Calcium Currents in Bullfrog Sympathetic Neurons

II. Inactivation

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ABSTRACT Calcium currents in bullfrog sympathetic neurons inactivate slowly and partially during depolarizations lasting 0.5–1 s. There is also a slower (minutes) inactivation process with a broad voltage dependence. An irreversible loss of current (rundown) is prominent with low concentrations of intracellular Ca$^{2+}$ buffers, with either Ca$^{2+}$ or Ba$^{2+}$ as the charge carrier. The extent and rate of the more rapid inactivation process are maximal near the voltage at which the peak inward current is generated, suggesting that inactivation might be Ca$^{2+}$ dependent. However, inactivation occurs with either Ca$^{2+}$ or Ba$^{2+}$ as the charge carrier, is not prevented by strong buffering of intracellular Ca$^{2+}$ with 10 mM BAPTA, and varies little as the peak current is changed 10-fold by changing the divalent ion concentration. That is, rapid inactivation is not explained by simple versions of voltage, Ca$^{2+}$- or current-dependent inactivation models. A model in which ion binding within the channel allows a slower, rate-limiting inactivation process fits some but not all of the observed features of inactivation. A purely voltage-dependent three-state cyclic model fits the data if microscopic inactivation is favored by hyperpolarization.

INTRODUCTION

Two common mechanisms of calcium current inactivation have been well documented: Ca$^{2+}$-dependent inactivation, due to buildup of free intracellular Ca$^{2+}$ in the immediate vicinity of the channel, and voltage-dependent inactivation (Eckert and Chad, 1984). The hallmarks of Ca$^{2+}$-dependent inactivation are a strong selectivity for Ca$^{2+}$ over Ba$^{2+}$, dependence on the amount of current (whether varied by voltage or by Ca$^{2+}$ concentration), and inhibition by moderate levels of intracellular Ca$^{2+}$ buffers (Eckert and Chad, 1984). This mechanism has been demonstrated in a variety of cell types, from Paramecium through mammals (Brehm et al., 1980; Eckert and Tillotson, 1981; Lee et al., 1985; Ganitkevich et al., 1987). It appears to be a general property of L-type calcium currents, although the inactivation rates vary quantitatively among preparations. A third mechanism for calcium current inactiva-
tion, local depletion of extracellular calcium (Almers et al., 1981) is not likely to be important here.

Since one of the identifying characteristics of N-type calcium currents is a more rapid inactivation (Nowycky et al., 1985), it is important to establish whether that inactivation is qualitatively or only quantitatively different from inactivation of L-type current. The ability of either Ca\(^{2+}\) or Ba\(^{2+}\) to trigger inactivation would suggest a distinct, voltage-dependent mechanism (Fox et al., 1987). However, a previous study of calcium current in frog sympathetic neurons proposed Ca\(^{2+}\)-mediated inactivation, on the grounds that inactivation was greatest at voltages that produced the maximal amount of Ca\(^{2+}\) entry (Adams, 1980).

The present results are most consistent with a purely voltage-dependent model. Counterintuitively, the microscopic rate constant for inactivation must increase with hyperpolarization.

A preliminary communication of some of these results has appeared (Marks and Jones, 1988).

**Materials and Methods**

Methods are generally as described in the accompanying paper (Jones and Marks, 1989). Lower sampling frequencies were used (1–2 kHz during long prepulses; generally 20 kHz otherwise), often using a "split-clock" protocol. 2 mM Ba\(^{2+}\) was used as the charge carrier, and 10 mM BAPTA was present in the patch electrode, unless otherwise noted. Records during long depolarizations were not leak subtracted, to avoid subjecting the cell to large hyperpolarizations for long times. Peak current values are leak subtracted, using measurements from brief hyperpolarizing steps.

**Results**

Fig. 1A illustrates the protocol generally used to investigate inactivation. Prepulses lasting 0.5 s were given to different voltages from a holding potential of −80 mV. Note that the current at +60 mV was essentially flat, suggesting that outward currents were effectively blocked. Furthermore, the extent of inactivation observed during the pulse to the point of peak inward current, −10 mV in this cell, is reflected in a corresponding decrease in the amplitude of the current during a subsequent brief test pulse to −10 mV. Measurement of tail currents gave equivalent results (not shown). These results suggest that the inactivation was genuine, rather than generation of, for example, a Ca\(^{2+}\)-dependent outward current. The prepulse to +60 mV produced much less inactivation than did the prepulse to −10 mV. This is not expected for a simple voltage-dependent mechanism, where the extent of inactivation would monotonically increase with depolarization.

Fig 1B illustrates current-voltage relations from the experiment of Fig. 1A. Roughly speaking, the amount of inactivation parallels the amount of inward current. However, the correspondence is only rough. Substantial inactivation can occur at voltages generating little inward current. A replot of the data, superimposing the fraction of maximal inactivation and the fraction of maximal inward current, shows that the inactivation curve is much broader (Fig. 2). In particular, at negative voltages, inactivation is shifted by at least 20 mV to the left. Half-maximal inactivation could be observed at voltages that produced <10% of peak inward current.
Inactivation of Calcium Current

**Figure 1.** Apparent current-dependent inactivation. (A) A voltage step to -10 mV generated an inward current that inactivated slowly and partially over 0.5 s (left). A step to +60 mV generated little current. Postpulses (right) to the point of peak inward current (-10 mV), given after a 15-ms delay, demonstrated that much less inactivation resulted from the prepulse to +60 mV than at -10 mV. Filtered at 2 kHz (postpulse) or 200 Hz (prepulse). (B) Inactivation resulting from 0.5-s prepulses was investigated over a broad voltage range by the protocol of part A. Inactivation, reflected in the decrease in current during the prepulse, is indicated by the difference between the peak current (△) and the current at the end of the prepulse (▽). Inactivation was also measured by the postpulse to -10 mV (□). Data are the averages of two runs in the same cell, with prepulse voltages given in ascending and in descending order, to correct for rundown. Cell a8128.

These experiments were complicated by a component of inactivation that recovered slowly if at all. This was controlled for in several ways, primarily by averaging the results of two runs: one in which the prepulses were given in ascending order from most negative to most positive, and one in descending order. With 10-s intervals between sweeps, and 10 mM BAPTA in the patch pipette, accumulated inactivation during the protocol was generally <10%. Equivalent results were obtained with a triple pulse protocol, where brief test pulses were given both before and after the long inactivating pulse, to control for the amount of activatable current at the start of each sweep.

The rate and extent of inactivation varied from cell to cell, and with time in an individual cell. The time course of inactivation was not always fit by a single exponential, due to a slow component that did not approach a steady-state during the longest pulses given (1 s). Inactivation appears to be most rapid near the point of peak inward current, but the change in kinetics with voltage is not strong (Fig. 3).

**Figure 2.** Inactivation occurs at voltages generating little inward current. Data from Fig. 1 B are replotted as the fraction of maximal inward current (△) and as the fraction of maximal inactivation (□).
Inactivation during 0.5–1-s pulses was generally 20–60% from a holding potential of $-80\text{ mV}$. The fraction of total current that inactivated increased with time for a given cell. This may be related to rundown, which appeared to be greater when many long depolarizing commands were given. The peak inward current decreased with time, with half-rundown times $>30\text{ min}$. Substantial currents with qualitatively similar properties could be recorded for over 2 h in many cells.

The data of Figs. 1–3 were from a cell dialyzed with 10 mM BAPTA, using 2 mM Ba$^{2+}$ as the charge carrier. The rate and extent of inactivation were similar with either Ca$^{2+}$ or Ba$^{2+}$, and with 10 mM BAPTA or no added Ca$^{2+}$ buffer (Fig. 4). Results with 1 mM EGTA were similar to those with no added buffer (not shown). As expected for the large diameter pipettes used, BAPTA was effectively introduced into the cell, as determined in separate experiments by elimination of the large-conductance Ca$^{2+}$-dependent potassium current, which can be $>100\text{ nA}$ in amplitude in these cells with 1 mM EGTA in the pipette (data not shown). However, recovery from inactivation did depend on the presence or absence of BAPTA. With BAPTA, $\sim99\%$ of the current recovered within 10 s after a 1-s pulse, whereas only $\sim90\%$ recovered without BAPTA. This effect was seen with either Ca$^{2+}$ or Ba$^{2+}$.

If the amount of calcium current is varied directly by changing the concentration of permeant ions, rather than indirectly by changing the membrane potential,

![Figure 3](imageurl)  
**Figure 3.** Kinetics of inactivation at different voltages. Single exponential fits to the time course of inactivation for the experiment of Figs. 1 and 2.

![Figure 4](imageurl)  
**Figure 4.** Dependence of inactivation on Ca$^{2+}$, Ba$^{2+}$, and BAPTA. Eight brief test pulses were given once per 10 s, with 0.9-s prepulses preceding the third and fourth test pulses (arrows), with all voltage steps to the point of peak inward current ($-10\text{ mV}$ or 0 mV in different cells). Currents are normalized to the average of the peak currents before the prepulses. Values are means, with error bars shown (±SEM) if larger than the size of the symbol. A compares the same three cells; B includes data from different cells.
fraction of current that inactivates is affected little. In the experiment of Fig. 5, the peak inward current was varied approximately 11-fold by changing the Ba\textsuperscript{2+} concentration from 0.5 to 10 mM. The inactivation rate is affected much less than would be expected from a simple current-dependent inactivation mechanism. Inactivation can also be observed with monovalent ions as the charge carrier, but then the extent of inactivation is less. However, this is difficult to establish quantitatively, as it is rare to hold a cell for more than a few minutes without millimolar concentrations of divalent cations externally.

Recovery from inactivation shows some degree of voltage dependence (Fig. 6). More negative holding potentials produce more rapid recovery. It is noteworthy

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\]
that a large fraction of the recovery from inactivation is relatively rapid, at least as rapid as the inactivation process itself.

Fig. 1 B indicates that substantial rapid inactivation can occur at voltages between -60 and -40 mV. This is partially responsible for a dependence of the peak current upon holding potential. It would also explain the observation that the extent of rapid inactivation is much less from more depolarized holding potentials. However, there is an additional, much slower inactivation process that takes minutes to develop and to recover (Fig. 7). That process is not well isolated kinetically from rundown. The slow inactivation process does not obviously depend on the nature of the permeant ion or on intracellular Ca²⁺ buffering, but that has not been investigated extensively. The slow time course of this process precludes true steady-state inactivation measurements, but additional current can be recruited by hyperpolarization even beyond -80 mV (data not shown).

In many cells, it is possible to demonstrate facilitation of calcium current by large, relatively brief depolarizing prepulses (Fig. 8). The effect was generally <10%, but larger effects were observed in at least three cells. Facilitation was not observed

Figure 7. Slow inactivation of the calcium current. Peak current amplitudes measured during brief test pulses to 0 mV are plotted vs. time subsequent to establishment of whole-cell recording. The holding potential was changed at the times indicated. Cell b7o08; 1 mM EGTA instead of BAPTA.

Figure 8. Facilitation of calcium current by large depolarization. A series of 16 brief (15 ms) test pulses were given every 5 s, with 60-ms prepulses to -10 or +60 mV given before the fifth and through the tenth test pulses of each series. The main figure plots the amplitude of each test pulse. The inset shows averaged currents superimposed, from runs before, during (arrow), and after the prepulses to +60 mV. The records were filtered at 2 kHz, with 250 μs blanked. Cell b8123; 1 mM EGTA instead of BAPTA.
after longer (0.5–1 s) pulses. At least small amounts of facilitation can be observed with 0.1 mM external EGTA, suggesting that facilitation is not due to relief of block by a contaminating di- or trivalent cation (see Jones and Marks, 1989).

**MODELS**

Some results (Fig. 1) suggest that inactivation depends on current through the calcium channel, while other experiments fail to confirm that interpretation (Figs. 4–6). Models in which the more rapid inactivation process depends on buildup of intracellular Ca$^{2+}$ or Ba$^{2+}$ appear untenable. Two models will be discussed further, representing the extreme cases of purely current- and purely voltage-dependent inactivation.

One model that explains some but not all of these results is high affinity binding of a permeant ion to a site within the channel, which permits a slower, rate-limiting inactivation step:

$$
\begin{align*}
C & \rightleftharpoons O \rightleftharpoons O \cdot Ba^{2+} \rightleftharpoons I \cdot Ba^{2+} \\
& \quad \quad \quad k_i \quad k_{-i}
\end{align*}
$$

(Scheme 1)

Placing the interaction within the channel would explain insensitivity to intracellular calcium buffers; a high affinity binding step would give a relatively broad apparent voltage dependence near the point of peak current; a slower isomerization step would permit the observed slow (~200 ms) inactivation rate, with little dependence on voltage or permeant ion concentration. If binding and unbinding are considered fast with respect to other processes, the model reduces to:

$$
\begin{align*}
C & \rightleftharpoons O \rightleftharpoons I \cdot Ba^{2+} \\
& \quad \quad \quad k_i' \quad k_{-i}
\end{align*}
$$

(Scheme 2)

where the effective rate constant $k_i' = k_i F$, $F = K/(1 + K) = $ fraction of open channels with bound Ba$^{2+}$, where $K = [O \cdot Ba^{2+}]/[O]$, with $K$ proportional to the current through the channel. Note that even at saturating current levels inactivation is not complete; the fraction of inactivation would be $k_i/(k_i + k_{-i})$. That is, incomplete inactivation on this model does not require the existence of multiple channel types. With appropriate parameters, this model does generate maximal inactivation with appropriate kinetics near the point of peak inward current, and little or no inactivation at very depolarized potentials.

However, there are important features of the data that are not fit by the model. In particular, the model does not predict substantial inactivation for voltages negative to the point of peak current. The macroscopic inactivation rate becomes limited by the rate of channel opening in the range of negative membrane potentials. With currents at or near saturation, the peak inactivation is actually shifted to the right on the voltage axis, as both channel opening and (microscopic) inactivation rates are near maximal at voltages just positive to peak current. Although saturation makes the model relatively insensitive to the amount of current, it predicts a stronger
dependence of inactivation on current than was observed experimentally (Fig. 5). Another problem with the model is that for parameters that generate large amounts of inactivation, the rate of recovery from inactivation becomes very slow. For example, at 50% maximal inactivation, $k_i = k_{-i}$. The maximal inactivation rate would be $(k_i + k_{-i})$, or twice the maximal recovery rate $k_{-i}$ (since recovery occurs at voltages where $k_i$ is small). Actually, the rate of recovery can be at least as fast as the rate of inactivation (Fig. 6). This problem was noted by Katz and Thesleff (1957) for a formally similar model of desensitization for the acetylcholine receptor. For these reasons, this model for calcium current inactivation must be rejected.

The opposite extreme, a purely voltage-dependent model, must explain the observation that inactivation decreases with voltage at extreme positive potentials. That can be achieved with a simple C→O→I model if the microscopic rate constant for inactivation decreases with depolarization, but less strongly than the rate constant for opening increases. Fig. 9A illustrates the steady-state probabilities for closed, open, and inactivated channels as a function of voltage on such a model. The voltage dependence of rate constants for opening ($k_i$) and closing ($k_{-i}$) are from Jones and Marks (1989); the rate constants in seconds$^{-1}$ for inactivation ($k_2$) and recovery ($k_{-2}$) are

$$k_2 = 2e^{-0.05V - 5}, \quad k_{-2} = 2e^{+0.05V - 5}. \quad (1)$$

On this model, inactivation is 100 times slower than activation, half as voltage sensitive, and the voltage dependence is shifted slightly. For simplicity, the voltage dependence of rate constants for inactivation and recovery have been constrained to be symmetrical. This model predicts inactivation that roughly parallels the amount of peak current (see also Fig. 10 below), but with substantial inactivation at relatively negative voltages that produce little activation (Fig. 2). It could also explain facilitation after large depolarizing prepulses (Fig. 8), as there is less steady-
state inactivation at extreme positive potentials. This model also predicts incomplete inactivation at all voltages.

One problem with this linear form of the voltage-dependent model is that rate constants for recovery from inactivation are too slow. Introduction of a pathway directly linking inactivated and closed channels solves this problem (see Katz and Thesleff, 1957). To explain the result of Fig. 6, $k_5$ (the rate constant for the inactivated-closed transition) must increase with hyperpolarization. For

$$k_5 = e^{-0.02(V-5)}$$

the time course of inactivation and recovery are fit reasonably well. On such a cyclic model, the rate constant for inactivating directly from the closed state ($k_{-3}$) is defined by the other five rate constants; with the parameters used, $k_{-3}$ increases steeply with depolarization (Fig. 9 B), but little inactivation occurs directly from the closed state as $k_1$ is favored by depolarization even more strongly. Introduction of $k_5$ and $k_{-3}$ do not affect the steady-state properties of the model (Fig. 9 A).

On this model, if channel opening and closing are assumed to be fast with respect to the inactivation (and recovery) processes, the probability of being in the inactivated state as a function of time is given by

$$I = \frac{a - (a - bI_0)e^{-bt}}{b}$$

where $I_0$ is the initial amount of inactivation, $a = k_3f + k_{-3}(1 - f), f = k_1/(k_1 + k_{-1})$, and $\tau$, the time constant for inactivation, is given by $1/\tau = b = a + k_{-2} + k_3$. Alternatively, if C and O are thought of as a single lumped kinetic state, then the effective rate constant for inactivation $k_1 = a$, and the reverse rate constant $k_{-1} = k_{-2} + k_3$. On that approach, $1/\tau = k_1 + k_{-1} = b$, and the steady-state inactivation level is $k_1/(k_1 + k_{-1}) = a/b$.

On this model, the time constant for inactivation is the same as the time constant for recovery from inactivation, at a given voltage. The dependence of $\tau$ on voltage is complex (Fig. 9 B), but fits at least qualitatively the observed data (Figs. 3 and 6).

Simulation of calcium currents using the above model produces results similar to the experimental data (Fig. 10). Time constants for inactivation and recovery agreed well with the expression for $\tau$ given above, except that the time constants measured from the simulated data were somewhat faster around $-40$ mV. This indicates that the assumption that activation is much faster than inactivation is a good approximation.

**Discussion**

**Mechanism of Inactivation**

The primary rapid (<1s) inactivation process emphasized here appears to involve a novel mechanism. Three simple models for calcium current inactivation are not consistent with the present data. Simple voltage-dependent inactivation can be ruled out by the nonmonotonic dependence of inactivation on voltage (Fig. 1). Simple calcium-dependent inactivation would be prevented by intracellular BAPTA, which buffers calcium rapidly and with high affinity (Fig. 4). Simple current-dependent
inactivation (i.e., channel block) would predict that the extent and rate of inactivation would depend strongly on the current flowing through an individual channel, as the concentration of permeant ions is varied (Fig. 5).

A variant of the channel block model reproduces some but not all features of the data. But the most satisfactory model is a three-state cyclic model with rate constants depending only on voltage (Figs. 9 and 10). The unique feature of the model is that microscopic inactivation increases with hyperpolarization; macroscopic inactivation reflects also the voltage dependence of the activation process. The model can explain several initially puzzling observations, including incomplete inactivation (Fig. 1 A), apparent current-dependence (Fig. 1 B), inactivation at voltages that produce little inward current (Fig. 2), a relatively flat dependence of the inactivation rate constant on voltage in the measurable region (Fig. 3), lack of sensitivity to the nature and concentration of the permeant ion (Figs. 4 and 5), more rapid recovery from inactivation at negative voltages (Fig. 6), and facilitation by steps to extreme positive voltage (Fig. 8). It is particularly remarkable that the apparent current dependence, so reminiscent of classical calcium-dependent inactivation, can be reproduced by a purely voltage-dependent model.

There are some potential problems with the cyclic model. In one sense, the model is quite simple in that it has no more than the minimum number of states (three) necessary to explain any form of inactivation. However, neither the form of the voltage dependence of the rate constants, nor the individual parameters of the model, are directly determined from the experimental data. It does not appear that the expressions presented here will be easy to test directly, as the overall time constant for inactivation and recovery reduces to a single rate constant only at extreme voltages (Fig. 9 B). It should also be noted that the experimental data for inactivation were not always well fit by a single-exponential time course, which might suggest the existence of additional states. A slow component of recovery from inactivation was particularly clear, and that slow process may well depend on the intracellular Ca²⁺ or Ba²⁺ concentration (Fig. 4). Nevertheless, the model seems to be a useful working hypothesis for calcium current inactivation in these cells. More complex models, perhaps involving a combination of current- and voltage-dependent steps, cannot be ruled out. But the only evidence here for current-dependent inactivation, the nonmonotonic dependence of inactivation on voltage, can be explained equally well by voltage-dependent inactivation (Figs. 9 and 10).

The mechanism proposed here might explain apparently Ca²⁺-dependent inactivation in some other cell types. However, the clear difference between inactivation in Ca²⁺ and Ba²⁺ in some preparations indicates that truly Ca²⁺- or current-dependent inactivation of calcium current does exist (Eckert and Chad, 1984). In many such cells, inactivation may be a mixture of current- and voltage-dependent mechanisms (Brown et al., 1981; Lee et al., 1985; Ganitkevich et al., 1987; Campbell et al., 1988).

Some of the results reported here have been previously observed in other preparations. Even sodium current inactivation, which is presumably purely voltage dependent, can be less complete at extreme positive potentials under some conditions (Chandler and Meves, 1970). Substantial inactivation at voltages producing little inward current has also been observed for calcium current in frog muscle
Inactivation of Calcium Current

(Cota et al., 1984), which was interpreted as evidence for voltage-dependent inactivation, but inactivation at positive membrane potentials was not investigated in that study. A voltage dependence of recovery has been reported in cases where inactivation appears on other grounds to be Ca²⁺-dependent (Ganitkevich et al., 1987). Facilitation of calcium current by large depolarizations has been previously reported (Hoshi et al., 1984), but has been explained by other mechanisms (Hoshi and Smith, 1987).

**Slow Inactivation**

Changes in the holding potential have dramatic effects on the amplitude of the calcium current, and on its inactivation kinetics. Some of this can be explained by the "rapid" inactivation process discussed above, as significant inactivation develops even within 1 s at voltages as negative as -60 mV. However, there is a largely reversible inactivation process with kinetics several orders of magnitude slower (Fig. 7). This is not surprising; slow inactivation processes have been reported for most voltage-dependent currents, including the classical sodium current (Fox, 1976), and the calcium current of cardiac ventricular cells (Schouten and Morad, 1988). The mechanism of slow inactivation has not been investigated here, but it is still present with 10 mM BAPTA.

**Rundown**

As in other cells, the peak amplitude of the calcium current decreased with time over tens of minutes under whole-cell conditions, even in the presence of MgATP. Rundown appeared to be more rapid when many long depolarizing steps were given, which could be explained by several mechanisms. Rundown was seen with either Ca²⁺ or Ba²⁺ as the charge carrier. The clearest effect on rundown was that recovery from inactivation was much more rapid and complete with internal BAPTA (Fig. 4). BAPTA, like EGTA, is highly selective for binding Ca²⁺ over Mg²⁺. However, EGTA binds Ba²⁺ reasonably well (Martel and Smith, 1979), and BAPTA is presumably similar in that regard. Several mechanisms for rundown have been pro-
posed, including activation of Ca\(^{2+}\)-dependent phosphatases and proteases (Chad and Eckert, 1986). It is not likely that Ba\(^{2+}\) would substitute for Ca\(^{2+}\), at least for phosphatase activation (Cheung, 1984).

**One Current or Two?**

Multiple types of calcium currents have been reported in a variety of cell types (Hagiwara et al., 1975; Carbone and Lux, 1984; Nowycky et al., 1985; Matteson and Armstrong, 1986). Sympathetic neurons appear to have N- and L-type currents, but lack the rapidly inactivating T-type current (Wanke et al., 1987; Hirning et al., 1988; Lipscombe et al., 1988). However, the N-type current of sympathetic neurons clearly inactivates much more slowly than that of dorsal root ganglia (Nowycky et al., 1985; Hirning et al., 1988; Lipscombe et al., 1988). Jones and Marks (1989) tentatively concluded that both N- and L-type calcium currents are present in bullfrog sympathetic neurons, but that the N-type current is predominant.

If there are both N- and L-type currents in these cells, which inactivation processes would correspond to which current? The rapid inactivation process, seen from more negative holding potentials, would have to be N current inactivation. Unlike the case in dorsal root ganglia (Nowycky et al., 1985), the N-type current of sympathetic neurons does not appear to inactivate totally during 1-s depolarizations; that is, the current remaining at the end of a 1-s pulse is not all L-type current (Hirning et al., 1988). The increase in proportion of rapidly inactivating current with time might suggest that L-type current runs down more quickly. The slow inactivation process would have to involve N-type current at least in part, since steady changes in holding potential continue to increase the availability of current even below −80 mV, while 1-s steps in the −120 to −80 mV region have little effect. Nearly full inactivation occurs after several minutes at −40 mV (Fig. 7). If significant L-type current is present, it must also therefore show a slow inactivation process. If there is a classical calcium-dependent inactivation process for an L-type current in these cells, it must be small or slow under the conditions examined here.

However, it is equally possible to explain the present results with a single current type. In particular, much of the difference in inactivation from holding potentials in the −80 to −40 mV range can be explained by the rapid inactivation process, which would quickly reach a steady-state level upon changes in the holding potential.

The initial goal of this work was to determine whether multiple calcium currents were present in bullfrog sympathetic neurons. That goal has not been fully reached. Kinetic and pharmacological criteria are consistent with the existence of a predominant current (Jones and Marks, 1989) resembling N-type calcium currents. Inactivation kinetics could be interpreted either as multiple inactivation processes for a single current type, or as multiple currents. But it is clear that the main, rapid inactivation process differs from inactivation mechanisms reported previously for L- and T-type calcium currents. This suggests that N-, L-, and T-type calcium currents inactivate by qualitatively different mechanisms.

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