Cellular and Molecular Neurobiology, Vol. 3, No. 4, 1983

Recovery of Ca Currents from Inactivation: The Roles of Ca Influx, Membrane Potential, and Cellular Metabolism

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Received October 3, 1983; revised October 31, 1983; accepted November 3, 1983

KEY WORDS: Ca current; voltage clamp; ATP; snail neuron.

SUMMARY

1. Ca currents were examined with regard to their recovery from inactivation. The experiments were done on isolated nerve cell bodies of *Helix aspersa* using a combined suction pippet, microelectrode method for voltage clamp, and internal perfusion. Ca currents were separated by suppressing K and Na currents.

2. The time course of recovery was determined by applying a test pulse at intervals ranging from 1 msec to 20 sec after prepulses varying from 20 to 3000 msec in duration. Each pair of pulses was preceded by a control pulse to ensure that the Ca currents had recovered before the next test pair was applied. Ba and Ca currents were compared and the effects of intracellular perfusion with EGTA, ATP, and vanadate were examined.

3. Ba currents recovered in two stages and this time course was well fit by a sum of two exponentials with amplitudes and time constants given by A_1 and τ_1 for the fast component and A_2 and τ_2 for the slow component. In Ba the time constants were unchanged when prepulse durations were prolonged from 70 to 700 msec, although the initial amplitudes A_1 and A_2, particularly A_2, were increased.

4. Comparable influxes of Ca during the prepulse caused much more inactivation, but interestingly the recovery occurred at the same rate. The time course of Ca current recovery was also fit by a sum of two exponentials, the time constants of which were both smaller than the time constants of Ba current recovery. However, the time constants of Ca current recovery were increased markedly when prepulse durations were prolonged. Increasing the extracellular Ca concentration had a similar effect.

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5. Increasing the Ba influx had no effect on the recovery time constants, and the Ba results are consistent with reversible inactivation gating of potential-dependent membrane Ca channels. The Ca results show that Ca influx enhances inactivation. Intracellular perfusion with EGTA resulted in less inactivation in the cast of Ca but it had no effect on Ba currents. Intracellular ATP increased the rate of recovery of Ca currents, and intracellular vanadate inhibited recovery. It is concluded that recovery of Ca channels depends upon both Ca influx and membrane potential and is modulated by agents which affect Ca metabolism.

INTRODUCTION

Recovery of Ca currents following a test pulse is a slow process that has been described by the sum of two exponentials (Tillotson and Horn, 1978; Adams and Gage, 1979; Plant and Standen, 1981). Inactivation of Ca currents during a single voltage clamp step and the onset of inactivation using a double-pulse protocol may also be described by the sum of two exponentials (Kostyuk and Krishtal, 1977; Brown *et al.*, 1981). The latter two measurements of inactivation were strongly affected by the influx of Ca but not Ba ions (Tillotson, 1979; Brehm *et al.*, 1980; Brown *et al.*, 1981) and comparison of Ba and Ca currents led to the conclusion that inactivation was both voltage dependent and Ca current dependent (Brown *et al.*, 1981). This being the case, two processes might be involved in the recovery from inactivation. Furthermore, agents which affect Ca metabolism might influence recovery. These two possibilities have led to the experiments and conclusions reported in this paper.

METHODS

The experiments were done on single isolated nerve cell bodies of *Helix aspersa*. The somata had diameters of 80–150 μm. The methods used for voltage clamp, internal perfusion, and separation of Ca currents were described fully by Brown *et al.* (1981). The solutions used are shown in Table I. ATP, cyclic AMP (Sigma), and Na orthovanadate (Fischer) were added as required. All experiments were done at a room temperature of 22–23°C. The following points are worth noting. The clamp circuit was described by Brown *et al.* (1983a) with the exception that in the present measurements we did not use shielded microelectrodes. Membrane input resistances were between 5 and 8 MΩ similar to the values obtained with two microelectrodes, giving shunt resistances ranging from 50 to 80 MΩ. Series resistance of the preparation was <5 kΩ and series resistance compensation was not applied since the maximum inward Ca currents were about 150×10^{-9} A. The voltage settled in <50 μsec, and the capacitative current transient was 95% complete in about 100 μsec. Linear components of the capacitive current transient and the leakage current were subtracted by adding currents from equal but opposite voltage clamp steps using a Nicolet 1170 signal averager.

The stimulus sequences consisted of a conditioning pulse of 20- to 3000-msec duration at a potential of $+10$ to $+20$ mV; this potential produced maximum Ca currents. The conditioning pulse was followed at varying intervals of milliseconds to

Table I. Solutions Used for Extra- and Intracellular Perfusion of *Helix* Neurons[a]

Solution		mM								
		NaCl	Tris–Cl	KCl	CsCl	TEA–Cl	4-AP	$CaCl_2$	$MgCl_2$	Glucose
External (pH 7.40)[a]	Normal solution	85	5	5	—	—	—	10	15	5.5
	Ca current solution	—	35	—	5	50	5	10[b]	15	5.5
		K aspartate			Cs aspartate			TEA–OH		
Internal (pH 7.30)[a]	Normal solution	135			—			—		
	CS aspartate solution[c]	—			135			10		

[a] pH was adjusted with Tris and HEPES buffer.
[b] $CaCl_2$ was replaced by $BaCl_2$ in Ba current experiments.
[c] EGTA was added at concentrations of 1, 5, and 10 m*M*, and the Ca concentration was buffered between 10^{-8} and 10^{-9} *M* (Martell and Sillen, 1972).

seconds by a test pulse of equal amplitude and a fixed duration of 30 msec. The interval between pairs of pulses was sufficiently long to ensure complete recovery before the next pair was initiated. This was always tested after 30 sec by a 30-msec pulse to the test potential, which in turn was followed by an interval of 30 sec before doing the next trial. The data were calculated as the ratio of the test pulse current peak amplitude following a conditioning or prepulse to the test pulse current peak amplitude in the absence of the prepulse. Results came from experiments using 50 cells and were typical of results obtained from a much larger number of cells.

The recovery data were analyzed using models consisting of a constant and a sum of one, two, or three exponentials. The fits were obtained by minimizing the sum of the squared error using a Marquardt-Levenberg optimization procedure as described by Bevington (1969). Global convergence was tested using various starting values; in most cases with a two-exponential fit there were few problems with local minima. When comparing models of different order, goodness of fit was estimated by an inspection of the residuals (Bard, 1974).

RESULTS

Recovery of Ca Currents

Figure 1 shows I_{Ca}'s produced by pairs of pulses with the test pulse following the prepulse at intervals varying from 10 to 4000 msec. The prepulse duration was 70 msec in the top row and 200 and 700 msec in the middle and bottom rows, respectively. The time course of Ca inactivation is slow (Brown *et al.*, 1981), and thus the prepulses used provided varying amounts of inactivation. The peak Ca current during the recovery test pulse increased in amplitude as the interval was increased and this is what we call recovery from inactivation. Recovery was quite slow. Complete recovery for a 70-msec prepulse required 6 sec, and recovery for a 700-msec prepulse required 20 sec. Also

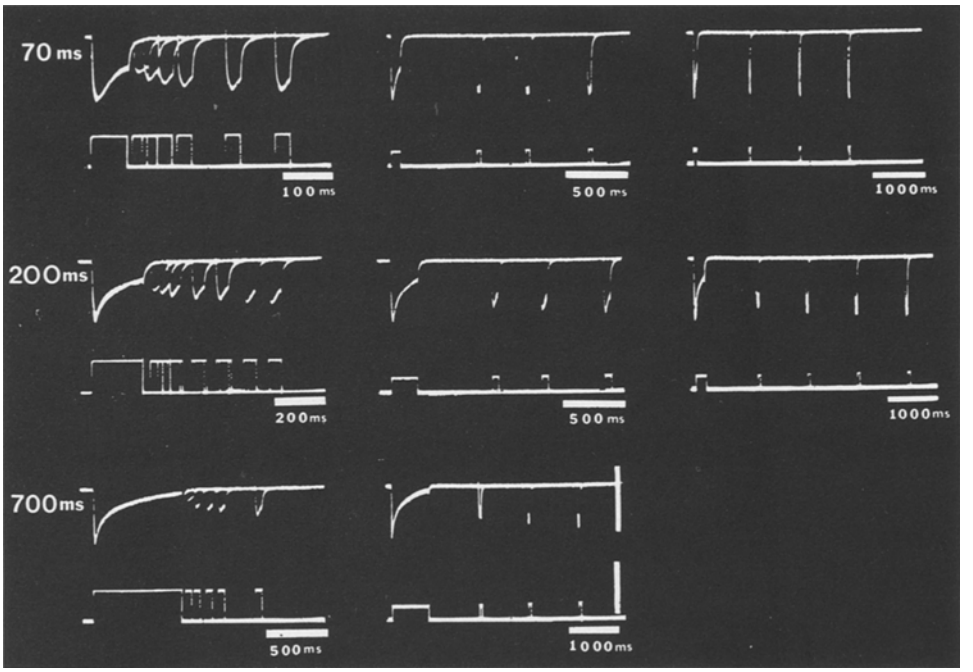


Fig. 1. Effects of different prepulse durations (70, 200, and 700 msec) on the rate of recovery of I_{Ca} from inactivation. The holding level was -50 mV and the conditioning and test pulses had amplitudes of $+70$ mV. The conditioning pulses (70, 200, and 700 msec) are shown at the extreme left of each panel and they act as the value for the control current. The first current trace in every segment is the control. Note that no correction has been made for leak or capacitive currents. Note the different time bases. The test pulse is initiated at various times after the end of the conditioning pulse. The vertical bars in the middle column are current and voltage calibration values and are 150 nA and 100 mV for the left panel and 200 mV for the middle and right panels, respectively. The test pulses gradually recover their amplitudes and shapes. Note that the rate of inactivation is slowed during the initial test pulses and then increased progressively.

note that the time course of the current during the test pulse changes; the rate of inactivation in the test pulse was slow initially and increased as the current recovered. The recovery time courses for these experiments, as determined by the ratio of the peak test pulse current to the control current, are plotted in Fig. 2. We found that a two-exponential model was the minimum sum-of-exponentials model that fitted the results satisfactorily, and thus we fit the data with

$$y = 1.0 - A_1 e^{-t/\tau_1} - A_2 e^{-t/\tau_2}, \tag{1}$$

where y is the ratio of the current in the test pulse to that in the control pulse; as we shall see, the recovery of Ba currents was fit by an identical model. Interestingly, the time course of recovery, as determined from the τ parameters of the exponential model, as well as the total time for recovery depended on the prepulse length. We found that the largest effect of prepulse on the recovery model was an increase in A_2. Both recovery time constants were increased as the prepulse duration was prolonged, the effect being more marked for τ_2. Values from such curve fits are shown in Table II and the results confirm the pattern described for the recovery curves shown in Fig. 2.

The observation that the recovery time constants were increased as the prepulse

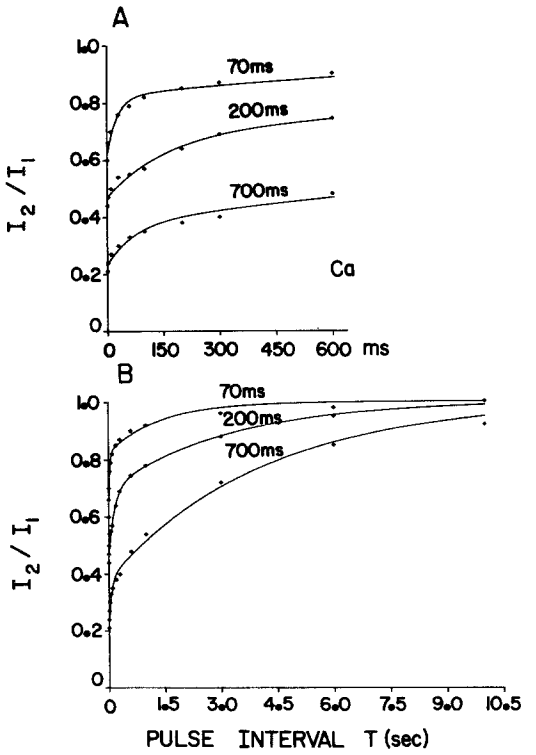


Fig. 2. Recovery of I_{Ca} following conditioning pulses lasting 70, 200, and 700 msec. Data were plotted after substraction of leak and capacitive currents. Peak Ca current during the test depolarization (I_2) was normalized by peak I_{Ca} during the immediately preceding conditioning pulse (I_1). The plot in B shows the data for 10 sec, and the initial points are shown in expanded form in A. The smooth curve is the least-squares fit with a two-exponential model [Eq. (1)]. The τ's for the fits increased markedly as a function of the prepulse duration. Parameters for the fits in A and B for a prepulse duration of 70 msec are $A_1 = 0.20$, $A_2 = 0.18$, $\tau_1 = 28$, and $\tau_2 = 1350$, where the τ values are in milliseconds; for a prepulse of 200 msec, the values are $A_1 = 0.14$, $A_2 = 0.30$, $\tau_1 = 42$, and $\tau_2 = 1700$; for a prepulse of 700 msec, the values are $A_1 = 0.15$, $A_2 = 0.62$, $\tau_1 = 76$, and $\tau_2 = 4000$.

duration was increased is not to be expected for a gating process regulated only by membrane potential. We did, nevertheless, examine recovery at potentials other than -50 mV, the usual holding and return potential in the present experiments. Although there was an identical amount of inactivation during the prepulse, there is a difference in recovery at -50 and -80 mV (Fig. 3). The largest difference occurs in the initial 200 msec. Interestingly, there was a large effect on the first point plotted at 1 msec. This result was also consistently found in experiments with Ba in the extracellular solution. This small potential-dependent process occurs much faster than the recovery from inactivation, and there was no readily apparent explanation based on tail current

Table II. Parameters from Fitting the Recovery of Ca and Ba Currents to Eq. (1)[a]

	Prepulse (msec)	A_1	τ_1 (msec)	A_2	τ_2 (msec)	
Ba	70	0.12 ± 0.01	310 ± 26	0.08 ± 0.01	3600 ± 500	(N = 8)
	200	0.14 ± 0.02	290 ± 60	0.20 ± 0.02	4300 ± 600	(N = 8)
	700	0.20 ± 0.02	360 ± 40	0.48 ± 0.02	4400 ± 400	(N = 6)
Ca	70	0.22 ± 0.03	25 ± 10	0.24 ± 0.04	900 ± 300	(N = 10)
	200	0.18 ± 0.03	26 ± 12	0.41 ± 0.06	2000 ± 400	(N = 10)
	700	0.24 ± 0.16	70 ± 22	0.60 ± 0.04	4000 ± 400	(N = 8)
10 Ca	70	0.18 ± 0.02	32 ± 10	0.28 ± 0.03	700 ± 300	(N = 3)
20 Ca		0.22 ± 0.03	68 ± 15	0.30 ± 0.02	1200 ± 200	(N = 3)

[a]Results are presented as a function of prepulse duration. Comparing the Ca and Ba data, there was a definite increase in the τ values as a function of the prepulse for the case of Ca, whereas in the case of Ba the τ values did not appear to be a function of the prepulse. Averages and standard deviations are given for the number of experiments, N. The bottom two entries compare three experiments in which two concentrations of Ca were used in the same cell. Note that there was an increase in the recovery τ's when Ca was increased.

or turn-on relaxations. We speculate that it may arise from a voltage-dependent block which is relieved quickly at negative potentials. It should be noted that steady-state inactivation is not present at potentials more negative than −50 mV (Brown *et al.*, 1981).

In Fig. 4 recovery of the Ca current following complete inactivation with a 3-sec prepulse is shown. Note that a delay such as that measured in the recovery of Na currents (Chiu, 1977) is not seen. Similarly, no such delay was measured in the recovery of Ba currents, in which case, as shown below, there were indications that inactivation followed normal voltage-dependent behavior.

We also examined the effects of increasing Ca_o on recovery. The test pulse amplitude was increased by 10 mV to take into account the effects of doubling Ca_o on

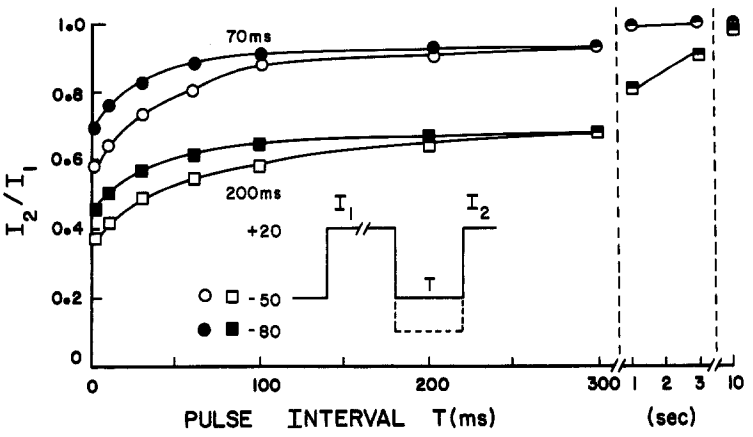


Fig. 3 Effects of hyperpolarization on recovery from inactivation. Ca current during the test depolarization (I_2) was normalized by I_{Ca} during the immediately preceding conditioning pulse (I_1). Open symbols show recovery at −50 mV and filled symbols show recovery at −80 mV. Recoveries are shown for relatively shorter intervals on the expanded scale; following the broken lines on the abscissa recoveries are for longer intervals.

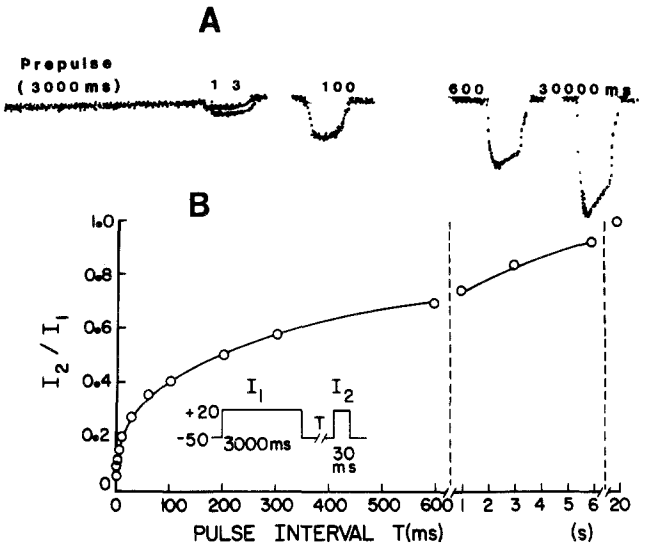


Fig. 4. Recovery of I_{Ca} following 3-sec prepulse which produced complete inactivation. (A) Ca current records 1, 3, 100, 600, and 30,000 msec following a 3-sec prepulse. Note that the current at the end of the prepulse is zero. Recovery begins within 1.0 msec and recovery shows faster rates of inactivation as their amplitude increases. (B) Peak Ca current during the test depolarization (I_2) was normalized by peak I_{Ca} during the immediately preceding conditioning pulse (I_1). There was complete inactivation of the Ca current, yet as noted, there was no delay visible in the recovery curve.

activation gating and peak Ca current (Wilson *et al.*, 1983). As shown in Table II, for 70-msec test pulses, inactivation was increased, recovery was slowed, and the amplitudes of both exponential functions were increased. The result with increased Ca_o further strengthened our suspicion that the amount of Ca influx during the prepulse was a factor in the recovery process. Therefore we applied two prepulses of different amplitudes and duration but which delivered similar amounts of Ca to the cell interior, as determined by integrating the Ca current. An initial protocol was estimated from a previously published graph of integrated currents (Brown *et al.*, 1981, Fig. 11) and this was later refined to give a similar amount of integrated current. A longer prepulse of 70 msec at -10 mV was associated with more inactivation than a briefer prepulse of 50 msec at $+10$ mV (Fig 5A). Additionally, the time course of recovery was slowed as seen in Fig. 5B when the curves were normalized to account for the different amounts of inactivation. Thus, inactivation and recovery were different despite the same Ca load; hence, Ca influx does not appear to be the only factor. A possible explanation of these recovery experiments seemed to involve a combination of Ca influx during the prepulse and voltage-dependent inactivation produced by the prepulse. A way of separating these two factors may be to study Ba rather than Ca currents. We have already shown that, unlike the case of Ca, perfusion with Ba_i at low concentrations or the addition of EGTA at high concentrations does not affect Ba currents (Brown *et al.*, 1981). This led to the conclusion that Ba current inactivation appears to be voltage-dependent.

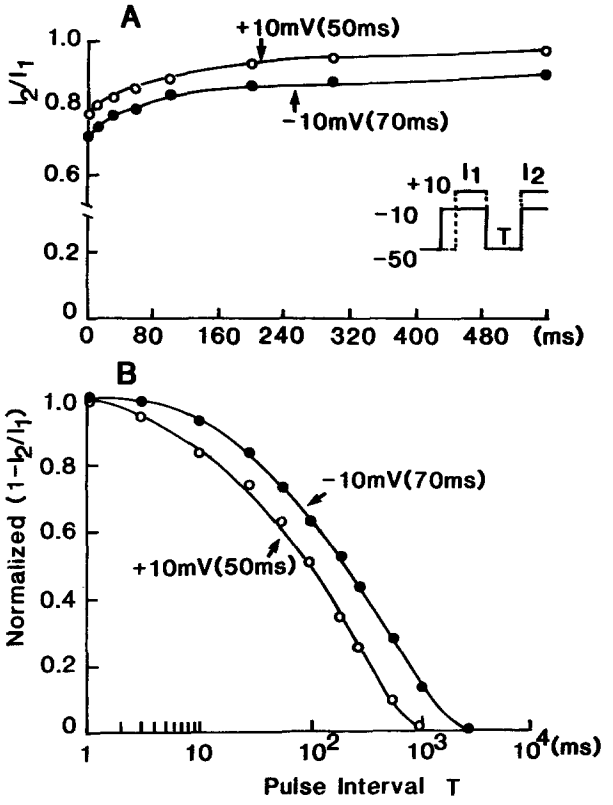


Fig. 5. Effects of prepulse voltage on recovery from inactivation. The duration and voltage of the prepulse were adjusted to obtain the same amount of integrated Ca influx. In A is a normal recovery plot as shown in Figs. 2 and 4. Because the amount of inactivation varied, we replotted the same data in B with the initial values normalized to 1.0. Note the logarithmic time axes.

Recovery of Ba Currents

The effects of Ba substitution on I_{Ca} (now I_{Ba}) were steady within 5 min. The peak Ba current amplitudes were 30 to 50% greater than the peak Ca current amplitudes at the test potential used (+10 mV), and the initial recovery test current was much larger (compare Fig. 6 to Fig. 1). The recovery time course of I_{Ba} is shown in Fig. 7; it was biexponential as for I_{Ca}, but for I_{Ba}, τ_1 and τ_2 were much larger. In fitting the Ba data we found that there was no consistent, significant dependence of the τ values on the prepulse duration. Thus, we fit the data in Fig. 7 with the two-exponential model, averaged the τ values reported in the figure legend, and fit the data again with the τ values constrained to the average values. The result of this fit is shown in Fig. 7. Results for other cells are shown in Table II, and they are consistent with the observation that the τ's are independent of the prepulse length.

The time course of the Ba recoveries suggests a voltage-dependent process for

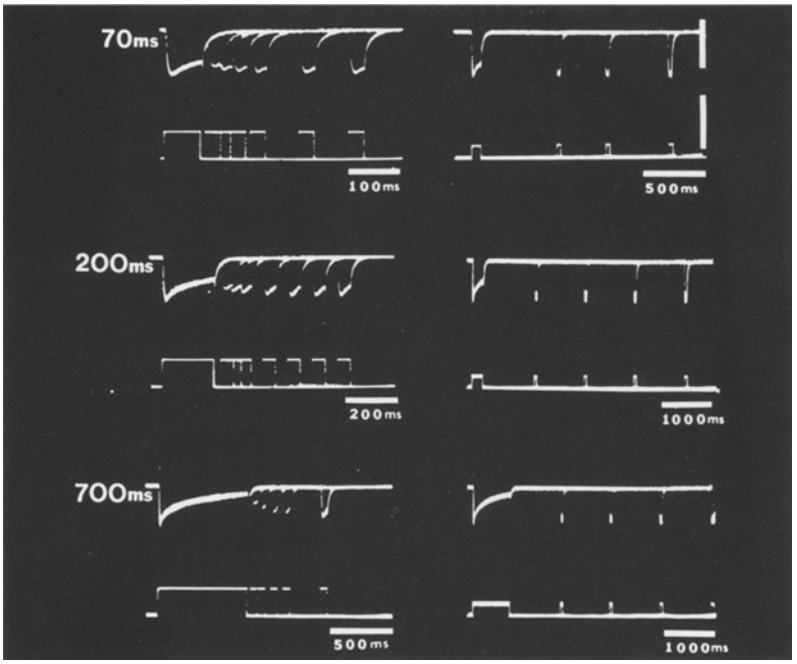


Fig. 6. Time course of recovery of Ba current from inactivation determined using the same double-pulse as in Fig. 1. Depolarizing pulse was to +10 mV rather than +20 mV because of the voltage shift of the peak current in Ba solutions. Equimolar Ba (10 m*M*) was substituted for Ca in the extracellular solution. No correction has been made for leak or capacitive currents. The vertical bars from top to bottom show 150 nA and 100 mV for left panels and 200 mV for right panels, respectively. Note the different time bases. The first current trace of each segment is the control current.

inactivation and recovery. The voltage-dependent component plus the role of Ca influx were examined in experiments in which $EGTA_i$ was perfused intracellularly.

Effects of Intracellular EGTA upon Recovery of Ca Currents

Intracellular EGTA reduced the amount of inactivation of Ca currents presumably by binding the free internal Ca (Brown *et al.*, 1981). The possibility that the time course of recovery of I_{Ca} might be affected by intracellular perfusion with EGTA was examined using Ca-EGTA-buffered intracellular solutions containing 1, 5, and 10 m*M* EGTA, with $[Ca]_i$'s ranging from 10^{-8} to 10^{-9} *M*. The peak currents increased slightly at the beginning of the perfusion and, after 15–20 min, became slightly smaller. Figures 8 and 9 show the time course of recovery from inactivation before and during the perfusion with intracellular EGTA, 5 m*M*. It is seen that intracellular EGTA decreased the amount of inactivation as reported earlier (Brown *et al.*, 1981) and the recovery time course resembled Ba recovery more closely. In three experiments curves were obtained after three removal of $EGTA_i$. Following the removal of $EGTA_i$ recovery reverted to the control pattern. $EGTA_i$ did not affect steady-state inactivation

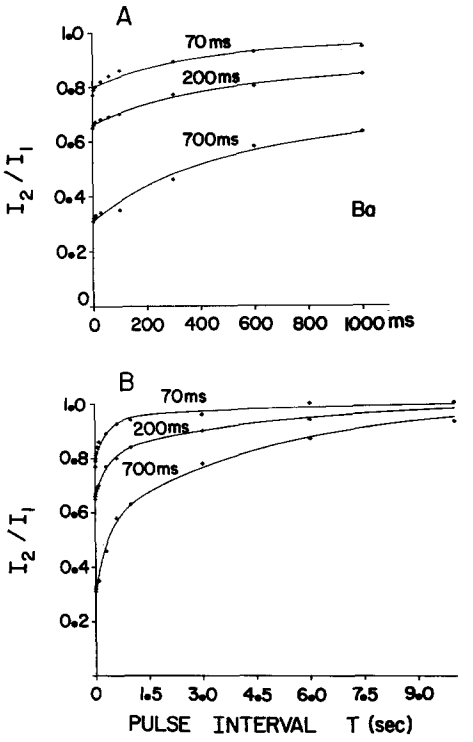


Fig. 7. Recovery of I_{Ba} with prepulses of 70, 200, and 700 msec from a different cell from that in Fig. 6. Ba current during the test depolarization (I_2) was normalized by I_{Ba} during the immediately preceding conditioning pulse (I_1). Initial curve fits of this data provided the following τ values (msec): τ_1 = 340, 360, and 340 and τ_2 = 4100, 5200, and 4800 for prepulses of 70, 200 and 700 msec, respectively. From this and other measurements on Ba currents, we inferred that there was no significant, consistent effect of prepulse duration on the recovery τ values. We therefore averaged the time constants associated with the three different prepulses and obtained new fits with the time constants constrained to the average values. The smooth curves shown here were drawn with τ_1 = 346 msec and τ_2 = 4700 msec and A_1 = 0.148, 0.152, and 0.247 and A_2 = 0.053, 0.187, and 0.444 for pulse durations of 70, 200, and 700 msec, respectively.

of Ba currents (Brown *et al.*, 1981), nor did it change either the time course of inactivation during a single pulse or the recovery from inactivation. The EGTA results point directly to a role for Ca influx and Ca_i accumulation as important factors in the recovery process.

Cellular Metabolism and Recovery from Inactivation

To examine the link between metabolism and recovery from inactivation, the effects of intracellular ATP and the Ca-ATPase inhibitor vanadate (DiPolo and Beauge, 1981) were studied. The same procedure used in Fig. 1 was repeated on 10 cells with and without the intracellular application of these chemicals. The duration of the conditioning pulses was 70 and 200 msec. Intracellular perfusion of ATP, 10^{-3} *M*, produced a 10 to 15% increase in I_{Ca} in 15–20 min (Yatani *et al.*, 1982). The increase occurred without any apparent changes in the activation kinetics of the Ca currents. Neither leakage nor nonspecific currents were affected. Changes in the time course of recovery from inactivation were slight, however (Fig. 10A). The major difference in the curves was a small reduction in the extent of inactivation which was reflected primarily by a decrease in the amplitude of A_2. Internal perfusion with 10^{-4} *M* cAMP produced similar results.

The effects of intracellular vanadate (10^{-3} *M*) were studied on four cells. Vanadate produced a reduction in I_{Ca} of about 25–30%, without affecting leakage and nonspecific currents. In the presence of vanadate, the amount of inactivation was

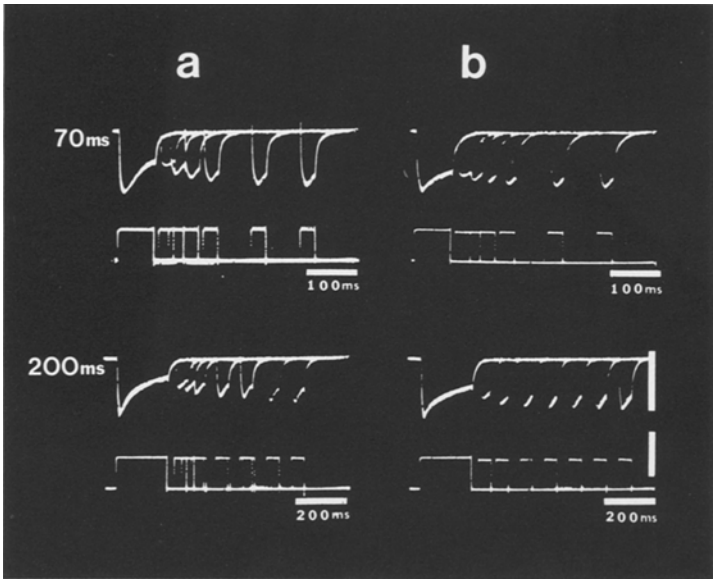


Fig. 8. Effect of EGTA (5 m*M*) applied intracellularly on the time course of recovery of Ca current from inactivation determined using the same double pulse procedure shown in Fig. 1. (a) Control; (b) after perfusion of EGTA (5 m*M*) intracellularly. No correction has been made for leak or capacitive currents.

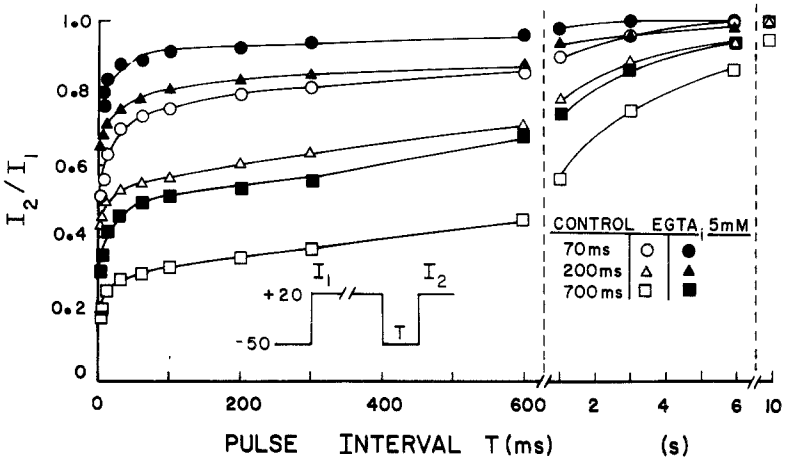


Fig. 9. Graphic representation of the EGTA data obtained using the procedure shown in Fig. 8. Recovery of Ca current before (open symbols) and after (filled symbols) the addition of 5 m*M* EGTA to the intracellular solution is shown. Ca current during the test depolarization (I_2) was normalized by I_{Ca} during the immediately preceding conditioning pulse (I_1).

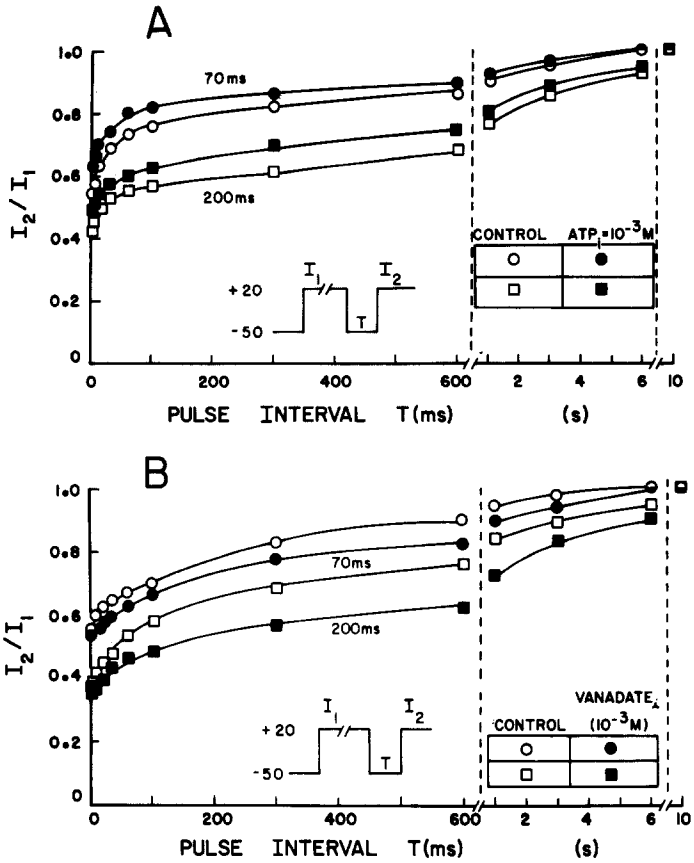


Fig. 10. (A) Effects of ATP (10^{-3} *M*) perfused intracellularly on the time course of recovery of Ca current. Ca current during the test depolarization (I_2) was normalized by I_{Ca} during the immediately preceding conditioning pulse (I_1). Controls are open symbols and results following ATP are filled symbols. (B) Effects of vanadate (10^{-3} *M*) perfused intracellularly on the time course of recovery of Ca current. Data are plotted as in A. Control data are open symbols, and vanadate results are given by filled symbols.

virtually unchanged as seen from the initial points in Fig. 10B, and recovery was clearly slowed. We tested for the possibility that a Na–Ca exchange mechanism could influence recovery. In these experiments 28 m*M* Na was substituted for Tris extracellularly, and the Na channels were blocked by 10^{-6} *M* TTX. The Na reversal potential (E_{Na}) was set at +45 mV by adding 5 m*M* Na to the intracellular fluid. Recovery was unchanged in these solutions.

In nine experiments, Ba was substituted extracellularly for Ca. Neither ATP nor vanadate changed the time course of recovery of I_{Ba}.

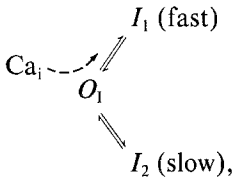
DISCUSSION

Recovery from inactivation of Ba currents is a biexponential process, as is the inactivation produced during a single voltage step. It is independent of Ba influx and is

largely, if not entirely, voltage dependent as we previously reported (Brown *et al.*, 1981). Recovery of Ca currents is more complicated and is influenced by Ca influx. In neither case do the activation kinetics interfere since the slowest component of activation is about 10 times faster than the fastest recovery component (Brown *et al.*, 1983a). Depletion of Ca_o is not a factor in recovery experiments and intracellular accumulation to significant levels, i.e., 10^{-3} *M*, is very unlikely given that internal perfusion was continuous and in some experiments $EGTA_i$ was present in millimolar amounts. It is most likely that Ca ions act upon an internal membrane site involved in inactivation. Increasing $EGTA_i$ to 5 and 10 m*M* reduced but did not abolish the effects of Ca_i.

The interaction between Ca and this site may be voltage dependent since the effect is relieved by hyperpolarization to −80 mV in the recovery period, whereas steady-state inactivation as measured from a two-pulse protocol is not affected by conditioning pulse potentials more negative than −50 mV, the usual resting potential of snail neurons. Recovery is much quicker than the rate at which overall Ca_i levels are returned to resting values following an I_{Ca} transient (Gorman and Thomas, 1980; Charlton *et al.*, 1982) and may reflect transfer of Ca to the nearby endoplasmic reticulum described by Akaike *et al.* (1983). Since Ca_i levels are under metabolic control, perfusions with ATP or vanadate might be expected to alter recovery rates, and although the effects were small, this was in fact observed.

A model of Ca current inactivation and recovery from inactivation should account for the following observations. (1) There is no discernable "delay" in the recovery curves as has been found for Na current, and this rules out a three-state sequential model of inactivation as was proposed by Chiu (1977) for Na current. (2) There is an increase in inactivation when Ca replaces Ba, but the total time for recovery of each is approximately the same. (3) There is a marked effect of a prepulse on the inactivation time course in subsequent pulses (see Fig. 1). (4) Recovery can be quite slow for only a moderately long prepulse (a 70-msec prepulse may require 2–3 sec for full recovery). The overall activation process can be neglected since we found previously that Ca current simply scaled by the amount of inactivation present without any change in the time course of relaxation (Brown *et al.*, 1983a). Moreover, as noted already, activation and inactivation kinetics occur over a time course on the order of 0.2–3 msec, which is an order of magnitude faster than the fastest kinetic processes discussed here (Brown *et al.*, 1983a). The much "slower" inactivation process requires as a minimum three states, and a possible scheme is



where state *O* is the open state, states I_1 and I_2 are the fast and slow inactivation states, respectively, and the arrows indicate the forward and backward rate constants. The effect of Ca on inactivation predominates on the fast inactivation process, and the dashed arrow is drawn to indicate this. Both state I_1 and state I_2 are connected

bidirectionally, because for small depolarizations the h_∞ curve does not reach zero (Brown *et al.*, 1981). This characteristic is also consistent with single-channel measurements. We find recurrent activity from a single channel at times when significant "fast" inactivation has occurred in the average patch current (Brown *et al.*, 1983b). We have not made a quantitative test, but observations 1, 2, and 3 above qualitatively account for this model. Observation 4 implies a voltage dependence of the rate constants associated with the I_2 state that gives a very slow time constant for recovery at negative potentials. A rigorous test of this model would require inactivation and recovery data over a range of potentials. It should be noted that the above intuitive arguments hold only because we assume large differences in the values of the inactivation rate constants for I_1 and I_2, as is expected. Normally the inactivation τ's would be a complicated function of all the rate constants.

The process of inactivation and recovery from inactivation provides the Ca channel with a very long-term memory mechanism. Both I_{Ba} and I_{Ca} may take as long as 2–4 sec to recover fully from a prepulse of 70 msec; this is a ratio of ~1:50. The slowness of recovery at the holding potential could indicate that, even though the Ca current inactivates quite slowly, there may be cumulative effects of inactivation even in cells with relatively short action potentials.

ACKNOWLEDGMENTS

This paper is dedicated to the late Tony Gorman, who contributed greatly to our understanding of calcium transients in excitable cells. We thank Mr. H. Henderson for making the illustrations, Ms. J. McLellan for helping with the manuscript, and Ms. S. Allison for typing it.

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