HERG-Like Potassium Current Regulates the Resting Membrane Potential in Glomus Cells of the Rabbit Carotid Body

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INTRODUCTION

Carotid bodies are sensory organs that regulate respiratory responses to alterations in arterial blood oxygen. Hypoxemia (low arterial O2) augments the sensory discharge of the carotid bodies, and the sensory information is conveyed via the carotid sinus nerve to respiratory neurons in the brain stem. Reflexes arising from the carotid bodies are important for maintaining homeostasis during hypoxemia that occur in many physiological situations including sojourn at high altitude, and in pathophysiological conditions such as sudden infant death syndrome (SIDS) (Perrin et al. 1984). Morphologically the carotid body is composed of neurotransmitter-enriched glomus (type I) cells and glial-like type II cells. There is much evidence that the glomus cells are the initial sites of sensory transduction. It has been proposed that hypoxia causes membrane depolarization in glomus cells causing influx of Ca2+ through voltage-gated Ca2+ channels, leading to release of neurotransmitter(s) that act on apposing afferent nerve terminals to increase sensory discharge in the carotid sinus nerve (for reviews see Fidone et al. 1990; Gonzalez et al. 1994; Prabhakar 1994).

The cellular basis for the initial depolarization of glomus cells during hypoxia remains poorly understood. Several studies have reported that hypoxia inhibits outward K+ currents in glomus cells. Therefore it was proposed that the hypoxia-sensitive K+ channels contribute to the initial depolarization that is essential for the transduction of the hypoxic stimulus (Lopez Barneo et al. 1988; Wyatt et al. 1995). However, recent studies have questioned whether inhibition of these K+ channels is central to the transduction process at the carotid body. First, these channels are not active at the reported resting membrane potentials of glomus cells (Gonzalez et al. 1994). Second, known blockers of these K+ channels had no effect on basal or hypoxia stimulated sensory activity of the carotid body or on intracellular Ca2+ ([Ca2+]i) in isolated glomus cells (Buckler 1997; Cheng and Donnelly 1995; Lahiri et al. 1998; however, see Wyatt et al. 1995). More recently, Buckler (1997) identified a K+ -selective “leak” conductance that is sensitive to hypoxia. However, its role in transduction remains elusive, because there is no known selective pharmacological blocker of this conductance. Consequently, which K+ current regulates the resting membrane potential and the role of K+ channels in the transduction process of the hypoxic stimulus remain uncertain. It follows that identification of specific channel(s) active at the resting membrane potential is of seminal importance to understand the basis for hypoxic depolarization in glomus cells.

Inwardly rectifying K+ channels contribute to the resting membrane potential in many different cell types. One such channel is the protein encoded by the human ether-a-go-go–related gene (HERG). HERG K+ channels were originally identified as molecular targets for mutations underlying one form of the long QT syndrome, a genetic disease with delayed cardiac repolarization (Curran et al. 1995). The main features of HERG current are a peculiar inward rectification mechanism and its unique sensitivity to methanesulfonanilide drugs such as E-4031 and dofetilide (Ficker et al. 1998; Jurkiewicz and...
Sanguinetti 1993; Smith et al. 1996; Snyders and Chaudhary 1996; Trudeau et al. 1995). HERG-like channels have also been identified in cells of neural crest origin, such as neuroblastoma cell lines, PC12 cells, and quail neural crest cells, where they regulate the resting membrane potential (Arcangeli et al. 1995, 1997; Bianchi et al. 1998; Shi et al. 1997). The goal of the present study was to determine whether carotid body glomus cells express HERG-like K+ current, and if so, to determine whether HERG-like currents regulate the resting membrane potential. However, identification of HERG-like currents in glomus cells is difficult due to the presence of a large outward K+ current. Therefore we took advantage of the unique characteristics of HERG channels to isolate the HERG-like current. If HERG current is present, stepping to hyperpolarized potentials from a holding potential of 0 mV (to inactivate the outward K+ current) should give rise to a rapidly activating (actually removal of inactivation) inward K+ current that decays in a time- and voltage-dependent manner as the channels close. This process forms a “nose” in the tail current that is characteristic of HERG current and should be blocked by dofetilide.

Based on the criteria above, our results show that glomus cells of the rabbit carotid body express HERG-like K+ current. Most importantly, the characteristics of this HERG-like current suggest that it is active at the resting membrane potential. Furthermore, block of HERG-like current depolarizes glomus cells, increases [Ca2+], and augments sensory activity under normoxia in an in-vitro carotid body preparation. This study is the first to show the molecular basis for a K+ current active around the resting membrane potential that is directly involved in controlling the resting membrane potential in glomus cells of the carotid body. Preliminary results from this study have been reported previously (Overholt et al. 1999).

**METHODS**

**Isolation of rabbit carotid body cells**

Carotid body cells were acutely isolated from adult male rabbits as described previously (Overholt and Prabhakar 1997). Dissociated cells were maintained at 37°C in a CO2 incubator in medium composed of a 50/50 mixture of Dulbecco’s modified Eagle’s medium (DMEM) and HAM F12 (GIBCO) supplemented with antibiotics (penicillin and streptomycin), 10% fetal bovine serum, and insulin, transferrin, and selenium (ITS, Sigma). Experiments were performed at room temperature. Cells were used within 4–36 h.

**Membrane current recording**

K+ currents were measured in the whole cell configuration of the patch-clamp technique (Hamill et al. 1981) using an Axopatch 200 amplifier (Axon Instruments). Patch pipettes had resistances of 2–5 MΩ when filled with (in mM) 100 K aspartate, 20 KCl, 2 MgCl2, 1 CaCl2, 10 EGTA, and 10 HEPES, pH 7.2, adjusted to 300 mosM with glucose. The standard extracellular solution was composed of (in mM) 140 NaCl, 5 KCl, 1 MgCl2, 1.8 CaCl2, 10 HEPES, and 10 glucose, pH 7.4. The K+ concentration in the extracellular solution was varied by equimolar replacement of NaCl with KCl. Unless indicated, current recordings were not corrected for leak. Current traces were sampled at 2.4 kHz and filtered at 1 kHz for off-line analysis. In some recordings, series resistance errors were compensated to at least 70%. PClamp programs (Axon Instruments) were used for data acquisition and analysis. The peak of the tail currents was determined by extrapolation of the deactivating tail current to the beginning of the voltage step. The extracellular solution was changed using a fast-flow device consisting of a linear array of borosilicate glass capillary tubings (Overholt and Prabhakar 1997).

**Membrane potential recording**

Membrane potential measurements were made using an Axopatch 200 amplifier in the current-clamp mode. The intracellular solution had the following composition (in mM) 120 K glutamate, 20 KCl, 5 Mg-ATP, 5 EGTA, 5 HEPES, and 0.1 Tris-GTP, pH 7.2. Cells were perfused with Krebs-Heinseliet buffer equilibrated with 5% CO2 in air (in mM): 120 NaCl, 4.8 KCl, 1.5 CaCl2, 2.2 MgSO4, 1.2 NaH2PO4, 25 NaHCO3, and 11 glucose, pH 7.4. Drugs were added to the extracellular perfusate. Input resistance was measured from hyperpolarizing current injections of −10 pA applied from the resting membrane potential.

**Measurements of [Ca2+]i**

Changes in [Ca2+]i in individual glomus cells were measured as described previously (Bright et al. 1996). Briefly, cells were plated on glass coverslips and preincubated in 3 ml of serum free DMEM medium containing 5 μM Indo-1-PE3 (Texas Fluorescence Lab) for 60 min at 37°C. Subsequently, they were placed in a gas-tight, temperature regulated chamber (Biopics) and superfused with Hank’s Balanced Salt Solution (HBSS) equilibrated with 21% oxygen and 5% CO2. Images were recorded with a Zeiss LSM-410 equipped with a UV laser. Excitation was 360 nm with emission at 408 and 475 nm. Data are expressed as ratio values due to unstable Rmin and Rmax values within the cell using either ionomycin or 8Br-A23187. Cells responding to hypoxia with increases in [Ca2+]i were considered to be glomus cells.

**Recording of carotid body sensory discharge in vitro**

The method for recording sensory discharge from isolated carotid bodies has been described previously (Prabhakar et al. 1995). Briefly, the carotid bifurcation along with the carotid sinus nerve was cannulated from anesthetized, adult rabbits. The carotid bifurcation was placed in a recording chamber, and the common carotid artery was cannulated and perfused with DMEM solution (pH 7.3; temperature, 36 ± 1°C) at a rate of 3.5 ± 0.5 (SE) ml/min. Chemoreceptor activity was recorded from the sinus nerve using platinum-iridium electrodes. Sensory discharge frequency is expressed as impulses/second. Hypoxia (PO2 = 38 ± 6 mmHg for 1–2 min) augmented the sensory activity, suggesting that the action potentials were of chemoreceptor origin. The superfusion medium was equilibrated with room air (normoxia). The partial pressure of oxygen (pO2) in the medium was measured using a blood gas analyzer.

**Drugs**

DofetilideN-[4-{-[4-(methanesulfonamino)-phenoxyl]-ethyl}ethyl]amino]ethylphenyl methansulfonamide was a gift from Pfizer Central Research. All other chemicals were purchased from Sigma.

**Data analysis**

Statistical analysis was evaluated by a paired or unpaired t-test, or one-way ANOVA combined with Tukey’s test, where appropriate. P values <0.05 were considered significant. Summary data are expressed as means ± SE.
**RESULTS**

**Glomus cells of the rabbit carotid body express a HERG-like K⁺ current**

Dissociated cells from carotid bodies contain both glomus and type II cells. Glomus, but not type II cells, express Na⁺ and large, outward, delayed rectifier-like K⁺ currents (Urena et al. 1989). Therefore we used the presence of large, outward K⁺ currents and inward Na⁺ current to distinguish glomus cells from type II cells (Overholt and Prabhakar 1997). Figure 1A shows typical currents elicited by 75-ms depolarizing voltage-clamp steps from a glomus cell held at −85 mV measured in an extracellular solution containing 5 mM K⁺. Rapidly inactivating, inward Na⁺ currents followed by large, outward K⁺ currents typical of glomus cells can be seen. Figure 1B shows the outward K⁺ currents elicited by longer (2.8 ms) depolarizing-voltage-clamp steps (note, the Na⁺ current cannot be resolved on this time scale). Outward currents were slowly decaying and could be described best by double exponential functions (see legend). Figure 1C shows the activation and inactivation properties of the outward K⁺ current. Activation properties were determined by analyzing peak current amplitudes using protocols shown in Fig. 1B. The threshold for activation was reached between −40 and −30 mV and was half-maximal at 4.7 mV. To determine steady-state inactivation, cells were held at +20 mV and stepped for 1 s from +20 to −130 mV in 10-mV increments. Peak currents were analyzed on return to +20 mV. Steady-state inactivation could be fitted by a Boltzmann equation with V₅₀ at −73 mV. The characteristics of the outward K⁺ currents are similar to those described in rabbit glomus cells by other investigators (Lopez-Lopez et al. 1993) and suggest that they would not be active at the resting membrane potential in glomus cells.

Once establishing that a recording was from a glomus cell, we tested whether the same cells also express HERG-like K⁺ current. HERG-like currents cannot be readily identified under the conditions in Fig. 1B, due to rapid inactivation at depolarized potentials, but can be substantially amplified at more hyperpolarized potentials by raising the concentration of extracellular K⁺ ([K⁺]₀). Figure 1D shows an example of currents elicited by the same protocol and in the same cell shown in Fig. 1B, but the extracellular solution was changed to one containing 70 mM K⁺ by equimolar replacement of NaCl by KCl. Elevating [K⁺]₀ did not change any of the kinetic parameters found in normal K⁺. The holding current measured at −85 mV showed only a minor increase from −12.8 ± 1.8 to −23.8 ± 2.1 pA (n = 7) in 5 and 70 mM [K⁺]₀, respectively. This small change in current amplitudes could not be prevented by 50 μM Ba²⁺, which should block current conducted by classical inward rectifier K⁺ channels in the KIR family. Rather, Fig. 1D shows that the most dramatic change observed in current recordings performed in elevated [K⁺]₀ was the appearance of a slowly deactivating tail current component (arrow), indicative of inward rectifying K⁺ current.

Figure 2 further characterizes this inward rectifier K⁺ current in glomus cells. Figure 2A shows that this current activates very slowly. After a 50-ms depolarization, tail currents elicited on return to −85 mV were instantaneous and decayed with a time constant of 22.1 ± 2.3 ms. More importantly, after a 3-s depolarization, a slow component appeared that showed delayed onset and decayed with a much slower time constant of 157.7 ± 1.4 ms (n = 3). The slow development of this component suggests that the current activates slowly (i.e., seconds, not milliseconds) as is characteristic for HERG channels. This “nose” that develops over time in tail currents recorded in elevated [K⁺]₀ indicates that the inward rectifier K⁺ current is conducted by a HERG-like K⁺ channel. Figure 2B shows current traces elicited by a 200-ms step from a holding potential of 0 to −120 mV in a glomus cell. This protocol is optimal for observing the HERG-like current in glomus cells, because HERG channels are maximally activated at 0 mV and the large, outward K⁺ currents are inactivated. It
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Pharmacological characterization of HERG-like currents in glomus cells

Heterologously expressed HERG channels are blocked with highest affinity and selectivity by class III antiarrhythmic methanesulfonanilide drugs such as dofetilide (Arcangeli et al. 1997). To further confirm that HERG channels underlie the HERG-like current in glomus cells, we tested the effect of dofetilide on this current. Figure 3A shows characteristic HERG-like tail currents elicited on hyperpolarization from 0 to −120 mV before and during exposure to 0.01 or 1 μM dofetilide in the extracellular solution. It can be seen that HERG-like K⁺ currents in glomus cells were blocked by dofetilide in a concentration-dependent manner. However, the effects of micromolar concentrations of dofetilide were not reversible. The effect of dofetilide on the HERG-like tail current is further characterized in Fig. 3B, which shows the relative effect of a range of concentrations of dofetilide on the current. From these experiments, we determined the IC₅₀ of dofetilide block to be 12.7 ± 4.2 nM (n = 11). In marked contrast, transient outward currents were not affected (1 μM dofetilide actually increased the outward current elicited on return to 0 mV by 7.1 ± 6.0%, see Fig. 3A; n = 8).

Cations such as Ba²⁺ have been used to differentiate HERG-like inward currents elicited in elevated [K⁺], from classical inward rectifier currents. HERG-like K⁺ currents, as well as heterologously expressed HERG channels, proved to be less sensitive to block by external Ba²⁺ than inward rectifiers in the Kir gene family (Kubo et al. 1993). In marked contrast to the strong block of classical inward rectifiers by 50 μM Ba²⁺, Fig. 3C shows that the HERG-like current in glomus cells is little affected by 50 μM Ba²⁺ (average reduction 7.5 ± 1.1% at −120 mV; n = 5). Figure 3C also shows that the HERG-like current is reduced by high (mM) concentrations of Ba²⁺. One and 10 mM Ba²⁺ reduced HERG-like currents at −120 mV by 34.1 ± 1.7 (n = 5) and 61.7 ± 4.2% (n = 4), respectively. Outward currents at 0 mV were only moderately reduced. Because TEA has been shown to block outward K⁺ currents in glomus cells, we tested the effects of TEA on the HERG-like current. Figure 3D shows that 10 mM TEA blocked a much larger proportion of the outward current elicited on return to 0 mV (77.3 ± 4.1%; n = 8) than of the HERG-like current elicited at −120 mV (35.9 ± 2.5%; n = 8). These results demonstrate that the pharmacological profile of the HERG-like current in glomus cells is similar to that described for HERG currents in other cells (Bianchi et al. 1998), and further support the idea that inward rectifier K⁺ current in glomus cells is conducted by HERG-like channels.

HERG-like K⁺ current regulates resting membrane potential in glomus cells

We next wanted to elucidate the functional role of the HERG-like current in glomus cells. To determine whether the HERG-like current is active at the resting membrane potential, we first examined the steady-state activation properties of this current. Figure 4A shows a family of current traces recorded on return to −100 mV after a range of 27.6 s test potentials from
a glomus cell exposed to an extracellular solution containing 70 mM K⁺. The results of these experiments are summarized in Fig. 4B, which shows normalized current measured on return to −100 mV. It can be seen that the data are well fit by a Boltzmann equation with $V_h$ at −44.0 ± 2.1 mV and slope conductance $k$ of −10.5 ± 2.1 ($n = 7$). The steady-state activation properties suggest that the HERG-like K⁺ current in glomus cells is active around −50 mV, which is close to the resting membrane potential of these cells (see Table 1).

To assess whether HERG-like current could regulate the resting membrane potential in glomus cells, we examined the effect of dofetilide on the resting membrane potential. For these current-clamp experiments we used nanomolar concentrations to demonstrate the reversibility of dofetilide effects. In addition, a bicarbonate buffered extracellular solution was used. Stable measurements of the resting membrane potential were recorded in 44 glomus cells current clamped in the whole cell configuration. Only five of these cells displayed spontaneous action potentials, which diminished over time during whole cell dialysis. On average, the resting membrane potential was −48.3 ± 1.9 mV (range −67.2 to −33.2 mV, $n = 32$), and the input resistance was 3.0 ± 0.3 GΩ ($n = 12$). Most importantly, 150 nM dofetilide caused a significant and reversible depolarization of −13 mV. In contrast, 10 mM TEA, which completely blocked the large, outward current, had no effect on the resting membrane potential. The results from these experiments are summarized in Table 1. These results show that the HERG-like current is involved in regulating the resting membrane potential in glomus cells.

**Dofetilide increases [Ca²⁺]ᵢ in glomus cells**

A depolarization-induced influx of Ca²⁺ through membrane Ca²⁺ channels is an essential step in chemotransduction at the carotid body. Therefore if the HERG-like current is active at the resting membrane potential, then inhibition of this current should cause depolarization and elevate [Ca²⁺]ᵢ in glomus cells. To test this possibility, we monitored the effect of dofetilide on [Ca²⁺]ᵢ in glomus cells using Indo-1-PE3, a calcium-sensitive fluorescent dye. As a control, we also tested the effect of tetraethylammonium (TEA) on [Ca²⁺]ᵢ. Figure 4C shows the effect of 10 mM TEA and 1 μM dofetilide on [Ca²⁺]ᵢ in a representative glomus cell. It can be seen that TEA had no effect on [Ca²⁺]ᵢ, whereas the same cell responded with a prompt increase in [Ca²⁺]ᵢ in response to dofetilide. [Ca²⁺]ᵢ returned slowly to baseline levels after wash out of dofetilide. Of the 43 cells tested, 33 responded with an increase in [Ca²⁺]ᵢ in response to dofetilide (change in ratio from 1.2 ± 0.02 to 2.0 ± 0.03), similar to that seen with the control response to hypoxia (PO₂ = 32 ± 4 mmHg; change in ratio from 1.2 ± 0.02 to 1.8 ± 0.02). In contrast, TEA caused an increase in [Ca²⁺]ᵢ in only one of these cells (Fig. 4D). These results suggest that the HERG-like current plays a functional role in glomus cells at the cellular level.
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FIG. 4. HERG-like current controls the resting membrane potential in glomus cells. A: family of current traces from a glomus cell elicited at −100 mV by 26.7-s prepulses from −80 to +10 mV in 7.5-mV increments (70 nM \([K⁺]₀ \); HP = −85 mV). Dotted line represents zero current level. B: average (n = 7), normalized \(I_{\text{K}}\) values (symbols) measured at −100 mV (protocol as in A) are plotted as a function of prepulse potential. The line shows that the data points are well fit by a Boltzmann equation with \(V_{\text{m}} = −44.0 ± 2.1 \text{ mV}\) and slope conductance \(k = −10.5 ± 2.1\). C: representative example of the change in cytosolic calcium concentration in response to tetraethylammonium (TEA) and dofetilide (DOF). Cells did not respond to 10 mM TEA (t = 1.5–5.6 min). Cells responded to 1 μM dofetilide with rapid and significant increases in cytosolic calcium (t = 6.8–10.1 min). D: summary of the response of cells to TEA and DOF (n = 43). Approximately 75% of the cells responded to DOF with >10% increase in indo-1 ratio, whereas <3% responded to TEA.

**Dofetilide increases baseline sensory discharge in the in-vitro carotid body**

To determine whether the functional aspects of the HERG-like current at the cellular level translate to the organ, we monitored the effects of dofetilide on sensory discharge of isolated carotid bodies using an in-vitro preparation. The in-vitro carotid body preparation avoids potential systemic effects of concentrations of dofetilide on baseline sensory discharge from the carotid sinus nerve in a representative preparation. As can be seen, dofetilide increased the sensory discharge in a concentration-dependent manner. As little as 0.3 μM dofetilide significantly enhanced the sensory activity, and at 3 μM, baseline activity was increased by 98 ± 6% (P < 0.01; n = 8). These results are summarized in Fig. 5B. The effects of dofetilide on baseline carotid body activity were first discernable between 3 and 5 min, had plateaued by 10 min, and did not return to control levels within 15 min after termination due to the high concentrations used. These results demonstrate that block of HERG channels mimics the effects of hypoxia on the sensory discharge of the carotid body and establish the functional significance of the HERG-like current at the organ level.

**DISCUSSION**

HERG channels are members of the voltage-gated ether-a-go-go K⁺ channel family and are characterized by an unusually slow current activation and deactivation, paired with a fast C-type inactivation mechanism. In the present study, we clearly identify a HERG-like current component in glomus cells using electrophysiological and pharmacological methods. We found HERG-like tail currents after long depolarizing steps under high \([K⁺]₀\) only in those cells that displayed the large, outward K⁺ current, characteristic of glomus cells (Urena et al. 1989). HERG-like current was not found in cells displaying only small outward K⁺ currents characteristic of type II cells, suggesting that expression of the HERG-like current in the carotid body is confined to glomus cells. Whether nerve and/or vascular tissue in the carotid body also express HERG-like current remains to be established. The HERG-like tail current in glomus cells increased for a few milliseconds before deac-
tivating at hyperpolarized membrane potentials, a characteristic unique to HERG channels. This “nose” clearly identifies this current as carried by HERG-like gene products because it results from a unique inactivation mechanism that recovers rapidly at negative potentials. The kinetics of the currents (especially Fig. 2C) are identical to HERG-like currents identified by electrophysiological and molecular means in other cell lines (Bianchi et al. 1998). Further, the HERG-like current is carried by K$^+$ ions (Fig. 2B), shows time- and voltage-dependent deactivation (Fig. 2C), slow current activation (Fig. 2A), and steady-state activation (Fig. 4B), all characteristics of HERG channel currents. However, it was not possible to characterize HERG-like outward currents in glomus cells because of the much larger outward K$^+$ currents and the rapid inactivation process at depolarized potentials. This precludes a quantitative analysis of the time course for activation of the HERG-like current.

The kinetic evidence that a HERG channel protein conducts the HERG-like current in glomus cells is further corroborated by pharmacological evidence. In contrast to conventional inward rectifier K$^+$ channels (Kubo et al. 1993), the HERG-like currents were little affected by micromolar concentrations of Ba$^{2+}$. However, they were inhibited by mM concentrations of Ba$^{2+}$, as reported for HERG-like channels studied in neuroblastoma and microglial cells (Arcangeli et al. 1995; Zhou et al. 1998). Furthermore, inward tails were blocked with nanomolar affinity by dofetilide, a potent and specific blocker of HERG K$^+$ channels (Ficker et al. 1998; Snyders and Chaudhary 1996). Consistent with previous reports we also found that the effects of dofetilide were only partially reversible at concentrations higher than 1 mM (Ficker et al. 1998). These results show that a HERG channel protein conducts the HERG-like current in glomus cells. HERG currents arise from expression of three closely related HERG genes, ERG1–3, which are widely expressed in nervous tissue (Shi et al. 1997; Warmke and Ganetzky 1994). However, which of the specific HERG gene(s) are expressed in glomus cells remain to be investigated.

Several observations in the present study show that the HERG-like K$^+$ current plays a functional role in regulating the resting membrane potential in rabbit glomus cells. Activation of the HERG-like K$^+$ current was half-maximal at −44 mV, and the threshold for current activation was reached between −70 and −60 mV (Fig. 4B). This is sufficiently negative to stabilize the membrane potential between −65 and −40 mV, as measured in current-clamp recording from glomus cells (Table 1). On the other hand, it is not expected that the O$_2$-sensitive, outward K$^+$ currents would be open at the resting membrane potential (Lopez-Lopez et al. 1993; Wyatt et al. 1995) (also Fig. 1C). Furthermore, TEA, which blocks the O$_2$-sensitive K$^+$ currents, neither depolarized nor increased [Ca$^{2+}$]$_i$ in glomus cells (Buckler 1997; Cheng and Donnelly 1995; Lahiri et al. 1998) (also Fig. 4C and Table 1). In contrast, dofetilide significantly depolarized glomus cells and increased [Ca$^{2+}$]$_i$. However, whether dofetilide affects hypoxia-induced depolarization and [Ca$^{2+}$]$_i$ in isolated glomus cells remains to be investigated. These results demonstrate that block of the HERG-like current, like hypoxia, causes depolarization and increases [Ca$^{2+}$]$_i$ in glomus cells.

The role of O$_2$-sensitive K$^+$ channels in the transduction process at the carotid body has also been questioned because known blockers of these channels (i.e., 4-aminopyridine, TEA, and charybdotoxin) had no affect on basal sensory discharge of intact carotid bodies (Buckler 1997; Cheng and Donnelly 1995; Lahiri et al. 1998). In contrast, dofetilide significantly augmented baseline sensory activity in the isolated carotid body in the present study. The fact that we have identified a HERG-like K$^+$ current in glomus cells that is blocked by dofetilide suggests that these effects are mediated by effects on glomus cells themselves. In support of this idea, another report showed that millimolar concentrations of Ba$^{2+}$ augment sensory discharge of the carotid body (Donnelly 1997). This augmentation could be due to block of the HERG-like K$^+$ current, because we found that mM Ba$^{2+}$ inhibits this current (Fig. 3C). In addition, the increase in nerve activity in response to hypoxia was qualitatively and quantitatively comparable with that seen in response to dofetilide. Hypoxia (PO$_2$ = 38 ± 6 mmHg) augmented the baseline sensory discharge by 87 ± 6%. However, given the constraints of the experimental conditions, we cannot rule out possible effects of dofetilide on sensory nerve endings. Nonetheless, this is the first study to show that block of a specific K$^+$ channel augments the sensory discharge of the intact carotid body. It should also be noted that the concentration of dofetilide required to produce augmentation of sensory discharge in the intact carotid body was relatively high and the response did not reach a plateau even with 3 mM. This is not unexpected because isolated cells and whole organs are very different preparations and the effective concentration near the glomus cell could be quite different because of the hydrophobic nature of dofetilide. It is possible that the effect of dofetilide did not reach a plateau because it has nonspecific effects at higher doses. Most importantly, the results from our physiological studies clearly show that modulation of the HERG-like current has a significant effect on the sensory discharge of the intact carotid body. This suggests that this current could be involved in the initial depolarization that is linked to the expression of the hypoxic response.

From the results of the present study, we cannot say whether or not the HERG-like current participates in chemosensing or is sensitive to O$_2$. However, recent studies suggest that the HERG channel protein is a prime candidate for O$_2$ sensing. For example, the HERG protein contains a PAS domain that is known to be sensitive to O$_2$ in other proteins (Pellequer et al. 1999). However, the mechanism by which O$_2$ modulates channel activity may not be straightforward. One possibility is that hypoxia could directly modulate K$^+$ channel activity. Alternatively, hypoxia could modulate K$^+$ currents by affecting the redox state of the cell and/or altering the level of reactive oxygen species (ROS) (Acker 1994). In line with this idea, ROS have been shown to modulate the activity of HERG channels expressed in Xenopus oocytes (Tagliatela et al. 1997). Therefore it is possible that O$_2$ chemosensing could involve effects of hypoxia on HERG either directly or indirectly through changes in ROS. These effects on HERG channel activity could contribute to the depolarization responsible for the initiation of sensory activity. We are currently investigating these possibilities.

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