constant of 24.5 s ($n = 7$). At hyperpolarized potentials, however, the recovery time course was better fit by the sum of two exponentials with time constants at -90 mV, for example, of ~ 1.7 and 9.8 s. Both time constants decreased with increasing hyperpolarization. These findings suggest that there may be two inactivated states of the channel and that recovery from the two inactivated states follows different time courses and has a different dependence on voltage. In the Discussion, we interpret the results in terms of the multistate model for K current inactivation developed by Aldrich (1981).

Inactivation was also influenced by the external K ion concentration. In high external K, inactivation of I_K during depolarization was decreased and recovery from inactivation on membrane hyperpolarization was more pronounced. Lowered external K had the opposite effect: it increased inactivation during depolarization and decreased recovery during hyperpolarizing conditioning pulses. High external K did not change the voltage dependence of recovery or the recovery kinetics. Instead, the increased recovery during hyperpolarization in high K appeared to result from an increase in the proportion of inactivated channels recovering over the faster recovery time course. High external K might therefore influence the distribution of channels between two inactivated states.

METHODS

Experiments were performed on neuron cell bodies isolated from the pleural ganglia of the dorid nudibranchs *Anisodoris nobilis* and *Doriopsilla albopunctata*. Ganglia were removed and treated for 1 h with a 0.5% solution of neutral protease (Dispase; Boehringer Mannheim Biochemicals, Indianapolis, IN) dissolved in normal saline, followed by a 2-h wash. After dissecting away the connective tissue sheath, small clumps of neurons were axotomized and removed from the posterior half of the pleural ganglia by undercutting with iris scissors. The enzyme treatment softened the connective tissue sheath over the ganglia, but in control experiments we found that it did not affect membrane currents.

Cells were studied using either a two-microelectrode voltage clamp, as described by Barish and Thompson (1983), or an internal perfusion voltage clamp, as described by Thompson (1982). The normal external bathing saline contained (in mM): 460 NaCl, 10 KCl, 10 CaCl₂, 50 MgCl₂, 20 Hepes (pH 8.0). 0-Ca solution was made by substituting MgCl₂ for CaCl₂ and adding 1 mM NiCl₂. For internal perfusion experiments, the normal internal medium was (in mM): 375 K-aspartate, 50 NaCl, 1 EGTA, 300 sucrose, 20 Hepes (pH 7.8). High external K solutions were prepared by equimolar substitution of KCl for NaCl. Low intracellular K solutions were made by substitution of Tris-aspartate for K-aspartate. External solution changes involved washing with 20 bath vol and data points were taken beginning 10 min after the wash. Internal solution changes were made by

switching the perfusion saline supplied to the cell. Measurements began when the reversal potential for outward current with the new internal saline had stabilized (~20 min). Measurements of reversal potential employed a two-pulse method to find the null potential for tail current in delayed K channels. Records of membrane voltage and current were taken on a strip-chart recorder (50 Hz full-scale bandwidth; Gould Inc., Cleveland, OH) or FM magnetic tape (2 kHz bandwidth; Tanberg/Sangamo, Sarasota, FL) and were subsequently digitized for analysis and display on a laboratory computer (PDP 11/23; Digital Equipment Corp., Marlboro, MA). All experiments were performed at 15°C.

Separation of Currents

The cells used in this study characteristically display three distinct types of K currents that are activated as a consequence of membrane depolarization (for review, see Adams et al., 1980). This study concentrates on the voltage-dependent delayed outward current called I_K by Adams et al. (1980), which is analogous to outward rectification in axonal preparations. It was important that I_K could be measured separately from both the transient K current, I_A , and the Ca-dependent K current, I_C . The Ca-dependent K current was eliminated in two ways. When internal perfusion was employed to introduce EGTA intracellularly, the activation of I_C was prevented as shown by the absence of a slow phase in the outward tail current that is characteristic of the Ca-activated K current (Aldrich et al., 1979a; Barish and Thompson, 1983). In experiments using the two-microelectrode voltage clamp, external Ca was replaced by Mg and 1 mM Ni was added to the bathing medium to block inward Ca currents during depolarizations. Again, the elimination of I_C was confirmed by the absence of slow outward tail currents on repolarization after a test pulse.

Separation of I_K from the transient K current, I_A , required a more complicated protocol. The voltage dependence of I_A activation and inactivation is such that this current will be strongly activated when test depolarizations are preceded by conditioning hyperpolarizations (Connor and Stevens, 1971b; see Adams et al., 1980). Since pulse schedules of this kind were used routinely, precautions had to be taken to ensure that I_A did not contaminate the records of I_K and did not confound interpretation of the voltage dependence of I_K inactivation. A temporal separation method was used that relied on differences in the inactivation and recovery kinetics for processes involving I_A and I_K . The transient K current is almost completely inactivated in the steady state at holding voltages more positive than about -40 mV, and at this voltage, inactivation is attained over an exponential time course with a time constant of 150–400 ms in different cells (Connor and Stevens, 1971b; Aldrich et al., 1979a; Serrano, 1982). It follows that if time is allowed for I_A inactivation to run to completion before a depolarizing test pulse is applied, then I_A will not contaminate the measurement of delayed outward current. For each of the experiments reported here, the voltage dependence and kinetics of I_A inactivation were measured over the full range of voltages prior to any further experiments so that conditions that might lead to contamination of delayed current by I_A could be avoided.

RESULTS

Voltage Dependence of Recovery from Inactivation

Short hyperpolarizing steps cause rapid recovery from inactivation of I_K . Fig. 1A shows the accumulation of outward current inactivation during a train of 300-ms depolarizing pulses to +10 mV presented at 1/s from a holding voltage of -40 mV. After 13 pulses, the peak outward current approached a steady

amplitude that was 0.2 times the peak amplitude during the first pulse caused by 80% inactivation of I_K . Results such as these are characteristic of the cumulative inactivation process described by Aldrich et al. (1979a). Fig. 1B shows the membrane current during a later part of the same pulse train. The interval between one pair of pulses was lengthened from 700 ms to 2 s while maintaining the voltage during the interval at -40 mV. The peak amplitude of outward current on the subsequent pulse was 1.2 times larger than the previous peak because of slightly greater recovery from inactivation during the lengthened

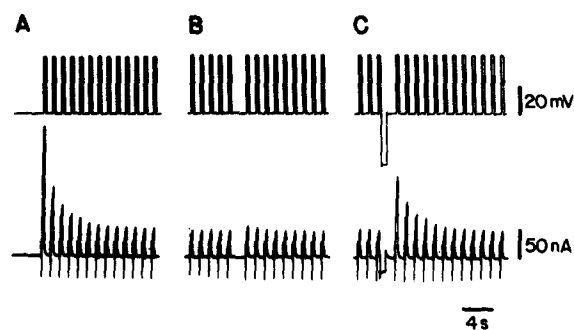


FIGURE 1. A short hyperpolarizing pulse causes rapid recovery from inactivation of delayed outward current. (A) Control record showing membrane currents (bottom trace) during a train of 300-ms depolarizing pulses to $+10$ mV from a holding voltage of -40 mV at a frequency of 1/s. After 13 pulses, the peak outward current had inactivated to 20% of the amplitude during the first pulse. (B) A 2-s delay between pulses was imposed during the train and resulted in a small (5%) recovery from inactivation on the next pulse. (C) A 1-s hyperpolarization to -80 mV presented during the 2-s interval caused a 55% recovery from inactivation on the next depolarization. Inactivation accumulated again during the continuation of the train in C and followed about the same time course as the onset of inactivation at the beginning of the pulse train. Inward current peaks were clipped by the recording device in these records. Perfusion voltage clamp: normal external and internal solutions.

interval. In Fig. 1C, a 1-s hyperpolarizing pulse to -80 mV was applied during a 2-s interval between two pulses later in the train. The hyperpolarization was followed by a threefold increase in peak current on the subsequent pulse, which indicates pronounced recovery from inactivation. Inactivation proceeded again from the newly recovered amplitude during continuation of the train. This experiment shows that short hyperpolarizing steps can lead to pronounced recovery from inactivation.

The steady state voltage dependence of recovery from inactivation was measured in a three-pulse experiment. First, the cell was held at conditioning voltages between 0 and -120 mV for 1 min. A second pulse to -35 mV was applied for 3 s to allow the transient K current, I_A , to activate and then inactivate completely. This was followed by a test pulse to $+10$ mV for 500 ms and then the peak outward current during the test pulse was measured. This pulse schedule was

repeated for different conditioning voltages at 2-min intervals during which the voltage was held at -40 mV. The peak amplitude of the outward current during each test pulse was normalized to the amplitude after a conditioning pulse to -100 mV; Fig. 2 shows the normalized currents plotted against the conditioning voltage for one cell. Recovery reached a minimum value of ~ 0.4 near 0 mV but increased as the conditioning voltage was made more negative. Half-recovery occurred at -45 mV, which is close to the resting potential of -40 mV in this cell.

Steady state recovery data from 11 cells are collected in Fig. 3. The points represent the peak amplitudes of currents during test pulses as in Fig. 2. For

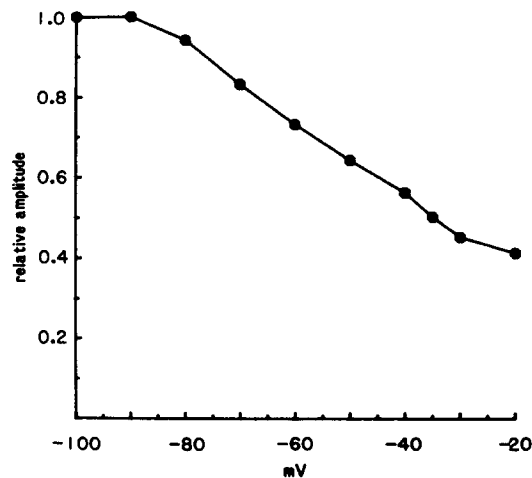


FIGURE 2. Voltage dependence of recovery from inactivation during 1-s hyperpolarizing conditioning pulses. Percent recovery was calculated for each conditioning pulse from the ratio of peak currents as described in the text. Microelectrode voltage clamp.

each cell, the peak currents at all of the test voltages were averaged and then each individual current peak was plotted relative to this average. This was done to minimize scatter in the plot, which results because some cells experienced more pronounced inactivation than others (see Aldrich et al., 1979a). The solid line in Fig. 3 represents the average recovery for all 11 cells at each voltage. In most cells, recovery tended toward a saturating value with hyperpolarizations to about -120 mV, but the amount of recovery during membrane hyperpolarization in different cells was quite variable, as indicated by the scatter in the collected data. There may be two or more types of inactivating delayed K channels differing in the kinetics or voltage dependence of inactivation and recovery. If several types of inactivating channels are expressed in different relative numbers in different cells, this could account for the variability. Our methods cannot resolve this point, but the results indicate a potentially important heterogeneity between cells.

The procedure used in this experiment might underestimate the amount of recovery during hyperpolarization if outward current inactivates further during the 3-s pulse to -35 mV that separates the conditioning pulse from the test pulse. The magnitude of this error is not great, however, since changes in inactivation occur slowly at -35 mV with a time constant of >30 s (see below). Because of this slow inactivation rate, the pulse protocol of Fig. 2 should not cause a significant error in the estimation of recovery.

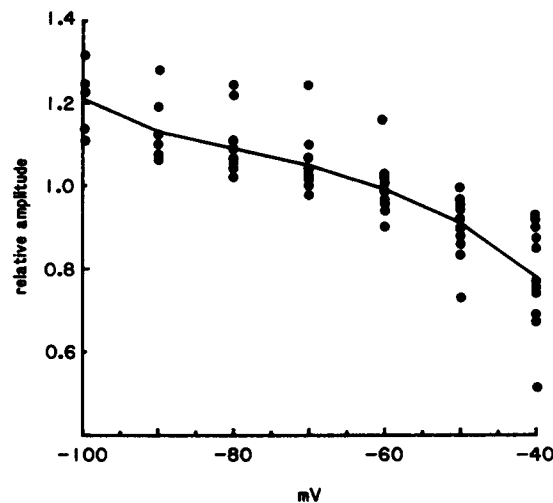


FIGURE 3. Voltage dependence of recovery from inactivation during 1-s conditioning pulses. The relative amplitude of recovered current is plotted against conditioning voltage by normalizing about the mean recovery current amplitude for each experiment ($n = 11$). The line represents the average relative current amplitude at each voltage.

Time Course of Recovery During Hyperpolarization

The time course of recovery from inactivation during hyperpolarization was measured with a nine-step protocol designed to avoid excessively long depolarizations. A train of seven 300-ms depolarizing pulses to $+10$ mV was applied from a holding voltage of -35 mV at a rate of 1/s in order to cause significant ($\sim 70\%$) inactivation of the peak outward current. After a 250-ms delay, a hyperpolarizing conditioning pulse of variable amplitude and duration was applied. The voltage was returned to -35 mV for 3 s before presenting a test pulse to $+10$ mV. The amount of recovery due to the conditioning pulse was calculated from the ratio $(P_t - SS)/(P_1 - SS)$, which is the ratio of peak current during the test pulse (P_t) minus peak current during the seventh pulse in the train (SS) to the peak current during the first pulse in the train (P_1) minus the amplitude during the seventh pulse (SS). The duration of the hyperpolarizing conditioning pulse was systematically lengthened and recovery was calculated for each conditioning voltage and duration. The time course of recovery is shown for one cell in Fig. 4 for

conditioning pulses to -40 , -70 , and -90 mV. These results are characteristic of eight experiments.

The rate of recovery increased as the conditioning pulse was made more negative. A recursive method (Marquardt, 1963) was used to fit either a single exponential or a double exponential to the data, and the smooth lines in Fig. 4 represent the fitted curves. The recovery time course at -40 mV was best fit by a single-exponential function with a time constant of 24.5 s. At more negative voltages, the recovery rate was better approximated by the sum of two exponentials. This is shown in Fig. 4 for conditioning pulses to -70 and -90 mV in the

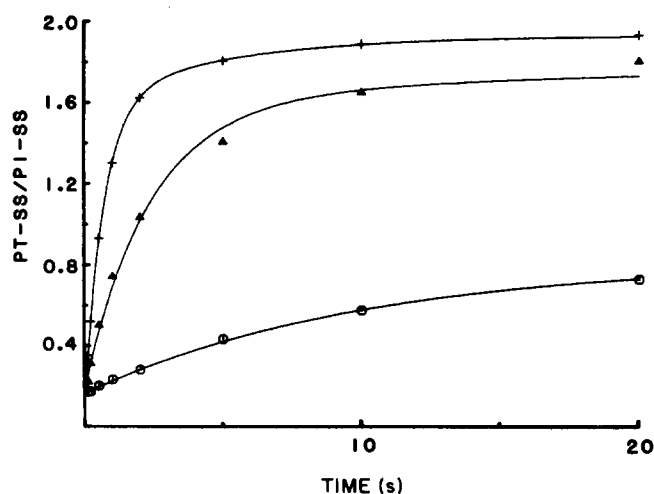


FIGURE 4. Time course of recovery at three different conditioning voltages: -40 (open circles), -70 (triangles), and -90 mV (crosses). The relative recovery is plotted as a function of conditioning pulse duration and is derived by the formula $(PT - SS)/(P1 - SS)$, where PT is the current amplitude during the test pulse (following step to the conditioning voltage), $P1$ is the current amplitude during the first of seven depolarizing steps, and SS is the steady state current amplitude measured during the seventh depolarizing step. Curves were fitted to points by a recursive method using either a single exponential (for data collected after -40 -mV conditioning pulses) or a double exponential (for data collected following -70 - and -90 -mV pulses).

same cell. At -70 mV, the two time constants were 9.16 and 17.8 s. Approximately 43% of the inactivated channels recovered by the faster time constant. For conditioning pulses to -90 mV, the time constants were 1.7 and 9.8 s, and at this voltage $\sim 30\%$ of the channels recovered with the faster time constant. This analysis indicates that there may be two inactivated states with the recovery from the two states proceeding at different rates. Recovery from both states becomes faster with greater hyperpolarization.

Effects of Changes in External and Internal K Concentration

We found, in agreement with Aldrich (1979), that I_K inactivates less during repetitive depolarizations when the cell is bathed in a high K solution. This

appears to result from the fact that a greater number of channels recover with the faster rate during the intervals between pulses in the train. Fig. 5 shows the results of an experiment where the cumulative inactivation of I_K was measured during trains of 300-ms pulses to +10 mV, presented at a rate of 1/s in external media containing either 10 (the control solution) or 200 mM K (K substituted for Na). The absolute amplitude of tail current relative to the steady holding current was measured 2 ms after the membrane voltage was returned to -40 mV following each depolarizing pulse in the train. These values were normalized by dividing by the amplitude of the tail current 2 ms after the first pulse. The normalized values were plotted against pulse number to show the sequential

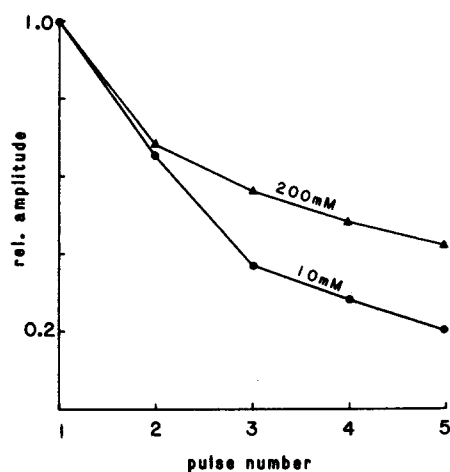


FIGURE 5. High external K decreases the accumulation of outward current inactivation during repetitive depolarizations. A train of 300-ms depolarizations to +10 mV from a holding voltage of -40 mV was imposed at a frequency of 1/s in normal (10 mM) K and then in a solution containing 200 mM K. The absolute amplitude of tail current relative to the steady holding current was measured 2 ms after each depolarizing pulse. These values were normalized by dividing by the amplitude of tail current after the first pulse and are plotted against pulse number to show the gradual accumulation of inactivation.

accumulation of inactivation. The accumulation of inactivation was less pronounced in the high K solution. After five pulses in high K, the tail current amplitude was 0.55 ($n = 3$) times the amplitude after the first pulse, whereas in normal K it was 0.14 ($n = 3$) times the first pulse amplitude. These effects were not due to the change in external Na concentration that accompanied the ion substitution, because when Tris instead of K was used as a substitute for Na, the accumulation of inactivation during repetitive depolarizations was independent of the Na concentration.

Extracellular K accumulation could lead to an overestimate of the difference in the amount of inactivation in high and low K salines, but the present results cannot be explained in this way. It was observed that the reversal potential for outward current changed by no more than 2 mV during trains of five pulses in

10 mM K, a saline solution that should maximize the effect of accumulation. This small change in reversal potential could account for, at most, an 8% change in tail current amplitude and is not sufficient to explain the 41% difference in the degree of inactivation in normal and high K solutions shown in Fig. 5.

The steady state voltage dependence of recovery from inactivation was measured in 10 and in 200 mM K saline. High K had little effect on recovery at the resting potential, but recovery became progressively greater than the control as the conditioning voltage was made more negative (see Fig. 6). When scaled to

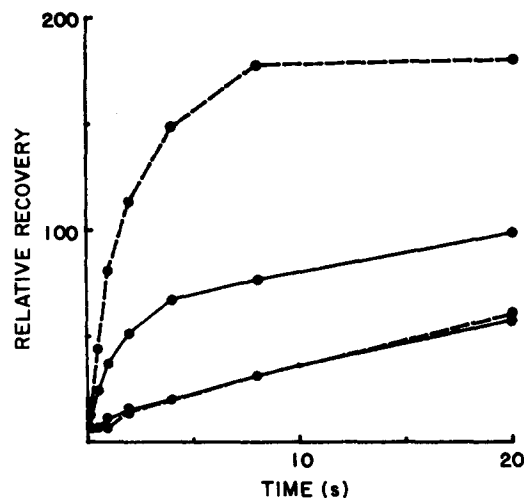


FIGURE 6. Time course of recovery from inactivation at -40 and -90 mV in normal external K (10 mM) and in 200 mM K. Recovery was measured using the protocol of Fig. 4. Test current amplitudes were normalized to the peak test current after a 20-s conditioning pulse to -90 mV in normal K^+ . Recovery is greater after a hyperpolarizing conditioning to -90 mV pulse in 200 mM K^+ (uppermost broken line) than in 10 mM K^+ (upper solid line). Recovery at -40 mV was the same in 10 mM external K^+ (lower solid line) and 200 mM K^+ (lower broken line).

the recovery at -100 mV, the two curves had the same shape, which indicates that high external K increased the amount of recovery during hyperpolarization but did not change the voltage dependence of recovery. Measurement of the time course of recovery at -40 and -90 mV showed that the time course at each voltage was similar in high and normal K (Fig. 6), but that a larger fraction of the inactivated channels recovered via the faster pathway during hyperpolarization in the high K medium.

Lowering of the external K concentration had the opposite effect of slightly increasing the accumulation of inactivation during repetitive pulses. Inactivation was also slightly decreased after internal perfusion with a low K medium.

Short Membrane Hyperpolarization Modifies Spike Duration

During repetitive firing, the action potential duration gradually increases toward a steady value that depends on the firing frequency (Aldrich et al., 1979b), and

this broadening of the spike results from inactivation of I_K (Aldrich et al., 1979a). The present experiments showed that K current inactivation can be removed by a short voltage-clamp hyperpolarization and it can also be shown that a hyperpolarizing current pulse can shorten the action potential during repetitive firing. In the example in Fig. 7, the cell was depolarized tonically to elicit a low repetitive firing rate of 2.2 Hz. The first spike in the train had a duration of 12 ms measured at one-half height. At a point during the repetitive train when the

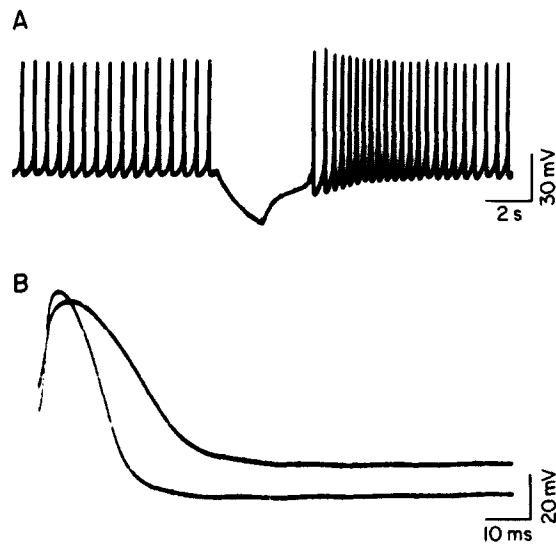


FIGURE 7. A brief membrane hyperpolarization causes a pronounced decrease in spike duration during repetitive firing. (A) The cell was tonically depolarized to elicit a repetitive firing rate of ~ 2 spikes/s. During this depolarization, the spike width gradually reached a steady value of 27.5 ms measured at one-half height. A 2-s current pulse was then applied which caused the cell to hyperpolarize to a maximum voltage of -55 mV. In B, the first spike after cessation of the hyperpolarizing current pulse is shown superimposed upon the last spike just before the hyperpolarizing pulse. The duration of the first spike after the hyperpolarization was 12 ms measured at one-half height.

action potential had reached a steady duration of 27.5 ms, a 2-s current pulse was applied that hyperpolarized the cell to a maximum voltage of -55 mV. The last spike in the train before the current pulse and the first spike after cessation of the pulse were superimposed and are shown in Fig. 7. The action potential duration was shortened from 27.5 to 12 ms as a result of the hyperpolarization. As repetitive firing continued after the hyperpolarizing pulse, the action potential gradually broadened again. This experiment showed that recovery from inactivation of the kind examined in the voltage-clamp experiments can occur in unclamped cells and, therefore, that physiological hyperpolarization by inhibitory synaptic input can result in significant modulation of the action potential duration.

DISCUSSION

The time course of recovery from inactivation suggested that there might be two parallel recovery pathways. A slower recovery pathway had a time constant of 9–40 s over the voltage range between -120 and -40 mV. During hyperpolarizing conditioning steps, a faster recovery process contributed increasingly more to recovery, and the rate of this faster process increased with hyperpolarization.

Aldrich (1981) presented a model for K current inactivation that with slight modification might explain the results presented here. His model assumes that individual outward current channels may exist in any of four states: a resting closed state, *R*; an open conducting state, *A*; and two separate inactivated states, Z_r and Z_a . Transitions between these states are assumed to be governed by voltage-dependent rate constants. During depolarization, resting channels may open and rapidly occupy state *A* or they may inactivate rapidly to state Z_r since these two transitions occur at comparable rates. If depolarization is maintained, channels that open may inactivate over a slower time course to a state Z_a . In this model, therefore, the probability that a channel will enter either state Z_r or Z_a depends on the voltage and the initial state of the channel. Because the rate of inactivation from the resting state is similar to the opening rate, a substantial fraction of channels undergo the transition from state *R* to state Z_r immediately on depolarization. Aldrich (1981) showed that a mathematical formulation of this model successfully predicts the time course of inactivation during depolarization, the time course of recovery from inactivation at the resting potential, and the accumulation of inactivation during repetitive depolarizations.

The model assumes that recovery from the two inactivated states follows the same time course. If we assume instead that the rate of recovery from state Z_r is more steeply dependent on voltage and becomes more rapid than recovery from state Z_a when the cell is hyperpolarized, then the present results might be explained in terms of Aldrich's model. In this formulation, then, recovery from state Z_r would be responsible for the rapid component of recovery that is especially evident during membrane hyperpolarization, while recovery from state Z_a would be responsible for the slower recovery process that predominates at the resting potential.

This idea was tested numerically by applying Aldrich's original four-state model for I_K gating using the parameter values he supplied for activation and inactivation and the time constants we measured for recovery. One cell was chosen as an exemplar and the time constants used in the model to approximate recovery were derived from it. The set of differential equations defining the four-state model can be found in Aldrich (1981). First, cumulative inactivation of K current was calculated for a repetitive train of 300-ms depolarizations from a holding voltage of -35 mV. At the end of the train, the occupancy of the four states (*R*, *A*, Z_a , Z_r) was noted. The time course of recovery at a conditioning voltage of -40 mV was then calculated for different conditioning pulse durations. It was assumed in this case that the recovery rate constant for the transition from state Z_r to *R* was 0 and the rate constant for recovery from Z_a was 0.1 s^{-1} . This means that in the steady state at the resting potential, about one-half of the

channels are in the inactivated state Z_r . Recovery at -40 mV is shown in Fig. 8. To simulate recovery after conditioning hyperpolarizations to -70 and -90 mV, it was assumed that the rate constant characterizing recovery from state Z_r was voltage dependent and was assigned a value of 0.42 s^{-1} at -70 mV and 1.4 s^{-1} at -90 mV. Recovery from state Z_a was also considered to be voltage dependent and was assigned rate constants of 0.061 s^{-1} at -70 mV and 0.209 s^{-1} at -90 mV. These rates were derived from the recovery time constants measured for the exemplar cell assuming that the reverse rate constants for these processes are 0 at -70 and -90 mV. The calculated time courses of recovery at

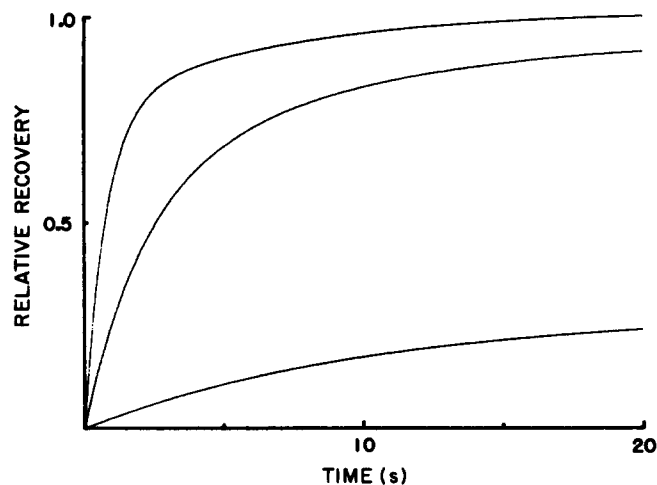


FIGURE 8. Simulated time course of recovery from inactivation at three conditioning voltages: -40 , -70 , and -90 mV. Delayed K^+ currents were simulated using Aldrich's (1981) model for cumulative inactivation. Relative amplitudes are normalized to the peak current amplitude during a test depolarization after full recovery, and are plotted as a function of conditioning pulse duration.

three voltages are shown in Fig. 8. These results should be compared with those of Fig. 4.

Our numerical solutions show that the kinetics of recovery from inactivation can be explained on the basis of voltage-dependent recovery rates for the transitions between two inactivated states and the resting state of the channel. We assumed that the recovery from Z_r was the faster of the two processes. This assignment seems appropriate since we note that initially resting channels inactivate more rapidly during depolarization than do open channels (Aldrich et al., 1979a). This means that during a repetitive pulse train, state Z_r becomes more populated than state Z_a and the difference in occupancy can be large. For example, if we start with 100 channels in state R , i.e., full recovery, after 10 depolarizing pulses to 10 mV, the occupancy of state Z_r is 57, while that of state Z_a is 24. In order to approximate the amplitude of the outward current observed during a test pulse after a conditioning hyperpolarization to -100 mV, the more

rapid recovery process would have to operate on the larger population of inactivated channels, i.e., those channels originally in state Z_r .

Inactivation of I_K in molluscan neuron cell bodies resembles in some ways the block of K channels by quaternary ammonium (QA) compounds in internally perfused squid axons (Armstrong, 1969, 1975). The similarities include the fact that QA-treated axons show a slow recovery time course from block, especially if the compounds have large hydrophobic regions (Armstrong, 1975). Membrane voltage during the interval between depolarizing test pulses affects the amount of block by QA; depolarization increases block, whereas hyperpolarization removes block. Also, raising the external K concentration relieves block in QA-treated axons. Armstrong suggests that at more hyperpolarized voltages, or at higher external K concentrations, the probability of QA ions being dislodged from a blocking site by K ions entering from the outside is increased, which leads to a removal of block. The fact that high external K antagonizes inactivation of I_K in the molluscan cell bodies suggests that normal inactivation may involve a removable blocker that can somehow enter the channel to inactivate the current.

The inactivation of inwardly rectifying K current in skeletal muscle (Katz, 1949), cardiac muscle (McAllister et al., 1975), and echinoderm eggs (Hagiwara and Takahashi, 1974; Ciani et al., 1978) is also dependent upon membrane voltage and the external K concentration. In these cases, as with the cell body K current, hyperpolarization and elevated external K decrease inactivation.

Several studies on the effects of K concentration on K conductance and maintenance of channels suggest the presence of regulatory binding sites for K that alter channel function (Almers and Armstrong, 1980). Dubois and Bergman (1977) found that K conductance increases as a Michaelian function of outside K concentration at the frog node of Ranvier and this effect is independent of voltage (Dubois, 1981). Dubois and Bergman (1977) concluded that both membrane depolarization and an increase in outside K concentration can lead to an increase in the number of open channels. They view this as evidence for the existence of K receptors or binding sites and suggest that binding is a necessary step for the formation of conducting channels and that the probability of a K ion reaching the receptor is higher for external K ions than for internal K. In the present study, high external K increased the recovery from inactivation during hyperpolarizing conditioning steps, possibly by increasing the number of inactivated channels recovering by the faster rate. One interpretation of this is that external K may act to stabilize one particular inactivated state of the channel.

In some types of molluscan cells, the removal of K activation by membrane hyperpolarization is readily observed during normal activity. Burst-firing pacemaker cells such as *Aplysia* cell R15 are an example. In these cells, the first spike in a burst is significantly shorter in duration than the last spike in the burst, presumably because of removal of inactivation during the hyperpolarized interburst interval. The experiment illustrated in Fig. 7 provides another example where membrane hyperpolarization can modify the spike duration by removal of K inactivation. Changes in the degree of inactivation of K currents can be important to the integrative function of the nerve cell since the duration of the action potential and hence the magnitude of Ca, Na, and K fluxes are affected.

Changes in spike duration could, therefore, modulate such cellular activities as synaptic transmission, repetitive firing, pacemaking, and metabolic rate.

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