Low-Voltage-Activated Calcium Current Does Not Regulate the Firing Behavior in Paired Mechanosensory Neurons With Different Adaptation Properties

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INTRODUCTION

Calcium currents (I_{Ca}) that activate in a low voltage range (LVA), and often transiently (T type), have been observed in a variety of neurons from vertebrate and invertebrate central and peripheral nervous systems. These currents activate at potentials low enough to gate the activity of other depolarizing voltage-activated ion channels and therefore their putative functional roles include lowering the threshold for spike generation (reviewed by Huguenard 1996), contribution to the depolarizing envelope that underlies neuronal burst firing (Llinás and Yarom 1981) and promotion of intrinsic oscillatory behavior (Lewis and Hudspeth 1983; McCormick and Huguenard 1992). LVA-Ca^{2+} channels have been shown to exist in the dendrites of several central neurons where they can be activated by synaptic potentials and may therefore enhance the synaptic input leading to action potentials (Magee and Johnston 1995). One important putative function of LVA channels is to increase intracellular Ca^{2+} concentration, which could lead to various Ca^{2+}-dependent secondary responses (McCobb and Beam 1991).

The lyriform slit sense organ VS-3 in the patella of the spider, Cupiennius salei, (nomenclature of Barth and Libera 1970) consists of seven to eight slits innervated by a pair of bipolar mechanosensory neurons. Because one neuron (Type A) in each pair usually fires only one or two action potentials after a step stimulus whereas the other (Type B) can fire a burst of up to several hundred milliseconds (Seyfarth and French 1994), the VS-3 organ provides an excellent model for examining the role of I_{Ca} in regulating excitability. The difference in spiking behavior can be explained only partially by differences in the time courses of the receptor potentials (Juusola and French 1998), and voltage-activated potassium currents do not contribute to spiking behavior (Sekizawa et al. 1999). We have discovered a large transient I_{Ca} in the somata of both types of VS-3 neurons, which activates in the range previously described for LVA currents. Here we examine the kinetics and pharmacological sensitivity of this current with the main focus being its possible functional significance in the spiking behavior of these two different types of neurons.

METHODS

Preparation

A laboratory colony of Central American wandering spiders, C. salei, was kept at room temperature (22 ± 2°C; mean ± SD). Legs from adult spiders of either sex were autotomized, and a piece of patellar cuticle containing slit sense organ VS-3 was dissected free in spider saline [which contained (in mM) 223 NaCl, 6.8 KCl, 8 CaCl_2, 5.1 MgCl_2, 5 sucrose, and 10 HEPES, pH 7.8] (Höger et al. 1997). The hypodermis preparation of VS-3 neurons described in detail by Sekizawa et al. (1999) was used in all experiments and is shown in Fig. 1. Briefly, the neurons were detached from the cuticle but remained embedded in an internal membrane (hypodermis), which then was spread onto a small coverslip that was either uncoated or coated with 5 µg/ml Collagen IV (Sigma, Oakville, ON). The coverslip then was placed on the preparation holder. The axons and dendrites were crushed at ∼100 µm from the somata to improve the voltage-clamp conditions.

Recording and stimulation

Current- and voltage-clamp recordings were performed by the discontinuous single-electrode method (Finkel and Redman 1984) with an SEC-10 I amplifier (NPI Electronic, Tamm, Germany). The conditions for successful single-electrode voltage and current clamp...
have been described in detail by Torkkeli and French (1994), and the same methods were used previously in VS-3 neurons to study voltage-activated currents (Sekizawa et al. 1999). Borosilicate microelectrodes (1 mm OD and 0.5 mm ID) were pulled with a horizontal puller (P-2000, Sutter Instrument, Novato, CA). For current-clamp experiments, the electrodes were filled with 3 M KCl, and for voltage-clamp experiments 3 M CsCl was used in the pipette, in both cases the electrode resistances were 45–85 MΩ.

With sharp microelectrodes, only a small amount of the pipette leakage subtraction. The mean values of input resistances after block-

In several experiments, extracellular CaCl₂ was replaced with equal (8 mM) concentrations of BaCl₂ or SrCl₂. We also tested several known blockers of I_Ca in current- and voltage-clamp experiments including CdCl₂, NiCl₂, nifedipine (initial dilution into dimethylsulfoxide as 100 mM) and ω-conotoxin-GVIA (ω-CgTX GVIA). The effect of blocking agents usually took place in 5–10 min. All chemicals were purchased from Sigma unless otherwise stated.

RESULTS

The main goals of this study were to isolate currents carried by Ca²⁺ in rapidly (Type A) and slowly (Type B) adapting mechanosensory neurons in the spider lyriform slit sense organ VS-3 and to determine their physiological functions. We examined the time- and voltage-dependent properties of I_Ca to relate it to the I_Ca described in other neurons and to learn if there was any difference in I_Ca between Type-A and Type-B neurons that could explain their different firing behaviors (Fig. 1). We also used well-known blockers to test for different components of I_Ca and determine their possible roles in the neurons’ dynamic behavior.

Voltage and time dependence of activation and inactivation of I_Ca

When voltage-activated I_Na and I_K were blocked (see methods), test potentials of −50–10 mV from a holding potential of
−100 mV produced a transient inward current (Fig. 2A) that was strongly dependent on voltage (Fig. 2B). This $I_{\text{Ca}}$ activated at about −45 mV and reached its maximum amplitude of $−1.79 \pm 0.65 \text{nA}$ at $−27.8 \pm 6.7 \text{mV}$ ($n = 9$) in Type-A neurons and $−1.67 \pm 0.63 \text{nA}$ at $−25.6 \pm 7.3 \text{mV}$ ($n = 9$) in Type-B neurons. Figure 2B shows the normalized mean (±SD) peak $I_{\text{Ca}}$ from nine neurons of each type at different test voltages. The amplitudes and ranges of voltage where $I_{\text{Ca}}$ operated were very similar in Type-A and Type-B neurons. Statistically significant differences were not found between the Type-A and Type-B neurons at any recording voltages.

Inactivation of $I_{\text{Ca}}$ was also voltage dependent. We determined the steady-state inactivation by using 50-ms conditioning pulses followed by test pulses of fixed amplitude (Fig. 3A). Currents then were normalized to the maximum current, plotted as a function of test potential (Fig. 3B) and fitted by the Boltzmann relation

$$I/I_{\text{max}} = 1/[1 + e^{(V - V_{50})/s}]$$

where $I$ is the current at the test potential $V$, $I_{\text{max}}$ is the maximum current, $V_{50}$ is the test potential giving the half-maximum current, and $s$ is the slope factor. Fitting was performed by the Levenberg-Marquardt general nonlinear fitting algorithm (Press et al. 1990). Boltzmann fits of eight Type-A and six Type-B neurons are shown in Fig. 3B. When the conditioning pulses were more negative than $−45 \pm 5.2 \text{mV}$ (Type A) or $−36.7 \pm 5.1 \text{mV}$ (Type B), there was no reduction in the $I_{\text{Ca}}$ amplitude, but it was entirely suppressed when conditioning pulses more positive than $−26.3 \pm 5.2 \text{mV}$ (Type A) and $−21.7 \pm 4.1 \text{mV}$ (Type B) were used. Between these two extremes, the relation between current and membrane voltage was sigmoidal and the $V_{50}$ values for inactivation of Type-A neurons were $−35.1 \pm 8.2 \text{mV}$ and for Type-B neurons $−30.3 \pm 4.8 \text{mV}$. These differences were not statistically significant. The resting potentials for VS-3 neurons are close to $−60 \text{mV}$, and these results indicate that the $I_{\text{Ca}}$ can activate from holding potentials well above the resting potential without any conditioning hyperpolarization. The slope factor for Type-A neurons was 4.0 mV, and for Type-B neurons it was 3.7 mV, both in the low range of slope factors reported for $I_{\text{Ca}}$ inactivation in other neurons (Huguenard 1996).

The time course of activation and inactivation of $I_{\text{Ca}}$ at different test potentials was fitted with the Hodgkin-Huxley equation for an inactivating current

$$I = I_c[1 - e^{−(V-V_{\text{m}})/V_{\text{m}}}]^n$$

where $I_c$ is the current level expected in the absence of inactivation, $V_{\text{m}}$ is the time constant of activation, $V_{\text{m}}$ is the time constant of inactivation, and $n$ is the integer exponent (Hodgkin and Huxley 1952). We used an $m$ value of 4 in all fits. The inset in Fig. 4A shows an example of this fit. Activation in both types of neurons was slightly more rapid at depolarized voltages, but there were no statistically significant differences between the neuron types (Fig. 4A). The $I_{\text{Ca}}$ inactivation was also dependent on test voltage (Fig. 4B), and it was faster in Type-A neurons than Type-B neurons, but there was a large variation in the rate of inactivation between neurons within both groups and therefore no statistically significant differences between the neuron types. We also measured the time-to-peak values at different test voltages, and in both Type-A and Type-B neurons, these values varied from $−13 \text{ms}$ at $−40 \text{mV}$ to $−5 \text{ms}$ at $10 \text{mV}$ without statistically significant differences between the neuron types.

When the membrane was held at a hyperpolarized potential, $I_{\text{Ca}}$ recovered from inactivation with an exponential time course. In the experiment shown in Fig. 5A, $I_{\text{Ca}}$ was activated with a test pulse to $−20 \text{mV}$ lasting 100 ms, and a similar test pulse was applied after varying periods at $−100 \text{mV}$. When peak $I_{\text{Ca}}$ elicited by the second test pulse was normalized to the peak $I_{\text{Ca}}$ obtained with the first test pulse and the normalized current was plotted against time between the two test pulses (Fig. 5B), the data indicated a fast time constant of recovery for both types of neurons. The mean (±SD) time constant for Type-A neurons was $23.2 \pm 5.2 \text{ms}$ ($n = 3$) and for Type-B neurons it was $29.0 \pm 3.3 \text{ms}$ ($n = 3$), and there were no
neurons, the peak amplitude was reached at 8M. When peak and steady-state I were plotted against membrane potential (Fig. 6C), they had very similar functional ranges, suggesting that Ba2+ channels (Tsien et al. 1986). However, both cations do block all types of Ca2+ channels when applied at sufficiently high concentrations. We did not find clear differences between the effects of Cd2+ and Ni2+ on the Ica of VS-3 neurons. In four experiments with 100 μM Ni2+, ~30% of the inward current remained, and the peak and steady-state currents were reduced similarly, as shown in Fig. 7A where the Ni2+ effect on Iba is illustrated. With long (>20 min) incubation times, the same concentration of Ni2+ blocked all of the inward current. The Ni2+ effect was always stronger at more negative potentials (Fig. 7B), and the reduction of inward current was smaller at more depolarizing test voltages. The effect of 50 μM Cd2+ on Ica is demonstrated in Fig. 8, A and B. In this cell, Ica did not have a clear steady-state component, but when Ba2+ was used as the current carrying ion in other experiments, there was a steady-state current and we did not see any difference between the effects of Cd2+ on peak and steady-state currents. The average amplitude of remaining inward current after 10 min in Cd2+ was 40% of control, and as with Ni2+, most of the current was blocked by application times of 20 min.

Because LVA-Ica has been suggested to be an important factor in the regulation of action potential firing frequencies (Llinas and Yarom 1981), we tested the effects of Ni2+ and Cd2+ on the voltage responses at several different holding potentials in both Type-A and Type-B neurons. Figure 7C illustrates the small effect that Ni2+ had on one Type-B neuron at a holding potential of −80 mV. In this typical experiment, the number of action potentials was not affected by application of 100 μM Ni2+ and the threshold current was not significantly changed either, although the potential at which action potentials were fired was slightly more positive in the Ni2+-treated cell than in the control situation. Figure 8C illustrates the effect of 50 μM

Ba2+ as the current carrier

When extracellular Ca2+ was replaced by an equimolar (8 mM) concentration of Ba2+, the inward current inactivated more slowly, revealing a steady component, but its amplitude did not change significantly (Fig. 6). Peak Iba was −1.73 ± 0.4 nA for Type-A neurons and −1.74 ± 0.4 nA for Type-B neurons (n = 8 in both cases), but there was a shift in the I-V curve to more negative potentials. In the case of Type-A neurons, the peak amplitude was reached at −41.3 ± 3.5 mV (n = 8) and in Type-B neurons, it was at −35.0 ± 7.6 mV (n = 8). When peak and steady-state Iba were plotted against test potentials (Fig. 6B), they had very similar functional ranges, suggesting that Ba2+ did not reveal a different group of Ca2+ channels. When the inactivation time constants of Ica and Iba were plotted against membrane potential (Fig. 6C), the inactivation of Ica was significantly different at all recording voltages (P < 0.05). Therefore part of the inactivation was dependent on the intracellular Ca2+ concentration. We also tested the function of 8 mM Sr2+ as a current carrying ion in four experiments, and it caused similarly long-lasting currents.

Blocking effect of divalent cations Ni2+ and Cd2+

Divalent cations have been shown previously to function selectively on different types of Ca2+ channels. Ni2+ usually blocks LVA channels more readily, whereas Cd2+ is a more potent blocker of different types of high-voltage-activated (HVA) Ca2+ channels (Tsien et al. 1986). In four experiments with 100 μM Ni2+, ~30% of the inward current remained, and the peak and steady-state currents were reduced similarly, as shown in Fig. 7A where the Ni2+ effect on Iba is illustrated. With long (>20 min) incubation times, the same concentration of Ni2+ blocked all of the inward current. The Ni2+ effect was always stronger at more negative potentials (Fig. 7B), and the reduction of inward current was smaller at more depolarizing test voltages. The effect of 50 μM Cd2+ on Ica is demonstrated in Fig. 8, A and B. In this cell, Ica did not have a clear steady-state component, but when Ba2+ was used as the current carrying ion in other experiments, there was a steady-state current and we did not see any difference between the effects of Cd2+ on peak and steady-state currents. The average amplitude of remaining inward current after 10 min in Cd2+ was 40% of control, and as with Ni2+, most of the current was blocked by application times of 20 min.

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![Image](http://jn.physiology.org/DownloadedFrom/10.1152/jn.1986.257.5.749.jpg)

**FIG. 5.** Ica recovery from inactivation. A: recovery from inactivation was studied by varying the duration (1–300 ms) between 2 similar test pulses of −20 mV. B: data from both types of neurons were well fitted by 1 exponential decay time constant. Example of a Type-A neuron (●) had a time constant of 21.9 ms and the Type-B neuron had a time constant of 27.8 ms. In both cases, full recovery was reached after ~100 ms interval between test pulses.

**FIG. 6.** Using Ba2+ as a charge carrier produced longer lasting currents. A: when Ca2+ was replaced with equimolar Ba2+ in the bath inactivation of the current became less evident. B: peak (○) and the decaying (●) Iba activated at similar voltages, indicating that the decaying current was not flowing through a different group of Ca2+ channels than the peak current, but the same current was slowly inactivating. C: inactivation time constants from 18 neurons where Ca2+ was the current carrying ion (●) and 18 neurons with Ba2+ as the current carrying ion (○) plotted against voltages. For this diagram, data from Type-A and Type-B neurons were pooled together.
Cd\(^{2+}\) on a Type-A neuron. In this recording, the neuron fired only one action potential after Cd\(^{2+}\) application, compared with two in the control situation. This was the strongest effect we ever saw after Cd\(^{2+}\) application. These results indicate that \(I_{\text{Ca}}\) does not affect the threshold of VS-3 neurons nor does it change the firing behavior in these neurons, because if this was the case, the threshold for action potentials would increase significantly after \(I_{\text{Ca}}\) is blocked. Although we did see small changes in the threshold in some experiments, they were in both directions. Similar changes often occur during long recordings, when the membrane seals better around the recording electrode or when the electrode clogs slightly.

Ni\(^{2+}\) and Cd\(^{2+}\) also would block \(I_{K(Ca)}\) under the same conditions as were used in the current-clamp experiments, which would be expected to accelerate firing. We did not observe any such effect. This results supports our observations with specific blockers of \(I_{K(Ca)}\), iberiotoxin, charybdotoxin, and apamin (results not shown), which have no effect on the dynamic behavior of these neurons. In addition, these neurons do not show the afterhyperpolarization at the end of a spike train that is usually produced by \(I_{K(Ca)}\).

**Ca\(^{2+}\) spikes**

VS-3 neurons fire Na\(^{+}\) spikes when they are bathed in normal spider saline (Seyfarth and French 1994). When 1 mM TTX is added to the saline, these neurons completely cease firing, but a small rapidly decaying depolarization is seen with high depolarizing voltages (e.g., Juusola and French 1998). When we blocked voltage-activated K\(^{+}\) currents with TEA and 4-AP, action potential amplitudes increased significantly and even Type-A neurons started firing several action potentials as shown in Fig. 9. This is a typical effect of blockade of repolarizing outward currents that is observed in many neurons. In all eight experiments here, the threshold for firing action potentials decreased when 4-AP and TEA were added to the saline. When we also added 1 mM TTX in addition to the K\(^{+}\)-channel blockers, Type-A and Type-B neurons still fired one large and wide action potential, but neither of them generated repetitive responses. The threshold for this action potential was always higher than for the Na\(^{+}\) spikes, but still lower than in control recordings. Although Ca\(^{2+}\) spikes in the presence of K\(^{+}\)-channel blockers are not a new finding (reviewed by Hagiwara and Byerly 1981), their uniformity in VS-3 neurons suggests that the difference in normal spiking behavior is not caused by \(I_{Ca}\).

**Effect of toxins on \(I_{Ca}\)**

To further explore the identity of \(I_{Ca}\) in the VS-3 neurons, we used two specific blockers of Ca\(^{2+}\) channels, nifedipine and \(\omega\)-CgTX GVIA, in several concentrations. These blockers have been shown to act specifically on HVA-Ca\(^{2+}\) channels, and \(\omega\)-CgTX GVIA even more specifically on the N-type channels commonly found in neurons (Swandulla et al. 1991). There are no specific blockers of LVA-\(I_{Ca}\), but their identification is commonly based on exclusion. \(\omega\)-CgTX GVIA at 3 and 5 mM concentrations did not have any effect on either current or voltage responses of VS-3 neurons confirming that no part of the \(I_{Ca}\) belonged to the N-type HVA group.

Nifedipine did not have any effect on \(I_{Ca}\) in VS-3 neurons when applied at low (10–50 mM) concentrations, but when 100 mM concentration was used it did block approximately one-half of the \(I_{Ca}\), as shown in Fig. 10, and in most experiments, this effect was at least partially reversible. In several experiments, 100 mM nifedipine also caused a loss of voltage control in the voltage-clamp experiments; this may have occurred because of increased activity. Nifedipine had a clear effect on

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**Fig. 7.** Effects of 100 mM Ni\(^{2+}\) on current and voltage responses. A: Ni\(^{2+}\) blocked more than half of the inward current. Test pulse in both traces was about 30 mV, and the current carrying ion in the control recording was Ba\(^{2+}\). Both the peak and steady-state current were affected by Ni\(^{2+}\). B: I-V plot from the recording shown in A at different test potentials. Ni\(^{2+}\) blocked the current more strongly at negative voltages than when the test potentials were close to 0 or positive. C: effect of Ni\(^{2+}\) on the voltage response was insignificant. In this typical recording from a Type-B neuron, the number of action potentials remained similar, and there was no change in the current amplitude needed to produce action potentials. Only a small increase in the level of depolarization was seen in the Ni\(^{2+}\) recording. Holding potential in this experiment was about 80 mV and action potentials were produced by a 500-pA stimulus.

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**Fig. 8.** Effect of 50 mM Cd\(^{2+}\) on current and voltage responses. A: Cd\(^{2+}\) blocked more than half of the \(I_{Ca}\). Test pulse in both traces was 20 mV. B: I-V plot from the recording shown in A at different test potentials. Blocking effect of Cd\(^{2+}\) was as strong at all potentials. C: typical recording from a Type-A neuron before and after 50 mM Cd\(^{2+}\) was added into the bath. This neuron was only able to fire 1 action potential after Cd\(^{2+}\) application, but there was no change in the amplitude of current needed for spike production and the depolarization level remained the same. Holding potential in this experiment was about 80 mV, and voltage responses were produced with a 750-pA current pulse.
CALCIUM CURRENT IN SPIDER MECHANORECEPTOR NEURONS

Dissipating bursting behavior. The presence of LVA-ternary by lowering the threshold for action potentials and in-
slightly longer durations than the control. When 100 neuron fired action potentials that had significantly higher amplitudes and
mesium chloride (TEA) and 25 mM 4-aminopyridine (4-AP) ( - - - ), this Type-A
excitatory synaptic input can activate
and somata (e.g., Huguenard 1996; Kaneko et al. 1989) where
often are localized at major sites of synaptic input; dendrites
connections from the CNS (Fabian-Fine et al. 1999a,b). Some
agonistic effects on them. LVA-Ca2
shaping the voltage response. What other functions could this
found that LVA-
Ca in VS-3 neurons does not play any part in
transient
Ca in cockroach dorsal unpaired median (DUM) neurons has Ca2+-dependent inactivation (Wicher and Penzlin
1997).

Recovery from inactivation is the most variable feature of
LVA- I Ca with its time constant varying from ~100 ms to
several seconds (Bossu and Feltz 1986; Carbone and Swandulla 1989; Huguenard 1996; Kaneko et al. 1989). In VS-3
neurons, the recovery was very rapid with a full recovery in
~100 ms and time constants of 20–30 ms, and there were no
statistically significant differences between the two neuron
types.

Steady-state inactivation of LVA-I Ca in VS-3 neurons had slope factors close to 4 mV (Fig. 3), in the low range of values
reported for central and sensory neurons (Gu and Spitzer 1993; Huguenard 1996; Liman and Corey 1996; Moolenar and Spec-
tor 1979; Tsien et al. 1986). Inactivation time con-
stants (Fig. 4B) in Type-A (6–38 ms) and Type-B (71–114 ms)
neurons were similar to values obtained from other sensory and
central neurons, where they vary between 10 and 400 ms (Bossu and Feltz 1986; Huguenard 1996; Liman and Corey
1996). When Ca2+- was replaced with Ba2+, the inactivation became significantly slower (Fig. 6), indicating its dependence
on intracellular Ca2+ concentration. Although Ca2+-dependent inactivation is a property of HVA-Ca2+ channels, it is not
usually associated with LVA channels (Fox et al. 1987). How-
ever, it has been observed in some invertebrate neurons, e.g.,
transient I Ca in cockroach dorsal unpaired median (DUM)
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1997).

Kinetic properties of I Ca in VS-3 neurons

I Ca in VS-3 neurons had rapid kinetics of activation and inactivation, and it also recovered from inactivation faster than in
most other neurons. I Ca activated at potentials positive to
about −45 mV and reached maximum amplitudes at −27.8
and −25.6 mV in Type-A and Type-B neurons, respectively
(Fig. 2). These values are in the range previously outlined for
LVA-I Ca (Gu and Spitzer 1993; Huguenard 1996; Kaneko et al.
1989; Liman and Corey 1996; Moolenar and Spector 1979;
Rennie and Ashmore 1991; Yoshii et al. 1998). The rate of
activation of I Ca in both types of VS-3 neurons was voltage
dependent and varied from 0.3 to 2 ms (Fig. 4A), somewhat
faster than values of 2–50 ms previously reported for LVA-I Ca
(Huguenard 1996; Tsien et al. 1986). Inactivation time con-
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ever, V50 values of steady-state inactivation in Type-A and

![Figure 9](image-url) Comparison of action potentials with and without the Na+ component. Control recording (—) was performed in normal spider saline (see METHODS). When repolarizing I k were blocked with 25 mM tetraethylammonium chloride (TEA) and 25 mM 4-aminopyridine (4-AP) ( - - - ), this Type-A neuron fired action potentials that had significantly higher amplitudes and slightly longer durations than the control. When 100 μM tetrodotoxin (TTX) was added ( - - - ), the same neuron was still able to fire 1 action potential that had an even larger amplitude than before TTX. Holding potential in all recordings was ~70 mV, and action potentials were produced by a current stimulus of 250 pA.

![Figure 10](image-url) Effect of 100 μM nifedipine on current and voltage responses. A: nifedipine blocked about half the inward current when applied in high concentrations. Control recording was done with Ba2+ as the current carrying ion and both current traces were produced with test potentials of ~30 mV. B: I-V plot from the recording shown in A at different test potentials. Nifedipine effect was similar at all test potentials. C: application of nifedipine reduced the threshold and increased the number of action potentials. This effect was more pronounced when neurons were held at hyperpolarizing potentials during current stimulation. For this recording, a Type-B neuron was held at −80 mV and stimulated with 500-pA current pulse. In the control situation, this cell did not produce action potentials when smaller stimuli were used, but the threshold decreased to 250 pA after Nifedipine.
Type-B VS-3 neurons were −35.1 and −30.3 mV, respectively, significantly more positive than the $V_{\text{th}}$ values of −50 to −95 mV reported for other neurons (Huguenard 1996; Kaneko et al. 1989; Liman and Corey 1996; Moolenan and Spector 1979; Yoshii et al. 1998), with the lower values being more common in sensory neurons.

The rapid kinetics of $I_{\text{Ca}}$ allows a fast neural response, a property that may be needed when VS-3 neurons respond to natural stimuli, such as substrate vibrations. Differences in the rate of inactivation and recovery from inactivation could be explained if Type-A neurons could respond more readily to high-frequency stimuli, whereas Type-B neurons would provide more time for $Ca^{2+}$ to enter at the cost of reduced high-frequency sensitivity.

**Ca$^{2+}$-channel blockers**

We found no evidence for Ca$^{2+}$-channel types other than LVA on the somata of VS-3 neurons. Peak and steady-state currents were equally sensitive to blockers, and their activation ranges were similar. $\omega$-cGTX GVIA had no effect on VS-3 neuron $I_{\text{Ca}}$. At high concentrations, Ni$^{2+}$ and Cd$^{2+}$ block all types of Ca$^{2+}$ channels as well as other voltage-gated channels. At lower concentrations, Cd$^{2+}$ is less effective on LVA than HVA channels (Mogul and Fox 1991; Ozawa et al. 1989) and Ni$^{2+}$ acts more strongly on LVA channels (Hagiwara et al. 1988). In VS-3 neurons, the effect of Ni$^{2+}$ was slightly stronger on LVA-$I_{\text{Ca}}$ than that of Cd$^{2+}$, but both blocked the current completely when application times were long. Current-clamp experiments with Cd$^{2+}$ and Ni$^{2+}$ indicated that $I_{\text{Ca}}$ does not decrease the threshold of VS-3 neurons (Figs. 7C and 8C). Ni$^{2+}$ and Cd$^{2+}$ also block $K_{\text{ca}}$ channels, so it is impossible to draw firm conclusions about their physiological effects when they are tested on neurons that have $I_{\text{K(Ca)}}$. Therefore, the somata of VS-3 neurons do not have $I_{\text{K(Ca)}}$. Thus, we could reliably show that although Ni$^{2+}$ and Cd$^{2+}$ both block $I_{\text{Ca}}$, the current does not control the spiking of these neurons.

Nifedipine like other dihydropyridines (DHP) is a blocker of HVA-$I_{\text{Ca}}$ (Tsien et al. 1986). However, at high concentrations DHPs can have nonspecific effects on Ca$^{2+}$ and also on Na$^{+}$ and K$^{+}$ channels (reviewed by Carbone and Swandulla 1989). DHP block is more efficient at depolarizing potentials, with hyperpolarizing potentials tending to remove the block. This might explain the excitatory effect of nifedipine on the voltage response of VS-3 neurons at hyperpolarizing potentials. DHP-sensitive LVA-Ca$^{2+}$ channels are found in, e.g., rat hypothalamic neurons (Akaike et al. 1989).

**Ca$^{2+}$ spikes**

Ca$^{2+}$ spikes are common in invertebrate muscle cells (Fatt and Katz 1953) and in developing neurons (Hagiwara and Byerly 1981). When quaternary ammonium ions are present, many vertebrate neurons also are able to fire Ca$^{2+}$ spikes (Fain et al. 1977; Horn 1978; Koketsu et al. 1959). Although both VS-3 neurons could fire one Ca$^{2+}$ spike when TEA and 4-AP were present (Fig. 9), this spike had higher threshold than Na$^{+}$ spikes under similar conditions and repetitive firing did not occur. In most other neurons, Ca$^{2+}$ spikes also have higher thresholds than Na$^{+}$ spikes (e.g., Llinas and Yarom 1981).

**Functional significance of LVA-$I_{\text{Ca}}$ in VS-3 neurons**

$I_{\text{Ca}}$ activated and peaked at slightly more negative potentials in Type-A than Type-B neurons, but this difference was not statistically significant. In Type-A neurons, the depolarization level at which the cells fired action potentials was about −31 mV and in Type-B neurons −39 mV. A similar, statistically significant, difference was found before when threshold depolarizations of large numbers of VS-3 neurons were studied under similar conditions (Sekizawa et al. 1999). These findings argue against the hypothesis of $I_{\text{Ca}}$ lowering the threshold for action potentials because in that case, $I_{\text{Ca}}$ in Type-B neurons would activate at lower voltages.

The consequence of lowering the threshold for Na$^{+}$ spikes in vertebrate central neurons is assumed to be promotion of burst firing (Huguenard 1996). However, LVA- $I_{\text{Ca}}$ is not only found in bursting neurons. In fact, its existence is not well correlated with firing behavior. For example, LVA-$I_{\text{Ca}}$ is present in mouse vomeronasal chemosensory neurons that fire tonically (Liman and Corey 1996) and in mouse retinal bipolar neurons (Kaneko et al. 1989) that do not fire action potentials. However, it is not found in cultured rat olfactory neurons (Trombley and Westbrook 1991) that only fire one action potential in response to a steady stimulus or amphibian olfactory neurons that fire phasically (Liman and Corey 1996).

Here, we found LVA-$I_{\text{Ca}}$ in two phasic sensory neurons.

In vertebrate saccular hair cells, $I_{\text{Ca}}$ and $I_{\text{K(Ca)}}$ interact to enhance the frequency tuning to mechanical stimuli. However, in contrast to VS-3 neurons, $I_{\text{Ca}}$ in hair cells flows through HVA channels, and these neurons do not fire action potentials (Hudspeth 1986). The rapid activation and inactivation kinetics of $I_{\text{Ca}}$ in VS-3 neurons seem likely to allow fast cellular responses, which may be significant when there are high stimulus frequencies. Using action potential waveforms as stimuli, McBobb and Beam (1991) demonstrated that LVA-$I_{\text{Ca}}$ activated early during the rising phase of the action potential and peaked after the spike peaks. This behavior makes it possible to rapidly increase intracellular Ca$^{2+}$ concentration during the brief spike.

In contrast to earlier suggestions that LVA-$I_{\text{Ca}}$ is an important regulator of neuronal firing properties, we found no evidence that LVA-$I_{\text{Ca}}$ is involved in controlling the firing patterns of Type-A and Type-B mechanosensory neurons in the spider VS-3 organ. This leaves two important questions that we will address in the future: what is the function of this prominent current in these sensory neuron pairs and what is the origin of their different firing patterns? These questions and their answers seem certain to have wider implications for other neurons.

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**REFERENCES**

