Interactions between hypoxia and hypercapnic acidosis on calcium signaling in carotid body type I cells

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Dasso, Leonardo L. T., Keith J. Buckler, and Richard D. Vaughan-Jones. Interactions between hypoxia and hypercapnic acidosis on calcium signaling in carotid body type I cells. Am J Physiol Lung Cell Mol Physiol 279: L36–L42, 2000.—The effects of hypercapnic acidosis and hypoxia on intracellular Ca²⁺ concentration ([Ca²⁺]i) were determined with Indo 1 in enzymatically isolated single type I cells from neonatal rat carotid bodies. Type I cells responded to graded hypoxic stimuli with graded [Ca²⁺]i rises. The percentage of type I cells responding was also dependent on the severity of the hypoxic stimulus. Raising CO₂ from 5 to 10 or 20% elicited a significant increase in [Ca²⁺]i in the same cells as those that responded to hypoxia. Thus both stimuli can be sensed by each individual cell. When combinations of hypoxic and acidic stimuli were given simultaneously, the responses were invariably greater than the response to either stimulus given alone. Indeed, in most cases, the response to hypercapnia was slightly potentiated by hypoxia. These data provide the first evidence that the classic synergy between hypoxic and hypercapnic stimuli observed in the intact carotid body may, in part, be an inherent property of the type I cell.

chemoreceptor; oxygen; carbon dioxide

THE ABILITY OF THE CAROTID BODY to sense changes in both blood O₂ and CO₂ was first reported by Heymans et al. (14). Subsequently, many studies (9, 11, 12, 15, 20) have described an apparent synergy between the effects of these two stimuli on carotid sinus nerve (CSN) discharge frequency. For example, increasing levels of CO₂ evoke a rightward shift in the P₀₂-CSN discharge response curve. Similarly, decreasing P₀₂ increases the slope of the relationship between PCO₂ and CSN discharge. Thus hypercapnia would appear to increase the sensitivity of the carotid body to hypoxia and vice versa.

Although synergy between these two stimuli is of obvious importance in the control of respiration, the molecular mechanism that underpins this interaction remains unexplained. Recent research indicates that transduction of hypoxic and hypercapnic stimuli have much in common. The primary site for the sensing of both chemostimuli is thought to be the type I cell (13). Hypoxia and hypercapnic acidosis individually evoke a rapid rise in intracellular Ca²⁺ and Ca²⁺-dependent neurosecretion in these cells (4, 6, 7, 17, 21), indicating that, as in many other cell types, intracellular Ca²⁺ concentration ([Ca²⁺]i) plays a pivotal role in stimulus-secretion coupling. Furthermore, in the rat type I cell, this Ca²⁺ signal is generated by a broadly similar mechanism for both stimuli, i.e., through a depolarizing receptor potential and voltage-gated Ca²⁺ influx (7, 8).

These observations suggest that synergistic interactions between the two stimuli could occur at or before the generation of the intracellular Ca²⁺ signal in the type I cell. Indeed, it has been reported that extracellular acidosis can augment the [Ca²⁺]i response to hypoxia in isolated adult rabbit type I cell clusters (4). To date, however, there have been no published studies on the effects of both hypercapnia and hypoxia, either singly or in combination, on intracellular Ca²⁺ in the same cell. Thus there has not even been a formal demonstration that both sensory modalities are present in each individual cell (as opposed to distinct populations of CO₂-sensing and O₂-sensing cells).

In the present study, we used fluorescence microscopy to examine the sensitivity of enzymatically isolated single type I cells to hypercapnic acidosis and hypoxia. Our results show that individual cells can sense both types of stimuli when presented independently and in combination. Moreover, the response to a hypercapnic stimulus was potentiated by hypoxia. It was noted, however, that the degree of stimulus interaction at the level of [Ca²⁺]i, signaling in the isolated type I cell was relatively weak compared with that found in the intact organ. This is the first evidence that suggests that the characteristic interaction between hypoxic and hypercapnic acidic stimuli in the intact chemoreceptor may, in part, result from an intrinsic property of a common primary receptor cell.

MATERIALS AND METHODS

Cell isolation. Isolation procedures have been previously described (6). Briefly, Sprague-Dawley rats of 11–16 days of age were anesthetized with 4% halothane, and their carotid bodies were excised and stored in ice-cold phosphate-buffered saline. The rats were then killed by decapitation while still deeply anesthetized. The carotid bodies were enzymatically digested at 37°C with 0.4 mg/ml of collagenase and 0.2 mg/ml

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of trypsin and mechanically dispersed. The cells were suspended in Ham’s F-12-based culture medium containing 10% fetal calf serum, 84 U/l of insulin, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin. The cell suspension was then plated onto poly-d-lysine-coated glass cover slips and kept in culture for 4–8 h until used.

\[\text{[Ca}^{2+}\text{]}_i, \text{measurement in single cells.} \] [Ca\(^{2+}\)] was measured with Indo 1. The method is essentially the same as that previously described (6). The cells were loaded with Indo 1 by incubation with 2.5 μM Indo 1-AM in culture medium for 1 h (added as a 1 mM stock in DMSO). The cover slips were then transferred to a perfusion chamber (–100 μl) mounted on the stage of an inverted microscope (Nikon Diaphot). The cells were continuously superfused with a standard bicarbonate-buffered Tyrode solution or a test solution at 35–37°C at a flow rate of 4 ml/min. The chamber was based on that described by Stern et al. (22). Argon was blown continuously (250 ml/min) over the surface of the recording chamber to isolate the perfusion saline from the atmosphere. Indo 1 fluorescence was measured at 405 ± 16 and 495 ± 30 nm with excitation at 340 nm. The data were integrated over 500-ms intervals. The conversion of fluorescence ratios into \([\text{Ca}^{2+}\]i] was accomplished by in situ calibration of a separate group of cells with ionomycin as previously described (6, 24).

**Solutions.** The standard (control) Tyrode solution contained (in mM) 117 NaCl, 4.5 KCl, 22 NaHCO\(_3\), 1 MgCl\(_2\), 2.5 CaCl\(_2\), and 11 n-glucose and was equilibrated with 5% CO\(_2\)-95% air (pH 7.4 at 37°C). Hypoxic solutions were equilibrated with 5% CO\(_2\) and 0, 1, or 2.5% O\(_2\), the remainder being N\(_2\). Hypercapnic solutions were equilibrated with 10% CO\(_2\)-90% air (pH 7.1) or 20% CO\(_2\)-80% air (pH 6.9). Hypoxic hypercapnic solutions were equilibrated with 10 or 20% CO\(_2\) and 0, 1, or 2.5% O\(_2\), the remainder being N\(_2\). Anoxia was achieved by equilibrating solutions with 5% CO\(_2\)-95% N\(_2\), 10% CO\(_2\)-90% N\(_2\), or 20% CO\(_2\)-80% N\(_2\) and adding 1 mM Na\(_2\)S\(_2\)O\(_4\).

**Measurement of PO\(_2\).** The PO\(_2\) values achieved in the bath under conditions identical to those used in the experiments were measured with a 3-mm-wide Clark-type O\(_2\) electrode fitted with a polytetrafluoroethylene membrane (Diamond General) placed in the chamber. The cathode was polarized at ~650 mV. Data were collected at 50 Hz and filtered at 10 Hz. The PO\(_2\) given by the solutions equilibrated with 5% CO\(_2\)-95% N\(_2\), 10% CO\(_2\)-90% N\(_2\), or 20% CO\(_2\)-80% N\(_2\) and containing 1 mM Na\(_2\)S\(_2\)O\(_4\) was defined as 0 Torr. The standard Tyrode medium was considered to have a PO\(_2\) of 150 Torr. The PO\(_2\) levels achieved in the chamber by equilibrating the perfusion medium with 0, 1, and 2.5% O\(_2\) (plus N\(_2\) and various concentrations of CO\(_2\)) were 2.3 ± 0.5 (n = 6 independent determinations), 9.5 ± 0.4 (n = 5 independent determinations), and 18.8 ± 0.9 (n = 5 independent determinations) Torr, respectively.

**Materials.** Poly-d-lysine, EGTA, Na\(_2\)S\(_2\)O\(_4\), trypsin type III, Ham’s F-12 medium, phosphate-buffered saline, insulin, L-glutamine, and penicillin-streptomycin were from Sigma. Fetal calf serum (heat inactivated) was from Gibco BRL. Indo 1-AM and ionomycin were from Calbiochem. Collagenase type I was from Worthington. All other chemicals were of the highest grade commercially available.

**Analysis of results.** All chemostimuli were applied for 2 min with the exception of those involving anoxia, which were applied for only 1 min to avoid subjecting the cells to extreme conditions for long periods of time. Changes in \([\text{Ca}^{2+}\]i], were integrated by calculating the area under the curve bounded by the mean resting \([\text{Ca}^{2+}\]i], determined before application of the stimulus and the actual \([\text{Ca}^{2+}\]i], during the stimulus. Changes in \([\text{Ca}^{2+}\]i], are therefore expressed as nanomoles per second (note that the anoxic response was multiplied by 2 for comparison with other stimuli). Data are expressed as ± SE. PO\(_2\)-\([\text{Ca}^{2+}\]i] response curves were fitted with InPlot 4.01 (GraphPad Software, San Diego, CA) to a four-parameter equation: Y = (B - A)/(1 + (PO\(_2\)/P50)nH) + A, where Y is the time-integrated \([\text{Ca}^{2+}\]i] response, B is the maximal \([\text{Ca}^{2+}\]i] response, A is the \([\text{Ca}^{2+}\]i] response at infinite PO\(_2\), P50 is the PO\(_2\) level causing 50% of the maximal response, and nH is the Hill coefficient. Significance of the effects of hypercapnia at any given level of PO\(_2\) was assessed with a two-tailed paired t-test. The specific hypothesis that hypoxia increases the response to CO\(_2\) was assessed with a one-tailed, one-way ANOVA a priori contrast test not assuming equal variances (with the SPSS statistical package).

**RESULTS**

Single rat type I cells were found to respond to hypoxia with a rapid elevation in \([\text{Ca}^{2+}\]i]. The \([\text{Ca}^{2+}\]i] response was dependent on the severity of the hypoxic challenge. All cells responded to anoxia with an increase in \([\text{Ca}^{2+}\]i]. With lesser degrees of hypoxia, it was found that progressively fewer cells responded with an elevation in \([\text{Ca}^{2+}\]i], and, when they did, the elevation was smaller (Fig. 1). A similar variability in the responsiveness of type I cells to hypoxic stimuli has also been observed in other studies (3, 7, 23).

To determine whether individual cells can sense both hypercapnic acidosis and hypoxia, a series of experiments was performed in which the cells were challenged with hypoxic stimuli, hypercapnic acidic stimuli, and combinations of the two. In these studies, a high proportion of single type I cells (i.e., >80%) responded to hypercapnic acidic or hypoxic and hypercapnic acidic stimuli (Fig. 1B). Of 10 cells that were exposed to a variety of stimuli (i.e., anoxia; hypoxia, 2.3 and 9.5 Torr; hypercapnia, 10% CO\(_2\), pH 7.1; or combinations of hypoxia and hypercapnia), the majority (i.e., 80%) responded to all of these stimuli. These data indicate that the same cells can sense both hypoxia and acidosis. In addition, in many cells, when hypoxia and hypercapnic acidosis were given in combination, the \([\text{Ca}^{2+}\]i] response was greater than the \([\text{Ca}^{2+}\]i] response to either stimulus given separately (e.g., Fig. 2A). This indicates that the responses to hypoxia and hypercapnic acidosis can be additive. Figure 3A summarizes data for the whole test group. On average, increasing PCO\(_2\) from 5 to 10% evoked a significant increase in \([\text{Ca}^{2+}\]i], under normoxic conditions and increased the \([\text{Ca}^{2+}\]i] response to hypoxia at 2 Torr (a nonsignificant increase is also evident at 9.5 Torr). Hypercapnic acidosis failed, however, to significantly increase the \([\text{Ca}^{2+}\]i] response in anoxia (P = 0.5; n = 22 cells). This failure to augment the anoxic response is not due to saturation of Indo 1 because the emission ratios observed are lower than those reached during calibration with ionomycin.

To examine the possible interactions between these two stimuli in generating the \([\text{Ca}^{2+}\]i] response more fully, a second series of experiments was conducted with five different levels of PO\(_2\), a stronger hypercapnic acidic stimulus (20%, pH 6.9), and a larger data set (Fig. 2B). In these experiments, the \([\text{Ca}^{2+}\]i] responses to hypercapnic hypoxia were significantly greater than
those to pure hypoxia alone at all levels of PO₂ including anoxia. This observation reinforces the conclusion that the effects of hypoxic and hypercapnic acidic stimuli on type I cell Ca²⁺ can summate. The data obtained in this series of experiments for hypoxic stimuli in normocapnic and hypercapnic acidic saline were also well fitted by a rectangular hyperbola (Fig. 3B). The P₅₀ values for the mean data were 1.4 and 1.6 Torr for the control and hypercapnic acidic curves, respectively. Calculation of P₅₀ values on a cell-by-cell basis yielded mean P₅₀ values of 2.73 ± 0.47 Torr for 5% CO₂ (n = 22 cells) and 2.66 ± 0.43 Torr for 20% CO₂ (n = 30 cells). These values were not significantly different, suggesting that hypercapnic acidosis does not change the affinity of the sensor for O₂.

The results shown in Fig. 3B have been replotted in Fig. 4A, which displays the rise of [Ca²⁺]ᵢ as a function of percent CO₂ recorded at different PO₂ levels. When plotted in this manner, the data show that hypercapnic acidosis increases [Ca²⁺]ᵢ at all levels of PO₂. Moreover, the hypercapnic acidic component of the [Ca²⁺]ᵢ response appears to increase in magnitude as PO₂ is decreased. This effect is more evident in Fig. 4B, which plots the difference in the [Ca²⁺]ᵢ response divided by the increment in CO₂ (i.e., the slope of the CO₂-response curve in Fig. 4A) as a function of PO₂. The response to hypercapnic acidosis at each level of hypoxia was compared with that obtained under normoxic conditions (150 Torr); the responses observed at PO₂ levels of 9.8 and 2.3 Torr were found to be significantly greater than the control response (P < 0.05 by 1-tailed, 1-way ANOVA a priori contrast test). Similarly, when all the data for the CO₂ acid responses under hypoxic or anoxic conditions were grouped, the slope [change in (Δ) [Ca²⁺]ᵢ/Δ%CO₂] of the averaged CO₂ acid response was significantly greater than control value (control, 0.073 ± 0.01 nM·s⁻¹·10⁻⁴%/; hypoxia or anoxia, 0.119 ± 0.015 nM·s⁻¹·10⁻⁴%/; P < 0.01 by 1-tailed t-test or 1-tailed 1-way ANOVA a priori contrast test). Thus hypoxia does potentiate the [Ca²⁺]ᵢ response to hypercapnic acidosis. To investigate this effect further, the responses to hypercapnic acidosis under hypoxic conditions were normalized on a cell-by-cell basis to the response recorded under normoxic conditions (note that cells that failed to respond to hypercapnic acidosis under normoxic conditions were consequently excluded from this analysis). The results of this normalization procedure are shown in Fig. 4C, from which it is evident that there was quite a strong potentiation of the hypercapnic acidic response at 2.3 and 9.8 Torr. The
response was also weakly, but significantly, potentiated at 20 Torr. At 0 Torr, however, the response was not significantly different from the control value (1-tailed 1-way ANOVA a priori contrast test).

DISCUSSION

Comparative aspects of the effects of hypoxic stimuli on type I cell [Ca\textsuperscript{2+}]i. In this and in other studies (4, 7), type I cell [Ca\textsuperscript{2+}]i only begins to rise significantly in response to hypoxic challenges when PO\textsubscript{2} is below 20–40 Torr. In contrast, the intact chemoreceptor displays a marked increase in neural discharge as arterial PO\textsubscript{2} falls below ~70–100 Torr (10). This difference in sensitivity probably reflects PO\textsubscript{2} gradients within the carotid body. For example, in the cat carotid body under normoxic conditions, the PO\textsubscript{2} of microvascular blood is ~50 Torr, i.e., approximately half the arterial level (16). In this same study, the neural response to hypoxia increased sharply only as microvascular PO\textsubscript{2} fell below ~40 Torr. Given that there may be a further decline in PO\textsubscript{2} between capillaries and cells, we believe the levels of hypoxia used in this study to approximate the cellular levels that lead to excitation in vivo.

It is notable that the O\textsubscript{2} sensitivity of [Ca\textsuperscript{2+}]i is somewhat less than that recently reported for the effects of hypoxia on background K\textsuperscript{+} currents in rat type I cells [half-maximal activation at ~12 Torr (5)]. Similar observations have also been made in isolated rabbit type I cells where the sensitivity of O\textsubscript{2}-sensitive K\textsuperscript{+} channels to hypoxia appears considerably greater than that of the [Ca\textsuperscript{2+}]i response (17). These differences probably reflect the fact that the cell must depolarize to a level sufficient to activate voltage-gated Ca\textsuperscript{2+} channels before [Ca\textsuperscript{2+}]i can rise; i.e., there is an apparent threshold above which changes in K\textsuperscript{+}-channel activity and membrane potential will have little effect on [Ca\textsuperscript{2+}]i. The sensitivity of type I cell [Ca\textsuperscript{2+}]i to hypoxia will therefore depend on the initial resting membrane potential of the cell. This suggests that other chemostimuli could influence the responsiveness to hypoxia by modulating the type I cell resting membrane potential.

The transduction of hypoxic and hypercapnic acidic stimuli converges within individual type I cells. One of the principal observations presented in this paper is that the same type I cell can respond to both hypercapnic acidic and hypoxic stimuli. Thus the transduction pathways for both O\textsubscript{2} and CO\textsubscript{2} acid coexist within a common primary receptor cell and must, therefore, converge at or before the generation of the [Ca\textsuperscript{2+}]i.
Indeed, convergence probably occurs before changes in \( [\text{Ca}^{2+}]_i \) because, in the rat, both stimuli have been shown to raise \( [\text{Ca}^{2+}]_i \) via a depolarizing receptor potential, electrical activity, and ensuing voltage-gated \( \text{Ca}^{2+} \) entry (7, 8). Moreover, for both stimuli, these electrophysiological events result from the inhibition of \( K_1 \) currents flowing through a resting \( K_1 \) conductance (5, 8) and a voltage- and \( \text{Ca}^{2+} \)-activated \( K_1 \) channel (18, 19). Thus the sensing of hypoxia and hypercapnia/acidosis involves many common steps. Some models of \( \text{O}_2 \) and \( \text{CO}_2 \) sensing propose that the effects of \( \text{CO}_2 \) and/or pH are mediated through changes in the affinity of an \( \text{O}_2 \) sensor (15). A prediction of this hypothesis would be that under anoxic conditions the receptor would be unable to detect changes in \( \text{PcO}_2 \). A strong hypercapnic acidic stimulus (20% \( \text{CO}_2 \)), how-

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Fig. 3. Time-integrated \( [\text{Ca}^{2+}]_i \) response to hypoxic and hypercapnic acidic stimuli in single type I cells. \( [\text{Ca}^{2+}]_i \) elevations were analyzed by determining area under curve of response elicited by chemostimuli in experiments similar to those shown in Fig. 2 as described in MATERIALS AND METHODS. Results are means \( \pm \) SE. Continuous lines are best fit for a rectangular hyperbola (see MATERIALS AND METHODS). A: 10% \( \text{CO}_2 \) hypercapnic stimulus \( (n = 22 \text{ cells at } \text{P}_{\text{O}_2} = 0 \text{ Torr, } 19 \text{ cells at } 2.3 \text{ Torr, } 18 \text{ cells at } 9.5 \text{ Torr, and } 16 \text{ cells at } 150 \text{ Torr}). \) For control curve (5% \( \text{CO}_2 \), \( \text{P}_{\text{O}_2} \) level causing 50% of maximal response \( (P_{50}) = 1.26 \text{ Torr and } \text{n}_H = 0.97 \); for hypercapnic curve, \( P_{50} = 1.85 \text{ Torr and } \text{n}_H = 1.27 \). B: 20% \( \text{CO}_2 \) hypercapnic stimulus \( (n = 48 \text{ cells at } 0 \text{ Torr, } 34 \text{ cells at } 2.3 \text{ Torr, } 33 \text{ cells at } 9.5 \text{ Torr, } 10 \text{ cells at } 19 \text{ Torr, and } 32 \text{ cells at } 150 \text{ Torr}). \) For control curve, \( P_{50} = 1.43 \text{ Torr and } \text{n}_H = 1.49 \); for hypercapnic curve, \( P_{50} = 1.63 \text{ Torr and } \text{n}_H = 0.9. \) P values indicate significant difference between hypercapnic and normocapnic responses at the same \( \text{P}_{\text{O}_2} \) was assessed by 2-tailed paired \( t \)-test. NS, not significant.

Fig. 4. Effects of hypoxia on \( [\text{Ca}^{2+}]_i \) response to hypercapnic acidosis. A: integrated \( [\text{Ca}^{2+}]_i \), under normocapnic (5% \( \text{CO}_2 \)) and hypercapnic (20% \( \text{CO}_2 \)) conditions at different levels of \( \text{P}_{\text{O}_2} \). Results are means \( \pm \) SE. Data are derived from those in Fig. 3B. B: effect of hypoxia on \( \text{CO}_2 \) sensitivity. Values are slopes [change in \( \Delta [\text{Ca}^{2+}]_i / \Delta \% \text{CO}_2 \)] of \( \text{CO}_2 \)-response curve shown in A (significance was assessed by 1-tailed, 1-way ANOVA a priori contrast test: \( P = 0.028 \) at 2.3 Torr; \( P = 0.0035 \) at 9.8 Torr). C: effects of hypoxia on relative \( \text{CO}_2 \) sensitivity. Hypercapnic acidic responses under hypoxic conditions were normalized to hypercapnic acidic response obtained under normoxic conditions (excluding those cells that failed to respond to hypercapnia under normoxic conditions). *Significant difference of responses obtained under each hypoxic condition relative to those obtained under normoxia was assessed by 1-tailed, 1-way ANOVA a priori contrast test (0 Torr: \( P = 0.32, n = 28 \text{ cells}; 2.8 \text{ Torr: } P = 0.022, n = 28 \text{ cells}; 9.8 \text{ Torr: } P = 0.015, n = 28 \text{ cells}; 20 \text{ Torr: } P = 0.048, n = 9 \text{ cells}).
ever, clearly increased $[\text{Ca}^{2+}]_i$ in anoxia (Figs. 2B and 3B). The model would also predict that the response to hypercapnic acidosis would be abolished at high PO$_2$ (i.e., where PO$_2$ is much greater than the affinity or the P$_{50}$ for the O$_2$ sensor). We find robust responses to hypercapnia/acidosis even at a PO$_2$ of 150 Torr. Moreover, there was no significant increase in the P$_{50}$ for hypoxic elevation of $[\text{Ca}^{2+}]_i$ under hypercapnic acidic conditions. Together, these results suggest that changes in the affinity of an O$_2$ sensor cannot account for CO$_2$ sensing. Consequently, it seems probable that the primary CO$_2$ and/or pH sensor is independent of the O$_2$ sensor but that the transduction pathways converge at the level of the cell membrane and the influx of Ca$^{2+}$.

**Stimulus interaction between hypoxic and hypercapnic acidic stimuli in the isolated type I cell.** One of the key features of the neural response to hypercapnic acidic stimuli is that it is strongly potentiated by hypoxia and vice versa (11, 15, 20). The data presented here (Figs. 3 and 4) provide the first indication that potentiation between hypoxic and hypercapnic acidic stimuli may be an inherent property of the type I cell, with hypoxia increasing the $[\text{Ca}^{2+}]_i$ response to hypercapnia/acidosis. This observation contrasts with that of Bamford et al. (2), who failed to detect any evidence of stimulus interaction in Ca$^{2+}$ measurements made from clusters of rat type I cells. In our study, the interactive effect is most evident when the $[\text{Ca}^{2+}]_i$ responses are normalized to those obtained under control conditions, indicating that interaction may be particularly prominent in some individual cells. When Ca$^{2+}$ responses are averaged across a population of isolated cells, however, the effect does not appear to be very strong; i.e., the slope of the mean $[\text{Ca}^{2+}]_i$-PCO$_2$ relationship increases by only ~85% when PO$_2$ is lowered from 150 to 10 Torr (Fig. 4B). These results compare with an increase in CO$_2$ sensitivity in the adult rat carotid body (measured as neural discharge frequency) of fivefold when PO$_2$ is lowered from 200 to 100 Torr (20; note that this study was conducted on a superfused intact rat carotid body, hence the higher levels of PO$_2$). The difference between the extent of interaction measured in the mean $[\text{Ca}^{2+}]_i$ response and that seen in neural discharge is unlikely to be due to the age of the rats used here because a recent study (25) on the effects of postnatal age on $[\text{Ca}^{2+}]_i$ signaling in isolated type I cells showed that responses to hypoxia are mature by 11 days of age; moreover, another recent study (2) failed to detect stimulus interaction in $[\text{Ca}^{2+}]_i$ signaling in older (14- to 21-day) rats. Direct quantitative comparison of the $[\text{Ca}^{2+}]_i$ response of isolated cells with neural discharge may not be entirely valid, however, for the following reasons. 1) We measured the average (integrated) $[\text{Ca}^{2+}]_i$; local intracellular Ca$^{2+}$ levels at the sites of exocytosis may differ significantly. 2) The $[\text{Ca}^{2+}]_i$ response of single type I cells to hypercapnic acidic and the milder hypoxic stimuli is rarely a sustained monotonic rise in $[\text{Ca}^{2+}]_i$, but often displays marked oscillations in $[\text{Ca}^{2+}]_i$ (e.g., Fig. 2; see also Ref. 6). How spatial gradients and different patterns of $[\text{Ca}^{2+}]_i$ signaling will affect the secretory response is unknown, but it is unlikely that these factors are without importance. In view of these considerations, it is difficult to evaluate precisely the role of stimulus interaction seen at the level of $[\text{Ca}^{2+}]_i$ signaling in determining the stimulus interaction seen in the whole organ. The data nevertheless establish the principle that some interaction does occur at the level of Ca$^{2+}$ entry into the type I cell.

There may, of course, be other factors involved in defining the extent to which hypoxia and hypercapnic acidosis appear to interact in the intact organ. Perhaps the most important consideration is the precise relationship between the amplitude of the $[\text{Ca}^{2+}]_i$ signal in the type I cell, neurosecretion, and the excitation of sensory nerve endings. Unfortunately, this part of the transduction cascade is poorly defined, but in the closely related chromaffin cell, the relationship between $[\text{Ca}^{2+}]_i$ and exocytosis displays a Hill coefficient of ~2 (1). If the same were true of the type I cell, then even additive $[\text{Ca}^{2+}]_i$, responses to hypoxia and hypercapnic acidosis could generate greater than additive neurosecretory responses. A significant part of the multiplicative interaction between CO$_2$ or pH and O$_2$ seen in the whole organ could therefore result from the intrinsic $[\text{Ca}^{2+}]_i$, sensitivity of exocytosis.

In conclusion, in this report, we have shown that individual type I cells are able to sense both hypoxic and acidic stimuli independently and in combination. Type I cells would therefore seem to be an ideal site for the integration of the sensory response to these independent stimuli. In this respect, it is of considerable interest that the $[\text{Ca}^{2+}]_i$ responses to hypoxia and hypercapnic acidosis are at least additive and, in most cases, multiplicative. This interaction between hypercapnic acidosis and hypoxia in defining Ca$^{2+}$ levels in the type I cell could therefore form the basis of the marked synergy between these two stimuli that is an important functional characteristic of the carotid body. Thus many of the sensory properties of the carotid body could be explained in terms of the physiology of the preneural receptor (type I) cells alone.

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