CO₂ and pH Independently Modulate L-Type Ca²⁺ Current in Rabbit Carotid Body Glomus Cells

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Summers, Beth A., Jeffrey L. Overholt, and Nanduri R. Prabhakar. CO₂ and pH independently modulate L-type Ca²⁺ current in rabbit carotid body glomus cells. J Neurophysiol 88: 604–612, 2002; 10.1152/jn.00767.2001. The carotid bodies respond to changes in arterial O₂, CO₂, and pH, and Ca²⁺ influx via voltage-gated Ca²⁺ channels is an important step in the chemoreceptor process. The objectives of the present study were as follows: 1) to determine whether hypercapnia modulates Ca²⁺ current in glomus cells, and if so, to determine if this modulation is secondary to changes in pH; 2) to examine the mechanism of CO₂ modulation of the Ca²⁺ current; and 3) to determine whether the effects of hypercapnia and hypoxia on Ca²⁺ channel activity in glomus cells are synergistic. The effects of CO₂ on Ca²⁺ current were monitored in glomus cells isolated from rabbit carotid bodies using both perforated and conventional patch-clamp techniques. Raising CO₂ in the extracellular solution from 5 to 10% (hypercapnia) reversibly augmented the whole-cell Ca²⁺ current. This augmentation was rapid and increased the whole-cell Ca²⁺ current similarly in both the perforated and the conventional patch configurations by 16 ± 2% (n = 5) and 15 ± 1% (n = 32), respectively. The following observations suggest that the effects of CO₂ are not secondary to changes in pH: 1) isohydric hypercapnia (pH maintained at 7.4) augmented the Ca²⁺ current by 24 ± 2% (n = 6); 2) decreasing the pH of the extra- or intracellular solutions decreased the Ca²⁺ current by 43 ± 4% (n = 8) and 13 ± 1% (n = 5), respectively; and 3) hypercapnia did not shift the half-maximal activation voltage (V₁/₂), whereas intracellular and extracellular acidosis alone caused shifts in V₁/₂. Furthermore, 100 nM of a membrane-permeable protein kinase A inhibitor prevented the augmentation by CO₂, and 500 μM 8-Br-cAMP mimicked the effect of CO₂ by augmenting the Ca²⁺ current by 10 ± 2% (n = 6). Also, cyclic AMP levels in carotic bodies increased from 1.98 ± 0.6 to 9.0 ± 0.2 pmol/μg protein in response to hypercapnia. In contrast, decreasing pH in the nominal absence of CO₂ did not affect cAMP levels in rabbit carotid bodies. Further, nisoldipine, but not α-conotoxin MVIIC, prevented augmentation of the Ca²⁺ current by CO₂. In addition, when combined, hypercapnia and hypoxia augmented the Ca²⁺ current by 26 ± 4% (n = 7), which is greater than either stimulus alone, suggesting the effects are additive. Taken together, these results indicate that L-type Ca²⁺ current is augmented by hypercapnia. The effect of CO₂ is not secondary to changes in pH and seems to be mediated by a protein kinase A-dependent mechanism. Furthermore, hypercapnia and hypoxia act additively in stimulating Ca²⁺ current in glomus cells.

INTRODUCTION

In addition to O₂, carotid body sensory activity is sensitive to the concentration of CO₂ in the arterial blood. Glomus cells are thought to be the principal elements in the carotid body for sensing changes in arterial O₂ and CO₂. While much progress has been made in our understanding of O₂ sensing mechanisms, relatively little is known about the cellular mechanism(s) associated with CO₂ chemoreception (Prabhakar 2000). It is generally acknowledged that Ca²⁺ influx via voltage-gated Ca²⁺ channels in glomus cells plays an important role in the transduction of hypoxia and CO₂ stimuli (Bisescu and Duchen 1990; Bright et al. 1996; Buckler and Vaughan-Jones 1993, 1994; Sato 1994). We have previously shown that O₂ modulates Ca²⁺ current in glomus cells (Summers et al. 2000). However, it is not known if high CO₂ affects Ca²⁺ current in glomus cells and thus contributes to the sensory response to hypercapnia. One problem with investigating the effects of CO₂ is that, under physiological conditions, changes in CO₂ cause changes in pH. Hence the effects of CO₂ may be secondary to changes in pH, which is known to affect Ca²⁺ current (Klockner and Isenberg 1994; Zhou and Jones 1996). Therefore, one objective of the present investigation was to determine whether hypercapnia modulates Ca²⁺ current in glomus cells, and if so, to determine if this modulation is due to a direct effect of CO₂ or secondary to changes in pH produced from hydration of CO₂.

Hypercapnia is one of the most potent vasodilating stimuli in the cerebral circulation of mammals. Interestingly, part of the vasodilating capabilities of hypercapnia is mediated through cAMP (Pelligrino and Wang 1998). There is also evidence that cAMP levels are increased during hypercapnia in the carotid body (Perez-Garcia et al. 1990). Protein phosphorylation elicited by cAMP-dependent protein kinase A (PKA) has been shown to modulate Ca²⁺ channel activity in a wide variety of cell types (Hartzell 1988; Hove-Madsen et al. 1996). Thus the second objective of the current study was to examine whether the effects of CO₂ on Ca²⁺ current are coupled to a cAMP/PKA signaling pathway.

A variety of studies have described an apparent synergy between the effects of high CO₂ (hypercapnia) and low O₂ (hypoxia) on carotid body sensory activity (Eyzaguirre and Lewin 1961; Fitzgerald and Parks 1971; Lahiri and Delaney 1975; Pepper et al. 1995). The cellular mechanism(s) that underlies this interaction has only recently been addressed. Several investigators have shown that when hypercapnic and hypoxic stimuli were given simultaneously, the rise in cytosolic Ca²⁺ was greater than the response to either stimulus given
alone (Dasso et al. 2000; Roy et al. 2000). Since the rise of cytosolic Ca$^{2+}$ is linked to influx through voltage-gated Ca$^{2+}$ channels, it is possible that CO$_2$ and O$_2$ may interact at the level of the Ca$^{2+}$ channel. Consequently, the third objective of the study was to determine whether the effects of hypercapnia and hypoxia on Ca$^{2+}$ channel activity in glomus cells are synergistic. Our results show that high CO$_2$ augments L-type Ca$^{2+}$ current through a PKA-sensitive mechanism that is independent of changes in pH. Moreover, CO$_2$ and O$_2$ augmented the Ca$^{2+}$ current in an additive manner, suggesting that they act synergistically on the Ca$^{2+}$ current.

METHODS

General procedures

Experiments were performed on glomus cells freshly isolated from the carotid bodies of adult rabbits killed with CO$_2$. Individual glomus cells were dissociated enzymatically as described previously (Overholt and Prabhakar 1997). Briefly, carotid bodies were incubated at 37°C in a media containing trypsin (type II, 2 mg/ml, Sigma Chemical, St. Louis, MO) and collagenase (type IV, 2 mg/ml, Sigma). The composition of the incubation medium (modified Tyrode’s) was as follows (mM): 140 NaCl, 5.4 KCl, 10 HEPES, 5 glucose, and the pH was adjusted to 7.4 with CsOH. The buffered extracellular solution had an osmolarity of 296 mOs and contained the following (mM): 115 CsCl, 20 TEA-Cl, 5 EGTA, 5 HEPES, and the pH was adjusted to 7.4 with NaOH.

Rundown of Ca$^{2+}$ current and the effects of drugs were monitored using a wash protocol (25-ms step to 0 mV, 10 s between steps). The effects of drug agents were compensated for rundown using a linear regression of the current decrease during the wash protocol in the absence of test compounds. Cells in which rundown was excessive or did not appear linear were excluded from the analysis. For comparison of I-V relations, Ca$^{2+}$ current at each potential was normalized to the maximum value recorded during the control I-V relation in individual cells (usually 0 mV).

For analysis of shifts in gating, activation curves were measured from peak tail current amplitudes measured on repolarization to −40 mV after termination of 20-ms depolarizing steps to voltages from −50 to +30 mV in 5-mV increments. The tail current amplitudes were normalized to the tail current measured after the depolarizing step to +30 mV. The data are fairly well fit by a single Boltzmann of the form

$$I_{\text{H max}} = \frac{1 + \exp(V - V_{1/2})}{k}$$

where $I_{\text{H max}}$ is the normalized current, $V$ is the holding potential, $V_{1/2}$ is the potential at which the channels are half-activated, and $k$ is the slope factor.

Perforated patch-clamp recording technique

Ca$^{2+}$ currents were recorded using the amphotericin-B perforated patch method as described by Rae and co-workers (Rae et al. 1991). Amphotericin-B (3 mg/0.1 ml; Sigma) was first dissolved in DMSO and then added to the internal pipette solution at a final concentration of 240 µg/ml. The internal pipette solution contained the following (in mM): 115 CsCl, 20 TEA-Cl, 5 EGTA, 5 HEPES, and the pH was adjusted to 7.2 with CsOH. For the formation of a gigaseal, the tip of the pipette was filled with amphotericin-B-free pipette solution and the pipette was backfilled with the amphotericin-B-containing pipette solution.

Solutions and drugs

CO$_2$/HCO$_3$-buffered extracellular solutions were made normoxic, hypoxic, normoxic hypercapnic, or hypoxic hypercapnic by continuously bubbling with either of the following (in %): 21 O$_2$, 5 CO$_2$; 1 O$_2$, 5 CO$_2$; 21 O$_2$, 10 CO$_2$; or 1 O$_2$, 10 CO$_2$, respectively (all gas mixtures balanced with N$_2$). CO$_2$/HCO$_3$-buffered extracellular solutions had pHs of 7.35 and 7.00 for normoxic and hypercapnic solutions, respectively. CO$_2$/HCO$_3$-buffered extracellular solutions were made isohydric hypercapnic (pH$_c$ = 7.35) by increasing the bicarbonate concentration from 25 to 50 mM (equimolar replacement of NMG-Cl) and the CO$_2$ from 5 to 10%. In addition, these experiments were performed in the presence of 500 nM tetrodotoxin so that Na$^+$ currents would not be activated in the presence of high sodium bicarbonate. The PO$_2$ and PCO$_2$ were routinely monitored using a blood gas analyzer (Laboratory Instruments) and found to be 35 ± 5 and 148 ± 3 mmHg (n = 5) for hypoxic and normoxic solutions, respectively. The PCO$_2$ was 40 ± 4 and 76 ± 5 mmHg (n = 5) for normocapnic and hypercapnic solutions, respectively. Table 1 summarizes the pH, PO$_2$, and PCO$_2$ values of the solution under various conditions.

Nisoldipine (Niso; Miles Laboratories) was prepared as a stock solution in polyethylene glycol (PEG, M$_r$ = 400, Sigma). PKA inhibitor 14–22 amide, myristoylated (Calbiochem), 8-Br-cAMP (Calbiochem), tetrodotoxin (Alomone Labs, Israel), and α-conotoxin MVIIIC (MVIIIC, Alomone Labs) stock solutions were prepared in sterilized, deionized water. Experiments were done in the dark when
TABLE 1. *A summary table of pH, Po₂, and Pco₂ values of a solution under the various conditions*

<table>
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<th>Stimulus</th>
<th>pH</th>
<th>Po₂</th>
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<tr>
<td>Hypoxia</td>
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<tr>
<td>Normoxic hypercapnia</td>
<td>7.00</td>
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<td>76</td>
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<td>Isotoxic hypercapnia</td>
<td>7.35</td>
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<tr>
<td>Hypoxic hypercapnia</td>
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pH, Po₂, and Pco₂ values in a bicarbonate-buffered solution are presented under normoxic, hypoxic, normoxic hypercapnia, isotoxic hypercapnia, and hypoxic hypercapnia conditions.

RESULTS

Augmentation of Ca²⁺ current by hypercapnia

In central neurons, the effects of CO₂ critically depend on intracellular buffering (Ritucci et al. 1998). For this reason, we compared the effects of hypercapnia on the Ca²⁺ current in a bicarbonate buffer in perforated versus conventional whole-cell patch-clamp methods, wherein the cell’s own buffering capacity is left intact in the former but not in the latter. An example illustrating the effects of increasing CO₂ from 5 to 10% (HC, pH 7.0) on the Ca²⁺ current and the time course of the response recorded from a glomus cell under a perforated patch condition is shown in Fig. 1, A and B. It is obvious from the current traces in B that hypercapnia augmented the Ca²⁺ current. B shows the time course for changes in Ca²⁺ current elicited every 10 s by a step to 0 mV from a holding potential of −90 mV. It can be seen that the effect of hypercapnia began within 10–20 s and returned to control levels within 60 s after terminating the hypercapnic challenge. Figure 1, C and D, show that CO₂ had similar effects on the current traces and a comparable time course under conventional patch conditions. On average, the Ca²⁺ current was augmented by 16 ± 2% (0 mV, n = 5, P < 0.05, paired t-test) under perforated conditions and by 15 ± 1% (0 mV, n = 32, P < 0.05, paired t-test) under conventional patch conditions. Augmentation of the Ca²⁺ current by hypercapnia appeared to be voltage-independent (see Fig. 3). Since the effects of hypercapnia were quantitatively

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**FIG. 1.** Hypercapnia (HC) augments the whole-cell Ca²⁺ current in rabbit glomus cells in a bicarbonate-buffered extracellular solution. A: raw, whole-cell Ca²⁺ current under perforated patch conditions elicited by a 25-ms step to 0 mV [holding potential (HP) = −90 mV] before [normoxic (N), 5% CO₂, pH 7.35] and during exposure to a hypercapnic extracellular solution (HC, 10% CO₂, pH 7.00). Broken line indicates 0 current level and subscripts correspond to numbers in B, indicating the position of the current traces in the time course. B: time course for changes in Ca²⁺ current elicited at 0 mV every 10 s under the conditions described in A. C: raw, whole-cell Ca²⁺ current under conventional patch conditions elicited by a 25-ms step to 0 mV (HP = −90 mV) before (N, pH 7.35) and during exposure to a hypercapnic extracellular solution (HC, pH 7.00). Broken line indicates 0 current level and subscripts correspond to numbers in D, indicating the position of the current traces in the time course. D: time course for changes in Ca²⁺ current elicited at 0 mV under the conditions described in C.
and qualitatively similar, subsequent experiments were performed under conventional patch conditions. These results suggest that the effect of CO2 on Ca2+ current is not dependent on the intracellular buffering capacity of glomus cells.

The effect of CO2 on Ca2+ current is not secondary to changes in pH

Since changes in CO2 cause changes in pH, we performed a number of experiments to determine whether the effect of CO2 on Ca2+ current is independent of pH. First, we tested the effect of isohydric hypercapnia on glomus cell Ca2+ current. In these experiments, pH was kept constant by increasing the bicarbonate concentration from 25 to 50 mM when changing CO2 from 5 to 10%. Under isohydric conditions, increasing the CO2 from 5 to 10% (50 mM bicarbonate, pHo = 7.35) augmented the Ca2+ current by 24 ± 2% (0 mV, n = 6, P < 0.05, paired t-test). An example of the effects of isohydric hypercapnia on the Ca2+ current is shown in the current traces and time course in Fig. 2, A and B, respectively. After washout of the isohydric hypercapnic solution, a hypercapnic solution caused a similar augmentation of the Ca2+ current in the same cell (Fig. 2B). The effect of isohydric hypercapnia on Ca2+ current also appeared to be voltage-independent (data not shown). However, the response of the Ca2+ current to isohydric hypercapnia is greater than that to hypercapnic acidosis (24 ± 2 vs. 15 ± 1% at 0 mV, respectively). This suggests that molecular CO2 itself augments Ca2+ current in glomus cells, and this effect is not secondary to changes in pH.

To further establish that Ca2+ current augmentation by CO2 is not due to secondary changes in extra- and/or intracellular pH (pHo and pHi, respectively), the following experiments were performed. If a decrease in pHo during hypercapnia causes augmentation of Ca2+ current, changing to an acidic extracellular solution should elicit a similar effect. Therefore, we tested the effect of an acidic extracellular solution (pH 6.8) in the nominal absence of CO2 on Ca2+ current in glomus cells. As can be seen in the normalized, average (n = 8) I-V relation in Fig. 3C, extracellular acidification in the absence of CO2 inhibited the Ca2+ current over a broad range of membrane potentials (43 ± 4% at 0 mV, P < 0.05, paired t-test). This effect is clearly different from the augmentation of the Ca2+ current by hypercapnia over the same voltage range shown in Fig. 3A. Exposing the cells to an alkaline extracellular solution (pH 8.0) augmented Ca2+ current by 19 ± 5% (at 0 mV, n = 8, P < 0.05, paired t-test). In another series of experiments, we determined whether intracellular acidification had a similar effect to hypercapnia on the Ca2+ current. For these experiments, 20 mM K+ acetate was added to the extracellular solution to decrease pHo without changing pHi. Under these conditions (pHo = 7.4), the Ca2+ current was inhibited by 13 ± 1% (0 mV, n = 5, P < 0.05, paired t-test). An example of the effects of intracellular acidosis on the Ca2+ current is shown in the current traces and time course in Fig. 4, A and B, respectively. Notice that the effects of intracellular acidosis on the Ca2+ current are slow for both onset and offset of the response, an effect inconsistent with any effects of acetate on free Ba2+ concentration. After washout of the intracellular acidosis solution, a hypercapnic solution caused a brisk augmentation of the Ca2+ current in the same cell. Again, the inhibitory effect of intracellular acidosis is clearly different from the augmentation of the Ca2+ current by hypercapnia. These observations suggest that a selective drop in intracellular pH produced by K+ acetate does not mimic the effect of hypercapnia on the Ca2+ current.

To further confirm that the free Ba2+ concentration was not being affected by the acetate and thereby causing the observed decrease in the Ca2+ current, we measured the Ba2+ concen-

**FIG. 2.** Isohydric hypercapnia (IH) augments the Ca2+ current in glomus cells. A: raw, whole-cell Ca2+ current elicited by a 25-ms step to 0 mV (HP = −90 mV) before (N, pH 7.35) and during exposure to either an IH (pH 7.35) or an HC (pH 7.00) extracellular solution. Broken line indicates 0 current level and subscripts correspond to numbers in B, indicating the position of the current traces in the time course. B: time course for changes in Ca2+ current elicited at 0 mV every 10 s as the extracellular solution is switched to and from a solution containing 5% CO2 and high bicarbonate (HB, 50 mM), 10% CO2 and HB (pH 7.35, i.e., IH), and 10% CO2 and normal bicarbonate (25 mM, pH 7.0, i.e., HC).

**FIG. 3.** pH, but not HC, causes shifts in the voltage dependence of activation. A: average (n = 32), normalized I-V relations elicited by 25-ms steps to test potentials over the range of −50 to +70 mV in 10-mV increments (HP = −90 mV) before (N) and during exposure to a HC extracellular solution (10% CO2). B: average (n = 7), normalized activation curves determined from peak tail current amplitudes measured on repolarization to −40 mV after termination of 20-ms depolarizing steps to voltages from −50 to +30 mV in 5-mV increments under the conditions in A. C: average (n = 8), normalized I-V relations in a HEPES-buffered extracellular solution at pH 7.4 and 6.8 elicited by the same protocol indicated in A. D: average (n = 4), normalized activation curves determined using protocols indicated in B and conditions indicated in C.
The effect of hypercapnia on Ca$^{2+}$ current involves a PKA-mediated mechanism

Hypercapnia has been shown to increase cAMP levels in glomus cells (Perez-Garcia et al. 1990). Hence, we tested if the effects of CO$_2$ on Ca$^{2+}$ current in glomus cells are coupled to a PKA-dependent pathway. To test whether PKA is involved with Ca$^{2+}$ current augmentation by hypercapnia, we examined the effects of hypercapnia in the presence of a cell-permeable form of a PKA inhibitor (100 nM PKAi). Figure 5A shows an example of the effect of hypercapnia in the absence and presence of PKAi on the current traces. It can be seen that PKAi had no effect on the Ca$^{2+}$ current alone ($-1 \pm 5\%$, $n=6$, $P>0.05$, paired t-test). More importantly, PKAi prevented the augmentation of the Ca$^{2+}$ current by hypercapnia ($-2 \pm 3\%$, $n=6$, $P<0.05$, ANOVA) even though hypercapnia augmented the Ca$^{2+}$ current by 17 $\pm$ 2% ($n=6$, $P<0.05$, paired t-test) in the absence of PKAi as shown in the time course in Fig. 5B from the same cell. Further, Fig. 5, C and D, shows that a cell-permeable cAMP analog (8-Br-cAMP, 500 $\mu$M) mimicked the effect of hypercapnia on the Ca$^{2+}$ current ($10 \pm 2\%$, $n=6$). In addition, 8-Br-cAMP occluded the effect of hypercapnia on the Ca$^{2+}$ current (Fig. 5D). These results support a role for cAMP in augmentation of the Ca$^{2+}$ current by hypercapnia.

To further establish a role for cAMP involvement in augmentation of the Ca$^{2+}$ current by hypercapnia, we examined...
the effect of hypercapnia on cAMP content in whole carotid bodies. The basal cAMP levels in carotid bodies exposed to a normoxic, bicarbonate-buffered medium (5% CO₂, 21% O₂) was 1.98 ± 0.6 pmol/μg protein. Exposing carotid bodies to a hypercapnic, bicarbonate-buffered medium (10% CO₂, 21% O₂) increased the cAMP level to 9.0 ± 2 pmol/μg protein. As a positive control, carotid bodies were exposed to 10 μM forskolin, a specific adenylate cyclase activator, for 5 min. Forskolin increased the levels of cAMP to 38 ± 7 pmol/μg protein, demonstrating the capacity of the carotid body to generate cAMP (data not shown). In another series of experiments we tested the effect of extracellular acidification (pH 6.8) in the nominal absence of CO₂ on cAMP levels in the carotid body. The control cAMP level was 3.58 ± 0.8 pmol/μg protein in carotid bodies exposed to a HEPES-buffered medium with a normal pH (7.4, 100% O₂). Exposing carotid bodies to an acidic HEPES-buffered medium (pH 6.8, 100% O₂) did not produce a significant change in tissue cAMP levels (5.0 ± 0.9 pmol/μg protein, paired t-test, \( P > 0.05 \)). Figure 6 summarizes the results under the various experimental conditions. These results show that hypercapnia increases cAMP levels in the carotid body and further support a role for cAMP in the augmentation of the Ca²⁺ current by hypercapnia.

**Hypercapnia specifically augments the L-type Ca²⁺ current in glomus cells**

Glomus cells express a number of different types of high-voltage-activated (HVA) Ca²⁺ channels. To determine whether hypercapnia specifically affects one or more types of Ca²⁺ channels, we tested the effect of hypercapnia on the Ca²⁺ current in the presence of 2 μM Niso, a specific blocker of L-type channels, or 2 μM MVIIC, a specific blocker of N- and P/Q-type channels. Figure 7A shows current traces elicited when the extracellular solution contained 5% CO₂ alone, 5% CO₂ and Niso, or 10% CO₂ and Niso. On average, Niso blocked 27 ± 5% (\( n = 10 \)) of the macroscopic Ca²⁺ current, confirming the presence of L-type channels. More significantly, hypercapnia was no longer able to augment the Ca²⁺ current (1 ± 4%, \( n = 6 \)) in the presence of Niso. Figure 7B shows current traces elicited when the extracellular solution contained 5% CO₂ alone, 5% CO₂ and MVIIC, or 10% CO₂ and MVIIC. As expected, MVIIC by itself blocked a portion of the basal Ca²⁺ current (25 ± 3%, \( n = 5 \)). More significantly, hypercapnia still augmented the Ca²⁺ current (22 ± 3%, \( n = 5 \)) in the presence of MVIIC. Average augmentation of the Ca²⁺ current by hypercapnia (10% CO₂) in the absence and presence of Niso or MVIIC are summarized in Fig. 7C. These results suggest that hypercapnia selectively augments the Ca²⁺ current conducted by L-type Ca²⁺ channels, not by N- or P/Q-type Ca²⁺ channels.

**The effects of hypoxia and hypercapnia on Ca²⁺ current are synergistic**

Several studies have shown that CO₂ and O₂ have a synergistic effect on carotid body activity (Eyzaguire and Lewin 1961; Fitzgerald and Parks 1971; Lahiri and Delaney 1975; Pepper et al. 1995) and on glomus cell cytosolic Ca²⁺ (Dasso et al. 2000; Roy et al. 2000). To determine if CO₂ and O₂ interact at the Ca²⁺ channel level, we monitored Ca²⁺ current in the presence of hypoxia, hypercapnia, and hypoxic hypercapnia in the same cell. Figure 8A shows the effect of each stimuli alone and then together (\( n = 7 \)) on the current traces from a representative glomus cell. Hypoxia and hypercapnia alone augmented the Ca²⁺ current as expected. Moreover, the current traces in Fig. 8A and the time course in Fig. 8B show that when given together hypoxia and hypercapnia augmented
Sensitivity of Ca\(^{2+}\) for L-type Ca\(^{2+}\) that is not secondary to changes in pH. In addition, the effect on Ca\(^{2+}\) current traces in the time course.

Average percentage augmentation of macroscopic Ca\(^{2+}\) current elicited at 0 mV every 10 s as the extracellular solution is switched to and from HX, an HC, and a hypoxic hypercapnic solution. Broken line indicates current level and subscripts correspond to numbers in B, indicating the position of the current traces in the time course. B: time course for changes in Ca\(^{2+}\) current elicited at 0 mV every 10 s as the extracellular solution is switched to and from an HX, an HC, and a hypoxic hypercapnic solution. C: comparison of the average percentage augmentation of macroscopic Ca\(^{2+}\) current at 0 mV under the various conditions indicated in A and B.

The Ca\(^{2+}\) current to a greater degree than with either stimulus alone. In addition, the effect of hypoxic hypercapnia on Ca\(^{2+}\) current appeared to be voltage-independent and could be prevented by Niso (data not shown, \(n = 3\)). The additive effect of hypoxia and hypercapnia are more clearly shown in Fig. 8C, which compares the average percentage augmentation of the Ca\(^{2+}\) current by hypoxia (12 ± 3%), hypercapnia (13 ± 2%), and hypoxic hypercapnia (26 ± 4%). These results indicate that hypercapnia and hypoxia interact synergistically on Ca\(^{2+}\) current in glomus cells of the carotid body.

**DISCUSSION**

The objective of the present study was to determine whether CO\(_2\), a natural stimulus to the carotid body, affects Ca\(^{2+}\) current in glomus cells, and if so, to determine the mechanism of this effect. This study presents a novel finding in which hypercapnia augments Ca\(^{2+}\) current in glomus cells, an effect that is not secondary to changes in pH. In addition, the effect is specific for L-type Ca\(^{2+}\) current and involves a PKA-mediated mechanism. Furthermore, the effects of CO\(_2\) (hypercapnia) and O\(_2\) (hypoxia) on Ca\(^{2+}\) current in glomus cells appear to converge synergistically on L-type Ca\(^{2+}\) channels.

**Sensitivity of Ca\(^{2+}\) current to CO\(_2\)**

It can be seen from our results that hypercapnia reversibly augments glomus cell Ca\(^{2+}\) current recorded in a CO\(_2\)/HCO\(_3\) -buffered extracellular solution. The onset of the effects was rapid (occurring within tens of seconds after application of hypercapnia) and reversible (Fig. 1). Furthermore, the augmentation of Ca\(^{2+}\) current by hypercapnia was qualitatively and quantitatively similar under perforated and conventional whole-cell patch methods. This suggests that augmentation of the Ca\(^{2+}\) current by CO\(_2\) is not dependent on a dialyzable factor in the cytosol of glomus cells. Furthermore, our data strongly suggest that an increase in molecular CO\(_2\) during exposure to hypercapnia is the primary cause for the augmentation of Ca\(^{2+}\) current in rabbit glomus cells based on the following considerations. First, isohydric hypercapnia (increasing PCO\(_2\) without changing pH) also augmented the Ca\(^{2+}\) current (Fig. 2). Second, a reduction in extracellular (Fig. 3) or intracellular pH (Fig. 4) did not mimic the effect of hypercapnia on the Ca\(^{2+}\) current. In addition, both extracellular and intracellular acidosis shifted the activation curve for the Ca\(^{2+}\) current, while hypercapnia and isohydric hypercapnia did not. Finally, the same level of hypercapnia (10% CO\(_2\)) has a greater effect on the Ca\(^{2+}\) current when the pH effects are minimized (i.e., isohydric hypercapnia). Thus these observations indicate that an increase in molecular CO\(_2\) rather than pH contribute to the augmentation of the Ca\(^{2+}\) current during hypercapnia.

**Sensitivity of Ca\(^{2+}\) current to pH**

The carotid body responds independently to a decrease in pH as well as hypoxia and hypercapnia. Biscoe and colleagues (1970) showed that a decrease in arterial blood pH excites carotid body activity even when PO\(_2\) and PCO\(_2\) are kept constant. The stimulating effect of acidosis on carotid body activity has been ascribed to inhibition of both Ca\(^{2+}\)-activated (Peers and Green 1991) and TASK-like (Buckler et al. 2000) K\(^+\) channels in glomus cells. In this context it is interesting that acidosis alone inhibited Ca\(^{2+}\) current in the present study (Figs. 3 and 4). This finding is different from that reported by Peers and Green (1991), where they found no effect of mild intracellular or extracellular acidosis on Ca\(^{2+}\) current in rat glomus cells. The reason for this discrepancy is beyond the scope of this investigation, but it may reflect a difference in the species used (i.e., rabbits vs. rats). However, a more likely explanation is that the experimental conditions differed between these two studies. For example, we tested extracellular acidosis at pH 6.8 versus pH 7.0 in the other study, and the concentrations of acetate used between the two studies differed (20 vs. 10 mM). In both cases, the stimulus was more severe in the present study than in the previous study and this could account for the differences observed. On the other hand, several studies have shown that HVA Ca\(^{2+}\) channels in other neuronal cells are sensitive to both extracellular and intracellular pH (Klockner and Isenberg 1994; Tombaugh and Somjen 1997; Zhou and Jones 1996). In these studies, moderate extracellular acidosis (pH 6.9–6.0) reversibly depressed HVA Ca\(^{2+}\) current amplitude and caused a shift in the activation curve to more positive membrane potentials. In addition, intracellular acidosis has been shown to reversibly inhibit Ca\(^{2+}\) current in several studies (Klockner and Isenberg 1994; Mironov and Richter 1998; Tombaugh and Somjen 1997). Our results are consistent with these observations in that we find similar inhibitory effects on Ca\(^{2+}\) current by both extracellular acidosis and intracellular acidosis in glomus cells. Furthermore, the effects of K\(^+\) acetate on the Ca\(^{2+}\) current, which was used to
selectively alter intracellular pH, appear to be related to a change in intracellular pH rather than altering free Ba\(^{2+}\) concentration because of the following reasons: 1) a metallochromic indicator showed no effect of K\(^+\) acetate on the free Ba\(^{2+}\) concentration; 2) K\(^+\) acetate had no effect on the reversal potential of the Ca\(^{2+}\) current; and 3) the slow time course of the acetate effect is not consistent with changes in free Ba\(^{2+}\) concentration. Nonetheless, the fact that acidosis alone has an effect opposite to hypercapnia further supports the idea that the effects of CO\(_2\) on Ca\(^{2+}\) current in glomus cells are independent of pH.

**CO\(_2\)** augmentation of Ca\(^{2+}\) current is dependent on protein kinase A

It has been suggested that hypercapnia, in part, promotes vasodilatation of cerebral vessels through a cAMP-dependent mechanism, implying the involvement of PKA (Pelligrino and Wang 1998). There is also evidence that cAMP levels are increased during hypercapnia in the carotid body, suggesting a role for PKA in hypercapnic chemotransduction (Perez-Garcia et al. 1990). A number of findings in the present study provide further evidence for the involvement of PKA in sensing a hypercapnic stimulus by the carotid body. First, a cell-permeable protein kinase A inhibitor (PKAi) prevented the hypercapnic-induced augmentation of the Ca\(^{2+}\) current (Fig. 5). In addition, the cell-permeable analog of cAMP (8-Br-cAMP) mimicked the effect of hypercapnia and occluded further activation of the Ca\(^{2+}\) current by hypercapnia (Fig. 5). Finally, hypercapnia increased cAMP content in the carotid bodies, while lowering pH in the nominal absence of CO\(_2\) had no effect (Fig. 6). These results are consistent with the study of Perez-Garcia (1990) and colleagues who reported increased cAMP levels in response to hypercapnia in the rabbit carotid body. Further, it is well known that cAMP stimulates L-type Ca\(^{2+}\) current in other cells (Hartzell 1988; Hove-Madsen et al. 1996). However, the present results differ from those reported by others who found that cAMP analogs had no effect on the Ca\(^{2+}\) current in rat glomus cells (Hatton and Peers 1996). The reason for this difference is not clear, but could be due to species-related differences. Taken together, these data provide further evidence that hypercapnia increases cAMP in glomus cells and this increase in cAMP is linked to augmentation of the Ca\(^{2+}\) current. How might CO\(_2\) affect cAMP levels? It is known that hydration of CO\(_2\) may produce HCO\(_3^-\) as well as H\(^+\). Recently, it has been shown that bicarbonate anions stimulate cAMP production in cells via soluble adenylyl cyclase and soluble adenylyl cyclase may function as a bicarbonate sensor (Chen et al. 2000). However, it remains to be seen if soluble adenylyl cyclase is present in glomus cells or if it plays a role in the chemotransduction process of hypercapnia. Further studies are needed to define the mechanisms by which CO\(_2\) activates a PKA-dependent pathway.

**L-type Ca\(^{2+}\)** current is specifically modulated by multiple natural stimuli of the carotid body

Rabbit glomus cells express a variety of HVA Ca\(^{2+}\) channels (Overholt and Prabhakar 1997). Therefore, we tested if the effect of hypercapnia was strictly confined to one type of Ca\(^{2+}\) channel in glomus cells. Our data indicate that hypercapnia affects the L-type Ca\(^{2+}\) current in glomus cells, not N-, P/Q-, or resistant-type currents (Fig. 7). It is becoming apparent that modulation of L-type Ca\(^{2+}\) current in glomus cells may contribute to the normal response of the carotid body to physiological stimuli. We have previously shown that hypoxia specifically augments the L-type current (Summers et al. 2000), and the current results demonstrate that CO\(_2\) also augments the L-type Ca\(^{2+}\) current in glomus cells (Fig. 7). Also interesting is the fact that hypercapnia and hypoxia converge at the level of the L-type Ca\(^{2+}\) channel. This is supported by our results, showing that hypoxic hypercapnia augmented the Ca\(^{2+}\) current in glomus cells more than either stimulus alone (Fig. 8), and this response can be prevented by the L-type Ca\(^{2+}\) channel blocker, Niso (n = 3). This synergistic effect implies that the effects of hypoxia and hypercapnia on the Ca\(^{2+}\) current work in concert to produce a cumulative response, which then could be reflected in neurochemical release. This finding is important in that several studies have suggested that the L-type Ca\(^{2+}\) current is involved in hypoxic- and hypercapnic-induced neurotransmitter release from glomus cells (Gomez-Nino et al. 1994; Obeso et al. 1992). In addition, a number of studies have shown that CO\(_2\) and O\(_2\) have synergistic effects on both carotid sinus nerve activity (Eyzaguirre and Lewin 1961; Fitzgerald and Parks 1971; Lahiri and Delaney 1975; Pepper et al. 1995) and cytosolic Ca\(^{2+}\) (Dasso et al. 2000; Roy et al. 2000). The results of the present study suggest that the synergistic effects of hypoxia and hypercapnia on L-type Ca\(^{2+}\) current could contribute to the greater rise of intracellular Ca\(^{2+}\) in response to hypoxic hypercapnia and thus result in augmented transmitter release (Dasso et al. 2000; Roy et al. 2000).

In summary, this study has shown that CO\(_2\), independent of its effect on pH, augments L-type Ca\(^{2+}\) current in rabbit glomus cells. Further, our data suggest that the effects of CO\(_2\) on Ca\(^{2+}\) current are associated with a PKA-dependent mechanism. In addition, this is the first study to show that pH affects Ca\(^{2+}\) current in glomus cells, and acidosis unexpectedly had an effect opposite to that of hypercapnia and did not increase Ca\(^{2+}\) levels in the carotid body. Interestingly, the differential effects of pH and CO\(_2\) on Ca\(^{2+}\) current as well as cAMP levels in the carotid body suggest that there may be fundamental differences in the sensing mechanisms for CO\(_2\) versus pH stimuli at the carotid body. These fundamental differences between CO\(_2\) and pH sensing in glomus cells ultimately require further investigation. Finally, this study provides the first indication that the CO\(_2\)-O\(_2\) interaction converges at the Ca\(^{2+}\) channel level (L-type) in glomus cells.

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