BK Channel Activation by Brief Depolarizations Requires Ca\(^{2+}\) Influx Through L- and Q-Type Ca\(^{2+}\) Channels in Rat Chromaffin Cells

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Prakriya, Murali and Christopher J. Lingle. BK channel activation by brief depolarizations requires Ca\(^{2+}\) influx through L- and Q-type Ca\(^{2+}\) channels in rat chromaffin cells. J. Neurophysiol. 81: 2267–2278, 1999. Ca\(^{2+}\)- and voltage-dependent BK-type K\(^{+}\) channels contribute to action potential repolarization in rat adrenal chromaffin cells. Here we characterize the Ca\(^{2+}\) currents expressed in these cells and identify the Ca\(^{2+}\) channel subtypes that gate the activation of BK channels during Ca\(^{2+}\) influx. Selective Ca\(^{2+}\) channel antagonists indicate the presence of at least four types of high-voltage-gated Ca\(^{2+}\) channels: L-, N-, P, and Q type. Mean amplitudes of the L-, N-, P-, and Q-type Ca\(^{2+}\) currents were 33, 21, 12, and 24% of the total Ca\(^{2+}\) current, respectively. Five-millisecond Ca\(^{2+}\) influx steps to 0 mV were employed to assay the contribution of Ca\(^{2+}\) influx through these Ca\(^{2+}\) channels to the activation of BK current. Blockade of L-type Ca\(^{2+}\) channels by 5 μM nifedipine or Q-type Ca\(^{2+}\) channels by 2 μM Aga IVA reduced BK current activation by 77 and 42%, respectively. In contrast, blockade of N-type Ca\(^{2+}\) channels by brief applications of 1–2 μM MnTC MVIIC or P-type Ca\(^{2+}\) channels by 50–100 nM Aga IVA reduced BK current activation by only 11 and 12%, respectively. Selective blockade of L- and Q-type Ca\(^{2+}\) channels also eliminated activation of BK current during action potentials, whereas almost no effects were seen by the selective blockade of N- or P-type Ca\(^{2+}\) channels. Finally, the L-type Ca\(^{2+}\) channel agonist Bay K 8644 promoted activation of BK current by brief Ca\(^{2+}\) influx steps by more than twofold. These data show that, despite the presence of at least four types of Ca\(^{2+}\) channels in rat chromaffin cells, BK channel activation in rat chromaffin cells is predominantly coupled to Ca\(^{2+}\) influx through L- and Q-type Ca\(^{2+}\) channels.

INTRODUCTION

Elevations of cytosolic calcium ([Ca\(^{2+}\)]\(_{i}\)) mediated by influx through voltage-dependent Ca\(^{2+}\) channels participate in a multitude of cellular functions, including exocytosis, excitation–contraction coupling, synaptic plasticity, ion channel gating, gene expression, and the growth and death of neurons (Bergeridge 1998). An unresolved question is how, if at all, specificity in signaling may arise when multiple kinds of Ca\(^{2+}\) channels with comparable activation properties may all contribute to the average elevation of submembrane [Ca\(^{2+}\)] within a cell. One possibility is that specificity in function of different Ca\(^{2+}\) signals may be encoded, in part, by the expression of different voltage-gated Ca\(^{2+}\) channels, each selectively coupled to particular Ca\(^{2+}\)-dependent processes and each functionally optimized to provide a localized pattern of Ca\(^{2+}\) influx that is appropriate for a particular Ca\(^{2+}\)-dependent process. As one step in addressing this issue, here we examine whether activation of large conductance Ca\(^{2+}\)- and voltage-dependent K\(^{+}\) channels (BK channels) in rat chromaffin cells may be selectively coupled to particular subtypes of Ca\(^{2+}\) channels.

In rat chromaffin cells, ≥10–20% of BK channels appear to be sufficiently tightly coupled to Ca\(^{2+}\) channels that the activation of those BK channels during brief Ca\(^{2+}\) influx steps is resistant to high concentrations of intracellular EGTA (Prakriya et al. 1996). During Ca\(^{2+}\) influx, these BK channels appear to be driven to high open probabilities with [Ca\(^{2+}\)] in the vicinity of those BK channels reaching ≥60 μM. This coupling of a portion of the BK channels to Ca\(^{2+}\) channels is essential to allow the BK channels to participate in the rapid repolarization of membrane potential during action potentials in the rat chromaffin cells (Solaro et al. 1995). Because chromaffin cells express multiple subtypes of Ca\(^{2+}\) channels (Ar talejo et al. 1994; Gandia et al. 1995; Hollins and Ikeda 1996), the observed functional coupling of BK channels to Ca\(^{2+}\) channels raises the question of whether BK current activation is dependent on Ca\(^{2+}\) influx through a particular subtype of Ca\(^{2+}\) channel.

We address two main issues. First, we confirm the identities of the various Ca\(^{2+}\) channel subtypes found in rat chromaffin cells (Gandia et al. 1995; Hollins and Ikeda 1996). Second, we examine their contribution in driving activation of BK channels. The results indicate that, of at least four classes of Ca\(^{2+}\) channels expressed in rat chromaffin cells, L-, N-, P-, and Q-type channels, BK current activation during brief Ca\(^{2+}\) influx steps characteristic of action potentials is coupled to Ca\(^{2+}\) influx predominantly through the L- and, when present, the Q-type channels. Because activation of BK current is important for the rapid termination of action potentials in rat chromaffin cells (Solaro et al. 1995), these results suggest that selective modulation of L- and Q-type Ca\(^{2+}\) channels by second messengers may affect the extent of BK current activation and hence cellular excitability.

METHODS

Chromaffin cell culture

Adrenal glands were removed from Sprague-Dawley rats and enzymatically digested with an enzyme cocktail containing 3% collagenase, 2.4% hyaluronidase, and 0.2% DNAse as described earlier (Neely and Lingle 1992). Chromaffin cells were plated on collagen-coated dishes and maintained at 37°C and 5% CO\(_2\) in a medium...
Electrophysiological methods

Whole cell recordings were performed at room temperature on cells 2–14 days after plating. In perforated patch-clamp cells, amphotericin was employed as the permeabilizing agent (Herrington et al. 1995). Whole cell currents were recorded with an Axopatch-1C amplifier (Axon Instruments; Foster City, CA) with a 500-MΩ feedback resistor. Whole cell voltage clamp was controlled with the Clampex program in the pClamp software package (Axon Instruments; Foster City, CA). Currents were obtained at a digitization rate of 2 kHz. Previous experiments (Prakriya et al. 1996) indicated that changing the sampling rate from 2 to 20 kHz produces only small effects on the estimates of the instantaneous BK current following the voltage step to +81 mV.

In some experiments involving Ca\(^{2+}\) channels, run-down of the Ca\(^{2+}\) current was noted. To prevent errors arising from run-down, only those cells were selected that satisfied the criteria that 1) Ca\(^{2+}\) currents exhibited stable responses during the control application of the Ba\(^{2+}\) solution, 2) the response to toxins displayed both a clear onset of block and an approximate exponential convergence toward a stable current level, and 3) recovery of response to reversible toxins such as nifedipine or brief applications of \(\omega\)-CnTx MVIC was complete.

Solutions

The standard extracellular solution contained (in mM) 140 NaCl, 5.4 KCl, 10 HEPES, 1.8 CaCl\(_2\), and 2.0 MgCl\(_2\) titrated to pH 7.4 with N-methylglucamine (NMG). In some experiments CaCl\(_2\) was excluded from the standard extracellular solution. The divalent concentration was kept constant by including 3.8 mM MgCl\(_2\) in this solution; 200 mM apanin was included in all experiments to block SK channels. For perforated patch-clamp experiments where both BK and Ba\(^{2+}\) currents were recorded from the same cell, the pipette saline contained (in mM) 120 K-aspartate, 30 KCl, 10 HEPES (H\(^+\)), and 2 MgCl\(_2\) adjusted to pH 7.4 with NMG. To isolate \(I_{\text{Ba}}\), the following extracellular solution was used: 10 BaCl\(_2\), 40 tetraethylammonium (TEA) chloride, 10 HEPES, and 90 NaCl with pH adjusted to 7.4 with NaOH. In experiments where only the Ba\(^{2+}\) currents were recorded, the composition of the internal saline was (in mM) 110 Cs-methane sulfonate, 14 phosphocreatine, 10 HEPES, 9 EGTA, 5 Mg-ATP, and 0.3 Tris-GTP. The pH was adjusted to 7.3 with CsOH. Osmolarity was measured by dew point (Wescor Osmometer) and adjusted between 290 and 310. Extracellular solution changes and drug applications were accomplished via a multibarrel perfusion system.

Drugs

Stock solutions of nifedipine (Sigma; St. Louis, MO) and Bay K 8644 (Bay K, Sigma) were prepared in ethanol at concentrations of 10 and 5 mM, respectively. \(\omega\)-Conotoxin MVIIIC (\(\text{CnTx MVIIIC}\)), \(\omega\)-conotoxin GVIA (\(\text{CgTx GVIA}\)) (both from RBI; Natick, MA), \(\omega\)-Agatoxin IVA (AgA IVA, gift of Pfizer), and \(\omega\)-Agatoxin TK (AgA TK, Peptide Institute; Japan) were dissolved in \(\text{dH}_2\text{O}\) at stock solutions of 500, 500, 100, and 100 \(\mu\)M, respectively. Final concentrations are given in the legends. In some experiments, L-type Ca\(^{2+}\) current was isolated by blocking P-, N-, and/or Q-type Ca\(^{2+}\) currents by preincubating chromaffin cells with a cocktail containing \(\omega\)-AgA IVA (100 \(\mu\)M), \(\omega\)-CgTx GVIA (2 \(\mu\)M), and \(\omega\)-CnTx MVIC (2 \(\mu\)M) for \(\geq 15\) min before electrophysiological recordings. P-type Ca\(^{2+}\) current and/or N-type Ca\(^{2+}\) was also preblocked in some experiments by exposure to \(\omega\)-AgA IVA (100 \(\mu\)M) and/or \(\omega\)-CgTx GVIA (2 \(\mu\)M) for \(\geq 15\) ins before recording.

RESULTS

Calcium current in rat chromaffin cells

Biophysical and pharmacological strategies were used to identify the various Ca\(^{2+}\) currents expressed in cultured rat chromaffin cells. Voltage-activated Ca\(^{2+}\) currents can be broadly classified into high-voltage-activated (HVA) and low-voltage-activated (LVA) currents (De Waard et al. 1996). LVA currents display rapid voltage-dependent inactivation, require relatively mild voltage stimuli for activation, and completely inactivate at depolarized holding potentials in the range of \(-60\) to \(-50\) mV. HVA currents in contrast lack rapid voltage-dependent inactivation and require more positive potentials for activation (Artalejo et al. 1991; Scroggs and Fox 1992). Therefore the presence of LVA current can be detected by comparing the I-V relationship at negative holding potentials to that at more depolarized holding potentials (Artalejo et al. 1991; Scroggs and Fox 1992). Here Ca\(^{2+}\) current I-V relationships were obtained at two holding potentials, \(-85\) and \(-50\) mV, and the difference current at each depolarizing step was determined. A typical Ca\(^{2+}\) current I-V relationship obtained at holding potential of \(-60\) mV in a rat chromatophin cell is shown in Fig. 1A. In 10 mM Ba\(^{2+}\), current activation occurred at voltages positive to \(-40\) mV and peaked around 0 mV. The I-V relationship was very similar in 10 mM Ca\(^{2+}\) except for a slight (~5 mV) shift to the right of the peak of the response \((n=4\) cells). As shown in Fig. 1B, when the holding potential was changed from \(-50\) to \(-85\) mV, the I-V relationship was unaltered at the more negative potentials, indicating minimal presence of LVA current in these cells (6/6 cells). Thus calcium current in the rat chromatophin cells studied here is predominantly of the HVA type.

HVA Ca\(^{2+}\) current can arise from several subtypes of Ca\(^{2+}\) channels, including L-, N-, P-, Q-, and R-type channels (De Waard et al. 1996). These can be distinguished from one another based on differential selectivity to various inhibitors.

L-type channels are sensitive to dihydropyridines (DHPs), N-type channels are irreversibly blocked by the snail toxin \(\omega\)-CgTx GVIA, and P-type channels are blocked by low concentrations of \(\omega\)-Aga IVA (for review see De Waard et al. 1996). Q-type channels can be blocked by high concentrations of \(\omega\)-AgA IVA or by the snail toxin \(\omega\)-CnTx MVIC (McDonough et al. 1996; Randall and Tsien 1995).

Figure 2A illustrates the effects of sequential application of \(\omega\)-CgTx GVIA, \(\omega\)-AgA IVA, and nifedipine on the Ba\(^{2+}\) current \((I_{\text{Ba}})\) evoked at 0 mV. \(\omega\)-CgTx GVIA (1–2 \(\mu\)M) and \(\omega\)-AgA IVA (0.1 \(\mu\)M) produced irreversible inhibition of some \(I_{\text{Ba}}\) in most cells, indicative of blockade of N- and P-type Ca\(^{2+}\) channels, respectively. Nifedipine (5 \(\mu\)M) produced a rapid (<20 s), reversible block of \(I_{\text{Ba}}\) in all cells, indicating the presence of L-type Ca\(^{2+}\) channels. There was substantial variability in the extent of block produced by each drug. The mean irreversible block produced by 1–2 \(\mu\)M \(\omega\)-CgTx GVIA applied for \(\geq 90\) s was \(24 \pm 3\%\) (SE; \(n=16\) cells). \(\omega\)-AgA IVA (0.1 \(\mu\)M) elicited only minor effects; the mean reduction of \(I_{\text{Ba}}\) by 0.1 \(\mu\)M \(\omega\)-AgA IVA applied for \(\geq 120\) s was only \(12 \pm 3\%\) (\(n=5\) cells). Nifedipine showed the most prominent reduction of \(I_{\text{Ba}}\) with a mean percentage block of \(33 \pm 3\%\) (\(n=13\) cells). A substantial fraction (30–40%) of the total \(I_{\text{Ba}}\) was unaffected by the combined actions of nifedipine, \(\omega\)-CgTx
The slow component of P- and Q-type Ca$^{2+}$ currents in rat chromaffin cells that was mostly complete within 40 s and was slowly reversible, whereas block of N-type current is fast and completely eliminated (6/6 cells), which suggested that it was mediated by N-type calcium channels. The slow component of $\omega$-CnTx MVIIC block was similar to the block of P- and Q-type Ca$^{2+}$ channels reported by McDonough et al. (1996). To examine if this arose solely from P-type Ca$^{2+}$ channels or whether Q-type channels were also present, we next tested the efficacy of $\omega$-CnTx MVIIC after preblocking N-type current by 1 $\mu$M $\omega$-CgTx GVIA and P-type current by 100 nM $\omega$-Aga IVA (Fig. 2C). Under these conditions, in 10 of 15 cells examined, $\omega$-CnTx MVIIC produced an additional slowly de-

FIG. 1. Rat chromaffin cells express predominantly high-voltage-activated (HVA) Ca$^{2+}$ currents A: current-voltage (I-V) relationship of Ca$^{2+}$ current in a rat chromaffin cell in 10 mM Ba$^{2+}$ and 10 mM Ca$^{2+}$. The cell was held at −60 mV and depolarized for 100 ms in increments of 10 mV. The peak of the I-V in Ca$^{2+}$ slightly right shifted compared with currents in equimolar Ba$^{2+}$. Current traces elicited between −20 and +20 mV are shown below. B: changing the holding potential from −85 to −50 mV does not reveal any low-voltage-activated current. Same cell as in A. Currents elicited at the holding potential of −50 mV were subtracted from the currents at −80 mV. The resulting difference current peaked at approximately +3 mV. Standard whole cell method. $R_{e} = 7 \text{ M}\Omega; C_{m} = 6 \text{ pF}; 60\%$ compensated.

$\omega$-CnTx MVIIC is a calcium channel antagonist that blocks P-, Q-, and N-type calcium channels (McDonough et al. 1996; Randall and Tsien 1995). In CNS neurons, the onset of block of P- and Q-type current by $\omega$-CnTx MVIIC is slow and only slowly reversible, whereas block of N-type current is fast and quickly reversible (McDonough et al. 1996). Figure 2B illustrates the effects of 2 $\mu$M $\omega$-CnTx MVIIC on $I_{pa}$ elicited at 0 mV in 6 mM Ba$^{2+}$. Effects of $\omega$-CnTx MVIIC were evaluated in 6 mM Ba$^{2+}$ rather than the 10 mM Ba$^{2+}$ used in previous experiments because of the observation that higher concentrations of Ba$^{2+}$ result in slower block of the P- or Q-type Ca$^{2+}$ current by this toxin (McDonough et al. 1996). Two kinetically distinct components of blockade by $\omega$-CnTx MVIIC could be detected, 1) a fast component (mean amplitude = 20 ± 3%; n = 14 cells) that was mostly complete within 40 s and was fully reversible and 2) a very slow component (mean = 27 ± 4%; n = 9 cells) that was largely irreversible over the time course of a typical experiment (Fig. 2B). When $\omega$-CgTx GVIA was applied, the fast reversible effect of $\omega$-CnTx MVIIC was consistently eliminated (6/6 cells), which suggested that it was mediated by N-type calcium channels. The slow component of

FIG. 2. Pharmacological analysis of HVA Ca$^{2+}$ current reveals the presence of L- N-, P-, and Q-type Ca$^{2+}$ current in rat chromaffin cells. A: N-, P-, and L-type Ca$^{2+}$ currents were recorded in the perforated patch-clamp mode by stepping to 0 mV for 15 ms from a holding potential of −60 mV every 10 s, and peak current amplitudes are plotted against time. The N-type antagonist $\omega$-CgTx GVIA (1.6 $\mu$M), the P-type antagonist $\omega$-Aga IVA (100 nM), and the L-type antagonist nifedipine (5 $\mu$M) were applied for the indicated durations. Standard whole cell method. $R_{e} = 6 \text{ M}\Omega; C_{m} = 9 \text{ pF}; 80\%$ compensated. B: $\omega$-CnTx MVIIC blocks N-type Ca$^{2+}$ current in a fast, fully reversible manner. Ca$^{2+}$ currents were activated by stepping to 0 mV every 10 s for 15 ms. The toxin $\omega$-CnTx MVIIC (2 $\mu$M) elicits 2 kinetically distinct blocking effects, a fast effect that is fully reversible and a very slow block that is largely irreversible in the time course of these experiments. The fast, reversible block is completely eliminated by blocking N-type current with $\omega$-CgTx GVIA (1.5 $\mu$M). Standard whole cell method. $R_{e} = 7 \text{ M}\Omega; C_{m} = 12 \text{ pF}; 80\%$ compensated. C: slowly developing block by $\omega$-CnTx MVIIC (2 $\mu$M) is present after blockade of P-type Ca$^{2+}$ channels by $\omega$-Aga IVA (100 nM), indicating the presence of Q-type Ca$^{2+}$ current. N-type current was preblocked in this experiment by exposing the cells to 1 $\mu$M $\omega$-CgTx GVIA for 15 min before recording. Perforated patch method. $R_{e} = 12 \text{ M}\Omega; C_{m} = 6 \text{ pF}; 80\%$ compensated. D: 2 $\mu$M $\omega$-Aga IVA produces significant block of Ca$^{2+}$ current consistent with effects on Q-type Ca$^{2+}$ channels. Cell was preincubated with 100 nM $\omega$-Aga IVA for 15 min before electrophysiological recording to block P-type Ca$^{2+}$ current. Standard whole cell method. $R_{e} = 6.5 \text{ M}\Omega; C_{m} = 7 \text{ pF}; 80\%$ compensated.
veloping block of some current, consistent with the presence of Q-type Ca$^{2+}$ channels.

The presence of Q-type channels was also confirmed by $\omega$-AgA IVA. This toxin blocks both P- and Q-type Ca$^{2+}$ channels, although the relative affinities are widely different; P-type channels are maximally blocked by 100 nM $\omega$-AgA IVA, whereas Q-type Ca$^{2+}$ channels are blocked only at micromolar concentrations of $\omega$-AgA IVA (Randall and Tsien 1995). Chromaffin cells were preincubated in 100 nM $\omega$-AgA IVA for 15 min before electrophysiological recording, and the effect of 2 $\mu$M $\omega$-AgA IVA was examined on the remaining Ba$^{2+}$ current. In 8 of 11 cells tested, 2 $\mu$M $\omega$-AgA IVA elicited a substantial block of the remaining $I_{Ba}$, indicating the presence of Q-type Ca$^{2+}$ channels in these cells (Fig. 2D).

In some cells, $\omega$-CgTx GVIA produced reversible block of a component of $I_{Ba}$ (data not shown). The amplitude of this current could be substantially reduced by simultaneously applying nifedipine, suggesting that this was arising from L-type channels sensitive to $\omega$-CgTx GVIA. We did not further characterize these currents. However, overlap between $\omega$-CgTx GVIA and DHP inhibitors of L-type Ca$^{2+}$ channels was reported in some cells (Kasai and Neher 1992), and it was suggested that the Ca$^{2+}$ channels underlying this current may be L-type channels arising from the $\alpha_{1D}$ pore-forming subunit (Williams et al. 1992). A more recent study, however, indicates that the L-type Ca$^{2+}$ channels in sympathetic neurons arising from the $\alpha_{1D}$ subunit are not $\omega$-CgTx GVIA sensitive (Lin et al. 1996). Thus the identity of this current remains to be elucidated.

In sum, the previous experiments demonstrate that rat chromaffin cells express four major classes of HVA Ca$^{2+}$ channels: L-, N-, P-, and Q-type channels. The average contributions of each type are summarized in Fig. 6. We also often observed a small residual Ca$^{2+}$ current even in the combined presence of all the calcium antagonists mentioned previously (not shown). This current may be similar to the R-type current reported in previous studies that is insensitive to all the known Ca$^{2+}$ channel toxins (De Waard et al. 1996).

**BK current in rat chromaffin cells**

Rat chromaffin cells express inactivating (BK$_{i}$) or noninactivating (BK$_{s}$) variants of BK current. Approximately 75% of the chromaffin cells express predominantly BK$_{i}$ current, and the rest express either noninactivating or a mixture of inactivating and noninactivating BK current (Ding et al. 1998; Solaro et al. 1995). All the data shown in this paper are from cells displaying BK$_{i}$ currents, although we observed no differences between the two cell types in the nature of BK–Ca$^{2+}$ channel coupling.

Depolarizing steps that elicit Ca$^{2+}$ influx through Ca$^{2+}$ channels can robustly activate BK current in rat chromaffin cells (Soloro et al. 1995). Here BK current activation was studied in perforated patch-clamped cells by stepping the voltage to a potential that activates Ca$^{2+}$ current followed by a test pulse to +81 mV where BK current activation was evaluated. Because minimal Ca$^{2+}$ influx occurs at +81 mV, the effects of conditioning steps that activate Ca$^{2+}$ influx can be easily distinguished from those that do not produce influx. Figure 3A shows the effect of stepping the voltage directly to +81 mV in the presence and absence of 1.8 mM [Ca$^{2+}$]$_{\text{ext}}$. Direct steps to +81 mV would be expected to only activate Ca$^{2+}$-independent voltage-activated K$^+$ currents (Solaro et al. 1995). Consistent with this, the current activated by a direct step to +81 mV in 1.8 mM [Ca$^{2+}$]$_{\text{ext}}$ is identical to that elicited in 0 [Ca$^{2+}$]$_{\text{ext}}$. In contrast, a 5-ms step to 0 mV before the +81-mV test pulse produces Ca$^{2+}$ influx through voltage-gated Ca$^{2+}$ channels and activates BK current. There are 2 components in the current that are activated at +81 mV, an instantaneous current that is simply the tail at +81 mV of the BK current activated at 0 mV (arrow) and a slow, time-dependent increase in current caused by the increased open probability of BK channels at +81 mV. Same cell as in A. C: increasing the influx step duration to 150 ms increases the instantaneous and the peak BK currents activated at +81 mV. Same cell as in A. Perforated patch method. $R_{e}$ = 12 MΩ; $C_{m}$ = 6 pF; 80% compensated.

A close examination of the BK current at +81 mV reveals two current components. First, there is an instantaneous cur-
CA2+ CHANNEL SUBTYPES INVOLVED IN BK CURRENT ACTIVATION

Recent studies have shown that the activation of Ca2+ channels at 0 mV (Fig. 3B, arrow) (for additional information about the instantaneous current see Prakriya et al. 1996). Second, there is a slower time-dependent increase in current that results from the activation of additional Ca2+ channels caused by the increased open probability of BK channels at +81 mV. The amplitudes of both these components of BK current increase as the duration of the influx step is increased (Fig. 3C). High concentrations of EGTA differentially affect these two components of BK current; the instantaneous current is unaffected by EGTA up to concentrations of 5 mM, whereas the slow component of BK current is eliminated by EGTA (Prakriya et al. 1996). These effects were interpreted to suggest the presence of two populations of BK channels at differing distances from Ca2+ channels, the instantaneous current possibly reflecting BK channels in close association with Ca2+ channels and exposed to high [Ca2+]i, and the more slowly activating BK current reflecting channels at greater distances from sites of Ca2+ influx and exposed to the much lower bulk [Ca2+]i. In this study, the contribution of influx through various subtypes of Ca2+ channels found in chromaffin cells to the activation of BK channels by brief influx steps is examined. Ca2+ influx steps were confined to brief duration (5 ms) because brief steps will favor elevation of the [Ca2+]i, in the immediate vicinity of the active Ca2+ channels while significantly altering the global [Ca2+]i. Thus BK channels activated by brief Ca2+ influx steps are more likely to be driven by Ca2+ elevations in the vicinity of Ca2+ channels.

Selective blockade of L- but not N-type Ca2+ current affects BK current activation

The role of the various subtypes of Ca2+ channels toward BK current activation was next examined with the previous Ca2+ channel inhibitors. Figure 4 shows an example of the effects of 5 µM nifedipine and 2 µM ω-CnTx MVIC on BK currents and Ba2+ currents activated by 5-ms Ca2+ influx steps. BK current was recorded in the standard external solution containing 1.8 mM [Ca2+], and Ba2+ current was recorded from the same cell by changing the standard external solution to one containing 10 mM Ba2+. Nifedipine caused a very substantial reduction in the amount of BK current activated by calcium influx. In contrast, applications of ω-CnTx MVIC lasting 40–50 s resulted in only minor effects on BK current activation (Fig. 4). The average nifedipine-induced reduction in the amplitude of instantaneous BK current activated by calcium influx was 77 ± 2% (mean ± SE; n = 15 cells), whereas the nifedipine-sensitive Ba2+ current comprised 33 ± 3% of the total IBa. In contrast, ω-CnTx MVIC reduced BK current activation by only 11 ± 3% (n = 9 cells), although the ω-CnTx MVIC-sensitive Ba2+ current in these cells comprised 21 ± 3% of the total IBa. Even in cells with comparable amplitudes of nifedipine and CnTx MVIC-sensitive Ba2+ currents, blockade of L-type Ca2+ channels by nifedipine evoked dramatically larger reductions in BK current than blockade of N-type Ca2+ channels by CnTx MVIC. Thus these results suggest that BK current activation during brief Ca2+ influx steps primarily occurs via influx through L- but not N-type Ca2+ channels.

We also determined the effects of the irreversible N-type blocker ω-CgTx-GVIA. ω-CgTx-GVIA (1–2 µM) inhibited BK current by 20 ± 4% (n = 15 cells) and IBa by 24 ± 3% (n = 16 cells). These effects on BK current are larger than those elicited by block of N-type channels by ω-CnTx MVIC. However, as pointed out earlier, ω-CgTx GVIA produced both irreversible and reversible effects on IBa in some cells, and much of the reversible effect could be blocked by nifedipine. Thus the larger effect of ω-CgTx GVIA on BK current relative to ω-CnTx MVIC may result from the reversible block by ω-CgTx GVIA of a rarely occurring, novel Ca2+ channel variant that is susceptible to nifedipine blockade.

In separate experiments, any direct effects of Ca2+ channel blockers on BK current were assessed by introducing 10 µM [Ca2+], into cells via the patch pipette to directly activate BK current. These experiments did not indicate any direct effects of the inhibitors on BK channels.

Blockade of Q- but not P-type Ca2+ channels affects BK current activation

The contribution of P-type channels toward BK current activation during calcium influx was evaluated with 100 nM ω-Aga IVA. Measurements of ω-Aga IVA–mediated inhibition of BK current activation and of the block of IBa were determined in different cells because of the irreversible nature of ω-Aga IVA block. Application of 100 nM ω-Aga IVA produced a small decrease in BK current activation in many cells (7/11 cells; no effect in 4 cells; Fig. 5A). The mean reductions in BK current and IBa were 14 ± 5% (n = 7 cells) and 12 ± 2% (n = 8 cells), respectively. These figures might be overestimates of the true contribution of P-type current because 100 nM Aga IVA would be expected to block Q-type Ca2+ current.
significant decrease in BK current activation. Regardless of the exact amount of Q-current, the results argue that, when Q-type channels are present, Ca\(^{2+}\) influx through these channels participates in initiating activation of BK channels.

Figure 6 summarizes the effects of all the Ca\(^{2+}\) channel antagonists used here on I\(_{Ba}\) and BK currents. Selective blockade of L-type Ca\(^{2+}\) channels had the greatest effect on BK current activation, whereas blockade of N- and P-type Ca\(^{2+}\) channels caused only minor effects. Blockade of Q-type Ca\(^{2+}\) channels by micromolar concentrations of \(\omega\)-Aga IVA also reduced BK current activation significantly.

Blockade of L- and Q-type Ca\(^{2+}\) channels inhibits BK current activation during action potentials

We next examined the role of Ca\(^{2+}\) influx during action potential waveforms on BK current activation in perforated patch-clamped chromaffin cells. Currents were activated by an action potential voltage-clamp waveform that was generated by digitizing a chromaffin cell action potential recorded in the current-clamp mode. The ability of the previous Ca\(^{2+}\) channel blockers to inhibit the action potential-induced BK current was estimated by comparing K\(^+\) currents obtained in 0 [Ca\(^{2+}\)]\(_{ext}\) solution with blockade of all Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels, which should block all the BK current induced by the action potential. Figure 7 shows typical examples of the effects of removing [Ca\(^{2+}\)]\(_{ext}\) and of the various Ca\(^{2+}\) channel blockers in perforated patch-clamped cells. In the majority of cells, a prominent shoulder or “hump” in the outward K\(^+\) current during the falling phase of the action potential is observed. When the external solution is replaced with a 0 [Ca\(^{2+}\)]\(_{ext}\) solution, the hump disappears, indicating that it arises because of the activation of BK current driven by Ca\(^{2+}\) influx during the action potential. In addition, the 0 [Ca\(^{2+}\)]\(_{ext}\) solution also reduces the peak outward K\(^+\) current in many cells, suggesting that BK current is also activated to some extent during the rising phase of the action potential.

Fig. 5. Blockade of P-type channels does not alter BK current activation, whereas Q-current blockade reduces BK current significantly. A: BK current activation was elicited by a 5-ms voltage step to 0 mV and followed by a step to +81 mV. Arrows point to the instantaneous current elicited at +81 mV by the 0-mV Ca\(^{2+}\) influx step. The trace shown here was obtained 150 s after the onset of drug application. Perforated patch method. \(R_s = 12.5\) M\(\Omega\); \(C_m = 8\) pF; 80% compensated. B: block of Q-current by 2 \(\mu\)M \(\omega\)-Aga IVA elicits a significant decrease in BK current activation. Same voltage protocol as in A. The cell was preincubated with 100 nM \(\omega\)-Aga IVA for 15 min before electrophysiological recording. Perforated patch method. \(R_s = 15\) M\(\Omega\); \(C_m = 9\) pF; 80% compensated.

Fig. 6. Summary of the pharmacological effects on BK and Ca\(^{2+}\) currents. BK current activation was elicited by 5-ms influx steps. Selective blockade or enhancement of L-type channels by nifedipine or Bay K 8644 have the greatest effects on BK current activation, whereas blockade of P-type channels by 100 nM \(\omega\)-Aga IVA and N-type channels by \(\omega\)-CgTx GVIA or by brief applications (<40 s) of \(\omega\)-CnTx MVIIC produces only minor effects. Blockade of Q-type channels by 2 \(\mu\)M \(\omega\)-Aga IVA also produces substantial reduction in BK current activation.
outward K current was completely eliminated by nifedipine. In fact, the outward K⁺ current profile was very similar to that elicited in

0 [Ca²⁺]oltage, indicating that nearly all BK current activation is prevented by the blockade of L-type Ca²⁺ channels during the action potential. In contrast, the same experiment done with transient (40–50 s) applications of ω-CnTx MVIIC to block N-type Ca²⁺ channels showed little or no effect in all cases (Fig. 7B). In these cells, the outward K⁺ currents were virtually identical to the control K⁺ currents activated in the normal [Ca²⁺]oltage solution. In four of the six cells that were examined in this way, the amplitude of the N-type Ca²⁺ current was then determined by 40-s applications of 2 µM ω-CnTx MVIIC with 10 mM Ba²⁺ as the charge carrier. The mean amplitude of the ω-CnTx MVIIC-sensitive I_{Ba} was found to be 24% of the total I_{Ba} in these cells. Thus the lack of an effect by ω-CnTx MVIIC on BK current activation in these cells was not because of the lack of ω-CnTx MVIIC-sensitive Ca²⁺ channels.

The role of P- and Q-type Ca²⁺ channels toward BK current activation during action potentials was assayed by blocking these Ca²⁺ channels with Aga TK. Analogous to Aga IVA, low concentrations of Aga TK selectively block P-type Ca²⁺ currents, whereas higher concentrations have been also shown to block Q-type Ca²⁺ currents (Teramoto et al. 1995). We used 100 nM and 2 µM Aga TK to selectively block P- and Q-type Ca²⁺ currents, respectively (Fig. 7, C and D). Aga TK (100 nM) had little or no effect on the outward K⁺ current in all cells tested (5/5 cells), indicating that P-type Ca²⁺ currents do not contribute to BK current activation during action potentials. In contrast, 2 µM Aga TK elicited a substantial inhibition of the outward K⁺ current in four of five cells tested (Fig. 7D).

Collectively, these data are consistent with results obtained by 5-ms Ca²⁺ influx steps and show that blockade of L- and Q-type Ca²⁺ channels results in significant inhibition of BK current activation during action potentials, whereas selective blockade of N- and P-type Ca²⁺ channels produces little or no effect.

**Bay K increases the BK current activated by calcium influx**

The effects of the DHP agonist Bay K 8644 (Bay K), which is known to increase the amplitude of the ensemble Ca²⁺ current by favoring long-duration openings of L-type Ca²⁺ channels (Kokubun and Reuter 1984; Nowycky et al. 1985), were next examined on the depolarization-evoked Ca²⁺ and BK currents.

Bay K elicited a significant increase in the amplitude of the peak Ca²⁺ current (peak I_{Ba} increased ~143% of control; n = 10 cells). To examine if this is a consequence of an increase in the number of L-type Ca²⁺ channels or an increase in the I_{open} of L-type Ca²⁺ channels, we used noise analysis (Sigworth 1980) to estimate the number of Ca²⁺ channels and their average P_{open} (Fig. 8). In these experiments, the contribution of non-L-type Ca²⁺ channels to the Ba²⁺ current was minimized by preincubating chromaffin cells for ≥15 min with a cocktail of 100 nM ω-Aga IVA, 1 µM ω-CnTx GVIA, and 1 µM ω-CnTx MVIIC. As shown in Table 1, the primary effect of Bay K was to increase the peak mean current with no obvious effects on average single-channel conductance or channel number. Thus this result argues that the increase in peak Ca²⁺ current occurs mainly by an increase in the P_{open} of L-type Ca²⁺ channels.

The consequences of the Bay K-mediated increase in L-type Ca²⁺ current on the BK current activated by brief influx steps
Bay K 8644 enhances L-type Ca\textsuperscript{2+} channel open probability. Ensemble variance analysis was performed on a train of 100 \textsuperscript{Ba}\textsuperscript{2+} current traces elicited at 0 mV every 6 s. Cells were exposed to 100 nM \textomega-Aga IVA, 1 \textmu M \omega-CgTx GVIA, and 1 \textmu M \omega-CaTx MVIIC for 15 min before electrophysiological recording to minimize the contribution of non-L-type Ca\textsuperscript{2+} channels. A and B: mean current and variance during the activation phase in control saline. C and F: variance plotted against the mean current. ———: fit of the relation $i = i_I - P/N + I_{BL}$, where $i$ is the single-channel current, $I$ is the mean current, $N$ is the number of channels, and $I_{BL}$ is the baseline current at 0 variance. $I_{BL}$ is simply a small correction factor to take into account the presence of a nonzero variance at the 0 current level and might arise because of contaminating currents before and during the voltage pulse. Fit parameters: control saline: $i_c = 0.41 \text{ pA}; N_c = 5.001 \text{ channels};$ Bay K: $i_c = 0.51 \text{ pA}; N_c = 4781$.

were also examined. Bay K produced very large increases in the amplitude of BK current activated by brief Ca\textsuperscript{2+} influx steps (Fig. 9A). Although the maximal peak BK current activated by long-duration influx steps was the same in the presence or absence of Bay K (Fig. 9B), the instantaneous BK current activated by brief influx steps increased 2.2-fold on average ($n = 5$ cells). In these cells, $I_{BL}$ increased 1.38-fold on Bay K treatment. Thus this result indicates that, when L-type Ca\textsuperscript{2+} channel open probability is increased, the fraction of BK channels activated by Ca\textsuperscript{2+} influx during brief Ca\textsuperscript{2+} influx steps also increases significantly.

We estimated the fraction of BK channels that can be activated by brief influx steps in the presence of Bay K by comparing the instantaneous BK current activated by 5-ms Ca\textsuperscript{2+} influx steps in the presence of Bay K with the total BK current present in the cell. For each cell, total current was estimated by measuring the peak BK current activated by 50 \textmu M muscarine at +81 mV, which activates nearly all the BK channels in the cell (Prakriya et al. 1996). This procedure indicated that Bay K increased the fraction of BK channels activated by brief influx steps twofold, from 17.4 ± 3% to 36 ± 5% ($n = 5$ cells). Thus $\geq 30–40\%$ of the BK channels in rat chromaffin cells are positioned sufficiently close to L-type Ca\textsuperscript{2+} channels so as to be influenced by Ca\textsuperscript{2+} influx through these Ca\textsuperscript{2+} channels during brief depolarizing steps.

Ca\textsuperscript{2+} influx through L- and Q-type Ca\textsuperscript{2+} channels is more effective than Ca\textsuperscript{2+} influx through other Ca\textsuperscript{2+} channels in driving BK current activation

The previous pharmacological experiments argue that BK channels are functionally associated with L- and Q-type but not the N- and P-type Ca\textsuperscript{2+} channels. How do these results compare with manipulations that do not discriminate among the various types of calcium channels but affect all of them? We examined this issue by lowering or raising $[Ca^{2+}]_{ext}$ to determine the dependence of BK and Ca\textsuperscript{2+} current activation on the $[Ca^{2+}]_{ext}$ (Fig. 10A). BK current at various $[Ca^{2+}]_{ext}$ was then plotted against the Ca\textsuperscript{2+} current amplitude at the respective $[Ca^{2+}]_{ext}$ (Fig. 10B). Note the sigmoidicity in the activation plot in Fig. 10B. This may represent the cooperative activation of BK channels by Ca\textsuperscript{2+}.

We found that, although \textomega-CnTx MVIIC, \textomega-CgTx-GVIA, and 100 nM \textomega-Aga IVA produced measurable reductions in BK current activation, these effects were not obviously different from those obtained by simply lowering the $[Ca^{2+}]_{ext}$ to produce an equivalent reduction in the overall Ca\textsuperscript{2+} current. However, the selective blockade of L-type Ca\textsuperscript{2+} channels by nifedipine or Q-type Ca\textsuperscript{2+} channels by 1.5–2 \textmu M \textomega-Aga IVA evoked reductions in BK current activation that were significantly greater than what could be produced by simply lowering the $[Ca^{2+}]_{ext}$ to produce an equivalent reduction in the overall Ca\textsuperscript{2+} current. The properties of Ca\textsuperscript{2+} channels were determined by ensemble-variance analysis of the \textsuperscript{Ba}\textsuperscript{2+} currents in the activation-phase of the \textsuperscript{Ba}\textsuperscript{2+} current. The ensemble-variance analysis was performed on a train of \textsuperscript{Ba}\textsuperscript{2+} current traces containing 100 stimuli. Cells were exposed to 100 nM \textomega-Aga IVA, 1 \textmu M \omega-CgTx GVIA, and 1 \textmu M \omega-CnTx MVIIC for 15 min before electrophysiological recording to remove the contribution of P-, N-, and Q-type Ca\textsuperscript{2+} channels. $i_c$ is the single-channel Ca\textsuperscript{2+} current in 10 mM \textsuperscript{Ba}\textsuperscript{2+}; $N_c$ is the number of Ca\textsuperscript{2+} channels in the cell. Filtering artifacts lead to underestimates of $i_c$ and overestimates of $N_c$, when the channel mean open times are small (Silberberg and Magleby, 1993). Bay K is known to increase the Ca\textsuperscript{2+} channel mean open time, the slight increase in $i_c$ and decrease in $N_c$ seen here with Bay K may simply reflect reduced effects of these errors.

### Table 1. Mean vs. variance analysis of Ca\textsuperscript{2+} channels

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>Control</th>
<th>Bay K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$i_c, \text{ pA}$</td>
<td>$N_c$</td>
</tr>
<tr>
<td>1</td>
<td>0.37</td>
<td>5770</td>
</tr>
<tr>
<td>2</td>
<td>0.43</td>
<td>3900</td>
</tr>
<tr>
<td>3</td>
<td>0.39</td>
<td>6223</td>
</tr>
<tr>
<td>4</td>
<td>0.41</td>
<td>5001</td>
</tr>
</tbody>
</table>

Ca\textsuperscript{2+} currents were measured in 10 mM \textsuperscript{Ba}\textsuperscript{2+}. The properties of Ca\textsuperscript{2+} channels were determined by ensemble-variance analysis of the \textsuperscript{Ba}\textsuperscript{2+} currents in the activation-phase of the \textsuperscript{Ba}\textsuperscript{2+} current. The ensemble-variance analysis was performed on a train of \textsuperscript{Ba}\textsuperscript{2+} current traces containing 100 stimuli. Cells were exposed to 100 nM \textomega-Aga IVA, 1 \textmu M \omega-CgTx GVIA, and 1 \textmu M \omega-CnTx MVIIC for 15 min before electrophysiological recording to remove the contribution of P-, N-, and Q-type Ca\textsuperscript{2+} channels. $i_c$ is the single-channel Ca\textsuperscript{2+} current in 10 mM \textsuperscript{Ba}\textsuperscript{2+}; $N_c$ is the number of Ca\textsuperscript{2+} channels in the cell. Filtering artifacts lead to underestimates of $i_c$ and overestimates of $N_c$, when the channel mean open times are small (Silberberg and Magleby, 1993). Bay K is known to increase the Ca\textsuperscript{2+} channel mean open time, the slight increase in $i_c$ and decrease in $N_c$ seen here with Bay K may simply reflect reduced effects of these errors.
Ca\(^{2+}\) current. Similarly, enhancing the L-type Ca\(^{2+}\) current by Bay K produced an increase in BK current amplitude that was far in excess of the increase that could be produced by increas-
ing the [Ca\(^{2+}\)]\(_{\text{ext}}\) to evoke a similar overall Ca\(^{2+}\) current. Thus the larger role of L-type Ca\(^{2+}\) current is not simply because this current comprises the largest Ca\(^{2+}\) current component. Instead BK current is more effectively activated when Ca\(^{2+}\) influx occurs through L- and Q-type Ca\(^{2+}\) channels than when it occurs through N- or P-type Ca\(^{2+}\) channels.

**DISCUSSION**

Multiple types of Ca\(^{2+}\) channels are frequently expressed in the same cellular compartment, and all contribute to the elevation of the average [Ca\(^{2+}\)]\(_{\text{cyt}}\). Under such conditions, an unresolved question is whether there is specificity in Ca\(^{2+}\) signaling triggered by influx through the various subtypes of Ca\(^{2+}\) channels. We addressed this question with the Ca\(^{2+}\)- and voltage-dependent BK-type channel on rat adrenal chromaffin cells and asked whether BK channels are specifically coupled to a particular Ca\(^{2+}\) channel subtype. Our results indicate, in fact, that BK channels appear to be preferentially activated by Ca\(^{2+}\) influx through L- and Q-type Ca\(^{2+}\) channels.

We assumed that the proportion of Ca\(^{2+}\) influx that occurs in the solutions used to record BK current (with 2 mM [Ca\(^{2+}\)]\(_{\text{cyt}}\)) is comparable with the Ca\(^{2+}\) current amplitudes we measured with 10 mM Ba\(^{2+}\). In trying to relate the amount of influx that would occur in 2 mM [Ca\(^{2+}\)]\(_{\text{cyt}}\) from the measured influx amounts in 10 mM [Ba\(^{2+}\)]\(_{\text{cyt}}\), two factors must be considered, 1) the increased permeability of HVA Ca\(^{2+}\) channels to Ba\(^{2+}\) over Ca\(^{2+}\) and 2) the saturable increase in the conductance of HVA Ca\(^{2+}\) channels as the divalent concentration is increased. If these effects occur in roughly the same proportion in all the HVA Ca\(^{2+}\) channel subtypes, the proportion of influx occurring at 10 mM [Ba\(^{2+}\)]\(_{\text{cyt}}\) through a channel subtype can be assumed to be the same as that occurring in 2 mM [Ca\(^{2+}\)]\(_{\text{cyt}}\).

Examination of the Ca\(^{2+}\)-Ca\(^{2+}\) permeability ratios of native L- and N-type channels from published studies show that these two currents do not differ significantly in their relative ability to conduct Ca\(^{2+}\) and Ba\(^{2+}\). The permeability ratio is roughly two- to threefold (Ba\(^{2+}\)/Ca\(^{2+}\)) in these channels (Fox et al. 1987; Kasai and Neher 1992; Smith et al. 1993). Moreover, the conductance dependence of native L- and N-type Ca\(^{2+}\) channels on the [divalent\(_{\text{cyt}}\)] sub saturating concentrations appear to be comparable and show a roughly twofold increase as the

**FIG. 10.** Dependence of the amplitude of BK and Ca\(^{2+}\) current on Ca\(^{2+}\) influx. A: titration curves for BK and Ca\(^{2+}\) current activation plotted against the external [Ca\(^{2+}\)] (0, 0.5, 1.0, 2.0, 4.0, and 8.0 mM). BK current was activated at +81 mV with a 5-ms Ca\(^{2+}\) influx step. Peak Ca\(^{2+}\) current was measured during a 10-ms depolarization to 0 mV. Currents were normalized for each cell to the 2 mM point and averaged. Each data point represents the average of 5–7 cells. The averaged data points were fit with a Hill equation (—). The fit for the Ca\(^{2+}\) current was constrained by making n = 1. Fit parameters were as follows: BK current I\(_{\text{max}}\) = 1.4; EC\(_{50}\) = 1.2 mM; n = 2.2; Ca\(^{2+}\) current: I\(_{\text{max}}\) = 2.65; EC\(_{50}\) = 3.28 mM. B: altering the Ca\(^{2+}\) current amplitude by nifedipine, Bay K, or high concentrations of 

- Aga IVA affects BK current activation to a much greater extent than altering the amplitude of the Ca\(^{2+}\) current by changing |Ca\(^{2+}\)|\(_{\text{cyt}}\). The data from A were redrawn here to plot the titration data for BK current activation against the titration data for Ca\(^{2+}\) current activation. The effects on Ca\(^{2+}\) and BK currents elicited by the various drugs were superimposed on this plot.
divalent concentration is increased from 2 to 10 mM (McNaughton and Randall 1997; Smith et al. 1993; Zhou and Jones 1995). Detailed information on permeation and the divalent concentration dependence of the current is unavailable for the P-, Q-, and R-type Ca$^{2+}$ channels. However, the Ba$^{2+}$-Ca$^{2+}$ ratio of the whole cell P-type current in Purkinje cells was reported to be $\approx 2$ (Regan 1991). Further, Bourinet et al. (1996) find that the Ba$^{2+}$-Ca$^{2+}$ conductance ratios of the cloned $\alpha$1A, $\alpha$1B, and $\alpha$1C channels, which may form the pore-forming subunits of the P–Q–, N–, and L-type Ca$^{2+}$ channels (De Waerdt et al. 1996), are $\approx 1.5, 1.5$, and 2, respectively. Their study also showed that the $[\text{Ca}^{2+}]_{\text{ext}}$ dependence of these channels is more or less similar. Collectively, these observations suggest that the relative amounts of influx through the L-, N-, P-, and Q-type Ca$^{2+}$ channels may be similar in 2 mM [Ca$^{2+}]_{\text{ext}}$ to that measured in 10 mM [Ba$^{2+}]_{\text{ext}}$. Although these are indirect arguments, there is no observation that requires us to reject this assumption.

Because blockade of L-type Ca$^{2+}$ channels had the greatest effect on BK current activation, we also directly examined the fraction of Ca$^{2+}$ current blocked by 5 $\mu$M nifedipine in 2 mM [Ca$^{2+}]_{\text{ext}}$. In two cells, $\approx 30\%$ of the Ca$^{2+}$ current in 2 mM [Ca$^{2+}]_{\text{ext}}$ was inhibited by nifedipine, which is virtually identical to the fraction of the DHP current in 10 mM Ba$^{2+}$ (Fig. 6). On the basis of the previous arguments, this result suggests that the proportion of the remaining Ca$^{2+}$ currents in 2 mM [Ca$^{2+}]_{\text{ext}}$ may also be comparable with our measurements in 10 mM [Ba$^{2+}]_{\text{ext}}$. However, irrespective of the precise proportion of influx contributed by the different Ca$^{2+}$ channels in 2 mM [Ca$^{2+}]_{\text{ext}}$, it does not affect the central conclusion that BK current activation is affected more significantly by the blockade of L- and Q-type Ca$^{2+}$ channels rather than N- and P-type Ca$^{2+}$ channels.

Ca$^{2+}$ currents in rat chromaffin cells

Our observations indicate that rat chromaffin cells express L-, N-, P-, and Q-type Ca$^{2+}$ channels. The presence of each of these currents is generally consistent with previous reports (Gandia et al. 1995; Hollins and Ikeda 1996). L-type current was the largest component ($\approx 33\%$) of total Ca$^{2+}$ current, whereas N-type current comprised $\approx 20–25\%$. Q-type current was found in $\approx 70\%$ of rat chromaffin cells, consistent with Gandia et al. (1995), but differing from Hollins and Ikeda (1996). P-type current appears to be a minor contributor to total $I_{\text{Ca}}$ in rat chromaffin cells.

We obtained no evidence of LVA current, contrasting with Hollins and Ikeda (1996), who found T-type current in $\approx 25\%$ of the cells they examined. It is possible that we did not sample enough cells to determine whether T-type currents are sometimes present. Alternatively, the Ca$^{2+}$ currents present in the different strains of rats used in the two studies may differ.

Activation of BK current by L- and Q-type Ca$^{2+}$ channels

Our results indicate that, although rat chromaffin cells express multiple types of HVA Ca$^{2+}$ channels, Ca$^{2+}$ influx through L- and Q-type channels dominates the activation of BK current. Although the precise physical mechanisms that mediate the specificity of this functional coupling will have to await future studies, in principle, it could arise if L- and Q-type Ca$^{2+}$ channels are located much closer to BK channels than N- or P-type Ca$^{2+}$ channels. Indeed the preferential targeting of ion channels to particular cellular locations was described in many cell types, and the underlying mechanisms may involve, at least in part, selective protein–protein interactions between ion channels and certain cytoskeletal proteins (Sheng and Wyszyinski 1997). Analogous mechanisms may be at work in implementing the functional specificity that we observe between the BK and Ca$^{2+}$ channels in rat chromaffin cells.

Selective functional association of Ca$^{2+}$-activated K$^{+}$ channels with particular Ca$^{2+}$ channel subtypes was described in several types of neurons, and the specificity of the coupling appears to differ among different cell types. For example, in chick ciliary ganglion neurons, BK current activation has been shown to occur via Ca$^{2+}$ influx through a L-type Ca$^{2+}$ current but not the N-type Ca$^{2+}$ current (Wisgirda and Dryer 1994). In contrast, in chick sympathetic ganglion neurons, the N-type current appears to dominate activation of BK current (Wisgirda and Dryer 1994). In rat superior cervical ganglion neurons, Ca$^{2+}$ influx through L-type channels preferentially activates a BK-type current, whereas influx through N-type Ca$^{2+}$ channels activates an SK-type current (Davies et al. 1996). In hippocampal neurons, BK channels are selectively coupled to N-type Ca$^{2+}$ channels, whereas SK channels are selectively coupled to L-type Ca$^{2+}$ channels (Marion and Tavalin 1998). Finally, in rat dorsal motor vagus nucleus neurons, N-type Ca$^{2+}$ channels are responsible for SK current activation, whereas BK current activation appears to be dependent on Ca$^{2+}$ influx through $\omega$-CgTx GVIA- and nifedipine-insensitive Ca$^{2+}$ channels (Sah 1995).

In neurons, the complement of Ca$^{2+}$ channels found in the various cellular compartments can be often markedly different. For example, N- and P-type Ca$^{2+}$ channels are primarily localized to the dendrites and synaptic terminals (Westenbroek et al. 1992), whereas L channels are primarily localized to the soma (Hell et al. 1993). Similar differences in Ca$^{2+}$ channel distributions occur in hippocampal neurons, in neurons of the cerebral cortex, and in the olfactory bulb neurons (Bischofberger and Schild 1995; Hell et al. 1993; Westenbroek et al. 1992). A consequence of this markedly different channel localization is that functional specificity in the association between Ca$^{2+}$ and Ca$^{2+}$-activated K$^{+}$ channels may result, at least in part, simply because of segregation of channels to different membrane compartments. In contrast, rat chromaffin cells are spherical cells without complex morphological features such as dendrites or axons. BK channels can be detected in virtually all patches pulled from chromaffin cells, suggesting that these channels are present, at least, to some degree in all parts of the cell membrane (unpublished observations). However, the failure of N- and P-type channels to activate BK current during single action potentials implies that the $[\text{Ca}^{2+}]_{\text{ext}}$ elevations resulting from influx through these channels can be exquisitely localized even in simple spherical cells. Chromaffin cells may thus prove to be of considerable benefit for questions addressing the molecular basis for how specificity in coupling between Ca$^{2+}$ channels and target proteins may occur.

Implications for cellular excitability

Ca$^{2+}$ channels are known to be targets of numerous kinases, phosphatases, and G-proteins that modulate their properties
and affect Ca\(^{2+}\) influx (Catterall 1997). Because BK current is the primary outward current involved in action potential repolarization in rat adrenal chromaffin cells (Solaro et al. 1995), an important implication of our results is that the properties of L- and Q-type Ca\(^{2+}\) channels might have important regulatory effects on action potential properties. Any second messengers that modulate the properties of L- and Q-type Ca\(^{2+}\) channels will also be able to dynamically regulate the extent of BK current activation and produce profound effects on cellular excitability. In fact, it is precisely because of the existence of selective coupling of particular Ca\(^{2+}\) channel variants to BK channel activation that modulation of Ca\(^{2+}\) current may be allowed to exert important effects on cellular excitability. If BK channel activation were not selectively coupled but influenced by the cumulative Ca\(^{2+}\) arising from all Ca\(^{2+}\) channel subtypes, regulation of any particular Ca\(^{2+}\) channel subtype would have only minimal consequences on both Ca\(^{2+}\) influx during action potentials and on any Ca\(^{2+}\)-dependent process. Thus selective coupling may provide a key mechanism that enables specific modulation of certain Ca\(^{2+}\) channel subtypes to exert more profound physiological consequences. The advantage of employing multiple Ca\(^{2+}\) channel variants, in this case the L- and Q-type, which may be differentially modulated by differing signaling cascades, would be an ability to regulate cellular excitability over a wide range of environmental conditions.

Are the Ca\(^{2+}\) channels involved in BK current activation the same as those involved in secretion?

L- and Q-type Ca\(^{2+}\) channels, which we show here to be involved in BK current activation, were also implicated in the control of exocytosis in bovine and cat chromaffin cells (Artalejo et al. 1994; Lopez et al. 1994a,b). This finding raises the interesting possibility that the Ca\(^{2+}\) channels involved in BK current activation may also be involved in the control of secretion. In fact, in the presynaptic nerve terminals of the frog neuromuscular junction and in auditory hair cells, it was reported that Ca\(^{2+}\) and Ca\(^{2+}\)-activated K\(^+\) channels are colocalized in such a way that the same Ca\(^{2+}\) signal that triggers transmitter release also activates BK channels (Roberts et al. 1990; Robitaille et al. 1993). Our finding that the Ca\(^{2+}\) channel subtypes involved in BK current activation may be the same as those implicated for catecholamine secretion raises the intriguing possibility that the Ca\(^{2+}\) channels might have important regulatory properties distinguishing three types of calcium currents in chick sensory neurones. J. Physiol. (Lond.) 394: 149–172, 1987.


