Analysis of K Inactivation and TEA Action in the Supramedullary Cells of Puffer

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ABSTRACT Under the voltage clamp condition, the K inactivation was analyzed in cells bathed in the isosmotic KCl *Lophius*-Ringer solution. After conditioning hyperpolarization, the cells respond to depolarizations with increased K permeability, which in turn is decreased during maintained depolarizations. The steady-state levels of the K inactivation as a function of the membrane potential are related by an S-shaped curve similar to that which describes the steady-state Na inactivation in the squid giant axon. TEA reduced the K conductance by a factor which is independent of the potential, and without a shift of the inactivation curve along the voltage axis. The rapid phase of the K activation is less susceptible to TEA than the slow phase of the K activation. Hyperpolarizing steps remove the K inactivation, the rate of the removal being faster the larger the hyperpolarization from the standard potential of about −60 mv.

INTRODUCTION

In the previous paper (16) on the voltage clamp analysis of the supramedullary cell (SMC) of the Atlantic puffer, *Spheroides maculatus*, the delayed currents which were produced upon sudden depolarizations were shown to decline during long lasting depolarizations. It was further shown that the changes of the current were almost entirely due to the conductance changes to potassium ion rather than to electromotive force changes of the membrane. Thus, the presence of the K inactivation process, which incompletely eliminated the K activation process, was confirmed. It was also shown that there were two inactivation processes, one developing relatively rapidly, the other occurring very slowly, with a K reactivation interposed between the two phases of K inactivation.

In the work reported here, the effect of the membrane potential on the steady-state values of the K inactivation was studied, and the action of

tetraethylammonium (TEA) on the potassium permeability was analyzed. It is shown that the effect of the drug is to diminish the component of K conductance which is independent of the membrane potential. An attempt is also made to analyze the kinetics of the K inactivation when the membrane is suddenly hyperpolarized to various levels.

METHODS

The methods of preparing the materials and for voltage clamping were described in the previous paper (16). All the experiments reported here are concerned with the

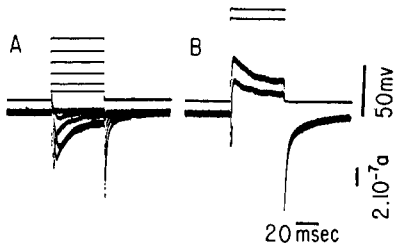

FIGURE 1. The occurrence of K activation and inactivation in the cells bathed in the isosmotic KCl solution. The membrane potential was preset to −75 mv, which corresponds to the base lines of the potential recordings. Depolarizing pulses induced increments of the inward currents (*A*); larger depolarizations (*B*) induced outward currents. The time course of the decline of these currents was similar to the decline of the delayed outward current in the normal medium. In all the records shown in this paper, the upper trace displays the potential, and the lower one the current. Upward deflection corresponds to the depolarizing direction of the potential and the outward direction of the current.

analysis of the K permeability in the SMC's which were immersed in an isosmotic KCl *Lophius*-Ringer solution. The performance of the same kinds of analysis on the cells bathed in the normal *Lophius*-Ringer proved to be impossible for the following reason. Application of conditioning hyperpolarizations to the cells bathed in the normal solution evoked a large inward current of more than 10 μa upon subsequent depolarizations probably due to the removal of inactivation of the initial current. This large current, often surpassing the limitation of the output of the feedback amplifier, resulted in an insufficient voltage clamping at the early phase of depolarization.

The TEA solutions were made by mixing the KCl *Lophius*-Ringer solution with the *Lophius*-Ringer solution in which all NaCl and KCl were replaced by equimolar concentrations of TEA chloride.

RESULTS

A. *Voltage-Current Relation in the KCl Solution*

When cells which had been depolarized by the KCl solution were hyperpolarized to about −60 mv for about 1 min, the membrane became responsive

to a subsequent depolarization with a permeability increase toward potassium ion. Thus, as shown in Fig. 1, under the voltage clamp condition a depolarization which exceeded about 25 mv but was still below the K equilibrium potential gave rise to an increment of inward current which represented an influx of potassium ion down the electrochemical gradient (*A*). A depolarization exceeding the K equilibrium potential evoked an outward current (*B*). As discussed in the preceding paper (16), these inward and outward currents declined with a time course similar to that of the initial phase of K inactivation observed in the cell in the normal medium.

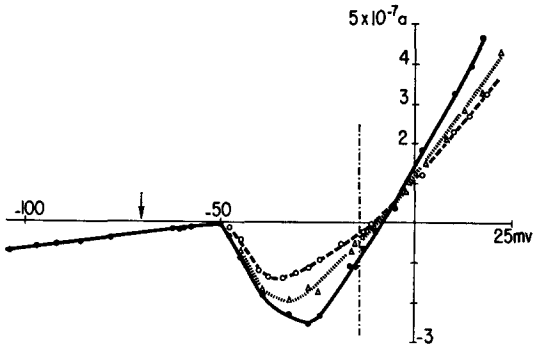

FIGURE 2. Current-voltage relationship of the cell immersed in the KCl solution. The resting potential was −14 mv (broken vertical line). The membrane potential was preset to −70 mv (arrow); then, depolarizing pulses were applied. Filled circles, measured at the peaks of the currents. Triangles, 80 msec after the onset of depolarization. Open circles, 860 msec. The point at which the three curves cross (about −5 mv) is the K equilibrium potential, which lies between resting membrane potential and outside zero potential.

Fig. 2 illustrates the current-voltage relations obtained from an experiment similar to that in Fig. 1. In this example, the membrane potential, which had been depolarized to −14 mv (broken vertical line), was hyperpolarized to −70 mv (arrow) for about 1 min. Depolarizing pulses were then superimposed on the conditioning hyperpolarization. The filled circles represent the current-voltage relation at the peak inward and outward currents, and the triangles and open circles give those at 80 and 860 msec, respectively. These three curves all passed through a point (about −5 mv), at which the current stayed unchanged in spite of possible changes of the conductance, and which represented the K equilibrium potential. Obviously, the conductances at the later phases are smaller than those at the initial phase over the entire range of the potentials at which the K activation occurs. This result is in keeping with the conclusion of the preceding paper (16) that the decline of the delayed currents is due to the change of conductance and not to a change of electromotive force.

B. *Steady-State Level of K Inactivation*

Fig. 3 shows the curves which illustrate how the membrane potential influences the availability of the K activation mechanisms. First, the membrane potential was set at a given value for at least 1 min. Then, varying magnitudes of depolarizing pulses of about 50 msec duration were superimposed on the conditioning potential. This gave the current patterns and current-voltage relation, which are illustrated in Figs. 1 and 2. From these records the K conductances at 5 msec after the onset of the pulse at the membrane potential of

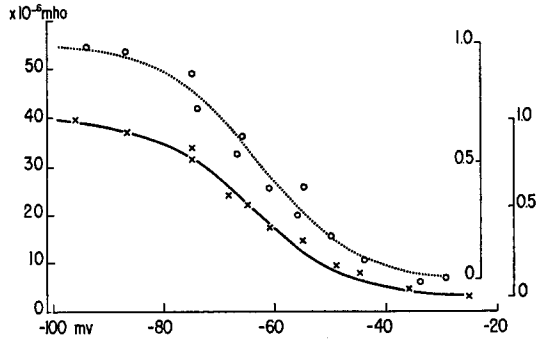

FIGURE 3. Steady-state values of the K inactivation as a function of the membrane potential in the cell bathed in the KCl solution, and the effect of TEA. The abscissa is the conditioning membrane potential. The left ordinate is the conductance at 5 msec after the onset of depolarization. The right ordinates are normalized units (k_∞). Circles, before application of TEA. Crosses, after application of TEA (40%). Further explanation in text.

0 to +10 mv, where the current-voltage relation was almost linear (Fig. 2), were determined.[1] The values of the conductances thus obtained were plotted on the ordinate of Fig. 3 with the conditioning potential on the abscissa. The right ordinates were determined by taking the maximum and minimum conductances as 1.0 and 0, respectively. We shall refer to these normalized values of K inactivation as k_∞. The circles are the data with the KCl solution, while the crosses were obtained after application of TEA. The broken line was drawn according to the equation:

$$k_\infty = \frac{1}{1 + \exp\{(E - E_k)/a\}} \quad (1),$$

where E = membrane potential, E_k = potential at half-inactivation (−63

[1] Here the potassium conductances were measured at a specified time (5 msec after the onset of depolarizing pulses). This procedure is a little different from the usual method for determining the Na inactivation, in which the peak conductances were measured (11, 12).

mv), and a = a constant which determines the steepness (8.5 mv). The upper half of Table I shows that the average values of E_k and a are −68 and 9.1 mv.

C. *Incompleteness of the K Inactivation*

The above procedure of normalizing the steady-state level of the K inactivation by the term k_∞ (0–1) did not take account of the fact that even after full

TABLE I

STEADY-STATE K INACTIVATION BEFORE AND AFTER APPLICATION OF TEA

No.	TEA	g_{min}	g_{max}	E_k	a
	%	mmho	mmho	mv	mv
133-1	0	0.0057	0.045	−73	9.0
133-3	0	0.0057	0.040	−64	6.5
137-2*	0	0.0068	0.057	−73	9.0
147-2	0	0.0023	0.035	−65	12.5
155-1‡	0	0.0068	0.054	−63	8.5
Mean		0.0055	0.046	−68	9.1
137-2*	30	0.0057	0.041	−75	9.0
137-3	30	0.0068	0.030	−74	8.3
137-4	30	0.0057	0.026	−72	7.5
148-2	20	0.0057	0.033	−66	8.5
155-1‡	40	0.0029	0.039	−64	8.5
155-2	40	0.0023	0.033	−66	9.5
Mean		0.0049	0.034	−70	8.6

g_{min} and g_{max} are the K conductances with the full development and full elimination of K inactivation. E_k and a are the parameters in equation (1). *, ‡ Two experiments before and after exposure of the same cell to TEA.

development of the K inactivation, the membrane conductance was still higher than the leak conductance (membrane conductance at about −60 mv). In other words, a large depolarization of long duration does not eliminate the K activation completely. For instance, in the case illustrated in Fig. 3 (circles), the leak conductance was 2.0 μmho, whereas the conductance at the minimum inactivation (k_∞ = 1.0) was 54 μmho and at the maximum inactivation (k_∞ = 0) was 6.8 μmho, respectively. Therefore, the K conductance after the full development of inactivation, 4.8 μmho (6.8–2.0), is about one-tenth the value of the K conductance at the full removal of the K inactivation. If we transf rm k_∞ into k'_∞, in which zero corresponds to the leak conductance, the empi cal equation becomes more complicated, but the half-inactivation pote ial (E_k) changes only about 1 mv.

D. *Effect of TEA on K Conductance*

The steady-state levels of K inactivation after the application of 40% TEA are shown by crosses in Fig. 3. In comparison with the steady-state values before the application of the drug (circles), the K activation was depressed by about 30% over the entire voltage range, but there was no shift of the curve along the abscissa. The values of E_k and a are −64 and 8.5 mv, respectively, indicating that the two normalized curves are almost identical. Table I summarizes the values of E_k and a before and after the application of TEA. The values of cells 137-2 and 155-1 are from the two successful cases, in which the

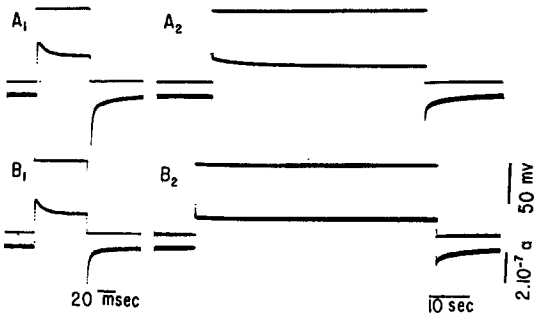

FIGURE 4. Effect of TEA on the time course of delayed outward current in a cell bathed in the KCl solution. The membrane potential was preset to −77 mv, which corresponds to the base lines of each potential recording. A_1 and B_1, fast sweep records. A_2 and B_2, slow sweep records. A, before application of TEA. B, after application of TEA (20%). Note the small changes in the recordings of the fast sweep in contrast to the marked effect on the slow sweep recordings.

measurements were done on the same cell before and after application of TEA. The table shows that the maximum K conductance decreased by 26% but that there were no appreciable differences in the values of E_k and a between the control and after TEA application.

Fig. 4 illustrates how TEA affects the sequential pattern of the K activation and inactivation. The controls before the application of TEA are shown in A_1 and A_2 at fast and slow sweeps, respectively. Depolarizing pulses were applied to the prehyperpolarized membrane (−77 mv). This produced the outward current which declined rapidly at first (A_1) and then slowly (A_2). These represent the rapid and slow phases of K inactivation. B_1 and B_2 were obtained after the application of TEA (20%), otherwise with the same procedure as in A_1 and A_2. The comparison of A_1 and B_1 shows that TEA decreased the K activation as represented by the peak of the current by about 9%. In contrast to the relatively small change in the fast sweep records (A_1 and B_1), the slow sweep records show a marked difference between the control (A_2) and after

the application of TEA (B_2). Although in the control a slow phase of K inactivation was clearly seen, after the application of TEA the K inactivation process completed rapidly, leaving almost no phase of slow inactivation. Although application of lower concentrations of TEA (5 to 10%) produced hardly any effect on the peak value of the K current, they always accelerated the time course of the slow phase of K inactivation. From these results we can conclude that the slow phase of K activation is more susceptible to TEA than the initial phase of K activation.

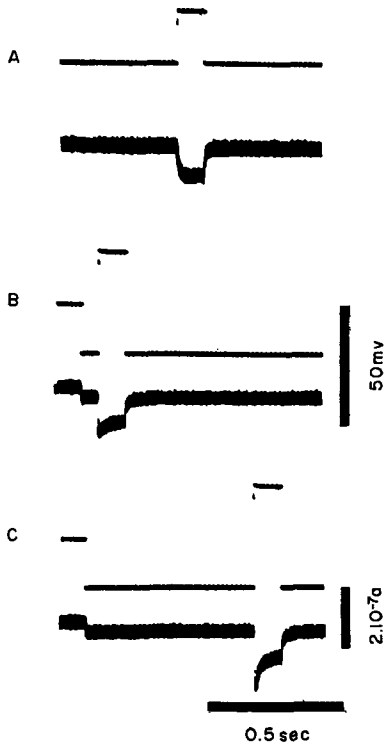

FIGURE 5. Removal of the K inactivation by hyperpolarization in the cells bathed in the KCl solution. Two-step experiment. The cell was hyperpolarized to −60 mv, which corresponds to the base line of each potential record. A test depolarizing pulse produced an inward current (*A*). The depolarization after the application of a hyperpolarizing step produced a larger inward current (*B*). The inward current became larger, the longer the hyperpolarization was applied (*C*).

E. *Kinetics of Removal of K Inactivation*

Fig. 5 *A* shows an experiment in which a depolarizing pulse of 22 mv was imposed on a KCl-treated cell, whose membrane potential had been set to −60 mv. This produced an inward current in addition to the small steady inward current which was needed for the conditioning hyperpolarization. As discussed in connection with Fig. 1 this increment of inward current is a result of an increase of K permeability evoked by the depolarizing pulse. As shown in Fig. 5 *B*, *C* and in the explanatory inset of Fig. 6, test depolarizing pulses (V_2) were applied at varying times (t) after a further conditioning hyperpolar-

izing step (V_1). Then, the magnitude of the inward current (I_K)[2] measured from the original level of the current records was increased by the additional hyperpolarization. The plot of I_K vs. t gives the time course of the removal of the K inactivation as a consequence of the added hyperpolarization. This is illustrated in Fig. 6. The ordinate gives potassium current (I_K) relative to the current with the test pulse (V_2) alone, while the abscissa shows the duration (t) of the conditioning hyperpolarization (V_1). The membrane potential had been preset to −60 mv for about 1 min before the application of V_1. The

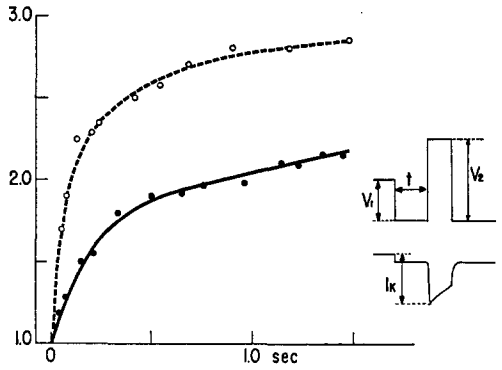

FIGURE 6. Time course of the removal of the K inactivation. Based on the data illustrated in Fig. 5. The inset diagram illustrates the experimental procedure. The abscissa is the duration (t) of the conditioning hyperpolarizations (V_1). The ordinate gives the inward current (I_K) relative to the current with the test depolarizing pulse (V_2) alone. The filled circles, V_1 = 19 mv. The open circles, V_1 = 39 mv. The initial 0.6 sec of the solid line and the initial 0.3 sec of the dashed line were drawn according to simple exponential functions with time constants of 0.24 and 0.08 sec, respectively.

additional hyperpolarizations (V_1) were 19 mv (filled circles) and 39 mv (open circles). The curves cannot be fitted by a simple exponential function. We can, however, regard these curves as having an initial exponential component followed by a much slower process. Actually the initial 0.6 sec of the continuous line and the initial 0.3 sec of the broken line of Fig. 6 were drawn according to a simple exponential function with time constants of 0.24 and 0.08 sec, respectively. The values of the time constant (τ_{ki}) of the initial phase of the removal of K inactivation (Table II) show that τ_{ki} is smaller, the higher V_1. This is analogous with the kinetics of the removal of the Na inactivation process in the squid giant axon (11).

Another way of following the recovery from the K inactivation is to use two successive depolarizing pulses (11). This is illustrated by Fig. 7. First, the potential was preset to −72 mv; next, a depolarizing pulse (53 mv) of long dura-

[2] I_K thus defined is smaller than the true potassium current by a constant amount of leak current. However, this procedure does not affect the estimation of the time constant of K inactivation.

tion (10 sec) was superimposed; after varying intervals from the termination of this depolarizing pulse, a second depolarization of smaller magnitude was applied. When the interval between the two pulses became long (*B*), the inward current (I_K) measured from the base line before the application of the first depolarization increased.

TABLE II

TIME CONSTANT OF THE RECOVERY OF THE K INACTIVATION

No.	Preset potential	τ_{hi}	
		V_1 = 19 to 20 mv	V_1 = 39 to 40 mv
	mv	*msec*	*msec*
71-3	−64	180	47
72-4	−65	75	55
73-1	−60	60	40
73-2	−60	240	80

τ_{hi}, the time constant of the initial phase of the recovery from the K inactivation.
V_1, conditioning hyperpolarization.

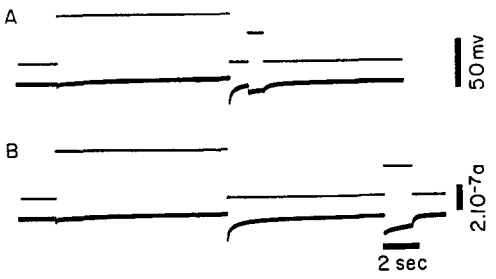

FIGURE 7. Removal of K inactivation in the KCl solution. Two-pulse experiment. The membrane potential was preset to −72 mv, which corresponds to the base line of each potential record. First, a long, large depolarizing pulse was applied in order to cause K inactivation. At varying times after the first pulse, a second test pulse was applied to measure the time course of recovery from the inactivation. In *B* larger inward current was produced than in *A*.

The relationship of the inward current (I_K) vs. the recovery time from K inactivation is plotted in Fig. 8. As can be seen from Fig. 7, cessation of the first depolarizing pulse induced an undershoot of about 5 sec duration in the current recording. This undershoot represents the recovery from K activation. Because of the presence of the undershoot, the time course of the recovery from the K inactivation was not determined accurately during the initial 5 sec. However, if this initial 5 sec is disregarded, the curve is reasonably well fitted by a simple exponential function with the time constant of 16 sec in this cell.

This slow rate of recovery is to be expected because this method allows only the second slow phase of the removal from K inactivation to be measured, whereas the two-step experiment (Figs. 5 and 6) revealed both the rapid and slow phases of the recovery process.

DISCUSSION

The analyses of the K inactivation have shown that the rate of recovery from the inactivation is faster, the more the membrane is hyperpolarized from the standard potential of about −60 mv. Thus, the qualitative aspects of the kinetics of the removal of K inactivation resemble those of the Na inactivation

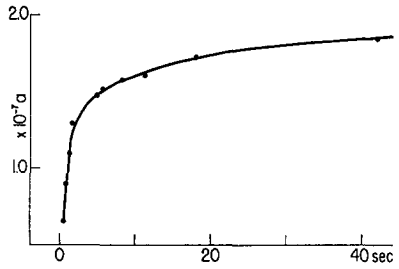

FIGURE 8. Recovery from K inactivation in the KCl solution. Based on the data illustrated in Fig. 7. Abscissa is the interval between the termination of the first pulse and the onset of the second pulse. Ordinate gives the inward current measured from the base line of the current recording. Except for the initial 5 sec, when an accurate measurement could not be made (see text), the line was drawn after a simple exponential function with a time constant of 16 sec.

of the squid giant axon (11). However, the kinetics of the removal could not be analyzed in terms of a simple exponential function; instead, we could more conveniently divide the process into two components: one a rapidly developing, and the other a slowly occurring component. This is in accord with the similar kinetics of the development of K inactivation in the SMC, dealt with in the preceding paper (16). Also in lobster axon more than one time constant was observed for the Na inactivation and reactivation (19).

The similarity between K inactivation and Na inactivation becomes more evident when we compare the steady-state behavior of the system as a function of the membrane potential. Not only was the empirical equation which represents the steady level of Na inactivation in squid giant axons and Ranvier nodes found to fit the steady-state behavior of the K inactivation, but also the values of the parameters of the equation were not very different: the values for E_k and a are −68 and 9.1 mv in the SMC (Table I), and the half Na inactivation potential (E_h) and a were about −60 and 7 mv in the squid axon (11, 12), and −61 and 8 mv in the Ranvier node (5, 6). Therefore, we may

speculate that the molecular mechanisms of the Na and K inactivations are basically similar.

On the other hand, the quantitative aspects of the kinetics of the K inactivation are quite different from those of Na inactivation, for the rate of development of K inactivation is much slower than that of Na inactivation. Thus, it seems more appropriate to regard Na activation and inactivation, and K activation and its inactivation as separate processes taking place in two different "sites" of the membrane rather than to assume a single channel with complex properties (8, 9). The discriminative action of tetrodotoxin on the Na activation process described by Narahashi et al. (20), Nakajima, Iwasaki, and Obata (15), Narahashi, Moore, and Scott (21), and Nakamura, Nakajima, and Grundfest (17) is also in keeping with the idea of the functional differentiation of the two activation systems.

Since the work of Tasaki and Hagiwara (23), TEA has been regarded as a depressant of the K activation mechanisms (8, 10, 27). The results of the present paper further suggest that the mode of TEA action is to depress a component involved in the K conductance which is independent of membrane potential. Therefore, this mode of TEA action is similar to the mode of action of the alcohols or local anesthetics on the squid giant axon (1,14, 22, 26). It differs, however, from the action of calcium on the Na activation mechanism (7), or from the effects of internal perfusion of the squid giant axon with solutions of low ionic strength (3, 4, 13, 18, 24, 25). In the latter two cases the effects are interpreted as the displacement of the Na activation and inactivation curves along the voltage axis.

Recently, Armstrong and Binstock (2) reported that the potassium channel showed an anomalously rectifying property after the internal application of TEA in the squid giant axon. In the SMC, however, the membrane after application of TEA did not show a marked rectifying property. Since we applied a high concentration of TEA in order to see obvious changes of outward current, subtle effects of lower concentrations of the drug on the inward current might have been overlooked, and further experiments on this point are necessary.

The mode of TEA action described above cannot explain all the aspects of the effects of the drug since TEA also altered the time course of the K inactivation processes. Although this cannot be analyzed in terms of a simple equation, the fact itself suggests that not only the population of the active sites for K permeability is reduced but also the kinetics of the individual site are affected by TEA.

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