ABSTRACT  Activation of distinct classes of potassium channels can dramatically affect the frequency and the pattern of neuronal firing. In a subpopulation of vagal afferent neurons (nodose ganglion neurons), the pattern of impulse activity is effectively modulated by a Ca2+-dependent K+ current. This current produces a post-spike hyperpolarization (AHPslow) that plays a critical role in the regulation of membrane excitability and is responsible for spike-frequency accommodation in these neurons. Inhibition of the AHPslow by a number of endogenous autacoids (e.g., histamine, serotonin, prostaglandins, and bradykinin) results in an increase in the firing frequency of vagal afferent neurons from <0.1 to >10 Hz. After a single action potential, the AHPslow in nodose neurons displays a slow rise time to peak (0.3–0.5 s) and a long duration (3–15 s). The slow kinetics of the AHPslow are due, in part, to Ca2+-discharge from an intracellular Ca2+-induced Ca2+ release (CICR) pool. Action potential-evoked Ca2+ influx via either L or N type Ca2+ channels triggers CICR. Surprisingly, although L type channels generate 60% of action potential-induced CICR, only Ca2+ influx through N type Ca2+ channels can trigger the CICR-dependent AHPslow. These observations suggest that a close physical proximity exists between endoplasmic reticulum ryanodine receptors and plasma membrane N type Ca2+ channels and AHPslow potassium channels. Such an anatomical relation might be particularly beneficial for modulation of spike-frequency adaptation in vagal afferent neurons.

Calcium regulation of a slow post-spike hyperpolarization in vagal afferent neurons

(spike frequency adaptation/ryanodine receptor/autacoids/allergic inflammation/mast cell)

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of the AHPslow. An analogous slow AHP has also been recently described in ≈25% of C type dorsal root ganglion neurons of the rat (9, 10).

The AHPslow in vagal afferent neurons influences cellular excitability and controls AP frequency over the physiological range from 0.1 Hz to 10 Hz (11, 12). One interesting property of the AHPslow is that its amplitude is tuned to both AP number and frequency. Over the range of 1–100 Hz, the amplitude of the AHPslow increases with the number of APs until it plateaus after ≈15 APs (Fig. 2); similar results were observed when the current underlying the AHPslow was monitored. For reasons still unresolved, 10 Hz (100-msec interspike intervals) consistently evokes the largest responses.

Table 1. Inflammatory mediators that block AHPslow in vagal afferent neurons

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Receptor type</th>
<th>EC50, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>B2</td>
<td>72</td>
</tr>
<tr>
<td>Histamine</td>
<td>H1</td>
<td>2,000</td>
</tr>
<tr>
<td>Serotonin</td>
<td>nd</td>
<td>300</td>
</tr>
<tr>
<td>PGD2, PGE2</td>
<td>nd</td>
<td>−20</td>
</tr>
<tr>
<td>Leukotriene C4</td>
<td>nd</td>
<td>−100</td>
</tr>
</tbody>
</table>

Bradykinin (26), histamine (27), serotonin (28), PGD2 and PGE2 (12), and leukotriene C4 (3) block the AHPslow, nd, not determined; PG prostaglandin.

The current generating the AHPslow (I_AHP) is a voltage-insensitive Ca2+-dependent K+ current (13, 14) that is unaffected by a wide range of K+ channel antagonists: 100 nM ampin, 10 μM D-tubocurarine, 5 mM Cs+, 30 mM tetraethylammonium, 10 mM Ba2+, 4 mM 4-aminopyridine, and 10 nM charybdotoxin. The magnitude of the AHPslow (or the I_AHP) is linearly related to the concentration of extracellular Ca2+ (Fig. 3) and requires a rise in cytosolic free Ca2+ ([Ca2+]i) for activation. Buffering intracellular Ca2+ with 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) abolishes the AHPslow (Fig. 4). Noise analysis of the I_AHP suggests a single-channel conductance of ≈10 pS (unpublished observations). These features are consistent with the properties of a small-conductance Ca2+-activated K+ channel (SK channel; ref. 8). Of the several SK channels recently cloned from mammalian brain (15), the hSK1 channel has a pharmacological and biophysical profile compatible with the K+ current underlying the AHPslow in nodose neurons.

Ca2+ Injection Evokes Two Temporally Distinct Outward Currents. To test whether the K+ channels associated with the AHPmedium and the AHPslow are directly activated by Ca2+-, we iontophoretically injected Ca2+ into nodose neurons. Independent of the AHPslow, a large outward current with rapid activation and decay kinetics was elicited by Ca2+ injection. This current (I_K-medium) was evoked at holding potentials between −2 mV and −45 mV. It was completely blocked by 5 mM tetrathylammonium but unaffected by inhibitors of the AHPslow (100 nM prostaglandins D2 or E2 or 1 μM forskolin). I_K-medium was strongly voltage-dependent, requiring membrane holding potentials more positive than −55 mV. Assuming a reversal potential of −80 mV, I_K-medium had an e-fold increase in peak conductance for each 8.0 ± 1.0 mV (mean ± SEM; n = 8) depolarization, as calculated from semilogarithmic plots of peak chord conductance versus voltage-clamp holding potential. These properties are similar to those of large-conductance BK (AHPmedium) channels.

In neurons that exhibited AHPslow, Ca2+ injection provoked a slowly developing and protracted outward current (I_Kslow).
Fig. 3. Effects of varying [Ca\(^{2+}\)]\_o on the amplitude of the AHP\(_{\text{slow}}\) recorded in isolated nodose neurons. (A) Sample traces of AHP\(_{\text{slow}}\) evoked by a train of four APs in the presence of different [Ca\(^{2+}\)]\_o. APs are evoked by transmembrane depolarizing current pulses (2 nA, 3 ms, 10 Hz) and are truncated. [Ca\(^{2+}\)]\_o was varied from 2.0 to 0.0 mM in 0.5 mM decrements. The AHP\(_{\text{slow}}\) is completely blocked when [Ca\(^{2+}\)]\_o is reduced to nominally zero. On returning to 2.0 mM [Ca\(^{2+}\)]\_o, the AHP\(_{\text{slow}}\) recovers to its original amplitude. (B) Relation between [Ca\(^{2+}\)]\_o and AHP\(_{\text{slow}}\) amplitude recorded in several neurons. Values are means ± SEM of the number of observations indicated near each data point. Data are normalized to the maximum response recorded in a given neuron. Linear regression analysis yields the solid line (r = 0.993).

Fig. 4 shows an overlay of the outward current responses evoked by Ca\(^{2+}\) injection in a single nodose C type neuron at holding potentials of −20 mV and −50 mV. The kinetic differences between I\(_{\text{K-medium}}\) and I\(_{\text{K-slow}}\) after Ca\(^{2+}\) injection are dramatic. In contrast to the rapid activation of I\(_{\text{K-medium}}\), the onset of I\(_{\text{K-slow}}\) is delayed, and the decay of I\(_{\text{K-medium}}\) is nearly complete before the peak amplitude of the I\(_{\text{K-slow}}\) is reached. These two outward currents mirror the temporal and pharmacological differences between AHP\(_{\text{medium}}\) and AHP\(_{\text{slow}}\). I\(_{\text{K-slow}}\), like the AHP\(_{\text{slow}}\), was blocked by 100 nM BAPTA/acetomethylester. The Ca\(^{2+}\) dependence of the AHP\(_{\text{medium}}\) is illustrated in C, where the neuron is superfused with 100 μM CdCl\(_2\) for 30 s, which blocks most of the AHP\(_{\text{medium}}\). The residual component of the AHP recorded in CdCl\(_2\) is the AHP\(_{\text{fast}}\), which is mediated by delayed rectifier K\(^+\) channels. (C) Depression of the AHP\(_{\text{slow}}\) markedly increases neuronal excitability. The average AP firing frequency induced by a current ramp protocol (1 nA, 2 s) increased from 1 to 5.5 Hz when the AHP\(_{\text{slow}}\) was blocked. Similar loss of spike-frequency adaptation was observed with bradykinin, prostaglandin D\(_2\), histamine, and other inflammatory autacoids (see Table 2). The scale bar represents 3 mV, 2 s in A; 15 mV, 0.25 s in B; and 15 mV, 0.5 s in C. The dashed line represents the resting membrane potential (−60 mV). Resting membrane input resistance was 70 MΩ. Data is from ref. 19 with permission from the American Physiological Society.

Fig. 5 shows an overlay of the outward current responses evoked by Ca\(^{2+}\) injection in a single nodose C type neuron at holding potentials of −20 mV and −50 mV. The kinetic differences between I\(_{\text{K-medium}}\) and I\(_{\text{K-slow}}\) after Ca\(^{2+}\) injection are dramatic. In contrast to the rapid activation of I\(_{\text{K-medium}}\), the onset of I\(_{\text{K-slow}}\) is delayed, and the decay of I\(_{\text{K-medium}}\) is nearly complete before the peak amplitude of the I\(_{\text{K-slow}}\) is reached. These two outward currents mirror the temporal and pharmacological differences between AHP\(_{\text{medium}}\) and AHP\(_{\text{slow}}\). I\(_{\text{K-slow}}\), like the AHP\(_{\text{slow}}\), was blocked by 100 nM BAPTA/acetomethylester. The Ca\(^{2+}\) dependence of the AHP\(_{\text{medium}}\) is illustrated in C, where the neuron is superfused with 100 μM CdCl\(_2\) for 30 s, which blocks most of the AHP\(_{\text{medium}}\). The residual component of the AHP recorded in CdCl\(_2\) is the AHP\(_{\text{fast}}\), which is mediated by delayed rectifier K\(^+\) channels. (C) Depression of the AHP\(_{\text{slow}}\) markedly increases neuronal excitability. The average AP firing frequency induced by a current ramp protocol (1 nA, 2 s) increased from 1 to 5.5 Hz when the AHP\(_{\text{slow}}\) was blocked. Similar loss of spike-frequency adaptation was observed with bradykinin, prostaglandin D\(_2\), histamine, and other inflammatory autacoids (see Table 2). The scale bar represents 3 mV, 2 s in A; 15 mV, 0.25 s in B; and 15 mV, 0.5 s in C. The dashed line represents the resting membrane potential (−60 mV). Resting membrane input resistance was 70 MΩ. Data is from ref. 19 with permission from the American Physiological Society.

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Ca\(^{2+}\) Released by the CICR Pool Is Essential for the Generation of the AHP\(_{\text{slow}}\). Single APs produce transient increases in [Ca\(^{2+}\)]\_i (ΔCa\(_i\)) as measured by the fluorescent indicator fura-2. The magnitude of the ΔCa\(_i\) depends on both [Ca\(^{2+}\)]\_o and the number of APs. Over the range of one to eight APs, there is an approximately linear relation between the magnitude of the ΔCa\(_i\) and the number of APs (Fig. 6). In the presence of drugs that block CICR but do not significantly affect AP-induced Ca\(^{2+}\) influx ([RY, 10 μM], 2,5-di-(t-butyl)hydroquinone (DBHQ, 10 μM), or thapsigargin (TG, 100 nM)), we found that at least eight APs were required to evoke a detectable ΔCa\(_i\) (Fig. 6). In the presence of RY, DBHQ, and TG, the ΔCa\(_i\)-AP relation exhibits slopes of 0.5, 1.1, and 0.8 nM per AP, respectively. When compared with the slope of 9.6 nM per AP in control neurons, Ca\(^{2+}\) influx produced by a single nodose AP is amplified by 5- to 10-fold by CICR (16). Nodose neurons demonstrate a relatively low stimulus threshold for eliciting CICR. For instance, a robust CICR response can be observed after a single AP stimulus in nodose neurons, whereas many tens of APs are required in dorsal root ganglion neurons (17). The greater CICR response in nodose neurons is not due to greater Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels (VDCCs); a single AP produces comparable Ca\(^{2+}\) influx in nodose and dorsal root ganglion neurons (39 vs. 49 pC, respectively; refs. 16 and 18). Rather, the more responsive CICR pool in nodose neurons...
may reflect either a closer proximity between plasma membrane Ca\(^{2+}\) influx channels and endoplasmic reticulum RY receptors or a more sensitive RY receptor.

By using physiological stimuli (APs) in conjunction with pharmacological manipulations of CICR, we have demonstrated that CICR is essential for the development of the AHP\(_{\text{slow}}\). Over the range of 1–16 APs, the magnitudes of the AP-induced AHP\(_{\text{slow}}\) and the ΔCa\(_{\text{Cat}}\) (a monitor of CICR in these neurons) were highly correlated (r = 0.985). Simultaneous recordings of ΔCa\(_{\text{Cat}}\) and AHP\(_{\text{slow}}\) before and during bath application of CICR inhibitors (RY, TG, DBHQ, or 10 \(\mu\)M cyclopiazonic acid) revealed that both responses were blocked in a parallel fashion (Fig. 7; see also Table 1 in ref. 19). These data indicate that a CICR pool is essential for the generation of the AHP\(_{\text{slow}}\). They also provide a potential explanation for the slow kinetics of the AHP\(_{\text{slow}}\), namely Ca\(^{2+}\) mobilization from CICR.

Effects of Changing [Ca\(^{2+}\)]\(_{\text{o}}\) on the AHP\(_{\text{slow}}\), ΔCa\(_{\text{Cat}}\), and Ca\(^{2+}\) influx. If the AHP\(_{\text{slow}}\) depends on Ca\(^{2+}\) released from the CICR pool triggered by AP-induced Ca\(^{2+}\) influx, it would follow that changes in [Ca\(^{2+}\)]\(_{\text{o}}\) should produce corresponding effects on both the AHP\(_{\text{slow}}\) and the ΔCa\(_{\text{Cat}}\). The data shown in Fig. 3A illustrate the effects of progressively lowering [Ca\(^{2+}\)]\(_{\text{o}}\) from 2.0 mM to nominally zero on the amplitude of the AHP\(_{\text{slow}}\) recorded in a single nodose neuron. As [Ca\(^{2+}\)]\(_{\text{o}}\) was decreased, the amplitude of the AHP\(_{\text{slow}}\) was reduced proportionally. When the results from this and five additional neurons were plotted (Fig. 3B), the relation between [Ca\(^{2+}\)]\(_{\text{o}}\) and the amplitude of the AHP\(_{\text{slow}}\) was linear (r = 0.993; n = 6, pooled data from three current-clamp and three hybrid voltage-clamp experiments).

![Fig. 5. Comparison of two outward K\(^{+}\) currents evoked by intracellular Ca\(^{2+}\) injection. Recordings were made in a single acutely isolated adult rabbit nodose neuron. A slow outward current (I\(_{\text{K,slow}}\)) was activated by a 5-nA, 1-s iontophoretic Ca\(^{2+}\) injection at a holding potential of ~50 mV. A second outward current (I\(_{\text{K,medium}}\)) was activated at ~20 mV (5 nA, 0.5 sec). I\(_{\text{K,medium}}\) activates and decays completely before I\(_{\text{K,slow}}\) reaches peak amplitude. I\(_{\text{K,medium}}\) was blocked by 10 nM tetraethylammonium; I\(_{\text{K,slow}}\) was blocked by 100 nM prostaglandin D\(_{2}\). The iontophoretic pipette was filled with a 0.2 M CaCl\(_{2}\) solution. Voltage-clamp currents were recorded with a second intracellular pipette. The discontinuous (switched) current injection mode of an Axoclamp II amplifier was used for both current- and voltage-clamp applications. The larger calibration value is for I\(_{\text{K,medium}}\). Population data is shown in Table 2.](image)

![Fig. 6. (Upper) Effect of RY on AP-induced Ca\(^{2+}\) transients. Traces are Ca\(^{2+}\) transients evoked by varying numbers of APs, as indicated below each trace. In control neurons, distinct Ca\(^{2+}\) transients can be elicited by very few APs. In contrast, in the presence of 10 \(\mu\)M RY, a CICR inhibitor, at least eight APs are required to generate a discernible change in [Ca\(^{2+}\)]\(_{\text{i}}\). Suppression of the Ca\(^{2+}\) transient by RY is due to its effect on CICR and not the result of nonspecific effects on Ca\(^{2+}\) channels; the kinetics and amplitude of I\(_{\text{Cat}}\) elicited by APs are completely unaffected by RY. (Lower) Effect of RY on the relation between the amplitude of Ca\(^{2+}\) transients and number of APs. ○ and ● are mean amplitudes of Ca\(^{2+}\) transients evoked by varying numbers of action potentials for control (n = 10) and for RY-treated nodose neurons (n = 3), respectively. Linear regression of data from control (○, action potentials) and RY-treated cells yielded slopes of 9.6 ± 0.01 and 0.5 ± 0.23 nM per AP, respectively. Comparison of the slopes illustrates that CICR is capable of amplifying the “trigger” Ca\(^{2+}\) resulting from AP-induced Ca\(^{2+}\) influx by 20-fold. Data is modified from ref. 16 with permission from Journal of Physiology (London).](image)

Next, we examined the relation between [Ca\(^{2+}\)]\(_{\text{o}}\) and the magnitude of the AP-induced ΔCa\(_{\text{Cat}}\). Fig. 8A illustrates ΔCa\(_{\text{Cat}}\) elicited by varying numbers of APs recorded from a single neuron in Locke solution containing 2.2 or 1.1 mM Ca\(^{2+}\). The population results relating the normalized amplitude of the ΔCa\(_{\text{Cat}}\) recorded in four neurons to the number of APs is shown in Fig. 8B. In 1.1 mM [Ca\(^{2+}\)]\(_{\text{o}}\), the first few APs did not elicit a measurable ΔCa\(_{\text{Cat}}\). For the neuron shown in Fig. 8A, at least eight APs were necessary to evoke a detectable ΔCa\(_{\text{Cat}}\). In three additional neurons, the minimum number of APs necessary to

Table 2. Comparison of I\(_{\text{K,slow}}\) and I\(_{\text{K,medium}}\)

<table>
<thead>
<tr>
<th>Current</th>
<th>Peak conductance, nS</th>
<th>Holding potential, mV</th>
<th>Time-to-peak, ms</th>
<th>Decay time constant, ms</th>
<th>Duration, s</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>I(_{\text{K,slow}})</td>
<td>27.9 ± 6.5</td>
<td>14</td>
<td>-55.4 ± 2.7</td>
<td>14</td>
<td>6,570 ± 1085</td>
<td>12</td>
</tr>
<tr>
<td>I(_{\text{K,medium}})</td>
<td>53.2 ± 16.5</td>
<td>6</td>
<td>-20 ± 3.7</td>
<td>6</td>
<td>958 ± 56</td>
<td>6</td>
</tr>
</tbody>
</table>

I\(_{\text{K,slow}}\) and I\(_{\text{K,medium}}\) are outward currents elicited by iontophoretic injection Ca\(^{2+}\) into acutely isolated nodose neurons of the rabbit. The peak conductance is the largest conductance elicited, independent of membrane potential. The holding potential is the potential at which the peak conductance was measured. The decay time constant was measured by fitting a line, by eye, to the log transform of the decay of the current. The duration was calculated from the onset of Ca\(^{2+}\) injection to the time at which the current had decayed to 20% of its peak value. Data are summarized as the mean ± SEM.
Fig. 7. Effect of DBHQ, a functional CICR inhibitor, on the AP-induced Ca\textsuperscript{2+} transient and on the AHP\textsubscript{slow} recorded simultaneously in an acutely isolated rabbit nodose neuron. Upper traces represent superimposed Ca\textsuperscript{2+} transients evoked by a train of four APs (10 Hz) recorded in control Locke solution and 7 min after switching to Locke solution containing 10 \textmu M DBHQ. The lower pair of traces shows AHP\textsubscript{slow}. DBHQ treatment completely blocked both the Ca\textsuperscript{2+} transient and the AHP\textsubscript{slow}. Resting [Ca\textsuperscript{2+}]\textsubscript{o} was 91 nM. Fluorescence data were acquired at 10 Hz. Resting membrane potential was \textapprox 67 mV. AP amplitudes are truncated. Data are from ref. 19 with permission from the American Physiological Society.

elicit a detectable \Delta C\textsubscript{a} ranged from 4 to 32. The \Delta C\textsubscript{a}–AP relation recorded in 1.1 mM [Ca\textsuperscript{2+}]\textsubscript{o}, as in Locke solution containing normal [Ca\textsuperscript{2+}]\textsubscript{o}, followed a hyperbolic relation (\chi^2 = 6.75 and 0.31; r = 0.988 and 0.999 for 2.2 and 1.1 mM Ca\textsuperscript{2+}, respectively; Fig. 8B and see also Fig. 1 in ref. 16). Given the hyperbolic nature of the \Delta C\textsubscript{a}–AP relation, deducing the effects of altered [Ca\textsuperscript{2+}]\textsubscript{o} on the magnitude of the \Delta C\textsubscript{a} clearly depends on where along this relation the comparison is made. At one extreme, there is a \approx 2-fold change when comparing the plateau phases of the curves in normal and one-half normal [Ca\textsuperscript{2+}]\textsubscript{o}. It is also possible to calculate the limiting initial slopes for the rising phase of the curves (dashed lines in Fig. 8B). The limiting slopes, which represent the full Ca\textsuperscript{2+} release potential of the CICR pool before any release has actually occurred, were 15 \pm 3.8 and 2 \pm 0.7 nM per AP in 2.2 and 1.1 mM Ca\textsuperscript{2+}, respectively. Thus, reducing [Ca\textsuperscript{2+}]\textsubscript{o} by a factor of 2 results in a reduction of the \Delta C\textsubscript{a} by a factor of 7 \pm 2.8 when the rising phases of the two curves are compared. The \approx 7-fold reduction of the \Delta C\textsubscript{a} associated with halving [Ca\textsuperscript{2+}]\textsubscript{o} is much larger than the 2-fold reduction in the AHP\textsubscript{slow} amplitude (Fig. 3), suggesting that some, but not all, of the Ca\textsuperscript{2+} released from the CICR pool is required for the generation of the AHP\textsubscript{slow}.

The disproportionate effect of reduced [Ca\textsuperscript{2+}]\textsubscript{o} on the \Delta C\textsubscript{a}, versus the AHP\textsubscript{slow} could arise from a nonlinear reduction of Ca\textsuperscript{2+} influx per AP and/or from a decreased Ca\textsuperscript{2+} release from CICR pool per unit Ca\textsuperscript{2+} influx. To study these possibilities, we examined the effect of lowering [Ca\textsuperscript{2+}]\textsubscript{o} on AP-induced Ca\textsuperscript{2+} influx. The amount of Ca\textsuperscript{2+} entering a neuron with each AP in normal and in reduced [Ca\textsuperscript{2+}]\textsubscript{o}, was determined by using a prerecorded AP as whole-cell voltage-clamp command under experimental conditions where the major inward charge carrier is Ca\textsuperscript{2+} (for details, see Fig. 2 in ref. 16). When [Ca\textsuperscript{2+}]\textsubscript{o} was decrementally reduced from 2 mM to nominally zero, the magnitude of the I\textsubscript{Ca} decreased proportionally. The charge movement caused by Ca\textsuperscript{2+} influx, normalized to cell membrane capacitance (pC/\rhoF), was plotted against varying [Ca\textsuperscript{2+}]\textsubscript{o}, for 12 neurons. Over the range of 0–2.0 mM [Ca\textsuperscript{2+}]\textsubscript{o}, Ca\textsuperscript{2+} influx varied linearly with [Ca\textsuperscript{2+}]\textsubscript{o} (r = 0.974). These results indicate that changes in Ca\textsuperscript{2+} influx alone cannot account for the disproportionate reduction in the \Delta C\textsubscript{a} relative to the AHP\textsubscript{slow} that is observed when [Ca\textsuperscript{2+}]\textsubscript{o} is reduced.

The disproportionate effect of reduced [Ca\textsuperscript{2+}]\textsubscript{o} on the \Delta C\textsubscript{a}–AHP\textsubscript{slow} relation could arise from a diminution in the amount of Ca\textsuperscript{2+} released from the CICR pool. Caffeine, a known agonist of CICR, is traditionally used to assess the releasable content of the CICR pool. In 8 of the 13 neurons studied, halving [Ca\textsuperscript{2+}]\textsubscript{o}, reduced the caffeine-induced \Delta C\textsubscript{a} by 20–79% (100% vs. 47 \pm 7.2% in 2.2 and 1.1 mM [Ca\textsuperscript{2+}]\textsubscript{o}, respectively; P = 0.0002). In other words, decreasing [Ca\textsuperscript{2+}]\textsubscript{o}, by a factor of 2 caused a 1.25- to 5-fold reduction in the caffeine response. On returning to normal Locke solution, the caffeine response was restored to near control values. In the remaining five neurons, the caffeine-induced \Delta C\textsubscript{a} was unaffected by reducing [Ca\textsuperscript{2+}]\textsubscript{o}, (100% vs. 112 \pm 8.4% in 2.2 and 1.1 mM [Ca\textsuperscript{2+}]\textsubscript{o}, respectively; P = 0.690). There was no significant difference in resting levels of [Ca\textsuperscript{2+}]\textsubscript{i} between these two groups of neurons (95 \pm 29.5 nM vs. 111 \pm 29.7 nM; P = 0.530). Unfortunately, the wide variability in the effects of reduced [Ca\textsuperscript{2+}]\textsubscript{o} on the caffeine responses prevents a meaningful interpretation of the effect of [Ca\textsuperscript{2+}]\textsubscript{o} on the releasable content of the CICR pool.

Ca\textsuperscript{2+} Influx Through N Type Calcium Channels Selectively Elicits AHP\textsubscript{slow}. Six types of VDCCs have been described in neurons: L, N, P, Q, R, and T (20). Nodose neurons express several types of VDCCs. By using a panel of pharmacologic reagents that are selective for different types of VDCCs, we tested the contribution of each to the total AP-induced Ca\textsuperscript{2+} current. Our results, summarized in Table 3, reveal that \approx 85%
of the AP-induced inward Ca$^{2+}$ current is shared by L and N type Ca$^{2+}$ channels (Fig. 9). P, Q, and T type Ca$^{2+}$ channel antagonists were ineffective, suggesting that the remaining Ca$^{2+}$ current is associated with Ca$^{2+}$ influx through R type channels. Nifedipine (10 $\mu$M), an L type Ca$^{2+}$ channel blocker, produced no measurable effect on either the AHP fast, the AHP medium, or the AHP slow. By contrast, $\omega$-conotoxin-GVIA (0.5 $\mu$M), a selective N type Ca$^{2+}$ channel blocker, always obliterated the AHP slow, and in $\approx 50\%$ of the neurons abolished the AHP medium (about half of the AHP medium are Ca$^{2+}$-sensitive, see above), while leaving the AHP fast unaffected (Fig. 9 and Table 4.). These results indicate that the SK and BK type K$^{+}$ channels are both regulated by Ca$^{2+}$ influx through N type channels. BK channels are gated by influx Ca$^{2+}$ directly (8), whereas SK channels are affected by influx Ca$^{2+}$ indirectly (i.e., Ca$^{2+}$ entering through N type VDCC triggers RY receptors to release Ca$^{2+}$ from CICR pools). Such a sequence implies a functional coupling between N type Ca$^{2+}$ channels

Table 3. Effects of Ca$^{2+}$ channel blockers on action potential-induced inward Ca$^{2+}$ currents

<table>
<thead>
<tr>
<th>Channel type</th>
<th>Channel blocker</th>
<th>Concentration $\mu$M</th>
<th>Reduction $%$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>Amiloride</td>
<td>500</td>
<td>0 $\pm$ 0</td>
<td>18</td>
</tr>
<tr>
<td>L</td>
<td>Nifedipine</td>
<td>10</td>
<td>44 $\pm$ 5.6</td>
<td>9</td>
</tr>
<tr>
<td>P/Q</td>
<td>$\omega$-AGA IVA</td>
<td>0.2</td>
<td>0 $\pm$ 0</td>
<td>2</td>
</tr>
<tr>
<td>Q</td>
<td>$\omega$-CTX MVIC</td>
<td>0.25</td>
<td>0 $\pm$ 0</td>
<td>6</td>
</tr>
<tr>
<td>N</td>
<td>$\omega$-CTX GVIA</td>
<td>1</td>
<td>40 $\pm$ 4.0</td>
<td>15</td>
</tr>
</tbody>
</table>

The blocking effect of amiloride, nifedipine, $\omega$-agatoxin (AGA) IVA, $\omega$-conotoxin (CTX) MVIC, and $\omega$-conotoxin (CTX) GVIA is expressed as percent reduction in the peak amplitude of the total calcium current $\pm$ SEM. $n$ corresponds to the number of cells for each condition.

of the AP-induced inward Ca$^{2+}$ current is shared by L and N type Ca$^{2+}$ channels (Fig. 9). P, Q, and T type Ca$^{2+}$ channel antagonists were ineffective, suggesting that the remaining Ca$^{2+}$ current is associated with Ca$^{2+}$ influx through R type channels. Nifedipine (10 $\mu$M), an L type Ca$^{2+}$ channel blocker, produced no measurable effect on either the AHP fast, the AHP medium, or the AHP slow. By contrast, $\omega$-conotoxin-GVIA (0.5 $\mu$M), a selective N type Ca$^{2+}$ channel blocker, always obliterated the AHP slow, and in $\approx 50\%$ of the neurons abolished the AHP medium (about half of the AHP medium are Ca$^{2+}$-sensitive, see above), while leaving the AHP fast unaffected (Fig. 9 and Table 4.). These results indicate that the SK and BK type K$^{+}$ channels are both regulated by Ca$^{2+}$ influx through N type channels. BK channels are gated by influx Ca$^{2+}$ directly (8), whereas SK channels are affected by influx Ca$^{2+}$ indirectly (i.e., Ca$^{2+}$ entering through N type VDCC triggers RY receptors to release Ca$^{2+}$ from CICR pools). Such a sequence implies a functional coupling between N type Ca$^{2+}$ channels

### Table 4. Actions of specific Ca$^{2+}$ channel blockers on the action potential-induced Ca$^{2+}$ transient and the AHP slow amplitude

<table>
<thead>
<tr>
<th>Channel type</th>
<th>Channel blocker</th>
<th>Ca$^{2+}$ transient Reduction, %</th>
<th>AHP slow amplitude Reduction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Nifedipine</td>
<td>57 $\pm$ 7.7</td>
<td>0 $\pm$ 0</td>
</tr>
<tr>
<td>N</td>
<td>$\omega$-CTX GVIA</td>
<td>39 $\pm$ 6.2</td>
<td>100 $\pm$ 0</td>
</tr>
<tr>
<td>T, R Nickel</td>
<td>nd</td>
<td>0 $\pm$ 0</td>
<td>100 $\pm$ 0</td>
</tr>
<tr>
<td>All Cadmium</td>
<td>100 $\pm$ 0</td>
<td>2</td>
<td>100 $\pm$ 0</td>
</tr>
</tbody>
</table>

The following concentrations of antagonists were used: nifedipine (10 $\mu$M), $\omega$-conotoxin GVIA (0.5 $\mu$M or 1 $\mu$M), nickel (50–500 $\mu$M), and cadmium (100 $\mu$M). nd, not determined.

![Fig. 9](image_url)

**Fig. 9.** Effects of VDCC antagonists on AP-induced calcium currents, AHP$_{slow}$ and AP-induced Ca$^{2+}$ transients. (A) Inward calcium currents recorded in isolated nodose neurons evoked by a prerecorded AP waveform from a holding potential of $-60$ mV. From Left to Right, control inward current in the presence of 2 mM [Ca$^{2+}$], and in the presence of 10 $\mu$M nifedipine. After reestablishing control conditions, the neuron was exposed to 1 $\mu$M $\omega$-conotoxin-GVIA. The effects of 500 $\mu$M cadmium were recorded in another neuron; the control current for this cell was similar to the first trace. (B) AHP$_{slow}$ evoked by a train of four APs (10 Hz) recorded in another nodose neuron. From Left to Right, AHP$_{slow}$ evoked in control conditions, in the presence of 100 $\mu$M CdCl$_2$, after washout, in the presence of 500 $\mu$M $\omega$-conotoxin-GVIA, and after washout. (C) AP-induced Ca$^{2+}$ transients recorded in two nodose neurons. From Left to Right, Ca$^{2+}$ transients evoked by a train of eight APs in normal Locke solution, and in Locke solution containing 10 $\mu$M nifedipine. In another neuron, 1 $\mu$M $\omega$-conotoxin-GVIA reduced the Ca$^{2+}$ transient $\approx 50\%$ (see Table 4.). APs were evoked by 2.5-ms, 10-Hz depolarizing current pulses.
DISCUSSION

Whether recorded in intact vagal sensory ganglia or in acutely isolated vagal afferent somata (nodose neurons), single APs can elicit an AHP\textsubscript{slow} that exhibits a delayed onset (50–300 ms), a slow time to peak amplitude (0.3–0.5 s), and a particularly long duration (2–15 s) (14, 21). Inhibition of the AHP\textsubscript{slow} by numerous inflammatory mediators (e.g., bradykinin, prostanooids, histamine, serotonin, leukotriene C\textsubscript{4}; see Table 1) results in an increased neuronal excitability and a loss of spike-frequency adaptation. Thus, modulation of the AHP\textsubscript{slow} by these mediators provides a mechanism for peripheral nociceptor sensitization that may underlie allergic inflammation-induced hyperalgesia.

One unresolved but important mechanistic question revolves around the delayed onset and protracted duration of the AHP\textsubscript{slow}. Many of our studies of nodose AHP\textsubscript{slow} were performed with acutely dissociated adult neurons, which are essentially spherical structures lacking dendritic and axonal processes. Thus, the delayed onset of the AHP\textsubscript{slow} cannot be due to slow diffusion of Ca\textsuperscript{2+} from distal sites of influx to somal SK channels. The high temperature coefficient (Q\textsubscript{10} > 3.0) for the rising phase and the decay time constant of the nodose AHP\textsubscript{slow} (14) also argues against simple Ca\textsuperscript{2+} diffusion as an explanation for the slow kinetics of the AHP\textsubscript{slow}. The time course of the AHP\textsubscript{slow} could arise from unusual channel kinetics of the SK channels. This also appears unlikely if SK channels in nodose neurons have activation kinetics similar to those cloned from rat brain (22). Recombinant SK channels from rat brain have activation time constants that are orders of magnitude shorter than the rise time of the AHP\textsubscript{slow}. It is more likely that the time course of the AHP\textsubscript{slow} is a consequence of the ΔCa\textsubscript{1} because of CICR.

If the AHP\textsubscript{slow} is directly dependent on Ca\textsuperscript{2+} released from the CICR pool, the AHP\textsubscript{slow} and the AP-induced rise in [Ca\textsuperscript{2+}], should display similar kinetics. Quantitative kinetic comparisons between these two variables are subject to some uncertainty, because the time course of the ΔCa\textsubscript{1} reflects global changes in [Ca\textsuperscript{2+}], whereas the kinetics of the AHP\textsubscript{slow} are determined by events at the plasma membrane. Nonetheless, we determined the time-to-peak and 10-to-90% decay time for both the AHP\textsubscript{slow} and the ΔCa\textsubscript{1} elicited by one to eight APs (19). The time-to-peak for AHP\textsubscript{slow} was significantly slower than the ΔCa\textsubscript{1} by nearly a factor of two (1.0 s vs. 1.9 s); the ΔCa\textsubscript{1} also decayed more rapidly than the AHP\textsubscript{slow} (3 s vs. 7 s). Although temporal discrepancies have been reported between the ΔCa\textsubscript{1} and AHP\textsubscript{slow} in vagal motoneurons (23). Such temporal differences suggest that Ca\textsuperscript{2+} released from CICR pools does not act alone to gate AHP\textsubscript{slow}. Cloned SK channels contain many potential phosphorylation sites (15); Ca\textsuperscript{2+}-dependent phosphorylation and/or dephosphorylation may thus be additional processes in the signal-transduction pathway of AP-evoked AHP\textsubscript{slow}.

Unambiguous data now exist showing that Ca\textsuperscript{2+} can directly activate SK channels in hippocampal neurons (24) and in Xenopus oocytes (22). In nodose neurons, it is less clear whether Ca\textsuperscript{2+} alone is sufficient to activate and sustain the AHP\textsubscript{slow} after an AP. In hippocampal neurons, flash photolysis of a “caged” Ca\textsuperscript{2+} chelator immediately truncates AP-induced AHP\textsubscript{slow}, suggesting that elevated intracellular Ca\textsuperscript{2+} is required to maintain the AHP\textsubscript{slow} (25). These results do not, however, distinguish between continuous Ca\textsuperscript{2+} gating of SK channel and the involvement of other Ca\textsuperscript{2+}-dependent factors sustaining the longevity of the AHP\textsubscript{slow}. It is also possible that Ca\textsuperscript{2+}-dependent factors act synergistically with Ca\textsuperscript{2+} to control SK channels (23). The nearly spherical morphology and large size of acutely isolated adult nodose neurons provide a favorable preparation to determine the nature of second messengers required to activate and sustain the AHP\textsubscript{slow}.

In conclusion, a subset of vagal primary afferent neurons possess a slowly developing and long-lasting spike afterhyperpolarization, the AHP\textsubscript{slow}, that can profoundly affect the discharge frequency of these visceral afferent neurons. Although AP-evoked Ca\textsuperscript{2+} influx via both L and N type Ca\textsuperscript{2+} channels triggers CICR, only Ca\textsuperscript{2+} influx through N type channels activates the CICR-dependent AHP\textsubscript{slow}. This type of specificity suggests that spatial coupling between N type Ca\textsuperscript{2+} channels and SK channels may be critical for the generation of the AHP\textsubscript{slow} in nodose neurons. The exact mechanism coupling ΔCa\textsubscript{1} to the AHP\textsubscript{slow} current remains to be determined.

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Fig. 10. Schematic diagram of the relation between plasma membrane Ca\textsuperscript{2+} channels, BK, and SK potassium channels and endoplasmic reticulum RY receptors in primary vagal afferent neurons. Single APs evoke Ca\textsuperscript{2+} influx through L and N type VDCCs. Ca\textsuperscript{2+} influx through either of these channels can trigger release of Ca\textsuperscript{2+} from the endoplasmic reticulum via RY receptors. Whereas BK channels are activated directly by Ca\textsuperscript{2+} entering the neuron via N type VDCC, SK channels are activated indirectly. SK channels require Ca\textsuperscript{2+} from CICR pools released after Ca\textsuperscript{2+} influx through N type channels.

