Ca\textsuperscript{2+} Current in Rabbit Carotid Body Glomus Cells Is Conducted by Multiple Types of High-Voltage–Activated Ca\textsuperscript{2+} Channels

JEFFREY L. OVERHOLT AND NANDURI R. PRABHAKAR

Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106-4970

Overholt, Jeffrey L. and Prabhakar, Nanduri R. \textit{Ca}\textsuperscript{2+} current in rabbit carotid body glomus cells is conducted by multiple types of high-voltage–activated \textit{Ca}\textsuperscript{2+} channels. J. Neurophysiol. 78: 2467–2474, 1997. Carotid bodies are sensory organs that detect changes in arterial oxygen. Glomus cells are presumed to be the initial sites for sensory transduction, and \textit{Ca}\textsuperscript{2+}-dependent neurotransmitter release from glomus cells is believed to be an obligatory step in this response. Some information exists on the \textit{Ca}\textsuperscript{2+} channels in rat glomus cells. However, relatively little is known about the types of \textit{Ca}\textsuperscript{2+} channels present in rabbit glomus cells, the species in which most of the neurotransmitter release studies have been performed. Therefore we tested the effect of specific \textit{Ca}\textsuperscript{2+} channel blockers on current recorded from freshly dissociated, adult rabbit carotid body glomus cells using the whole cell configuration of the patch-clamp technique. Macroscopic \textit{Ba}\textsuperscript{2+} current elicited from a holding potential of \textminus80 mV activated at a \textit{V}\textsubscript{m} of approximately \textminus30 mV, peaked between 0 and +10 mV and did not inactivate during 25-ms steps to positive test potentials. Prolonged (≈2 min) depolarized holding potentials inactivated the current with a \textit{V}_{1/2} of \textminus47 mV. There was no evidence for \textit{T}-type channels. On steps to 0 mV, 6 mM \textit{Ca}\textsuperscript{2+} decreased peak inward current by 97 ± 1% (mean ± SE). Nisoldipine (2 μM), 1 μM \textit{ω}-conotoxin GVIA, and 100 nM \textit{α}-agatoxin IVa each blocked a portion of the macroscopic \textit{Ca}\textsuperscript{2+} current (30 ± 5, 33 ± 5, and 19 ± 3% after rundown correction, respectively). Simultaneous application of these blockers revealed a resistant current that was not affected by 1 μM \textit{ω}-conotoxin MVIIIC. This resistant current constituted 27 ± 5% of the total macroscopic \textit{Ca}\textsuperscript{2+} current. Each blocker had an effect in every cell so tested. However, the relative proportion of current blocked varied from cell to cell. These results suggest that \textit{L}, \textit{N}, \textit{P}, and resistant channel types each conduct a significant proportion of the macroscopic \textit{Ca}\textsuperscript{2+} current in rabbit glomus cells. Hypoxia-induced neurotransmitter release from glomus cells may involve one or more of these channels.

INTRODUCTION

Carotid bodies are sensory organs that detect changes in arterial oxygen. Hypoxemia (low arterial O\textsubscript{2}) augments carotid body sensory activity, and the ensuing reflexes are vital for maintaining cardiorespiratory homeostasis during hypoxemia. Carotid body tissue is composed of neurotransmitter-enriched glomus (type I) cells and glial-like type 2 cells. Glomus cells, of neural crest origin, are believed to be the initial sites of sensory transduction. Glomus cells, like other excitable cells, express voltage-gated \textit{Ca}\textsuperscript{2+}, Na\textsuperscript{+}, and K\textsuperscript{+} channels that are important in chemotransduction. The mechanism of sensory transduction in the carotid body remains unknown. However, it is generally agreed that sensory transduction by the carotid body involves a hypoxia-induced increase in intracellular \textit{Ca}\textsuperscript{2+} in glomus cells. This leads to a \textit{Ca}\textsuperscript{2+}-dependent release of neurotransmitter(s) from glomus cells, which activates sensory fibers of the carotid sinus nerve. Several lines of evidence support the idea that extracellular \textit{Ca}\textsuperscript{2+} is involved in transduction of the hypoxic stimulus. Removal of extracellular \textit{Ca}\textsuperscript{2+} abolished or attenuated the sensory response to hypoxia (Shirahata and Fitzgerald 1991) and diminished the hypoxia-induced neurotransmitter release in in vitro preparations (Obeso et al. 1992) and isolated glomus cells (Montoro et al. 1996; Urena et al. 1994). These studies showed that hypoxia evokes a secretory response in glomus cells; extracellular \textit{Ca}\textsuperscript{2+} is required for this response and voltage-gated \textit{Ca}\textsuperscript{2+} channels are involved.

It is well-known that neuronal cells express multiple types of \textit{Ca}\textsuperscript{2+} channels in a single cell, which can be involved in neurotransmitter release (Dunlap et al. 1995). Traditionally, \textit{Ca}\textsuperscript{2+} currents have been categorized as either high- (HVA) or low- (LVA) voltage–activated. HVA and LVA currents can be distinguished readily using whole cell patch clamp based on their differing voltage dependencies. There are a number of distinct channels that underlie HVA currents. However, identifying specific channels underlying HVA conductance based on their biophysical characteristics can be misleading (Plummer et al. 1989). Generally, channels underlying HVA conductances are identified pharmacologically when using whole cell patch (e.g., Randall and Tsien 1995). Using specific toxins, HVA channels have been identified as \textit{L}, \textit{N}, \textit{P}/\textit{Q}, and \textit{R} type based on their sensitivity to dihydropyridines, \textit{ω}-conotoxin GVIA, and \textit{ω}-agatoxin IVa, respectively. \textit{ω}-Conotoxin MVIIIC, a relatively less specific toxin, also blocks \textit{N}- and \textit{P}/\textit{Q}-type channels. \textit{Q}-type channels, like \textit{P}-type, arise from expression of the class A α\textsubscript{1} subunit, but differ in their biophysical characteristics and pharmacological sensitivities (Sather et al. 1993). Other proposed types include dihydropyridine and toxin-resistant \textit{E}- and \textit{R}-type channels (for reviews see Dunlap et al. 1995; Olivera et al. 1994). \textit{E}-type channels have been cloned and expressed (Soong et al. 1993), but the diversity of \textit{R}-type currents identified suggests that they may arise from a heterogeneous group of channels.

Electrophysiological studies of glomus cells have been done primarily in rabbit and rat. These studies have identified differences in the population of channels expressed in rabbit and rat carotid body glomus cells. Previous studies have reported that both rabbit and rat glomus cells exhibit HVA, but not LVA \textit{Ca}\textsuperscript{2+} current (e.g., Urena et al. 1989; e Silva et al. 1995) respectively. It is generally agreed that HVA \textit{Ca}\textsuperscript{2+} current is sensitive to dihydropyridines in both rabbit (Obeso et al. 1992) and rat (e Silva and Lewis 1995; Fieber...
RESULTS

Our results suggest that there are differences in the population of Ca\(^{2+}\) channels that underlie the macroscopic Ca\(^{2+}\) current in rabbit and rat glomus cells. In rabbit glomus cells, Ca\(^{2+}\) current is conducted by L-, N-, and P/Q-type channels. In addition, a significant proportion of the current is conducted by a dihydropyridine-insensitive, toxin-resistant channel. These results provide information necessary to determine the involvement of membrane Ca\(^{2+}\) channels in carotid body glomus cell function and neurotransmitter release. Preliminary results from this study have been reported previously (Overholt and Prabhakar 1996a,b, 1997).

METHODS

Experiments were performed on glomus cells freshly isolated from carotid bodies of adult male rabbits killed with CO\(_2\). Individual glomus cells were dissociated enzymatically from carotid bodies using the method of Gomez-Nino et al. (1994). Briefly, cells were incubated at 37°C in a solution that contained trypsin (type II, 2 mg/ml), collagenase (type IV, 2 mg/ml), and DNase (0.5 mg/ml) and was composed of (in mM) 140 NaCl, 5 KCl, 10 N-2hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 5 glucose, pH 7.2. Single cells were obtained by gentle triturating during the incubation. After the incubation, cells were maintained at 37°C in a CO\(_2\) incubator in medium composed of a 50/50 mixture of Dulbecco’s modified Eagle’s medium (DMEM) and HAM F12 supplemented with antibiotics and 10% fetal bovine serum. Experiments were performed at room temperature, and cells were used within 24 h.

Membrane Ca\(^{2+}\) current was monitored using the whole cell configuration of the patch-clamp technique (Hamill et al. 1981). Pipettes were made from borosilicate glass capillary tubing and had resistances of 4–5 M\(\Omega\). Currents were recorded using an Axopatch 200A voltage-clamp amplifier, filtered at 5 kHz, and sampled at a frequency of 10 kHz using an IBM compatible computer with a Digidata 1200 interface and pClamp software (Axon Instruments). Currents were not leak subtracted. Current-voltage (I-V) relations were elicited from a holding potential of −80 mV using 25-ms steps (5 s between steps) to test potentials over the range of −50 to + 70 mV in 10-mV increments. Current at each potential was measured as the average over a 2.5-ms span at the end of the 25-ms step. Current rundown and drug effects were monitored using a wash protocol (25-ms step to 0 mV, 10 s between steps). The effects of channel-blocking agents were compensated for rundown using a linear regression of the current decrease during the wash protocol in the absence of test compounds. Rundown was negligible compared with drug effects over the same time period (e.g., 0.6 ± 0.3% per minute, mean ± SE, \(n = 15\), for the AGA + GVIA + NISO group). Cells in which rundown was excessive or did not appear linear were not used in this study.

For comparison, current at each potential was normalized to the maximum value recorded during the control I-V relation in individual cells (usually 0 mV). All values are reported as mean ± SE.

Ca\(^{2+}\) current was isolated by using K\(^+\) and Na\(^+\)-free intra- and extracellular solutions of the following compositions (in mM): 130 CsCl, 20 tetraethylammonium chloride (TEA-Cl), 5 MgATP, 0.1 tris(hydroxymethyl)aminomethane guanosine triphosphate (TrisGTP), 5 ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), and 5 HEPES, pH adjusted to 7.2 with CsOH; 140 NMCl, 5.4 CsCl, 10 BaCl\(_2\), 10 HEPES, and 11 glucose, pH adjusted to 7.4 with CsOH, respectively. In some experiments, 115 mM CsCl and 10 mM phosphocreatine was used in place of the 130 mM CsCl in the intracellular solution. The extracellular solution was changed using a fast-flow apparatus consisting of a linear array of borosilicate glass tubes (Overholt et al. 1995). In these experiments Ba\(^{2+}\) was the charge carrier. For simplicity, Ba\(^{2+}\) current conducted by Ca\(^{2+}\) channels will be referred to as Ca\(^{2+}\) current. Stock solutions of channel-blocking agents were prepared in water (ω-conotoxin GVIA and ω-conotoxin MVIIIC, Alamone Labs; and agatoxin IVa, gift of Dr. Nicolas Sarcomano, Pfizer Laboratories), or PEG-400 (NISO, Miles Laboratories) and diluted 1:1000 in the extracellular solution. Solutions containing agatoxin IVa contained 0.1% cytochrome C.

RESULTS

Freshly dissociated cultures of carotid body cells contain glomus and type 2 cells. Rabbit carotid body glomus cells, like other neurons, are excitable and reportedly exhibit Ca\(^{2+}\), K\(^+\), and Na\(^+\) current, whereas type 2 cells exhibit only a small outward K\(^+\) current (Urena et al. 1989). These characteristics were used to identify glomus cells used in this study (i.e., the presence of Ca\(^{2+}\) current indicated a glomus cell). Figure 1A shows an example of membrane currents recorded in an isolated glomus cell under physiological conditions (see figure legend for solution composition). The membrane potential was stepped from the holding potential of −80 to test potentials from −50 to +70 mV in 10-mV increments. Under these conditions, two separate components are apparent in the raw, whole cell current: a large, rapidly inactivating, inward Na\(^+\) current; and a large, sustained, outward K\(^+\) current. Figure 1B shows that a relatively smaller, inward Ca\(^{2+}\) current is revealed when recording the membrane current under conditions designed to isolate Ca\(^{2+}\) current (Na\(^+\)- and K\(^+\)-free intra- and extracellular solutions, see METHODS for composition of solutions). The characteristics of this current shown in Fig. 1A, 1B, and C suggest that it is carried by HVA type(s) of channels. These factors prompted us to identify the type(s) of channels underlying the macroscopic Ca\(^{2+}\) current in rabbit glomus cells.
To identify type(s) of channels underlying the macroscopic current, we tested the effects of more specific Ca\(^{2+}\) channel blockers on the macroscopic current. Previous reports have suggested that the current is carried by L-type channels. To confirm this, we tested the effect of the dihydro-erythronate, NISO, on the current. NISO (2 \(\mu\)M) completely blocks HVA Ca\(^{2+}\) current in cardiac myocytes (i.e., Overholt et al. 1993). To confirm that this concentration is maximal in carotid body glomus cells, we tested the effect of three concentrations of NISO on the macroscopic Ca\(^{2+}\) current. Figure 3A shows that increasing the concentration of NISO from 0.2 to 2 \(\mu\)M further attenuated the current recorded at 0 mV. However, increasing the concentration to 20 \(\mu\)M resulted in no further increase in block. Therefore in the following experiments 2 \(\mu\)M NISO was used to decrease the chance of nonspecific effects. Figure 3B shows the time course for changes in current elicited at 0 mV as the extracellular solution is changed to one containing NISO. Onset of block by NISO was rapid and stabilized within 1–2 min. The effect of a 5-min exposure to NISO on average, normalized I-V relations pooled from 11 cells is shown in Fig. 3B. NISO blocked a similar proportion of the current at the membrane potentials tested, and the effect of NISO was reversible (data not shown). Macroscopic Ca\(^{2+}\) current was decreased by NISO in all cells tested. However, the amount of current

potential. Figure 2A (left panel) shows an example of the current elicited by 25-ms steps to 0 mV (wash protocol) as the holding potential is changed from −90 to 0 mV in 30-mV increments. Current slowly decreases over ≈1 min and then stabilizes. The right panel in Fig. 2A plots the relative current at 0 mV as a function of membrane holding potential. The data are fairly well fit by a single Boltzman

\[ I_n = \frac{I_{\text{max}}}{1 + \exp \left( \frac{V - V_{1/2}}{k} \right)} \]

where \(I_n = I/I_{\text{max}}\), \(V\) is the holding potential, \(V_{1/2}\) is the potential at which the channels are half inactivated, and \(k\) is the slope factor. The solid line shows the fit with \(V_{1/2} = -47.1\) and \(k = 12.8\) mV. However, the deviations in the fit by a single Boltzman suggest that the current may be carried by more than one channel. Figure 2B shows that the current is nearly completely blocked by 6 mM Co\(^{2+}\), a nonspecific Ca\(^{2+}\) channel blocker. The left panel shows raw, whole cell current recorded at 0 mV in the presence and absence of Co\(^{2+}\). The right panel shows average, normalized I-V relations in the presence and absence of Co\(^{2+}\) in data pooled from nine cells. Block by Co\(^{2+}\) further confirms that the macroscopic current is carried by Ca\(^{2+}\) channels.

**FIG. 1.** Whole cell current from rabbit carotid body glomus cells is composed of at least 3 components. A: raw, whole cell current from a freshly dissociated rabbit carotid body glomus cell showing Na\(^{+}\) and K\(^{+}\) current. The intracellular and extracellular solutions contained (in mM) 120 K-glutamate, 20 KCl, 5 MgATP, 0.1 tris(hydroxymethyl)aminomethane gluconate triphosphate (TrisGTP), 5 ethylene glycol-bis(\(\beta\)-aminoethyl ether)-N,N′,N′,N′-tetraacetic acid (EGTA), and 5 N-2-hydroxyethylpiperazine-N′,2-ethanesulfonic acid (HEPES); 140 NaCl, 5.4 KCl, 2.5 CaCl\(_2\), 0.5 MgCl\(_2\), 5.5 HEPES, and 11 glucose, respectively. The membrane potential was stepped from −80 mV to test potentials from −50 to +70 mV for 75 ms. B: raw, whole cell currents from a freshly dissociated rabbit carotid body glomus cell under conditions designed to isolate Ca\(^{2+}\) current (see METHODS). Protocol similar to A, but 25-ms steps. Macroscopic Ca\(^{2+}\) current in glomus cells of the rabbit carotid body is noninactivating, high-voltage–activated (HVA) Ca\(^{2+}\) current (note absence of rapidly inactivating, inward current). C: current-voltage (I-V) relation derived from B (at time indicated by solid line).

**FIG. 2.** A: effect of holding potential on the macroscopic Ca\(^{2+}\) current. Left panel: time course for changes (slow inactivation) in the current elicited at 0 mV as the holding potential is changed from −90 to −60, −30, and 0 mV. Right panel: steady-state inactivation curve. Data points are average data pooled from 5 or 6 cells (except −40, \(n = 2\)), and solid line is the fit of a simple Boltzman: \(I_n = \frac{I_{\text{max}}}{1 + \exp \left( \frac{V - V_{1/2}}{k} \right)}\), where \(I_n = I/I_{\text{max}}\). V is the holding potential, \(V_{1/2}\) is the potential at which the channels are half inactivated, and \(k\) is the slope factor. Solid line shows the fit with \(V_{1/2} = -47.1\) and \(k = 12.8\) mV. B: block of macroscopic Ca\(^{2+}\) current by application of 6 mM CoCl\(_2\) in the extracellular solution. Left panel: raw currents elicited by a 25-ms step to 0 mV in the presence and absence of 6 mM CoCl\(_2\). Right panel: average (\(n = 9\), normalized I-V relations in the presence and absence of CoCl\(_2\).
Figure 3. Effect of nisoldipine (NISO) on whole cell Ca$^{2+}$ current. A: raw, whole cell currents elicited by a 25-ms step from \(-80\) to 0 mV before and after a 5-min exposure to 0.2, 2, or 20 \(\mu\)M NISO in the extracellular solution. B: time course for changes in \(\text{Ca}^{2+}\) current elicited at 0 mV during wash in of 2 \(\mu\)M NISO. C: average (\(n=11\)), normalized I-V relations recorded before and after a 5 min exposure to 2 \(\mu\)M NISO in the extracellular solution. Currents are corrected for rundown and normalized to the maximum (usually 0 mV), inward control current recorded in individual cells.

blocked by NISO at 0 mV varied from 11 to 65% in the 11 cells studied (average of 30 \(\pm\) 5%). These results suggest that part of the whole cell \(\text{Ca}^{2+}\) current in rabbit glomus cells is conducted by L-type \(\text{Ca}^{2+}\) channels, but the relative proportion of L-type channels may vary from cell to cell.

To further elucidate the channels underlying the macroscopic \(\text{Ca}^{2+}\) current in rabbit carotid body glomus cells, we tested the effects of 1 \(\mu\)M \(\omega\)-conotoxin GVIA (GVIA) and 100 nM \(\omega\)-agatoxin IVa (AGA) on this current. These concentrations have been shown to be maximally effective for N- and P/Q-type \(\text{Ca}^{2+}\) channels in other mammalian neurons (Boland et al. 1994 and Mintz et al. 1992, respectively). Figure 4A shows the time course for changes in current elicited at 0 mV as the extracellular solution is changed to one containing GVIA (left panel) or AGA (right panel). Onset of block by AGA required a longer exposure than GVIA or NISO and required 3–5 min to stabilize. Figure 4B shows the effect of a 5-min exposure to GVIA (left panel) and AGA (right panel) on average, normalized I-V relations pooled from 8 and 7 cells, respectively. GVIA and AGA, specific blockers of N- and P/Q-type \(\text{Ca}^{2+}\) channels, respectively, also partially attenuated the macroscopic \(\text{Ca}^{2+}\) current in rabbit glomus cells. Like NISO, GVIA and AGA blocked a portion of the current in all cells tested (average of 33 \(\pm\) 5% and 19 \(\pm\) 3% at 0 mV, respectively), and the degree of block was variable. Block by GVIA and AGA varied from 11 to 51% and 5 to 26%, respectively in the cells tested. However, unlike NISO block, block by GVIA and AGA was irreversible (data not shown) and was greater with increasing membrane depolarization. These results suggest that part of the whole cell \(\text{Ca}^{2+}\) current in rabbit glomus cells is also conducted by N- and P/Q-type \(\text{Ca}^{2+}\) channels, and that the relative proportion of N- and P/Q-type channels varies from cell to cell.

Addition of the effects of the individual blockers (\(\approx 81\%\) at 0 mV) suggests that a fourth, resistant (non-L, non-N, non-P/Q) channel(s) type is also conducting current. This was tested by simultaneous application of NISO, GVIA, and AGA (Fig. 5). Figure 5A shows raw currents before (left panel) and 5 min after (middle panel) simultaneous application of these three blockers. Simultaneous application of NISO, GVIA, and AGA attenuated, but did not completely block the macroscopic \(\text{Ca}^{2+}\) current. The remaining current could still be blocked by the relatively nonspecific, inorganic \(\text{Ca}^{2+}\) channel blocker Co\(^{2+}\) (right panel). Figure 5B shows the effect of simultaneous application of NISO, GVIA, and AGA on average, normalized I-V relations pooled from 15 cells. The characteristics of the resistant current are consistent with that conducted by an HVA type of \(\text{Ca}^{2+}\) channel (i.e., noninactivating, activated at relatively depolarized membrane potentials, blocked by Co\(^{2+}\)). This supports the idea that \(\text{Ca}^{2+}\) current is also conducted by a non-L, non-N, non-P/Q type of HVA \(\text{Ca}^{2+}\) channel(s). Further, this resistant channel
carries a significant proportion of the current (21–67% of the macroscopic current remained in the presence of NISO, GVIA, and AGA).

\( \omega \)-Conotoxin MVIIC (MVIIC) is a less specific toxin that blocks N- and P/Q-type channels. To test the possibility that the resistant current is due to incomplete block of N- or P/Q-type channels, we applied 1 \( \mu \)M MVIIC in the presence of NISO, GVIA, and AGA. Figure 6A shows the time course for current elicited at 0 mV as extracellular solutions containing AGA, GVIA, and NISO; AGA, GVIA, NISO, and MVIIC; and CoCl\(_2\) are washed in consecutively. Figure 6B compares the block of the current before and 5 min after addition of MVIIC at 0 mV in data pooled from seven cells. Although MVIIC slightly increased block at more positive potentials (data not shown), Fig. 6 shows that MVIIC had relatively little effect on the resistant current. This supports the idea that the concentrations of blockers used in this study are maximally effective concentrations for blocking L-, N-, and P/Q-type current in rabbit carotid body glomus cells. Most importantly, a significant proportion (27 ± 5%) of the current still remained.

Figure 7A compares the individual block by NISO, GVIA, or AGA at 0 mV with the current remaining in the combined presence of NISO, GVIA, AGA, and MVIIC. These values correspond to the proportion of the macroscopic Ca\(^{2+}\) current conducted by L, N, P/Q, and resistant channels. Although there are no statistically significant differences, this figure suggests that N, L, and resistant type channels conduct the largest percentage of the macroscopic Ca\(^{2+}\) current. Figure 7B further characterizes the currents attributable to L, N, P/Q, and resistant currents in rabbit glomus cells. This figure shows I-V relations derived by subtracting currents in the presence of individual blockers (NISO, GVIA, or AGA) from control currents. These difference currents should represent the current attributable to the blocked channel type. These currents are also compared with the resistant current (current remaining in the presence of NISO, GVIA, AGA, and MVIIC). The shape of the I-V relations is similar; however, they are shifted slightly along the voltage axis. The resistant current would predominate with smaller depolarizations, whereas L, N, and P/Q currents would be recruited with further depolarization. Taken together, the above data suggest that Ca\(^{2+}\) current is conducted by at least four types of HVA Ca\(^{2+}\) channels in rabbit carotid body glomus cells, each with distinctive characteristics. Moreover, each type appears to conduct a significant portion of the current.

**DISCUSSION**

It is noteworthy that there were detectable L, N, P/Q, and resistant currents in every cell so tested. This suggests that macroscopic Ca\(^{2+}\) current in rabbit carotid body glomus cells...
is more complex than in neonatal (Peers et al. 1996) or adult rats (Silva and Lewis 1995; Fieber and McCleskey 1993). In neonatal rats, GVIA had effects in some cells but was without effect in the majority of cells (Peers et al. 1996). In adult rats there are two conflicting reports. GVIA did not affect macroscopic Ca$^{2+}$ current in one study (Fieber and McCleskey 1993), but it partially blocked this current in all cells tested in another (Silva and Lewis 1995). In addition, the study by Silva et al. (1995) identified an inactivating resistant current but found no evidence for P or Q type channels. In their study, they suggest that the resistant current may be due to incomplete block of L-type channels by nimodipine. However, the results of the present study suggest that the resistant current in rabbit glomus cells does not arise from incomplete block of L-type channels. The current was not affected by higher concentrations of NISO (Fig. 3A), and the voltage dependence of the I-V relation of the difference current was distinct from that attributable to L-type current (Fig. 7B). The differences between rabbits and rats are most likely due to species differences, because there are other differences in the populations of channels found in rabbit and rat carotid body glomus cells. For example, rabbit (Urena et al. 1989), but not rat (Fieber and McCleskey 1993) carotid body glomus cells reportedly have Na$^+$ current and can fire Na$^+$-dependent action potentials (Duchen et al. 1988). The existence of P and noninactivating resistant Ca$^{2+}$ currents in rabbit carotid body glomus cells may reflect further differences in the channel populations found in rat and rabbit carotid bodies.

It is also noteworthy that the proportion of current blocked by the drugs tested varied in individual cells. Similar variable effects of nifedipine and GVIA have been reported in neonatal rat carotid body glomus cells (Peers et al. 1996). This suggests that the relative proportion of different HVA Ca$^{2+}$ channel types may vary in individual cells. This could explain the results of a recent study in our laboratory that showed that hypoxia increases intracellular Ca$^{2+}$ in a cell-specific manner in carotid body glomus cells (Bright et al. 1996). The response patterns were heterogeneous in individual cells with respect to the kinetics of the changes in intracellular Ca$^{2+}$ in response to hypoxia. This variation is also important with respect to regulation of Ca$^{2+}$ current. It is well-known that neurotransmitters and neuromodulators can affect specific Ca$^{2+}$ channels. This variation would therefore allow differential regulation of Ca$^{2+}$ influx in specific carotid body glomus cells. Further, the resting membrane potential reported in carotid body glomus cells of approximately −40 mV lies on the steep part of the steady-state inactivation curve near the $V_{1/2}$ (Fig. 2A). This would produce the maximum effect from a neurotransmitter or neuromodulator that affects the activation curve for Ca$^{2+}$ current, such as G proteins (Jones and Elmslie 1997). It is too early to say whether this heterogeneity reflects a functional significance, yet it is interesting to speculate that this might be the case.

One advantage of using whole cell patch is that one can determine the relative contributions of the underlying channels to the macroscopic current. As defined by individual block by NISO, GVIA, and AGA, our results suggest that L, N, P/Q, and resistant type channels.

FIG. 7. Comparison and characteristics of current carried by the different channels identified in rabbit carotid body glomus cells. A: relative proportion of the macroscopic Ca$^{2+}$ current blocked at 0 mV by 2 μM NISO (n = 11), 1 μM GVIA (n = 8), or 100 nM AGA (n = 7) and the proportion of current remaining in the presence of these 3 blockers and 1 μM MVIC (n = 7). B: I-V relations for difference currents derived by subtracting currents recorded in the presence of NISO, GVIA, or AGA from control currents, and the current remaining in the presence of these 3 blockers and MVIC. These I-V relations represent currents conducted by L, N, P/Q, and resistant type channels.

The existence of P and noninactivating resistant Ca$^{2+}$ currents in rabbit carotid body glomus cells may reflect further differences in the channel populations found in rat and rabbit carotid bodies.

It is also noteworthy that the proportion of current blocked by the drugs tested varied in individual cells. Similar variable effects of nifedipine and GVIA have been reported in neonatal rat carotid body glomus cells (Peers et al. 1996). This suggests that the relative proportion of different HVA Ca$^{2+}$ channel types may vary in individual cells. This could explain the results of a recent study in our laboratory that showed that hypoxia increases intracellular Ca$^{2+}$ in a cell-specific manner in carotid body glomus cells (Bright et al. 1996). The response patterns were heterogeneous in individual cells with respect to the kinetics of the changes in intracellular Ca$^{2+}$ in response to hypoxia. This variation is also important with respect to regulation of Ca$^{2+}$ current. It is well-known that neurotransmitters and neuromodulators can affect specific Ca$^{2+}$ channels. This variation would therefore allow differential regulation of Ca$^{2+}$ influx in specific carotid body glomus cells. Further, the resting membrane potential reported in carotid body glomus cells of approximately −40 mV lies on the steep part of the steady-state inactivation curve near the $V_{1/2}$ (Fig. 2A). This would produce the maximum effect from a neurotransmitter or neuromodulator that affects the activation curve for Ca$^{2+}$ current, such as G proteins (Jones and Elmslie 1997). It is too early to say whether this heterogeneity reflects a functional significance, yet it is interesting to speculate that this might be the case.

One advantage of using whole cell patch is that one can determine the relative contributions of the underlying channels to the macroscopic current. As defined by individual block by NISO, GVIA, and AGA, our results suggest that L, N, P/Q, and resistant type channels. In their study, they suggest that the resistant current may be due to incomplete block of L-type channels by nimodipine. However, the results of the present study suggest that the resistant current in rabbit glomus cells does not arise from incomplete block of L-type channels. The current was not affected by higher concentrations of NISO (Fig. 3A), and the voltage dependence of the I-V relation of the difference current was distinct from that attributable to L-type current (Fig. 7B). The differences between rabbits and rats are most likely due to species differences, because there are other differences in the populations of channels found in rabbit and rat carotid body glomus cells. For example, rabbit (Urena et al. 1989), but not rat (Fieber and McCleskey 1993) carotid body glomus cells reportedly have Na$^+$ current and can fire Na$^+$-dependent action potentials (Duchen et al. 1988). The existence of P and noninactivating resistant Ca$^{2+}$ currents in rabbit carotid body glomus cells may reflect further differences in the channel populations found in rat and rabbit carotid bodies.

It is also noteworthy that the proportion of current blocked by the drugs tested varied in individual cells. Similar variable effects of nifedipine and GVIA have been reported in neonatal rat carotid body glomus cells (Peers et al. 1996). This suggests that the relative proportion of different HVA Ca$^{2+}$ channel types may vary in individual cells. This could explain the results of a recent study in our laboratory that showed that hypoxia increases intracellular Ca$^{2+}$ in a cell-specific manner in carotid body glomus cells (Bright et al. 1996). The response patterns were heterogeneous in individual cells with respect to the kinetics of the changes in intracellular Ca$^{2+}$ in response to hypoxia. This variation is also important with respect to regulation of Ca$^{2+}$ current. It is well-known that neurotransmitters and neuromodulators can affect specific Ca$^{2+}$ channels. This variation would therefore allow differential regulation of Ca$^{2+}$ influx in specific carotid body glomus cells. Further, the resting membrane potential reported in carotid body glomus cells of approximately −40 mV lies on the steep part of the steady-state inactivation curve near the $V_{1/2}$ (Fig. 2A). This would produce the maximum effect from a neurotransmitter or neuromodulator that affects the activation curve for Ca$^{2+}$ current, such as G proteins (Jones and Elmslie 1997). It is too early to say whether this heterogeneity reflects a functional significance, yet it is interesting to speculate that this might be the case.

One advantage of using whole cell patch is that one can determine the relative contributions of the underlying channels to the macroscopic current. As defined by individual block by NISO, GVIA, and AGA, our results suggest that L, N, and P/Q type channels contribute, on average, ≈30, 33, and 19% of the total macroscopic Ca$^{2+}$ current, respectively, at 0 mV (Fig. 7). In addition, elimination of L, N, and P/Q-type currents revealed a current that constitutes, on average, 27% of the total current (Fig. 5). It should be noted that addition of these four components yields a value >100%. This is at least in part due to the wide range of effects of these drugs in individual cells. Also, we cannot rule out the possibility that these drugs are not perfectly selective and there is some overlap in the types of channels blocked. For instance, it has been reported that high concentrations of GVIA (10−15 μM) can block the class D $\alpha_1$ subunit, one of the $\alpha$-subunits that forms dihydropyridine-sensitive L-type channels (Williams et al. 1992). If rabbit carotid body glomus cells express the class D $\alpha_1$ subunit, the relative contribution of N-type channels may be overestimated. This is supported by the finding that block by NISO appeared to be relatively less when applied after GVIA (data not shown). However, the most likely conclusion is that L, N, P, and resistant channels each conduct a significant proportion of the macroscopic current.

What is the nature of the resistant current? One possibility is that this current is conducted by L, N, P, or Q type channels that are incompletely blocked. However, our data indicate otherwise. First, our data suggest that L-type currents are completely blocked, because increasing the concentration 10-fold (2−20 μM) over that used in these experiments did not further attenuate the current (Fig. 3). Second, we
used concentrations of GVIA and AGA shown to be maximally effective in other neurons for N- and P-type channels (Boland et al. 1994; Mintz et al. 1992). Furthermore, application of MVIIC (which blocks N-, P-, and Q-type currents) in the presence of GVIA and AGA did not further block the resistant current (Fig. 6). Q-type channels are 10-fold more sensitive to MVIIC than P-type (Sather et al. 1993). This suggests that N-, P-, and Q-type currents were completely blocked by GVIA and AGA. Additionally, the voltage dependence of the I-V relation for the resistant current was distinct from those attributable to the other channel types (Fig. 7B). These findings indicate that the resistant current is not conducted by one of these known dihydropyridine- or toxin-sensitive channels.

The existence of currents resistant to dihydropyridines, GVIA, AGA, and MVIIC is not without precedent in neuronal cells and may be conducted by a heterogenous group of channels. A resistant current, termed R type, was identified in rat cerebellar granule cell neurons (Randall and Tsien 1995), which resembles current generated by the E class of α1 subunit (Soong et al. 1993). However, the resistant current in rabbit carotid body glomus cells shown in Fig. 5A (middle) differs in that it does not exhibit rapid (ms time scale) inactivation. This suggests that the resistant current in rabbit carotid body glomus cells is not conducted by a channel similar to the E class. However, the resistant current in rabbit carotid body glomus cells is similar to a noninactivating, dihydropyridine-, GVIA-, and AGA-resistant current identified in other neurons (Mintz et al. 1992). Taken together, these results suggest that carotid body glomus cells may express a unique, noninactivating, HVA Ca2+ channel that is resistant to NISO, GVIA, AGA, and MVIIC. This channel could be formed by an as yet unidentified class of α1 subunit or a unique arrangement of previously identified subunits.

Some studies have suggested that L-type current is involved in hypoxia-induced NT release from glomus cells (Gomez-Nino et al. 1994; Obeso et al. 1992). The results of the present study suggest that N, P/Q, and resistant (non-L, non-N, non-P/Q) Ca2+ channels are also present in rabbit carotid body glomus cells. It is generally believed that N- and P/Q, but not L-type Ca2+ channels are involved in neurotransmitter release in neurons (Dunlap et al. 1995). In chromaffin cells, however, the relative contribution of L-type channels to catecholamine release is much greater than their contribution to the macroscopic Ca2+ current (Artalejo et al. 1994). Given these caveats, it would be misleading to speculate as to the relative contribution of these other channel types to neurotransmitter release based on their relative contribution to the macroscopic current. Further studies are required to determine which of these channel(s) are involved in neurotransmitter release and their roles in glomus cell function. The results of the present study can serve as a basis from which to begin these studies.

In summary, the present study is the first to provide substantial evidence for the involvement of more than one Ca2+ channel type in conducting the macroscopic Ca2+ current in rabbit carotid body glomus cells. The results suggest that the macroscopic Ca2+ current in rabbit carotid body glomus cells is more complex than in rat glomus cells and is conducted by L-, N-, and P/Q-type channels. A significant proportion of the current is also conducted by a unique, noninactivating, dihydropyridine- and toxin-resistant channel. The data also indicate that, although all cells can conduct each type of current, the relative proportion of different channel types varies from cell to cell.

The authors thank Drs. S. W. Jones and R. D. Harvey for many helpful discussions during this study and in preparation of the manuscript. This work was supported by National Heart, Lung, and Blood Institute Grants HL-07288 and HL-25830. Address reprint requests to J. L. Overholt.

Received 28 March 1997; accepted in final form 5 June 1997.

REFERENCES


