Effect of firing rate on the calcium permeability in adult neurons during spontaneous action potentials

Michele Mazzanti, Aurelio Galli, and Arnaldo Ferroni
Dipartimento di Fisiologia e Biochimica Generali, Laboratorio di Elettrofisiologia, Universitá Statale di Milano, Milano, Italy

ABSTRACT Calcium channels in neurons mediate a wide variety of essential functions. In addition to contributing to action potential shape, they furnish a substrate that acts as an intracellular second messenger. This study shows that the shape of the neuronal action potential has characteristics that promote long openings of L-type (high threshold) calcium channels. We also present evidence that a change in the firing rate of isolated neurons modulates gating of single calcium channels. This mechanism could be important in modulating neuron excitability and providing a rise in intracellular Ca, when needed.

INTRODUCTION

Dihidropiridine sensitive (high-threshold or L-type) Ca channels have different kinetics of opening (Hess et al., 1984; Nowycky et al., 1985; Pietrobon and Hess, 1990). The question arises whether these diverse opening modes have a physiological significance (Bean, 1990). Suggestions regarding modulation of cardiac action potential plateau or fast increases of intracellular calcium ions in neurons were made by various authors (Hess et al., 1984; Nowycky et al., 1985; Pietrobon and Hess, 1990). Several cytoplasmic functions are, in fact, activated by cytoplasmic divalent ions. Skeletal and cardiac muscle cells take advantage of long action potentials and large sarcoplasmic stores to provide intracellular elevation of calcium ions. A neuronal excitation wave lasts only a few milliseconds; thus, it must have a different mechanism to increase calcium permeability, when needed. Neurons utilize as a communication pattern different numbers of action potentials per unit time. Increased firing frequency, for example, is commonly used to trigger neurotransmitter release, which, like other neuronal functions, requires an intracellular increase of Ca ions (Mulkey and Zucker, 1991; Grover and Teyler, 1990).

We hypothesize that an increase in calcium permeability not only depends on voltage, but also on action potential frequency, thereby providing a means to increase intracellular calcium at higher firing frequencies. According to this hypothesis, spontaneous neuronal action potentials increase the occurrence of long-lasting, high open probability (high-p_o) states of L-type Ca channels by a "prepotentiation" process (Hoshi and Smith, 1987; Pietrobon and Hess, 1990). Furthermore, an increase in the firing frequency would maintain the high-p_o state, preventing the channel from reverting to its fast gating pattern before the occurrence of the next action potential.

In our experiments, like in most single-channel studies, the permeating ion is barium. During the spontaneous cardiac action potential, different modes of opening were observed using either 10 mM Ba or Ca (Mazzanti and DeFelice, 1990). Here we use 20 mM barium in the recording pipette, because the probability that the channel will stay open throughout the action potential is high. Another manipulation we used in our experiments is to prolong the action potential by partially blocking potassium conductance with tetraethilammonium. This operation acts only during the second phase of the repolarization, extending the plateau and not interfering with the prepotentiation that occur earlier. A longer plateau phase also facilitates the measurement of single-channel conductance.

Our results suggest that two different mechanisms, one intrinsic to the action potential and one triggered by the firing frequency, concur to increase Ca current at the single-channel level, causing an exponential rise of Ca ion influx relative to an increase in firing rate. This mechanism, during high frequency potential oscillations, would produce a causal relationship between firing frequency and the permeability of the nerve membrane to Ca.

METHODS

Dorsal root ganglion (DRG) cells of adult rat were prepared by enzymatic digestion, following the procedure of Ferroni et al. (1989). After 12 to 24 h in tissue culture medium, and immediately preceding the experiments, we washed the cells in bath solution (in mM units: 142 NaCl, 5 KCl, 2 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, adjusted to 7.35 pH). The whole-cell electrode for measuring voltage contained an intracellularlike solution, consisting of 120 KAsp, 20 tetraethylammonium (TEA), 10 NaCl, 2 MgATP, 0.1 BAPTA, 10 HEPES, adjusted to 7.35 pH. The cell-attached electrode for measuring patch currents contained (in mM): 20 $BaCl_2$, 70 NaCl, 1 EGTA, 0.003 tetrotodotoxin (TTX), 5 TEA, 10 4-aminopyridine (4-AP), 10 HEPES, adjusted to 7.35 pH. All experiments were performed at room temperature.

In these experiments we used two electrodes to record the patch current and the whole-cell voltage. The data were bandlimited at 1,000 Hz, and we have subtracted the capacitive transient from all current traces. For a detailed description of the two-electrode recording method used during action potentials, see Mazzanti and DeFelice (1987, 1990) and Wellis et al. (1990).

Dorsal root ganglion neurons in culture fired spontaneously, but they also often stop. We utilized this behavior to compare the single-channel currents from both active and quiescent cells from the same culture (Fig. 1).

Address correspondence to Dr. Mazzanti.

0006-3495/92/10/926/09 $2.00

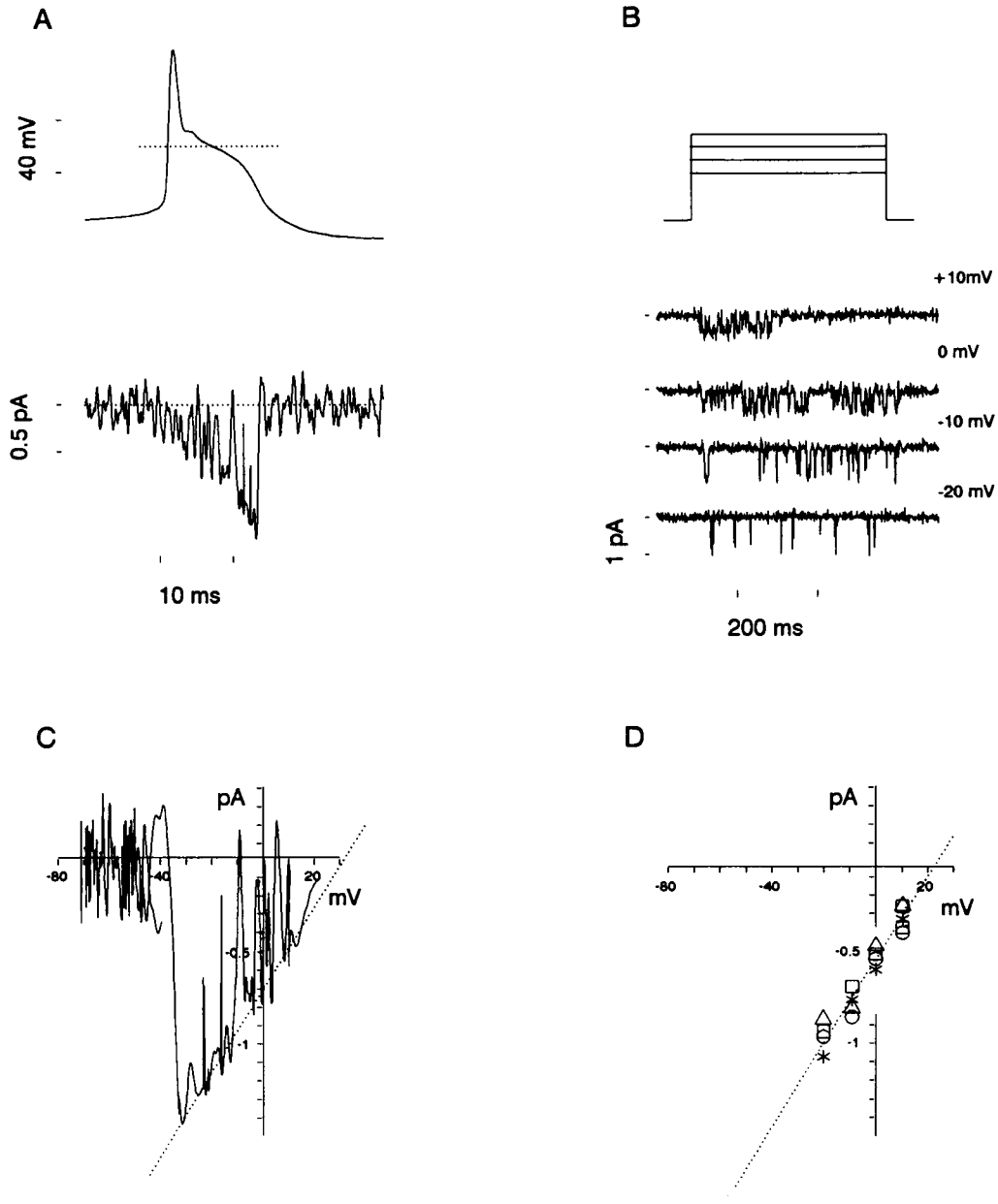


FIGURE 1 Characterization of a single calcium channel carrying 20 mM barium. Different applications of the two-electrode technique during (*A*) the nervous action potential and (*B*) the steady-state condition. Panel A shows a spontaneous action potential (*top*) recorded simultaneously with the action current (*bottom*) after capacitance-current subtraction. Instantaneous current/voltage relationship in *C* was obtained by plotting the voltage wave against the ionic current. The dotted line was obtained from the steady-state *i/V* curve (*D*) and adapted to the open channel profile. *B* depicts a steady-state experiment using the same experimental configuration. A whole-cell electrode clamped the cell at −50 mV while voltage steps of different amplitudes (*top*) were delivered to the membrane patch via the cell-attached electrode. Below are plotted single channel current traces at four test potentials (value above each trace) in the range of the plateau phase of the action potential (*left*). In *D* a current/voltage relationship was generated by plotting current values obtained from amplitude histograms of four experiments. The difference in the extrapolated reversal potential between *i/Vs A* and *B* reflects different interpolating methods. Current values obtained from histogram peaks (*B*) represent mean single channel open amplitudes. In *A*, on the other hand, the dotted line lies on top of the single open channel noise.

The electrodes were made from borosilicate glass using a modified puller (model 700 C; David Kopf Instruments, Tujunga, CA). After coating with Sylgard (Dow Corning) and fire polishing the tip to 1–2 μm internal diameter, the electrodes had resistances of 4–10 megaohms (MΩ). Axopatch and List EPC7 amplifiers were used to measure the voltage and current, respectively. We stored the data on a video cassette recorder, and analyzed it on a Nicolet 310 oscilloscope and a Mitsuba 386SX, using programs developed by William Goolsby, Department of Anatomy and Cell Biology (Atlanta, GA).

RESULTS

Fig. 1 *A* shows a double-electrode experiment (Mazzanti and DeFelice, 1987, 1990; Wellis et al., 1990) on isolated rat dorsal root ganglion (DRG) neurons. The top trace is a spontaneous action potential. Below that is the ionic current obtained after capacitance-current subtraction.

The inward current, recorded with a second electrode in cell-attached mode on the same cell, is carried by barium ions flowing through the calcium channel (Mazzanti and DeFelice, 1990). To obtain the rare event of a single calcium channel in the open state throughout the action potential, we prolonged the excitation wave, particularly the repolarization phase. The action potential was generally slowed down by working at room temperature, and the plateau phase was increased by including 20 mM tetraethylammonium (TEA) in the whole-cell electrode solution. Long plateaus are required to measure single Ca channel conductance during spontaneous activity. Fast repolarization will close the voltage-dependent channel too quickly, making conductance measurement impossible. *B* depicts a steady-state experiment using the same double-electrode technique. The membrane voltage was clamped with the whole-cell electrode to −50 millivolts (mV) whereas a step protocol (*top*) was delivered by the cell-attached pipette. The potential steps were chosen to cover the range of voltages experienced by the channel during the action potential plateau. Below, in *C* and *D*, are two single-channel current/voltage (*i/V*) relationships. On the left, the instantaneous *i/V* was obtained by plotting the action potential against the action current. On the right, the experimental points were calculated from amplitude histogram analysis of four single-channel experiments like the one in Fig. 1 *B*. The dotted line in *D* is the least-square linear fit to experimental data. In *C*, the line was transposed from *D* to determine whether the instantaneous *i/V* plot had the same slope conductance. The line adequately fits the open channel profile of the instantaneous *i/V*. The conductance calculated from the line slope is 22 picosiemens (pS). Single calcium channels activated in the voltage range described above (Hess et al., 1984; Fox et al., 1987), with a conductance around 20 pS in 20 mM barium (Yue and Marban, 1990; Mazzanti et al., 1991), are classified as "high threshold" or "L-type".

To investigate whether increase in firing frequency is directly responsible for a rise of calcium influx (Hoshi et al., 1984; Artalejo et al., 1991*a, b*), two-electrode experiments were carried out at different firing rates. Occurrences of action potentials were controlled by the whole-cell electrode. In a spontaneously firing neuron, injection of hyperpolarizing current slows down frequency, whereas depolarizing the membrane results in a firing-rate increase. Fig. 2 shows three action potential averages superimposed. The corresponding action current averages are plotted below. Each trace was obtained from 60 consecutive action currents at firing rate of 0.5 Hz (*A*), 1 Hz (*B*), and 4 Hz (*C*). In voltage trace *C*, the action potential foot appears to be depolarized a few millivolts, which is essential to increase action potential occurrences. The average current increases 20% between 0.5 and 1 Hz and shows more than 100% increase at 4 Hz.

The increase of Ca current with action potential frequency rate, in our experiments, cannot be due to the

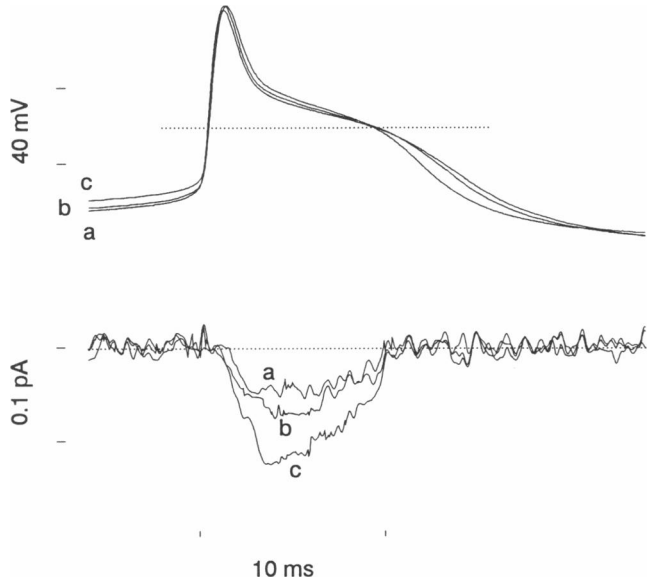


FIGURE 2 Single channel current averages at different firing rates. The figure shows, on the top, three superimposed action potential averages from the same experiment. Trace *C* is slightly depolarized and belongs to the 4 Hz action potential train. Below are the corresponding current averages obtained from 60 consecutive action currents.

activation of a greater number of Ca channels. The presence of more than one channel would be easily detected as multilevels with the cell-attached electrode. Other sources of single-channel current increment could be provided by an increase of probability of opening or an increase in the open time. In the experiment shown in Fig. 2, as in three other experiments, the number of action currents in which we observed opening of single Ca channels was not significantly different within the same experiment among the diverse frequency rates. In more than three experiments, in which the total number of action potentials at different frequency rates was between 200 and 280, we observed channel openings in 62 ± 0.6% of action currents. Consequently, the probability of opening, from one action potential to the next (*Po*), cannot account for a 40–100% current increase within the firing-rates explored. On the other hand, L-type calcium channels have the ability to switch between their normal gating pattern of brief openings to a state characterized by long openings (also called "mode 2", Hess et al., 1984; Nowycky et al., 1985). To increase the occurrence of "long-opening" mode, the channels need to be conditioned by a prepulse to positive voltages before reaching the test potential, usually around 0 mV (Hoshi and Smith, 1987; Pietrobon and Hess, 1990). The phenomenon, defined as "prepotentiation" (Pietrobon and Hess, 1990), dramatically increased the occurrence of high-p_o of Ca channels. Fig. 3 shows the activity during a nervous action potential. The shape of the spontaneous excitation wave simulates the prepotentiation step by driving the voltage up from −50 mV to ~70 mV

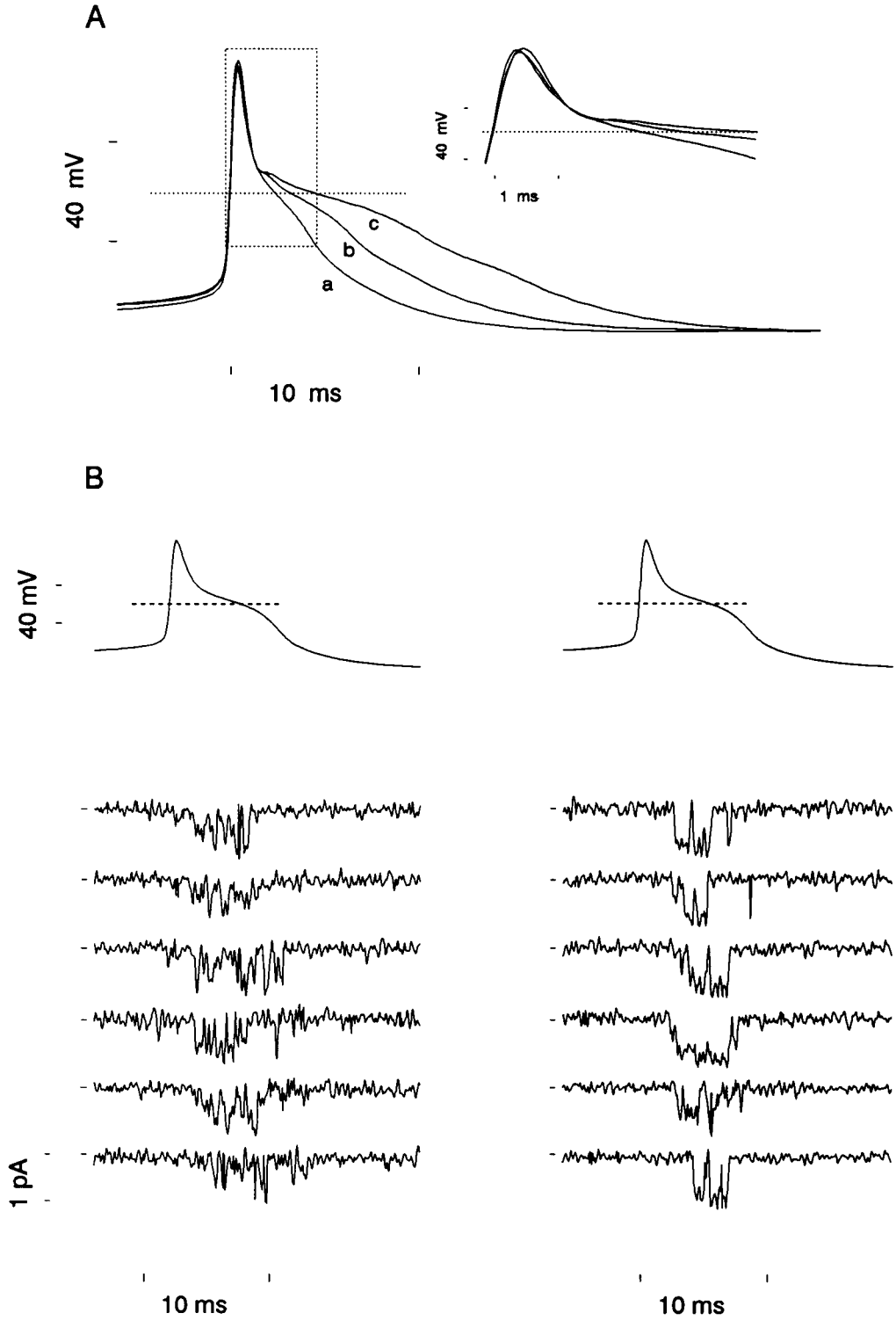


FIGURE 3 Existence of different gating patterns during spontaneous nerve cell activity. Fig. 3 *A* shows the effect of 20 mM TEA perfusion over time on the action potential shape. The voltage waves show the first action potential recorded after whole-cell configuration was reached (*a*), after 3 minutes (*b*), and after 6 min of electrode solution perfusion (*c*). After 5 min, the action potential profile usually remained constant. Potassium channel blockers act only on the plateau phase and not on the early fast depolarization or repolarization responsible for the prepotentiation (*insert on top right*). Fig. 3 *B* (*top*) shows two action potentials from the same experiment. Below (*left*) are six action currents in which the calcium channel opens with fast kinetics. On the right are selected current traces showing the channel in the long opening and high open probability state.

and then to a plateau phase, starting at ~10 mV. The prepotentiation wave described above, fast depolarization and rapid repolarization, takes place in 1–2 ms (*insert, upper right,* Fig. 3 *A*) and is independent of potassium channel blocker perfusion, which increases only the plateau duration (Fig. 3 *A*). Fig. 3 *B* (*left*) shows a spontaneous action potential and, below, six action currents after capacitance-current subtraction. Current traces show single calcium channels opening with fast kinetics. Fig. 3 *B* (*right*), underneath the same action potential, is a different set of action currents selected from the same experiment. In this case, the channel has a longer open state. Channel openings with fast and slow kinetics occurred randomly during low frequency (0.1 Hz) firing of the single neuron. To determine whether two distinct open kinetics are present, we create open-time histograms for a series of consecutive traces containing one calcium channel. Open-time values cannot be compared with steady-state experiments because the voltage dynamically changes in the activation region of a voltage-dependent channel. Also, no comparison can be made between experiments with different action potential shapes, in which the time course of the voltage wave plays an important role in the channel kinetics. However, they are useful for comparison within the same experiment, in which action potentials do not change appreciably throughout the recording. To prevent a phenomenon known as "facilitation" of calcium current due to stimulus frequency (Hoshi et al., 1984; Artalejo et al., 1991*a, b*), single-action potentials were triggered by small depolarizations in a quiescent cell every 10 s (Fig. 3 *B*). In this way, we show that during the nervous action potential with addition of a fast open kinetics, high-p_o mode appears spontaneously as a gating pattern of Ca channels. An open-time histogram of a single Ca-channel shows, as a consequence, two-exponential distribution. Time constants reflect two different kinetics: fast (τ = 0.19) and slow (τ = 1.32). The two modes of opening, calculated as the relative area delimitated by the two exponential fittings, occur with probabilities Po_{m1} and Po_{m2} of 76 and 24%, respectively (averaged over 50 action potentials, when the channel was active), with a ratio Po_{m2}/Po_{m1} of 0.315.

After obtaining the open-time constants and the ratio between modes at a firing frequency where supposedly no Ca-current facilitation occurs, we compared single calcium channel open-times at different firing rates. Fig. 4 (*left*) shows action potential trains firing at 0.5, 1, and 4 Hz. Next to each voltage record (*right*), we plotted single Ca channel open-time histograms from 80 concatenated action currents at each firing frequency. Two-exponential functions were used to fit the points. The fast time constant appears unchanged and is comparable to steady-state experiments under similar conditions (Hess et al., 1984; Mazzanti et al., 1991). The slow component that will be analyzed here, increases substantially as the firing rate increases. In Fig. 5 *A* we plot normalized current charge (Q/Q_{max}) and slow open-time (τ/τ_{max}) on a semi-logarithmic plot as a function of firing frequency for four experiments. All the data came from current averages of single-channel records, where the action potentials can be superimposed within the same experiment at every firing frequency. Current charge and τ behave in a similar manner as the frequency increases. Normalized experimental values are constant between 0.1 and 0.8 Hz. At higher action potential occurrences, these values increase. As confirmation, the ratio Po_{m2}/Po_{m1} increases from 0.33 at 0.5 Hz to 0.4 at 1 Hz and 0.61 at 4 Hz. The relationship between firing frequency and appearance of high-p_o is analyzed in Fig. 5 *B*. Here we observed the occurrence of mode 2 during 120 consecutive action potential trains for three different firing rates. The separation between action currents in which the channel opens with a mode 1 and mode 2 kinetics, was done by plotting for each current trace the open time histogram. The sweeps in which the slow time constant was predominant (more than 50% of the area delimited by the exponential fitting) were considered in high-p_o state. For each frequency, we plot two columns, representing the state occupied by the channel at that particular moment (one channel closed or in mode 1; two channels in mode 2). The progression of the experiment is from bottom to top. Occurence of high-p_o not only increases at higher firing frequencies, but also, once the channel switches to mode 2, the long opening mode persists in the subsequent action potentials. Clusters of high-p_o state appear evident at higher frequencies.

DISCUSSION

The mechanism and extent of calcium influx during the nervous action potential is not yet clear. Nervous cells produce an excitation wave that lasts a few milliseconds. In particular, the time spent by the voltage in the activation range of the calcium channel is less than a millisecond. The increase of intracellular calcium cannot, therefore, be within a single-action potential but may occur by a cooperation of events over a burst of upstroke potentials.

Other authors have already shown in diverse preparations an increase of Ca current that is dependent on stimulation frequency or a depolarizing prepulse (Hoshi et al., 1984; Hoshi and Smith, 1987; Ikeda, 1991; Artalejo et al., 1991*a, b*). To explain the mechanism of current "facilitation" three hypotheses were suggested: a shift of the channels from a "reluctant" to a "willing" state (Bean, 1989; Ikeda, 1991); a voltage dependent equilibrium between different gating patterns of dyhidropiridine sensitive Ca channels (Pietrobon and Hess, 1990); or a recruitment of a Ca channel similar to the long-lived openings exhibited by L-type channels (Artalejo et al., 1991*b*).

We believe that one of the problems with single-channel observations is correlated with the permeating ions.

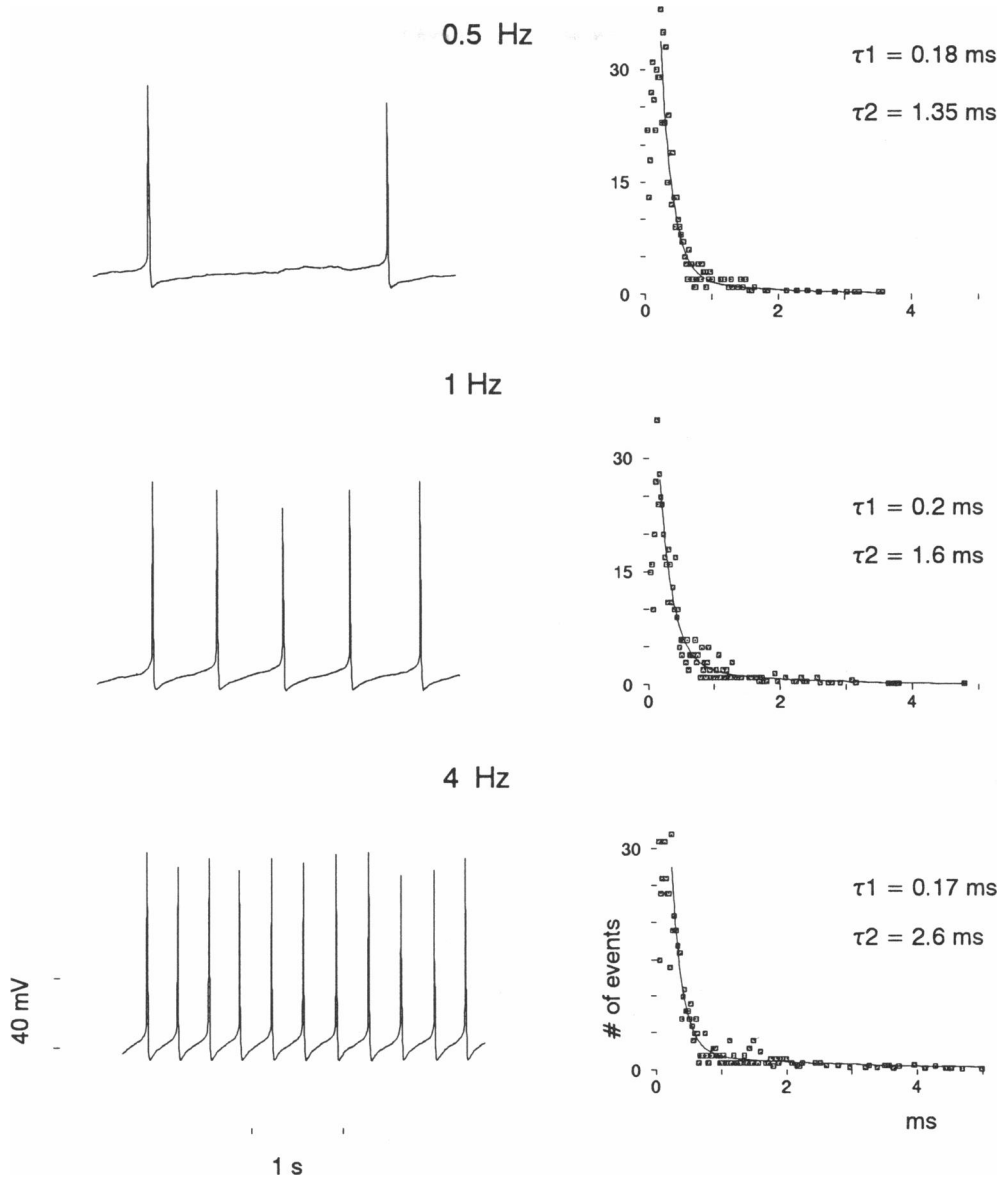
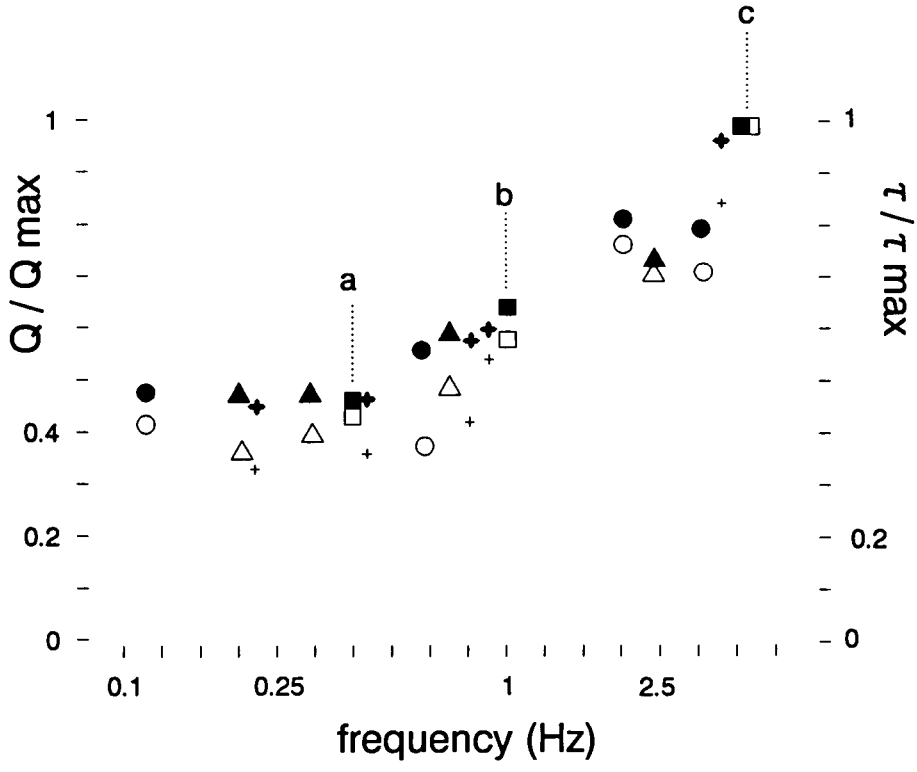


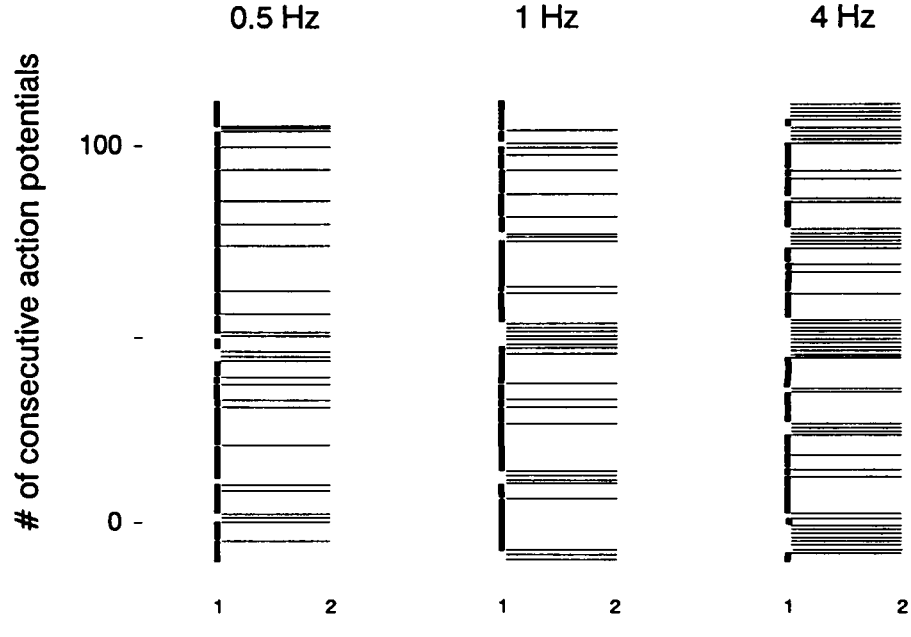
FIGURE 4 Comparison of single-channel open times at various action potential frequencies. On the left are action potential trains at various rates. On the right are the nonsteady state single Ca-channel open-time histograms, from 80 concatenated action currents at each corresponding frequency. The points were fitted with a two-exponential function. The resulting values are on the upper right corner of each plot. While $\tau 1$ remains unchanged, $\tau 2$ almost doubles from 0.5 to 4 Hz.

In the experiments on the function of single Ca channels, Ba at high concentration almost always replaces Ca, because using physiological Ca concentrations, the channels are too small to be detected. However, the substitution causes modification in the channel. For example, in the experiments performed during the cardiac action potential, Ba not only doubles the conductance, but also increases the probability that the channel will stay open throughout the plateau (Mazzanti and DeFelice, 1990). Being the first study of this kind in neurons, we used in the recording pipette 20 mM Ba, an experimental situation in which it was relatively easy to observe the single channels. In the same sense, we view the TEA in the intracellular solution as a means to help observe the phenomenon, even though it clearly perturbs it. To compare single-channel openings at different firing frequencies, we must be sure that we have only one channel, and that it does not change its intrinsic characteristics, like conductance, during the experiment. From Fig. 3, it is possible to observe that, although the partial block of potassium conductance with TEA has promoted a more pronounced plateau, it does not interfere with the early part of the action potential. It will become essential to reproduce the same experiments using normal concentrations of Ca and a physiological action potential. At the present time we can, however, speculated how Ca channels function, and we can discuss the eventual modification that would occur in physiological conditions.

A


c
a
b
1
0.4
0.2
0
Q / Q max
τ / τ max
0.1
0.25
1
2.5
frequency (Hz)

B


0.5 Hz
1 Hz
4 Hz
# of consecutive action potentials
100
0
1
2

Our findings suggest that the channel does not change its intrinsic characteristics, such as conductance or probability of opening, but changed instead, the relation between open-channel kinetics from one action potential to the next.

Fig. 3 indicates that prepotentiation, used by the cell to promote high-p_o mode in 24% of the cases, is a characteristic of each action potential. In our experiments, the brief time spent at the peak of depolarization appears to be sufficient to induce high-p_o, and this seems to be a physiological peculiarity of neurons. In the heart, longer times of depolarizing prepulse are required to produce long openings of Ca channels (Pietrobon and Hess, 1990).

When frequency of action potential increases above 0.8 Hz (Fig. 5 *A*), there is, in addition to the increment of high-p_o events, a clustering of the long openings from beat to beat (Fig. 5 *B*). A clustering of modal gating channel behavior has been described in steady-state experiments in DRG chicken neurons (Nowycky et al., 1985). The tendency for similar sweeps to follow each other has been suggested as an inclination of individual channels in the patch to remain in the same mode from one depolarization to the next. In cardiac myocytes, according to Pietrobon and Hess (1990), the rate constant (K_b) of L-type channels switching from high-p_o to low-p_o kinetics is in the order of hundreds of milliseconds, well within the range of voltage in which the channel is active. At negative potential, K_b became faster. However, in neurons, clustering of long opening events has been observed even in pulse trains at low frequency (Nowycky et al., 1985), suggesting a much slower mechanism. In our experiments, spontaneous action potentials probably accentuates this channel behavior. Physiological excitation waves could play an important role, such as in cardiac cells, where long opening of Ca channel conducting Ba persists along the entire action potential plateau (Mazzanti and DeFelice, 1990). During neuronal action potential, it is possible that due to a slower K_b the channel would be closed by the repolarization in a voltage dependent manner, maintaining high-p_o mode during voltage oscillations.

There are many factors that influence Ca channel kinetics. Voltage, time, and equilibrium between modes interact dynamically during the spontaneous action potential. Hess et al. (1984) propose a model for L-type Ca-channels in which each open state (mode 1 or mode 2) has the possibility to switch to a closed condition. From the closed state the channel can change mode, but the transition is slower than in the open state. We have shown that, when the channel opens in mode 2, the closer the next action potential, the higher the probability that the channel will maintain high-p_o open kinetics. Therefore, Ca current at higher firing frequency increases not only because of more action potentials per unit time but also because a high-p_o state persists following each upstroke, allowing more influx of Ca ions. The presence of longer clusters of high-p_o events at higher firing frequencies as shown in Fig. 5 *B* supports this hypothesis.

Increasing firing frequency induces a linear increase of Ca current as a consequence of more action potentials per unit time. "Facilitation" would appear as a deviation in this linearity. Fig. 5 *A* shows that frequency-induced "facilitation" of calcium current begins to be substantial between 0.8 and 1 Hz. These values could be considered a threshold for significantly modifying calcium inflow in our preparation.

According to this mechanism, the action potential upstroke alone induces high p_o in 20–25% of the action currents where the channel opens (prepotentiation). At firing frequencies >0.8–1 Hz, the channel maintains a high-p_o state between one action potential and the next, thus, increasing exponentially the total calcium inflow over time (facilitation).

The significant discrepancy in the frequency ranges that trigger substantial modifications (Mulkey and Zucker, 1991; Grover and Teyler, 1990) may be the result of using different experimental conditions (room temperature as opposed to 35°C, and barium ions versus calcium [Hess and Tsien, 1984]), or different preparations. Our results suggest that, because firing frequencies that regulate various functions vary in different physiological conditions, different cells may use diverse frequency thresholds to modulate distinct calcium-dependent cell behaviors.

In conclusion, we report here a mechanism by which calcium permeability starts to increase exponentially at a defined firing rate threshold. Higher frequency may overload the cell with calcium, triggering and modulating a variety of key cellular responses.

Our results provide a mechanism for increasing intracellular calcium during action potential bursts. High-frequency action potential trains, according to our data, would act as a single cooperative object, in calcium permeation. Oscillations in the voltage during potential bursts would play the role of amplifier in a positive feedback system.

FIGURE 5 (*A*) Nonlinear relation between current charge and channel opentime as a function of firing frequency. A semi-logarithmic plot shows the relationship of normalized charge (Q/Q_{max}) and slow open-time constant (τ/τ_{max}) against action potential frequency for four different experiments. Both charge and τ follow the same course; they are constant for frequencies below 0.4–0.5 Hz and increase following a single exponential, eventually reaching a plateau. (*B*) Appearance of high-p_o state during 120 action potential trains at different firing rates. For each frequency we plot two columns, representing (1) the close and "mode 1" state and (2) "mode 2" state, occurring in temporal order during voltage oscillations. The switch between modes is represented as a horizontal line from on column to the other. Clusters of high-p_o openings increase at higher frequencies.

We thank Mr. G. Mostacciulo for excellent technical assistance. We also thank Drs. E. Wanke and A. Becchetti for reading and Winnie Scherer for editing the manuscript.

Received for publication 23 March 1992 and in final form 15 June 1992.

REFERENCES

Artalejo, C. R., M. K. Dahmer, R. L. Perlman, and A. P. Fox. 1991*a*. Two types of Ca currents are found in bovine chromaffin cells: facilitation is due to the recruitment of one type. *J. Physiol. (Lond.).* 432:681–707.

Artalejo, C. R., D. J. Mogul, R. L. Perlman, and A. P. Fox. 1991*b*. Three types of bovine chromaffin cell Ca channels: facilitation increases the opening probability of a 27-pS channel. *J. Physiol. (Lond.).* 444:213–240.

Bean, B. 1990. Gating for the physiologist. *Nature (Lond.).* 348:192–193.

Ferroni, A., E. Mancinelli, S. Camagni, and E. Wanke. 1989. Two high voltage-activated calcium currents are present in isolation in adult rat spinal neurons. *Biochem. Biophys. Res. Comm.* 159:379–384.

Fox, A. P., M. C. Nowycky, and R. W. Tsien. 1987. Single-channel recordings of three types of calcium channels in chick sensory neurones. *J. Physiol. (Lond.).* 394:173–200.

Grover, L. M., and T. J. Teyler. 1990. Two components of long-term potentiation induced by different patterns of afferent activation. *Nature (Lond.).* 347:477–479.

Hess, P., and R. W. Tsien. 1984. Mechanism of ion permeation through calcium channels. *Nature (Lond.).* 309:453–456.

Hess, P., J. B. Lansman, and R. W. Tsien. 1984. Different modes of Ca channel gating behavior favoured by dihydropyridine Ca agonists and antagonists. *Nature (Lond.).* 311:538–544.

Hoshi, T., J. Rothlein, and S. J. Smith. 1984. Facilitation of Ca-channel currents in bovine adrenal chromaffin cells. *Proc. Natl. Acad. Sci. USA.* 81:5871–5875.

Hoshi, T., and S. J. Smith. 1987. Large depolarization induces long openings of voltage-dependent calcium channels in adrenal chromaffin cells. *J. Neurosci.* 7:571–580.

Ikeda, S. R. 1991. Double-pulse calcium channel current facilitation in adult rat sympathetic neurones. *J. Physiol. (Lond.).* 439:181–214.

Mazzanti, M., and L. J. DeFelice. 1987. Na channel kinetics during the spontaneous heart beat in embryonic chick ventricle cells. *Biophys. J.* 52:95–100.

Mazzanti, M., and L. J. DeFelice. 1990. Ca channel gating during cardiac action potentials. *Biophys. J.* 58:1059–1065.

Mazzanti, M., L. J. DeFelice, and Y. M. Liu. 1991. Gating of L-type Ca channels in embryonic chick ventricle cells: dependence on voltage, current and channel density. *J. Physiol. (Lond.).* 443:307–334.

Mulkey, R. M., and R. S. Zucker. 1991. Action potentials must admit calcium to evoke transmitter release. *Nature (Lond.).* 350:153–155.

Nowycky, M. C., A. P. Fox, and R. W. Tsien. 1985. Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc. Natl. Acad. Sci. USA.* 82:2178–2182.

Pietrobon, D., and P. Hess. 1990. Novel mechanism of voltage-dependent gating in L-type calcium channels. *Nature (Lond.).* 346:651–655.

Yue, D. T., and E. Marban. 1990. Permeation in the dihydropyridine-sensitive calcium channel. Multi-ion occupancy but no anomalous mole-fraction effect between Ba and Ca. *J. Gen. Physiol.* 95:911–939.

Wellis, D. P., L. J. DeFelice, and M. Mazzanti. 1990. Outward sodium current in beating heart cells. *Biophys. J.* 57:41–48.