Behavior of Delayed Current under Voltage Clamp in the Supramedullary Neurons of Puffer

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ABSTRACT Depolarizations applied to voltage-clamped cells bathed in the normal solution disclose an initial inward current followed by a delayed outward current. The maximum slope conductance for the peak initial current is about 30 times the leak conductance, but the maximum slope conductance for the delayed current is only about 10 times the leak conductance. During depolarizations for as long as 30 sec, the outward current does not maintain a steady level, but declines first exponentially with a time constant of about 6 msec; it then tends to increase for the next few seconds; finally, it declines slowly with a half-time of about 5 sec. Concomitant with the changes of the outward current, the membrane conductance changes, although virtually no change in electromotive force occurs. Thus, the changes in the membrane conductance represent two phases of K inactivation, one rapidly developing, the other slowly occurring, and a phase of K reactivation, which is interposed between the two inactivations. In isosmotic KCl solution after a conditioning hyperpolarization there occurs an increase in K permeability upon depolarization. When the depolarizations are maintained, the increase of K permeability undergoes changes similar to those observed in the normal medium. The significance of the K inactivation is discussed in relation to the after-potential of the nerve cells.

INTRODUCTION

In addition to the original three factors of spike electrogenesis, Na and K activations and Na inactivation (28), the presence of K inactivation has been demonstrated in the electrically excitable membranes of electroplaques (3, 6, 7, 20, 39), Ranvier node (18, 31), skeletal muscle fiber (36), squid giant axon (12), and Purkinje fibers (9, 27). In nerve cells, however, voltage clamp studies on spike electrogenesis (1, 13, 14, 25, 26) have failed to demonstrate

the presence of K inactivation probably because the analyses have been confined to the events which occur during a relatively short time, whereas K inactivation is a process which usually occurs more slowly. In *Onchidium* nerve cell bathed in K-rich solution, Hagiwara, Kusano, and Saito (24) reported a decay of the membrane conductance during depolarizations; this indicates the occurrence of K inactivation in the nerve cell.

The present paper is largely concerned with long-duration voltage clamp of the nerve cells, the supramedullary cells (SMC) of the Atlantic puffer,

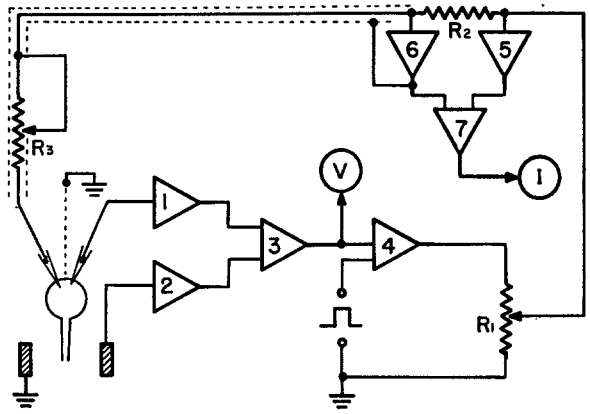

FIGURE 1. Schematic diagram of the voltage-clamping circuit. 1, 2, neutralized capacity input stages for recording membrane potential. 3, 4, differential dc amplifiers for applying feedback; voltage gains are ×5 and ×1000 to 5000, respectively. 5, 6, cathode follower stage for recording the currents. 7, differential dc amplifier for recording the currents. R_1 = 50 k Ω, R_2 = 1 MΩ, R_3 = 22 MΩ.

Spheroides maculatus. The presence of K inactivation, which slowly diminishes the K activation, is shown in the normal environment and in a K-rich solution.

A preliminary account has been published (37).

METHODS

The experimental procedures were the same as those described by Bennett, Crain, and Grundfest (4). The Atlantic puffer, *Spheroides maculatus*, collected off the coast near the Marine Biological Laboratory, Woods Hole, weighing from 100 to 400 g, was used. After exposure of the cluster of nerve cells, *Lophius*-Ringer's solution (44) was applied, and two glass capillary microelectrodes were introduced into the same cell under visual observation, one for passing current (resistance 1 to 5 MΩ, filled with 2 M K citrate), the other for recording potential (about 10 MΩ, filled with 3 M KCl). The isosmotic KCl *Lophius*-Ringer was made by replacing NaCl of the normal *Lophius*-Ringer with equimolar KCl.

The circuit for voltage clamping (Fig. 1) is essentially the same as that used by Hagiwara and Saito (25). In the experiments of short-duration clamping, single-

ended potential recording was employed, and for the long-duration clamping, in order to minimize drifts, the potentials were recorded differentially with the two neutralized capacity amplifiers 1 and 2, and the differential amplifier 3. A calomel electrode placed in the bath surrounding the nerve cells served as the indifferent electrode, and a silver plate was connected to the ground. The currents were measured with the differential amplifier 7 with cathode follower inputs 5 and 6, which had a dynamic range of about ± 100 v and a grid current about 10^{-9} a. This magnitude of grid current produced potential changes in the membrane less than 1 mv because the total resistance of the cells was below 1 MΩ. The membrane potential (E) is expressed as internal potential minus external potential, and the outward current is regarded as a positive quantity (+ I). The experiments were performed at room temperature (20–25°C).

Possible Sources of Errors

A. SERIES RESISTANCE

In the case in which the membrane potential was recorded single-ended, the resistance in series with the membrane was less than 500 Ω, the larger part of which was probably due to the skin resistance of the fish. Thus, an inward current of 5 μa would produce a voltage of 2.5 mv across this series resistance. For analyzing the delayed current, with differential recording of the membrane potential employed, the errors were probably negligible because the series resistance became less, and the current was below 2 μa.

B. CAPACITATIVE ESCAPE OF THE CURRENT

The recorded currents included the capacitative escape from the current-injecting microelectrode to the ground. Assuming the capacity to be 4 picofarads and the electrode resistance 5 MΩ, this effect would make the recorded peak initial inward current (in the case of Fig. 2 F) higher by about 2% than the real current flowing through the membrane. The errors caused by this effect are negligible in measuring the delayed currents, which changed more slowly.

C. SPACE-CLAMP INSUFFICIENCY

Because of the lack of dendrites, and because the series resistance caused by the convergence of current is negligible compared with the membrane resistance, the SMC probably offers the most favorable neuron in so far as uniform potential distribution throughout the soma membrane is concerned. However, the current escaping to the axon would cause some error. When both soma and axon are excited, this error would be less than 10%. This is based on the observation that depolarizations under voltage clamping sometimes produced repetitive small inwardly directed current superimposed on the much larger delayed outward current. These small currents probably represented the neurite activity, and their magnitude was below 10% of the peak inward current, which represented the soma activity. When both soma and axon are quiescent, it is possible that the current spread toward the axon is larger than 10% because the resting membrane resistance of both axon and soma is larger than that

during excitation. Nevertheless, a subthreshold step current produced a voltage transient which showed a fairly good fit to a simple exponential function. Nor did small de- or hyperpolarizations under voltage clamp produce a large residual current transient (Fig. 2 *B*), which reflected the charging process of the dendrite capacity (41). A residual current was observed in the cat's spinal motoneuron (1) and in electromotoneurons of the electric cat fish (S. Nakajima and Bennett, unpublished data).

RESULTS

A. *Experiments in the Normal Solution*

The action potential elicited by intracellularly applied currents had the same characteristics as those described by Bennett, Crain, and Grundfest (4, 5)

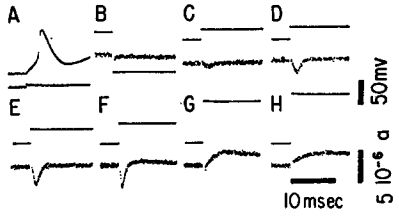

FIGURE 2. Short duration voltage clamp in normal *Lophius*–Ringer's solution. *A*, action potential evoked by an intracellularly applied constant current. *B*, hyperpolarizations were associated with an instantaneous capacitative surge followed by a leak current. *C–F*, depolarizing steps were associated with an initial phase of inward current followed by a smaller outward current. *F* and *G* show a peak in the delayed outward current. In all the records shown in this paper, the upper beam displays the potential, and the lower beam the current. An upward deflection corresponds to a depolarizing potential and outward current.

The spike potential was usually not followed by an after-hyperpolarization, which was reported to be present in the SMC of the Pacific puffer (25) and the SMC of the sargassum fish (8).

1. SHORT-DURATION VOLTAGE CLAMP

Fig. 2 shows a voltage clamp experiment in the normal *Lophius*–Ringer solution. As is shown in Fig. 2 *B*, a hyperpolarizing step was simply associated with a brief capacitative current followed by a steady inward current. Depolarizations over about 30 mv, however, evoked an initial inward current, followed by an outward current (*C–F*). We did not perform experiments to determine the ionic nature of the initial current. However, the delayed current is probably carried by potassium ion as will be shown later. Thus, we shall refer to the initial current as I_{in} and the conductance responsible for it as g_{in}, and to the delayed current and conductance as I_K and g_K, respectively.

Records *F* and *G* show that the delayed outward currents did not maintain

the steady-state values, but declined from a maximum, which occurred about 5 msec after the onset of depolarization. This is an indication of K inactivation, which will be dealt with in more detail below. A very large depolarization (more than 100 mv) often suppressed the development of K inactivation (*H*).

In Fig. 3 current-voltage relations are plotted at the phase of the peak inward current (filled circles) and the peak delayed current (open circles). It is evident that the maximum slope conductance for the initial current ($g'_{in} = \partial I_{in}/\partial E$) is greater than that for the delayed current ($g'_K = \partial I_K/\partial E$). Table I, summarizing the data obtained by the short-duration voltage clamp,

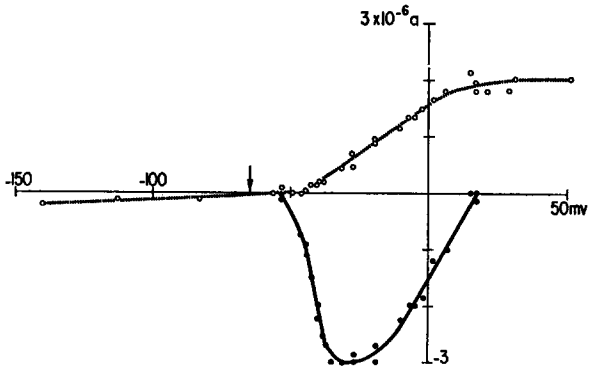

FIGURE 3. Current-voltage relationship under short-duration voltage clamp in the normal solution. The data were derived from the experiment of Fig. 2. The filled circles represent the peak initial current. The open circles show the peak delayed outward current and the leak current. The arrow indicates the resting potential.

shows that while g'_{in} increased by a factor of 30 over the leak conductance (g_l), g'_K was only about 10 times the leak conductance. A conductance lower for the delayed current than that for the initial current was also observed in the lobster axon (30), and is in contrast to the results obtained in other excitable cells (1, 11, 14, 15, 25, 29), in which almost the same conductance increase for the initial and delayed currents has been obtained.

The other feature illustrated in Fig. 3 is that the delayed current is curved in the region above about +20 mv, and sometimes over a narrow range of voltage even a slight tendency to negative conductance is obtained (compare *G* and *H* of Fig. 2). This differs from the characteristic of delayed current in the squid giant fiber, which shows a linear relation over a wide range of depolarization (29). A curvature in the current-voltage relation, which could not simply be attributed to the nonlinearity of the constant field equation (19), was reported for the K current in amphibian myelinated nerve fibers (16).

2. LONG-DURATION VOLTAGE CLAMP

Fig. 4 *A* shows voltage clamp recordings with long-duration pulses at a slower sweep speed and with a higher magnification of current recording. With small depolarizations the outward current stayed almost unchanged as long as depolarizations were maintained ($A_{2\text{-}4}$). When the depolarization exceeded about 40 mv, however, the outward current began to show an early peak, followed by a decline which lasted for about 20 msec ($A_{5\text{-}7}$). When a stronger

TABLE I

SHORT-DURATION VOLTAGE CLAMP DATA

No.	Resting potential	Spike height	Minimum peak inward current	g_l	g'_{in}	g'_K	g'_{in}/g_l	g'_K/g_l
	mv	*mv*	*μa*	*mmho*	*mmho*	*mmho*		
35-2	−53	90	−4.8	0.0050	0.119	0.032	23.8	6.4
35-4	−49	80	−5.4	0.0044	0.178	0.047	40.5	10.7
35-5	−65	77	−3.0	0.0035	0.067	0.029	19.1	8.3
47-1	−60	92	−5.9	0.0140	0.145	0.028	10.4	2.0
52-3	−44	81	−4.0	0.0025	0.097	0.040	38.8	16.0
60-1	−64	66	<−13.0*	0.0195	0.441	0.085	22.6	4.4
74-1	−62	110	−3.5	0.0017	0.049	0.036	28.8	21.2
74-2	−56	98	<−8.1*	0.0046	0.246	0.043	53.5	9.4
Mean	−57	87	−4.4‡	0.0069	0.168	0.043	29.7	9.8

g_l is the leak conductance obtained from anodal polarization of the membrane. g'_{in} is the maximum slope conductance for the initial current (the maximum slope of the solid line in Fig. 3 minus g_l). g'_K is the maximum slope conductance for the delayed outward current (the maximum slope of the dotted line in Fig. 3 minus g_l).

* Could not be measured accurately owing to the saturation of the feedback amplifier due to large inward currents.

‡ Did not include the values of 60-1 and 74-2.

depolarization was applied, the current, which had first decreased, again began to increase (A_7). Fig. 4 B_1, which was recorded with a still slower time base, illustrates clearly the initial phase of decrease and the secondary increase of the current.

If these marked changes of the outward current had been caused by "polarization", changes of electromotive force, a sudden restoration of the potential to the original level should have given rise to an undershoot of the current such as was observed in the squid giant axon (17); the magnitude and the time course of the undershoot should be similar to those of the changes in the outward current. As shown in Fig. 4 $A_{5\text{-}7}$, B_1, however, the restoration of the voltage was associated with only a brief capacitative surge, and never with a slow undershoot of the current. This observation indicates that the

changes of the outward current are not due to changes of electromotive force, but to changes of the membrane conductance.

Records 2 and 3 of Fig. 4 *B* show the currents associated with hyperpolarizations. When hyperpolarizations exceeded a certain magnitude, the inward current declained (B_3); this decline might be partly due to hyperpolarizing K inactivation (22, 42).

When the sequence of the current was observed at a still slower sweep speed, the outward current, which first declined and then increased, was found to

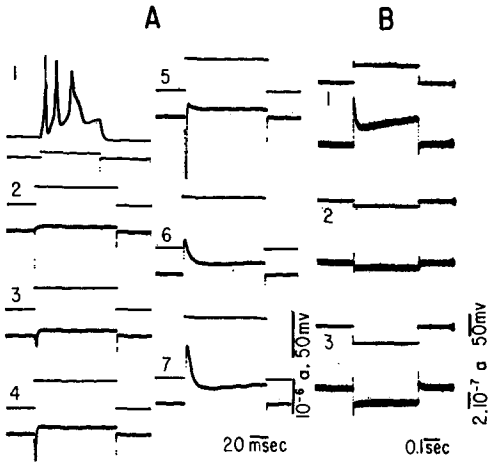

FIGURE 4. Long-duration voltage-clamp experiments on two cells (*A*, *B*) in the normal solution. A_1, repetitive action potentials elicited by an intracellularly applied constant current. A_2–A_7, voltage clamp records. The decline of outward current after the early maximum is displayed in A_5–A_7. A secondary rise in the outward current after the decline is recorded at a slower sweep speed in B_1.

decrease again very slowly (Fig. 5). At a depolarization of 25 mv (A_1), which did not induce either the rapid phase of inactivation or the secondary activation, the outward current simply declined at a slow rate. With larger depolarizations ($A_{2,3}$) the whole sequence of the initial rapid decrease of the current, the reaugmentation, and the slower decline was evident. Fig. 5 *B* shows data on a different cell, in which the secondary rise of the outward current was not as marked as in the case of *A*. Indeed, the secondary rise seemed to be a labile process and was easily diminished by repetitive depolarization. It was, nevertheless, always present in cells which were judged to be in good condition.

Again the restoration of the potential to the original level did not produce a slow undershoot of the current (Fig. 5 $A_{1,2,4}$, B_2), which should have occurred if the preceding slow decrease of the outward current had been due to polarization. Regardless of whether the restoration was made at an earlier

phase of the decline (A_4), or almost near the completion of the decline (A_2), it was not associated with an inward current. A small undershoot, however, occurred after a large depolarization (A_3, B_3). This represents a change of electromotive force induced by the long, large depolarizations, which probably altered the ionic composition of the space just outside the membrane (17). But the magnitude of the undershoot is much smaller than that of the decrease of outward current, and the change of electromotive force alone cannot account for the total decline of the outward current.

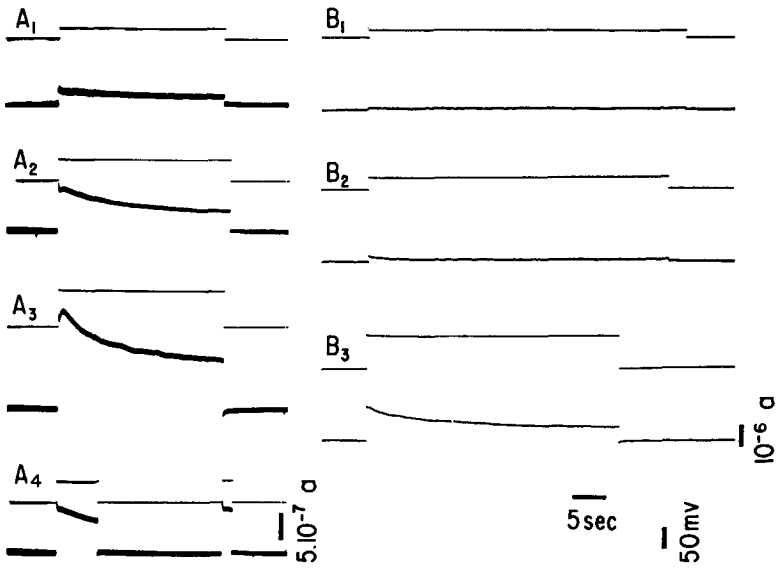

FIGURE 5. Long-duration voltage clamp in the normal solution. Experiments with two cells (A, B). In A_2 and A_3 the initial decline and the subsequent rise are followed by a very slow decrease of the outward current. In A_1 the outward current simply declined slowly. In A_3 the repolarization was accompanied by an undershoot of the current, while in A_1, A_2, A_4 no undershoot was observed. In B_2 and B_3 the secondary rise of the outward current was not so marked as in A_2 and A_3.

In summary, almost all the changes of the outward currents are to be attributed to the changes of membrane conductance, and we can conclude that depolarization produces K activation, which is followed by the initial rapid K inactivation, then, K reactivation occurs, and finally, there develops the secondary slow phase of K inactivation.

3. DOUBLE-STEP VOLTAGE CLAMP

The changes of conductance and electromotive force associated with the changes of the currents can be measured with double-step voltage clamp experiments, as illustrated by the inset records of Fig. 6. First, the membrane

was depolarized to −20 mv, resulting in an initial inward current followed by an outward current. Then, varying magnitudes of repolarizing steps were superimposed on the conditioning depolarization. In record *A* the repolarizing steps were applied near the peak of the outward current, whereas in *B* the steps were applied after the outward current had declined by about 50%.

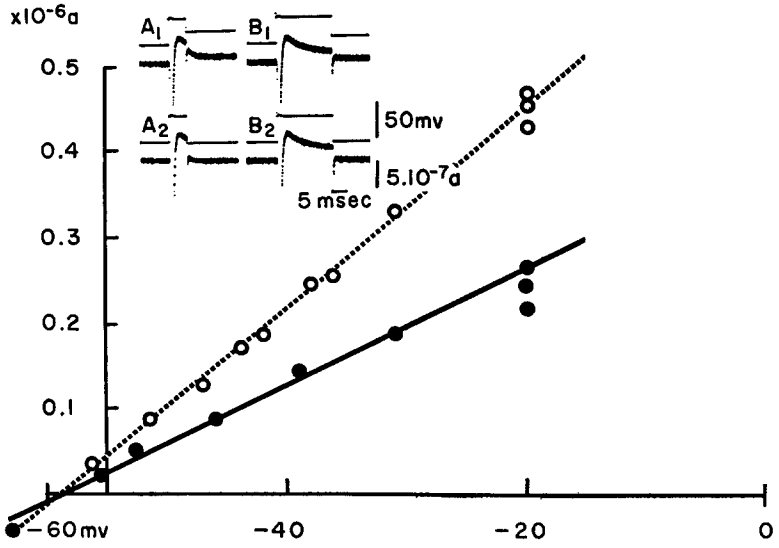

FIGURE 6. Instantaneous current-voltage relation at two different phases of the delayed outward current in the normal solution measured by double-step voltage clamp, based on data like those shown in the inset. First, a conditioning depolarization was applied. Then, varying magnitudes of repolarizing steps were applied. The membrane potentials after the superimposition of repolarizing steps are shown on the abscissa. The currents at 1 msec after the application of repolarizing steps are plotted on the ordinate. The points at −20 mv show the currents without the repolarizing step. Open circles and dotted line, the duration of the conditioning depolarization is 6 msec (inset *A*). Filled circles and solid line, conditioning for 20 msec (inset *B*). The ordinate crosses the abscissa at the level of the resting potential. Note the decrease of the chord conductance concomitant with the decrease of delayed current.

Because of the presence of a capacitative surge associated with sudden changes of the voltage, it was difficult to measure the currents at the instant of the application of the repolarizing step. Thus, in Fig. 6 the currents at a fixed time (1 msec) after the onset of the steps are plotted on the ordinate, with the membrane potentials during the second step on the abscissa. The open circles give the current-voltage relation at the period of the nearly maximum outward current (corresponding to records *A* of the inset), and the filled circles at the period of almost full development of the initial K inactivation (*B* of the inset). The current-voltage relation is approximately linear in

both cases. The equilibrium potentials, indicated by the intersections of the two straight lines with the abscissa, are almost the same in both cases, while there is a decrease in the slope, indicating a decrease of the conductance. In many cases deviations from a linear current-voltage relation were observed, particularly when the repolarizing pulses were given at a phase when the outward current was changing rapidly. However, because of the limited frequency response of the system, we could not determine whether or not the nonlinearity represented the inherent membrane property as in the case of amphibian Ranvier nodes (16).

4. KINETICS OF THE K INACTIVATION PROCESS

The rate of development of the initial K inactivation was much slower than that of the K activation. This is evident from Fig. 4 $A_{5\text{-}7}$, B_1, which shows that the maximum of the outward currents was attained almost instantaneously in comparison with the subsequent decline of the current. We can, therefore, regard the time course of the outward current decrease as representing the time course of K inactivation without much complication from the kinetics of K activation. The time course of the inactivation approximately fitted a first-order kinetics. Table II shows the time constant of the initial K inactivation (τ_{ki}) at varying magnitudes of depolarization. There was a decrease of τ_{ki} as the depolarization became larger. However, at a large depolarization (72 mv) τ_{ki} again increased slightly. The latter phenomenon could be ascribed to the influence of the secondary potassium activation. In some cells, however, τ_{ki} remained almost constant regardless of the magnitude of depolarization. In 5 cells the minimum τ_{ki} was on the average 5.9 msec, ranging from 4.2 to 8.6 msec.

TABLE II

TIME CONSTANT (τ_{ki}) OF THE INITIAL PHASE OF THE K INACTIVATION

Depolarization, *mv*	22	32	42	50	61	72
τ_{ki}, *msec*	7.2	7.4	5.5	5.5	5.4	6.0

The time course of the secondary slow phase of K inactivation could not be fitted by a simple kinetics. The half-time of the process during depolarization of 70 to 80 mv was on the average 5.2 sec in 3 cells.

B. *Experiments in Isosmotic KCl Solution*

1. REGENERATIVE RESPONSE UNDER CONSTANT-CURRENT CONDITIONS

Application of the isosmotic KCl *Lophius*–Ringer solution to the cluster of SMC produced depolarization, which reached a steady level in about 10 min.

The membrane potentials after full depolarization were between −10 and −25 mv. By applying a conditioning hyperpolarization to these KCl-treated cells, they became capable of producing a regenerative response to a depolarizing current (Fig. 7). Some of the responses had a small spikelike phase followed by a slow component (*B*), while others had only a slow component (*A*). Estimation of the membrane resistance by applying small recurring currents showed an initial decrease followed by a gradual increase (*C*). These regenerative responses are analogous to those observed in various electrogenic membranes under conditions in which the electrochemical K gradient is inward (2, 24, 31, 33, 34, 40, 43). As pointed out by Moore (33), Lüttgau (31), Grundfest (22), and Ooyama and Wright (40), the response can be

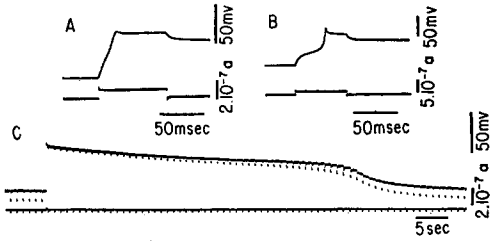

FIGURE 7. Regenerative electrogenesis under constant-current conditions in cells bathed in an isosmotic KCl solution. Hyperpolarizing conditioning currents were applied to bring the membrane potential to −68 mv (*A*) and −71 mv (*B*); then, depolarizing pulses were superimposed. *C*, a short depolarizing pulse, applied to a cell which was hyperpolarized to −60 mv, evoked a long lasting electrogenesis. Recurring small constant current pulses were superimposed to show the resistance change during the response.

interpreted as representing K activation, which allows an inward K current to flow down the electrochemical gradient. The spontaneous termination of the response is to be attributed to the K inactivation process.

2. K CURRENT UNDER VOLTAGE CLAMP

Voltage clamp recordings in the KCl-treated cells are shown in Fig. 8. The membrane potential was set to −75 mv (*A*) and −72 mv (*B*). Depolarizations of certain magnitudes evoked an inwardly directed current (A_2), which developed and diminished much more slowly than the initial inward current in the normal solution. A larger depolarization evoked a slowly declining outward current (A_4). The time courses of the development and decline of the inward and outward currents were similar to those of the delayed outward current seen in the normal solution. Thus, it is reasonable to assume that the inwardly and outwardly directed currents in Fig. 8 are produced by the same mechanisms as those which are responsible for the delayed outward currents in the cells in the normal solution. The mechanism responsible is the per-

meability increase to potassium, which is the only ion whose equilibrium potential was changed in such a way as to produce the inward current at certain magnitudes of the depolarizing pulse. The decline of the inward and outward currents should be attributable to the initial phase of the K inactivation process as analyzed for the cells in the normal solution. When the membrane potential at the depolarizing pulse coincided with the K equilibrium potential, the current remained constant in spite of possible changes of the membrane conductance (A_3). The current patterns under similar conditions are displayed in B on a slower time base.

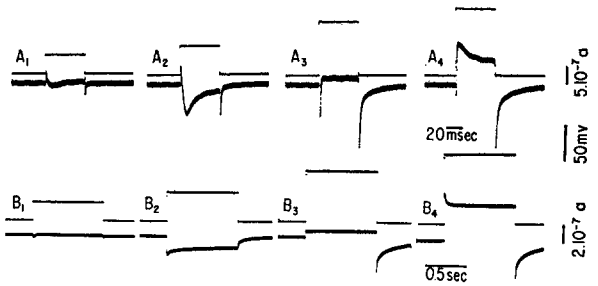

FIGURE 8. Voltage clamp experiment in the KCl solution. Examples with two cells (A, B). The base lines for the voltage traces correspond to the preset membrane potentials of −75 mv (A) and −72 mv (B). In A_1 and A_2 the increments of the inward current, which declined slowly, are illustrated. In A_4 the outward current declined with a time course similar to that of the decline of the inward current shown in A_2. In A_3 the current stayed at a constant level: the potential level corresponds to the K equilibrium potential. B shows an experiment on another cell recorded at a slower sweep speed.

For the records of Fig. 9 the membrane had been hyperpolarized to −73 mv and depolarizing pulses of about 50 sec duration were superimposed. Records B and C show that both the inward and outward currents declined at a slow rate similar to that of the secondary slow phase of the K inactivation observed in the normal solution (Fig. 5). In D recurrent voltage pulses were superimposed under voltage clamp. A concurrent decrease of the conductance during the slow decrease of the outward current is evident. The conductance measured in this way would give values corresponding to the slope conductance ($\partial I/\partial E$) because the sweep speed is too slow to show the rapid transient of the current record. However, in the isosmotic KCl solution the potassium equilibrium potential would be near zero, and at +25 mv, where the conductance was measured in Fig. 9 D, the slope conductance would give almost the same value as the chord conductance because the current-voltage relationship is approximately linear over this region of the membrane potential. Thus, we can conclude that the slow decrease of the potassium current

in the KCl solution is again due to changes of the conductance rather than to electromotive force changes.

As shown in Figs. 8 and 9, a feature of the current pattern in the KCl solution is the absence of the secondary rise of the current, which was usually observed in the normal solution. Thus, the K reactivation seems to be a labile process, which is diminished not only by repetitive depolarization but also by application of KCl.

3. KINETICS OF THE K INACTIVATION PROCESS

The time courses of the initial phase of K inactivation in the KCl solution were somewhat slower than those observed in the normal solution. The

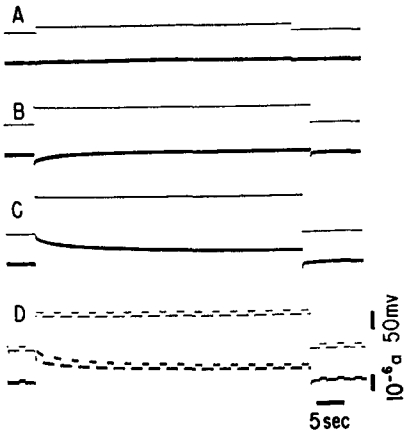

FIGURE 9. Long-duration voltage clamp in a cell bathed in the KCl solutions. The membrane potential was preset to −73 mv, which corresponds to the base line of each potential recording. In *B* and *C* the inwardly and outwardly directed currents declined very slowly. In *D* small recurrent potential pulses were superimposed under the voltage clamp condition. The corresponding changes of the currents became smaller as the outward current decreased, indicating a decrease in conductance.

minimum value for the τ_{ki} for the average of 6 cells was 9.2 msec, ranging from 4.1 to 14 msec. The half-time of the secondary slow K inactivation process ranged from 3.1 to 4.8 sec (4 cells) with an average of 3.9 sec.

DISCUSSION

The present investigation has revealed that in the SMC the K activation mechanism is poorly developed in comparison with the activation mechanism for the initial current. A similar phenomenon was reported by Julian, Moore, and Goldman (30) in lobster axons. In addition to the poorly developed K activation mechanisms in the SMC, the cells are characterized by the rapid decrease of K conductance during maintained depolarizations; this elimination of the K activation is the K inactivation, which is the counterpart of the Na inactivation in the Hodgkin-Huxley theory.

The existence of K inactivation in electrically excitable membrane was first suggested by Grundfest (20–22) to account for data in eel electroplaques. Frankenhaeuser and Waltman (18) suggested the presence of K inactivation

in KCl-depolarized myelinated nerve fibers, and Lüttgau (31) actually observed a slowly developing increase in resistance with a half-time of 15 sec after cessation of a hyperpolarizing current.

Nakajima, Iwasaki, and Obata (36) observed the conversion of delayed rectification into anomalous rectification in skeletal muscle fibers during depolarizations, and ascribed this to K inactivation. A decline of the conductance during depolarization in the KCl-treated *Onchidium* neurons was observed by Hagiwara, Kusano, and Saito (24). An increase in resistance during the peak of the spike in the electroplaques of gymnotid fishes has been observed (3, 6), and voltage clamp studies in various gymnotid fishes including the electric eel have demonstrated K inactivation (7, 39). The occurrence of K inactivation has been observed also in squid giant axon (12), crayfish stretch receptor (S. Nakajima, data to be published), and mammalian Purkinje fibers (9, 27). Thus, these findings support the view that K inactivation is a general property of electrically excitable membrane (23).

There are, however, wide differences in the kinetics of K inactivation processes. In myelinated nerve fibers (31) and squid giant axons (12) the rate of development of the process appears to be slower than that in skeletal muscle fibers (36) and the SMC. In skeletal muscle fibers (36) the K activation is completely abolished by maintained depolarizations, while in myelinated nerve fibers (31) and in the SMC the inactivation process is incomplete, so that some enhanced K conductance is always left even after full development of K inactivation (35).

In the squid giant axon Frankenhaeuser and Hodgkin (17) attributed the decrease of the outward current induced by maintained depolarizations largely to the accumulation of potassium ion in the Schwann cell space. However, in the SMC the changes of the delayed currents are almost entirely to be ascribed to conductance changes rather than to changes in electromotive force. In other words, depolarizations hardly produce redistribution of ions outside or inside the membrane in this material. This is in accord with the similar conclusion by Dodge and Frankenhaeuser (10), and Meves (32) on Ranvier nodes, by Julian, Moore, and Goldman (30) on lobster axons, and by S. Nakajima on stretch receptor neurons of Crustacea (data to be published). Electron micrographs of the SMC by Y. Nakajima, Pappas, and Bennett (38) reveal that the nerve cell membrane is completely surrounded by glial cells with intervening extracellular space of about 200 A. Therefore, we may suppose that the permeability of the glial cell membrane surrounding the SMC is greater than that of the Schwann cell sheath of squid giant axons. Another important factor which would account for the apparently great permeability of the glial cell is the presence of numerous submicroscopic foldings and digitations in the nerve cell membrane. These would result in a very much greater membrane area than that calculated from the assumption of a simple spheroid structure.

In agreement with the observation by Bennett, Crain, and Grundfest (4, 5), the majority of the SMC of the Atlantic puffer did not exhibit an after-hyperpolarization. This is attributable to the poorly developed K activation and to the K inactivation. In fact, over the physiological range of membrane potential (-70 to $+20$ mv) g'_K is only 10 times the leak conductance, and by 10 to 15 msec after the onset of the spike, when the repolarization from the spike completes, g'_K would be further decreased (*a*) by the development of the rapid phase of K inactivation, and (*b*) by the recovery from the K activation which is induced during the repolarizing phase of the spike. Thus, it is quite possible that the spike is not associated with after-hyperpolarization even though the potassium equilibrium potential is more negative than the resting potential. However, it would be necessary to compute the wave form of the action potential from the data obtained by the voltage clamp in order to determine this point definitely.

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