

pH Regulation in Adult Rat Carotid Body Glomus Cells

Importance of Extracellular pH, Sodium, and Potassium

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ABSTRACT The course of intracellular pH (pH_i) was followed in superfused (36°C) single glomus (type I) cells of the freshly dissociated adult rat carotid body. The cells had been loaded with the pH-sensitive fluorescent dye 2',7'-(2-carboxyethyl)-5 (and -6)-carboxyfluorescein. The high K^+ -nigericin method was used for calibration. The pH_i of the glomus cell at pH_o 7.40, without CO_2 , was 7.23 ± 0.02 ($n = 70$); in 5% $\text{CO}_2/25 \text{ mM HCO}_3^-$, pH_i was 7.18 ± 0.08 ($n = 9$). The pH_i was very sensitive to changes in pH_o . Without CO_2 , $\Delta\text{pH}_i/\Delta\text{pH}_o$ was 0.85 (pH_o 6.20–8.00; 32 cells), while in $\text{CO}_2/\text{HCO}_3^-$ this ratio was 0.82 irrespective of whether pH_o (6.80–7.40; 14 cells) was changed at constant PCO_2 or at constant $[\text{HCO}_3^-]_o$. The great pH_i sensitivity of the glomus cell to pH_o is matched only by that of the human red cell. An active Na^+/H^+ exchanger (apparent $K_m = 58 \pm 6 \text{ mM}$) is present in glomus cells: Na^+ removal or addition of the amiloride derivative 5-(*N,N*-hexamethylene)-amiloride induced pH_i to fall by as much as 0.9. The membrane of these cells also contains a K^+/H^+ exchanger. Raising $[\text{K}^+]_o$ from 4.7 to 25, 50, or 140 mM reversibly raised pH_i by 0.2, 0.3, and 0.6, respectively. Rb^+ had no effect, but in corresponding concentrations of TI^+ alkalization was much faster than in K^+ . Reducing $[\text{K}^+]_o$ to 1.5 mM lowered pH_i by 0.1. These pH_i changes were shown not to be due to changes in membrane voltage, and were even more striking in the absence of Na^+ . Intrinsic buffering power (amount of strong base required to produce, in the nominal absence of CO_2 , a small pH_i rise) increased from 3 to $\sim 21 \text{ mM}$ as pH_i was lowered, but remained nearly unchanged below pH_i 6.60. The fitted expression assumed the presence of one "equivalent" intracellular buffer (pK 6.41, 41 mM). The exceptional pH_i sensitivity to pH_o suggests that the pH_i of the glomus cell is a link in the chemoreceptor's response to external acidity.

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INTRODUCTION

The carotid body, a small organ perched at or near the bifurcation of each of the two common carotid arteries, monitors oxygen pressure and acid–base conditions of the blood. De Castro (1928) was the first to demonstrate that its innervation by a branch of the glossopharyngeal nerve (“carotid body nerve”) is afferent in nature, from which he deduced that the carotid body senses alterations in the blood. Since that time, a wealth of data has been accumulated on the neural response, and also on the properties of some of the cells of the organ (for recent work see, for instance, Duchen, Caddy, Kirby, Patterson, Ponte, and Biscoe, 1988; López-López, González, Ureña, and López-Barneo, 1989; Ureña, López-López, González, and López-Barneo, 1989; Biscoe and Duchen, 1990; Buckler, Vaughan-Jones, Peers, and Nye, 1991*a*; Buckler, Vaughan-Jones, Peers, Lagadic-Gossmann, and Nye, 1991*b*; Peers and Green, 1991; for reviews see Verna, 1979; Fidone and González, 1986). Yet the precise mechanisms whereby the carotid body translates chemical changes of the blood into nerve activity are still far from clear.

In early *in vivo* studies from this laboratory (Hornbein and Roos, 1963) in which we recorded the integrated electrical activity of the cat carotid body nerve, we demonstrated the preeminence of an elevated arterial H ion concentration, rather than of PCO₂, as a chemoreceptor stimulus. We raised the possibility that this response to acidity might be mediated via changes of “H ion concentration within the chemosensing cells” of the carotid body. At the time, little information was available about the physiology of its various cell types, and there was no standard method for measuring intracellular pH (pH_i). There is now reasonable (though not unanimous and, admittedly, largely circumstantial) evidence that the so-called glomus cells (also known as type I cells), which are by far the most numerous (Verna, 1979), serve a transducer function, the nerve endings in their immediate vicinity being the “postsynaptic” structures (see, for instance, Verna, 1979; Duchen et al., 1988). Also, optical methods make it possible to follow pH_i in relatively small cells such as glomus cells (~10 μm diameter). In this work we have studied the relationship between extracellular pH (pH_o) and pH_i in isolated adult rat glomus cells in both the presence and (nominal) absence of CO₂. We also examined the effect on pH_i of changes in extracellular [Na⁺] and [K⁺].

A preliminary report on this work has been published (Wilding, Cheng, and Roos, 1991).

METHODS

Adult Sprague-Dawley rats (~300 g; Harlan Sprague-Dawley, Inc., Indianapolis, IN) were anesthetized with sodium pentobarbital (1 mg/kg). The two carotid bodies were dissected free and put in 2 ml of enzyme-containing salt solution (pH ~7.4) of the following composition (in millimoles per liter): 140 Na⁺, 4.7 K⁺, 2 Mg²⁺, 3 *N*-methyl-D-glucammonium⁺ (NMDG⁺), 146.7 Cl⁻, 10 HEPES, and 5 glucose (NMDG⁺ was substituted for Ca²⁺, which was omitted to prevent clumping of the cells). The solution contained 4 mg each of class II collagenase (Worthington Biochemical Corp., Freehold, NJ) and type XIV protease (Sigma Chemical Co., St. Louis, MO). The cells, kept at 37°C, were triturated twice and then centrifuged for 10 min at 800 *g*. The

pellet was resuspended in 2 ml of the same salt solution containing 4 mg each of class II collagenase and hyaluronidase (Sigma Chemical Co.), 10 mg of albumin (Sigma Chemical Co.), and 1.4 mg of DNase I (Sigma Chemical Co.). Trituration was repeated and the suspension was again centrifuged. The pellet was resuspended, washed with enzyme-free salt solution, and centrifuged twice. This procedure is a modification of that of Biscoe, Duchen, Eisner, O'Neill, and Valdeolmillos (1989). An aliquot of the cell suspension was placed on a coverslip, coated with the adhesive polyphenolic protein Cell Tak (Collaborative Research, Inc., Bedford, MA), that formed the bottom of a shallow chamber (surface area $\sim 75 \text{ mm}^2$, depth $\sim 1 \text{ mm}$) fastened to the stage of a Nikon Diaphot inverted microscope. After a 30-min period required for the cells to settle, most of the chamber's contents was replaced by a salt solution that now contained 2 mM Ca^{2+} and 10 μM of the acetoxymethyl ester of 2',7'-bis(2-carboxyethyl)-5- (and -6) carboxyfluorescein (BCECF; Molecular Probes, Inc., Eugene, OR) (Rink, Tsien, and Pozzan, 1982). The ester readily enters the cell where esterases remove the acetoxymethyl groups, trapping an ion whose fluorescence is pH sensitive. The dye was stored at -20°C as a 10-mM solution in water-free DMSO. After 30 min of dye-loading, superfusion by gravity at a rate of 4 ml/min was started with dye-free solution from one of an assembly of six 140-ml syringes which were kept in a thermostatically controlled water bath. Chamber temperature, continuously monitored with a miniature probe, was $36\text{--}36.5^\circ\text{C}$. Some studies were done at room temperature ($\sim 22^\circ\text{C}$); these will be separately described. CO_2 -impermeable Saran tubing connected the syringes through a manifold to the chamber. The CO_2 -free experiments were usually started with "normal" superfusate (pH 7.40) of the following composition (millimoles per liter): 140 Na^+ , 4.7 K^+ , 2 Mg^{2+} , 2 Ca^{2+} , 146.7 Cl^- , 10 HEPES, and 5 glucose (modified Tyrode's). The effect on pH_i of raising or lowering pH_o , of pulsing with NH_4Cl , or of changing Na^+ , Cl^- , or K^+ concentrations was then studied. At $\text{pH}_o < 6.80$ the buffer was 10 mM PIPES; at $\text{pH}_o 8.00$ the buffer was 10 mM glycylglycine. When $[\text{Na}^+]_o$ was reduced, NMDG⁺ was used as substitute. When $[\text{Cl}^-]_o$ was reduced or omitted, glucuronate was the substitute (except that the Ca^{2+} and Mg^{2+} salts were gluconates; to compensate for binding, the Ca^{2+} concentration was increased to 5 mM). In most experiments in which $[\text{K}^+]_o$ was raised, $[\text{Cl}^-]_o$ was kept constant; in a few, $[\text{Cl}^-]_o$ was reciprocally reduced to maintain $[\text{K}^+]_o \times [\text{Cl}^-]_o$ constant. The thallium (Tl^+) Tyrode was made entirely from nitrate salts. K^+ and Tl^+ were substituted for Na^+ .

The intrinsic (i.e., nonbicarbonate) buffering power, β (Roos and Boron, 1981), was measured in 32 cells (14 rats) between pH_i 5.90 and 7.70. These pH_i 's were achieved by varying pH_o (6.20–8.20). At a stable pH_i value, buffering power was evaluated with small pulses of NH_4Cl (0.8–5 mM, the higher concentrations at the more acidic pH_i 's), which were applied for 1–2 min. After the pulses pH_i always returned to the original value.

In experiments with CO_2 the syringes' contents were continuously gassed with 1, 5, or 10% CO_2 in O_2 . Preliminary experiments showed negligible loss of CO_2 between syringe and chamber. The effect of hypoxia was examined by switching to a superfusate gassed with 2% O_2 , the remainder being either N_2 or a N_2/CO_2 mixture. The effect of 0.5 mM SITS (4-acetamido-4'-isothiocyanatostilbene 2,2'-disulfonic acid; Pfalz and Bauer Inc., Waterbury, CT) on pH_i recovery from CO_2 was studied by preexposing the cells to the drug for 1 h. This should result in irreversible binding to the membrane. The subsequent solutions were SITS-free since the drug's fluorescence interferes with pH_i measurements. The effect on pH_i of 5-(*N,N*-hexamethylene)-amiloride (HMA; Research Biochemicals Inc., Natick, MA), a powerful inhibitor of Na^+/H^+ exchange (Simchowit and Cragoe, 1986), was also examined. Here, the problem of the drug's fluorescence cannot be solved so easily, since its reversible binding properties require it to remain present in the superfusate. We could not use concentrations greater than 15 μM without noticeable optical interference. Since, in 140 mM Na^+ , the drug at this low

concentration had no effect on pH_i when an effect could reasonably be expected, HMA was always added at reduced $[\text{Na}^+]_o$ (50 and sometimes 20 mM).

The optical arrangement was, in principle, that described by previous workers (Thomas, Buchsbaum, Zimniak, and Racker, 1979; Thomas, 1986; Boyarsky, Ganz, Sterzel, and Boron, 1988). The intracellular dye was alternately excited at 490 and 440 nm. The emitted light over a bandwidth of 520–560 nm was sensed by a phototube (Nikon P1). At 490 nm excitation the intensity of the emitted light is much more sensitive to changes in pH than at 440 nm. The ratio of the two emitted intensities, a measure of pH_i , should be quite insensitive to variations in dye concentration.

The light source was a 75-W xenon lamp. The light passed through a heat filter and then, alternately, through two band-pass filters (490 ± 0.5 and 440 ± 0.9 nm) mounted on a disk that was spinning at 8 s per revolution. The disk's movement, actuated by a stepper motor, was computer-controlled and synchronized with a shutter (located immediately after the disk) that opened for 0.05 s at each wavelength. In this time interval, 20 samples of the phototube's output were taken, digitized, and averaged. After each disk revolution, throughout the experiment, the computed ratio of the two averages was plotted on a monitor and stored. After the experiment the ratios were converted to pH_i values using the calibration curve described below. The course of pH_i thus obtained was printed. Neutral density filters, placed after the shutter, reduced the exciting light by >95%; this light reached the bottom of the chamber, to which the cells were attached, via a dichroic mirror placed at a 45° angle. The light emitted by the cells, passing through the mirror and a 520–560-nm band-pass filter, was directed to the phototube. The cells could also be observed by directing the emitted light to an eyepiece. A circular field diaphragm limited the illuminated area; a second, rectangular diaphragm, placed in the path of the emitted light, allowed the light to be collected from one selected cell and its immediate surrounding.

All measurements were made on single glomus cells. These cells are by far the most numerous ones in the carotid body (Verna, 1979). They could be identified by their relatively large size (10–12 μm). No fluorescence could be detected in the medium between the cells. Also, there was no autofluorescence, probably because of the low intensity of the exciting light. This, in conjunction with the brief periods of illumination, allowed us to make observations for as long as 45 min.

At the end of each experiment a single calibration procedure was performed by superfusing the cells with a solution at pH 7.10 containing (mM) 140 KCl, 4 MgCl_2 , 10 HEPES, 5 glucose, and 10 μM nigericin (Thomas et al., 1979). Intracellular pH closely approximates extracellular pH when glomus cells are exposed to nigericin-containing 140 mM K^+ (Buckler and Vaughan-Jones, 1990). In preliminary experiments, a complete calibration curve was constructed from 88 measurements obtained from 19 glomus cells. The cells were exposed to nigericin/140 mM K^+ -containing solutions buffered to various pH's (5.60–8.00). In each cell a measurement at 7.10 was included. The fluorescence excitation ratios thus obtained were normalized, taking the ratio at pH 7.10 as unity. The normalized ratios, E_{490}/E_{440} , were then fitted to a curve of the form:

$$E_{490}/E_{440} = (10^{\text{pH}_i - \text{pK}} + A)/B (1 + 10^{\text{pH}_i - \text{pK}})$$

This represents a combination of the Henderson-Hasselbalch expression (pK is the apparent ionization exponent of the intracellular dye) and Beers's law applied to the dye's protonated and deprotonated forms. A and B are constants. Nonlinear least-squares fitting yielded: $\text{pK} = 7.14 \pm 0.022$, $A = 0.227 \pm 0.0053$, and $B = 0.589 \pm 0.0070$. Fig. 1 shows the goodness of the fit.

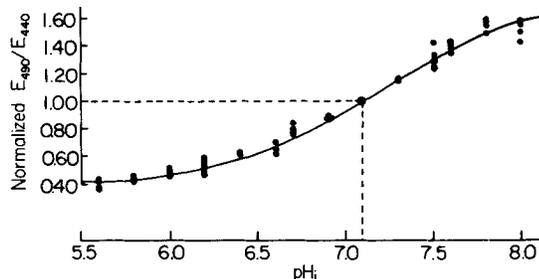


FIGURE 1. Intracellular calibration of BCECF: relationship between pH_i and the ratio of emitted light intensities at excitation wavelengths 490 and 440 nm. The emitted light was measured at 520–560 nm; the ratios were normalized at pH 7.10. 88 measurements from 19 glomus cells. The curve is described by: $E_{490}/E_{440} = (10^{pH_i - pK} + A)/B(1 + 10^{pH_i - pK})$, where A and B are constants. $pK = 7.14 \pm 0.022$, $A = 0.227 \pm 0.0053$, and $B = 0.589 \pm 0.0070$.

RESULTS

Experiments in the Nominal Absence of CO₂

Relation between pH_i and pH_o . In solutions buffered with 10 mM HEPES to pH 7.40, the pH_i of the glomus cells was 7.23 ± 0.02 (mean \pm SEM; $n = 70$). When pH_o was changed from 7.40 to values ranging from 6.20 to 8.00 (32 cells), pH_i changed in the same direction and attained values between 6.08 and 8.18. The new steady state was reached in 5–15 min and was reversible (Fig. 2). At pH_o values less than 6.90 pH_i and pH_o were nearly identical; at higher values pH_i was slightly lower than pH_o . The sensitivity of pH_i to changes in pH_o was striking: the ratio $\Delta pH_i/\Delta pH_o$ amounted to 0.85 over the entire pH range studied. The relationship is given by $pH_i = 0.99 + 0.85(pH_o)$, with a correlation coefficient of 0.995 (Fig. 3).

Effect of Na^+ removal and of HMA. Upon removal of Na^+ (replacement with NMDG⁺), pH_i fell in ~ 15 min from the control value (7.27 ± 0.07 ; $n = 11$) to a new steady value of 6.35 ± 0.10 . When Na^+ was returned, pH_i recovered to close to its original value. Reducing $[Na^+]_o$ to 50 or 20 mM reversibly lowered pH_i to 7.06 ± 0.03 ($n = 9$) or 6.86 ± 0.05 ($n = 10$), respectively. In nine cells bathed in 50 mM Na^+ , the effect of 15 μ M HMA, an amiloride derivative, was compared with that of Na^+ removal. The fall in pH_i induced by HMA was always slower and of less magnitude (Fig. 4). It is not clear to what extent this difference is due to incomplete

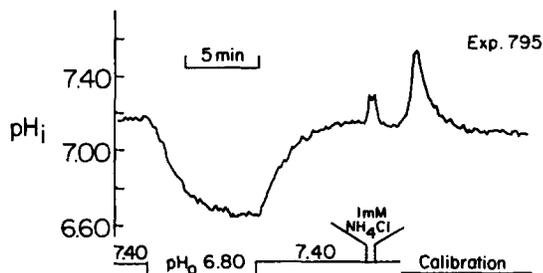


FIGURE 2. Response of pH_i to external acidification and to NH_4Cl . The plots in this figure and in Figs. 4, 6, 7, and 9 were derived from the optical measurements as described under Methods. When, in the nominal absence of CO_2 , pH_o is transiently lowered from 7.40 to 6.80, pH_i falls by 0.54 to a steady value and then returns. This is followed by an NH_4Cl pulse (for measuring buffering power). The experiment ends with a calibration in 140 mM K^+ and 10 μ M nigericin, pH 7.10. Note the pH_i peak that precedes the stable final calibration value.

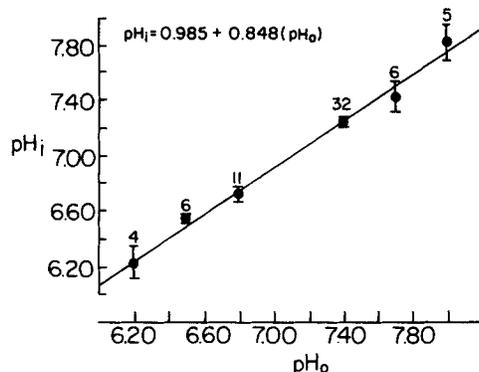


FIGURE 3. Relationship between pH_i and pH_o in the nominal absence of CO_2 . Means \pm SEM; 32 cells. Correlation coefficient = 0.995. Each experiment was started at pH_o 7.40.

inhibition by the drug (higher concentrations could not be used since they interfere with the optical measurements; see Methods) or to transient reversal of the Na^+/H^+ exchanger upon external Na^+ removal. The observed cell acidification might be due to the unmasking either of metabolic H ion production, a mechanism that exchanges external H^+ for internal K^+ (see Discussion), or a combination of these.

The effect of various Na^+ concentrations on the rate of pH_i recovery relative to the rate of recovery at 140 mM Na^+ was determined after the cells had been acidified by Na^+ removal. All measurements were made at nearly the same pH_i (6.43–6.54). Intervals at different $[\text{Na}^+]_o$ were separated by Na^+ -free periods. 25 cells from 13 rats yielded 64 measurements. When expressed by simple Michaelis-Menten kinetics, the apparent K_m of the $[\text{Na}^+]_o$ -dependent recovery was 58 ± 6 mM (Fig. 5). The rate of removal of H^+ equivalents from the cells (millimoles per liter cell water per minute), obtained as the product of the nonnormalized pH_i recovery rate and intrinsic buffering power (19–21 mM; see below), amounted to 7.1 ± 1.2 ($n = 26$) at 140 mM Na^+ , 5.3 ± 1.0 ($n = 11$) at 40 mM Na^+ , and 1.0 ± 0.17 ($n = 7$) at 10 mM Na^+ .

Effect of changing $[\text{K}^+]_o$. When the calibration solution containing 140 mM K^+ and nigericin (pH 7.10) was applied at the conclusion of each experiment, the eventually stable pH_i reading was often preceded by a transient rise in pH_i (see Figs. 2 and 6). To analyze this phenomenon, we examined the effect on pH_i when, at pH_o 7.40, $[\text{K}^+]_o$ was raised to 140 mM without nigericin. This resulted in a reversible rise of pH_i from 7.38 ± 0.08 to 7.98 ± 0.16 ($n = 6$) in ~ 5 min. The results were the same at either constant $[\text{K}^+]_o \times [\text{Cl}^-]_o$ product or constant $[\text{Cl}^-]_o$. Smaller elevations of $[\text{K}^+]_o$ (50 and 25 mM) reversibly raised pH_i by smaller amounts: 0.34 ± 0.04 ($n = 7$) and 0.21 ± 0.03 ($n = 11$), respectively (Fig. 6). The K^+ -induced alkalinization also occurred in cells whose pH_i had first been raised (to 7.80) or lowered (to 6.80) by changing pH_o .

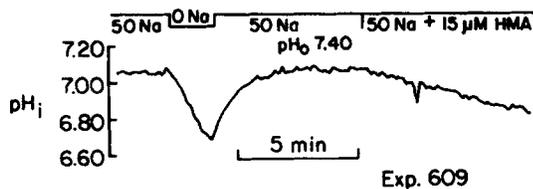


FIGURE 4. The pH_i of a cell in 50 mM Na^+ falls rapidly upon Na^+ removal, and then returns when Na^+ is readmitted. Upon applying 15 μM HMA the pH_i again falls, but the fall is slower and less pronounced.

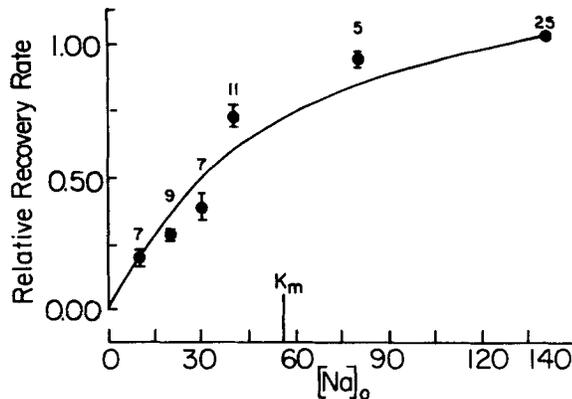


FIGURE 5. Effect of Na^+ concentration on the pH_i recovery rate relative to the recovery rate in 140 mM Na^+ . 25 cells were initially acidified by exposure to Na^+ -free medium. All measurements are at pH_i 6.43–6.54. Measurements on each cell included one in 140 mM Na^+ . The apparent K_m for Na^+ (Michaelis-Menten kinetics) is 58 ± 6 mM.

The question arose whether, by reducing external potassium below its normal value, pH_i could be driven in the opposite direction. Indeed, lowering $[\text{K}^+]_o$ from 4.7 to 1.5 mM reversibly reduced pH_i by 0.11 ± 0.03 ($n = 5$). In the absence of Na^+ (which lowers pH_i as described above) all these $[\text{K}^+]_o$ effects were more pronounced: 50 mM K^+ increased pH_i by 0.66 ± 0.05 ($n = 3$), while 1.5 mM K^+ reduced it by 0.40 ± 0.06 ($n = 6$). (The studies in 1.5 mM K^+ /0 Na^+ were done at pH_o 7.70 [$\text{pH}_i \sim 6.9$], rather than at 7.40, in order to obviate intracellular acidity beyond calibration range [see Fig. 1]).

These responses of pH_i to changes in external K^+ were not the result of changes in membrane voltage that almost certainly must have occurred. Depolarization in either gramicidin (1 μM ; Sigma Chemical Co., St. Louis, MO) or Ba^{2+} (2 mM) in Cl^- -free medium had no effect on pH_i (for the depolarizing effect of gramicidin, see Silman and Karlin [1968] and Podleski and Changeux [1969]; for the effect of Ba^{2+} in Cl^- -free solutions, see Astion, Obaid, and Orkand [1989]). Moreover, in cells exposed to 2 mM Ba^{2+} /0 Cl^- for 30 min or more, the rise of pH_i with 50 mM K^+ was similar to that in normal chloride without Ba^{2+} , and the fall in pH_i upon reducing $[\text{K}^+]_o$ to 1.5 mM was also unaffected.

Effect of NH_4Cl . When we pulsed cells (pH_o 7.40) for ~ 5 min with 20 mM NH_4Cl (substituted for NaCl) we observed the expected steep rise of pH_i , always followed by "plateau acidification" (i.e., a slow fall). Upon NH_4Cl removal, the pH_i "undershoot" below the initial value was followed by near-complete recovery. Qualitatively similar transients were obtained in 140, 50, and 20 mM Na^+ ; of course, the initial values before the pulse were substantially lower at the reduced Na^+ concentrations (7.06 at 50 mM and 6.86 at 20 mM). When the solution after the pulse was Na^+ -free, there was no recovery. In the presence of 50 mM Na^+ , 15 μM HMA, added

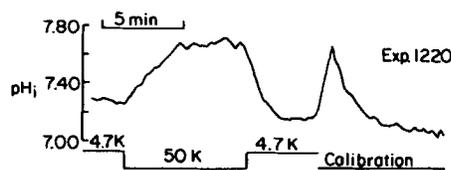


FIGURE 6. The pH_i transiently rises by 0.44 when $[\text{K}^+]_o$ is transiently increased from 4.7 to 50 mM at constant $[\text{Cl}^-]_o$ (pH_o 7.40, nominal absence of CO_2). The end of the record shows the calibration.

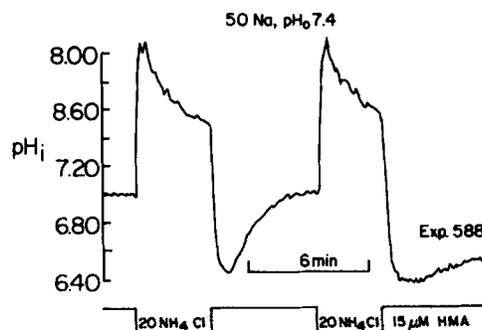


FIGURE 7. Course of pH_i in response to two successive pulses of NH_4Cl (20 mM). The solution after the second pulse contained $15 \mu M$ HMA, which greatly slowed recovery.

immediately after the pulse, greatly slowed recovery but only rarely abolished it completely (Fig. 7).

Intrinsic buffer power. The intracellular buffering power, β (amount of strong base required to produce, in the nominal absence of CO_2 , a small pH_i rise), was measured at pH_i 5.90–7.70 (32 cells) with small pulses of NH_4Cl (see Fig. 2). The results are shown in Fig. 8. The scatter could be due in part to individual variations among the glomus cells. Upon simple inspection, it appears that β increases from 3 mM to ~ 21 mM as pH_i is reduced from 7.70 to ~ 6.60 , but remains nearly unchanged as pH_i is lowered further. Thus, the traditional linear regression (dashed line in Fig. 8; see Discussion for references) does not seem suitable to describe our results. We fitted our data to an expression that assumed the presence of a single “equivalent” intracellular buffer, $\beta = 2.3 KCH/(K + H)^2$ (Koppel and Spiro, 1914; Roos and Boron, 1980), with dissociation constant $K = 10^{-6.41}$ and concentration $C = 41$ mM (Fig. 8, curved line). (The limited number and scatter of the data do not support the presence of multiple buffers.) Though based on a simplified premise, this fit is superior to the linear one: the sum of the squared residuals for the “equivalent” curve is 312 and for the straight line, 413.

When Na^+ was absent from the superfusate, the relationship between β and pH_i (7.10 to 6.00, $n = 12$) was not significantly altered.

Studies at room temperature. Some studies were performed at room temperature ($\sim 22^\circ C$). At pH_o 7.40, pH_i was 7.31 ± 0.03 ($n = 44$), slightly higher than at $36^\circ C$.

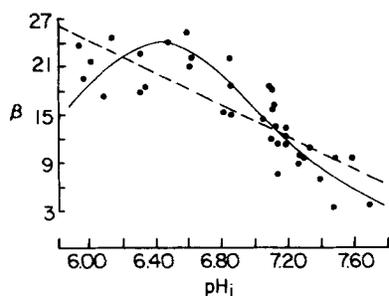


FIGURE 8. Relationship between intrinsic buffering power, β , and pH_i . Buffering power was measured with small (0.8–5 mM) pulses of NH_4Cl (nominal absence of CO_2). The best linear fit ($\beta = 83.7 - 9.9 pHi$; dashed line) is shown, as well as a curvilinear fit constructed on the assumption of the presence of only one intracellular buffer, $\beta = 2.3 CKH/(K + H)^2$, where dissociation constant $K = 10^{-6.41}$ and

concentration $C = 41$ mM. The curvilinear fit is superior: the sum of squared residuals is 312, whereas that of the linear fit is 413.

The pH_i - pH_o relationship in the pH_o range 6.20–8.00 could be expressed by $\text{pH}_i = 2.32 + 0.645 (\text{pH}_o)$ ($n = 13$). Thus, pH_i at room temperature is not quite as sensitive to pH_o as at body temperature. Upon removal of Na^+ , the fall in pH_i averaged 0.58 ($n = 4$), somewhat less than at body temperature. The sensitivity of intrinsic buffering power to pH_i between 6.10 and 7.40 (pH_o 6.20–8.00; $n = 13$) was nearly the same at room temperature as at 36°C. Again, Na^+ removal was without effect.

Experiments with CO_2 -containing Solutions

Relation between pH_i and pH_o in the presence of CO_2 . With cells in $\text{CO}_2/\text{HCO}_3^-$ -buffered media (36°C), we examined the relationship between steady-state pH_o and pH_i , just as we had with cells in CO_2 -free solutions. The pH_o was changed either at constant PCO_2 or at constant $[\text{HCO}_3^-]_o$. (a) In 5% $\text{CO}_2/95\%$ O_2 , a reduction of $[\text{HCO}_3^-]_o$ from ~25 to 5 mM (pH_o 7.40 and 6.80) promptly lowered pH_i from 7.18 ± 0.08 to 6.69 ± 0.06 ($n = 9$). This is illustrated in Fig. 9 A. (b) At 5 mM $[\text{HCO}_3^-]_o$, an increase in PCO_2

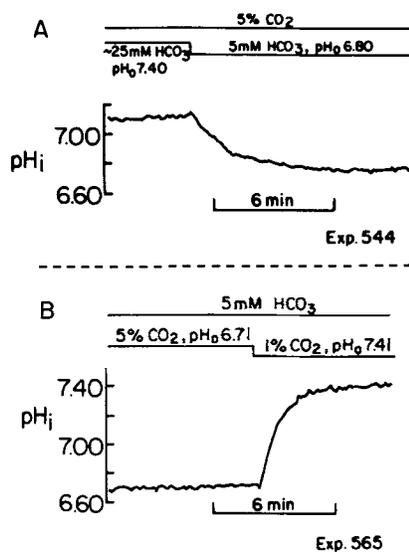


FIGURE 9. (A) The pH_i falls by 0.4 when, at constant PCO_2 (5%), pH_o is lowered by 0.6 when HCO_3^- concentration is reduced from ~25 to 5 mM. (B) The pH_i rises by 0.66 when, at constant HCO_3^- concentration (5 mM), pH_o is raised by 0.7 when PCO_2 is reduced from 5 to 1%.

from 1 to 5% (pH_o 7.42 and 6.71) promptly lowered pH_i from 7.26 ± 0.04 to 6.67 ± 0.03 ($n = 5$). Fig. 9 B shows an experiment in which the sequence of these conditions was reversed. Both at constant PCO_2 and at constant HCO_3^- the responsiveness of pH_i to pH_o ($\Delta\text{pH}_i/\Delta\text{pH}_o$) was 0.82. This ratio is nearly the same as that obtained (over a wider pH range) in the nominal absence of CO_2 (0.85; Fig. 10).

When, at unchanged pH_o 7.40, both bicarbonate concentration and PCO_2 were increased 10-fold, from 1% $\text{CO}_2/5$ mM HCO_3^- to 10% $\text{CO}_2/50$ mM HCO_3^- , the pH_i (7.17 ± 0.05 ; $n = 8$) transiently fell by an average of 0.25, but then returned to the original value. Complete recovery was also observed when PCO_2 was increased fivefold, from 1% $\text{CO}_2/5$ mM HCO_3^- to 5% $\text{CO}_2/25$ mM HCO_3^- .

Effect of changing from CO_2 -free to CO_2 -containing media. Changing the buffer from 10 mM HEPES (nominal absence of CO_2) to 5% $\text{CO}_2/25$ mM HCO_3^- at unchanged

pH_o 7.40 transiently reduced pH_i from 7.29 ± 0.04 to 7.05 ± 0.04 ($n = 12$). Within 5–15 min pH_i usually partially recovered to a new steady state (7.17 ± 0.05), which was slightly but significantly lower than the original value in HEPES ($P < 0.005$). A similar pH_i course was observed upon switching from HEPES to 10% CO₂/50 mM HCO₃⁻: a transient fall from 7.25 ± 0.05 to 6.87 ± 0.06 ($n = 11$) was followed by recovery to 7.11 ± 0.09 , 0.14 less than the initial value. Thus, the steady-state values in 1, 5, and 10% CO₂ are nearly the same and somewhat lower than under nominally CO₂-free conditions at the same pH_o (7.40).

Similar experiments (10 mM HEPES to 5% CO₂/25 mM HCO₃⁻) were done after the cells had been exposed for 1 h to HEPES-buffered medium containing 0.5 mM SITS; the subsequent solutions were SITS-free. The initial pH_i was 7.25 ± 0.06 ($n = 14$), nearly the same as without SITS. The average CO₂-induced initial fall (0.27) was also similar to the fall without SITS. The subsequent pH_i recovery of 0.06 was half of that seen without SITS, but the difference was not quite significant ($0.05 < P < 0.1$).

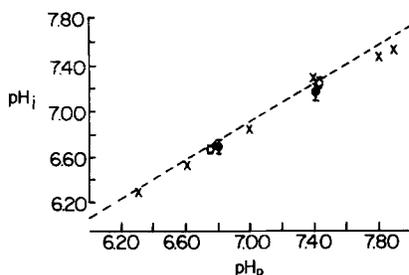


FIGURE 10. Comparison of pH_i-pH_o relationships in glomus cells and red cells. The circles pertain to glomus cells in CO₂/HCO₃⁻: ○, pH_o was changed at constant HCO₃⁻ concentration (5 mM); ●, pH_o was changed at constant PCO₂ (5%). X represents data obtained from oxygenated human red cells; pH_o was changed either by changing PCO₂ or with NaOH

or HCl (from Funder and Wieth, 1966). The six points have been arbitrarily chosen using these authors' regression, $[H^+]_o/[H^+]_i = 3.094 - 0.335 \text{ pH}_o$ (correlation coefficient = 0.995 ± 0.015 ; pH_o range 6.32–8.64). The dashed line (same as in Fig. 3) represents the pH_i-pH_o relationship in glomus cells in the nominal absence of CO₂.

Effect of Cl⁻ removal. Under Cl⁻-free conditions (pH_o 7.40) the course of pH_i upon changing from HEPES to 5% CO₂/HCO₃⁻-buffered superfusate was nearly the same as in the presence of Cl⁻: pH_i fell from 7.35 to 7.13 and then partially recovered to 7.21 ($n = 3$). In agreement with recently published work by Buckler et al. (1991a), Cl⁻ removal in the midst of the exposure to CO₂ raised pH_i from 7.05 ± 0.04 to 7.52 ± 0.12 ($n = 8$), a value higher than the preceding pH_i in Cl⁻ free HEPES-buffered medium (7.23 ± 0.07). This effect was reversible and abolished by pretreatment with 0.5 mM SITS.

High [K⁺]_o. In two cells, raising K⁺ concentration from 4.7 to 25 mM in the presence of 5% CO₂/HCO₃⁻ (pH_o 7.40) resulted in a pH_i rise from 7.06 to 7.41, similar to the rise seen in the absence of CO₂.

Studies at room temperature. Changing the buffer from HEPES to 5% CO₂/HCO₃⁻ (pH_o 7.40) resulted in a pH_i fall from 7.29 ± 0.05 to 7.00 ± 0.03 ($n = 10$), not different from our findings at 36°C. However, in six of these cells there was no recovery. At room temperature, as at 36°C, removal of Cl⁻ during CO₂ exposure led to a rise in pH_i that could be blocked by 150 μM DIDS (4,4'-diisothiocyanatostilbene

disulfonic acid). At this concentration, the drug's fluorescence does not interfere with the pH_i measurement.

Effect of Hypoxia

When cells, originally in 10% CO_2 , 50 mM HCO_3^- (pH_o 7.40), were exposed to a hypoxic gas mixture (2% O_2 , 10% CO_2 , remainder N_2), no change in pH_i was observed. In the nominal absence of CO_2 , hypoxia (2% O_2 , 98% N_2) also had no effect either at pH_o 7.40 or 6.80.

DISCUSSION

The average pH_i of freshly dissociated adult rat glomus cells in HEPES buffer (pH_o 7.40, 36°C) was 7.23. This is in reasonable agreement with the pH_i of neonatal rat cells (7.30) after overnight culture in HEPES (Buckler et al., 1991a). In 5% $\text{CO}_2/\text{HCO}_3^-$ (pH_o 7.40) the pH_i was 7.17. This closely resembles the pH_i of most other mammalian cells, such as mouse and rat skeletal muscle fibers, sheep Purkinje cells, guinea pig vas deferens smooth muscle (see Aickin, 1984 for references), human neutrophils (Simchowicz and Roos, 1985), and human red cells (Funder and Wieth, 1966). Rat renal mesangial cells have a slightly higher pH_i (7.26; Boyarsky et al., 1988).

Sensitivity of pH_i to pH_o

A principal feature of our work is the striking responsiveness of the pH_i of the glomus cell to changes in external pH in both the absence and presence of CO_2 . For instance, reducing pH_o in CO_2 -free solution from 7.40 to 6.20 caused pH_i to fall from 7.23 to 6.23; raising pH_o to 8.00 increased pH_i to 7.84. The ratio $\Delta\text{pH}_i/\Delta\text{pH}_o$ amounted to 0.85 and was constant over the entire pH_o range (6.20–8.00; Fig. 3). Nearly the same high ratio (0.82) was found in media buffered with $\text{CO}_2/\text{HCO}_3^-$. Here, pH_i alterations achieved either by changes in $[\text{HCO}_3^-]_o$ at constant Pco_2 or by changes in Pco_2 at constant $[\text{HCO}_3^-]_o$ had the same effect on pH_i (Fig. 10). Similar observations in $\text{CO}_2/\text{HCO}_3^-$ -containing media have been made by Buckler et al. (1991b).

This sensitivity of pH_i to changes in pH_o is quite unusual, as is shown by a comparison with other cells. For example, $\Delta\text{pH}_i/\Delta\text{pH}_o$ in guinea pig smooth muscle is 0.38 (Aickin, 1984); in mouse soleus muscle, 0.39 (Aickin and Thomas, 1977); in sheep Purkinje fibers, 0.40 (Ellis and Thomas, 1976); and in human neutrophils, 0.30 (Simchowicz and Roos, 1985) (all measurements in $\text{CO}_2/\text{HCO}_3^-$). The human red cell stands out as an exception. In this cell, the $\Delta\text{pH}_i/\Delta\text{pH}_o$ ratio, as well as the actual pH_i values at the respective pH_o 's (Funder and Wieth, 1966), closely match those of the glomus cell. Fig. 10 (see legend for details) shows the comparison. Yet the mechanisms of H ion distribution in the two cell types are probably quite different. In the red cell, H, Cl, and HCO_3^- ions are at electrochemical equilibrium. Their distributions (and membrane voltage, V_m) are determined by the net charge concentrations of the impermeant ions on both sides of the membrane (Donnan equilibrium). In glomus cells, on the other hand, the H ions are almost certainly not at equilibrium. This is indirectly demonstrated by our observation that either Na^+ removal or application of

HMA, an inhibitor of Na^+/H^+ exchange, sharply reduced pH_i (Fig. 4), indicating that in "normal," Na^+ -containing media a vigorous H^+ -removing mechanism is in force. We have not measured membrane voltage; V_m measurements by others (for references see Peers and Green, 1991), obtained by impaling these small cells, must be looked upon with some skepticism because of possible cell damage. L. A. Fieber and E. W. McCleskey have measured the V_m of dissociated adult rat glomus cells without disrupting the cell membrane (unpublished observations). They used on-cell patch pipettes to measure currents (I) through single Ca^{2+} channels at different pipette voltages (V). From the I - V relationship, the cells' resting voltage could be derived by repeating the procedure in 140 mM K^+ that was assumed to depolarize the cell's resting V_m to zero (the assumption was validated by the unchanged I - V relationship of the excised patch in 140 mM K^+). At pH_o 7.30, resting voltage in six cells ranged between -11 and -23 mV; in the remaining four, between -43 and -62 mV. If the voltage heterogeneity is due to the damaged condition of some of the cells, the more negative values should most accurately reflect *in vivo* conditions; if the heterogeneity were also to exist *in vivo*, the H^+ ions in the low-voltage cells would be in near-equilibrium. Such equilibrium would be hard to explain in view of the presence of an active Na^+/H^+ exchanger as we just discussed. These direct measurements indicate that in some of the glomus cells, if not in all, the H^+ ions are not in equilibrium.

The HCO_3^- ion is not required for the pH_i responsiveness to pH_o , since the pH_i - pH_o relationship with and without $\text{CO}_2/\text{HCO}_3^-$ was nearly the same. That leaves at least three mechanisms to account for the sensitivity of pH_i to changes in pH_o : (a) Electrodiffusion of H^+ ions. For example, reducing pH_o from 7.4 (pH_i 7.2) to 6.8 establishes (or increases) an inward electrochemical H^+ ion gradient. Assuming that $V_m = -60$ mV and cell diameter = 10 μm , a very large H^+ permeability, $3 \times 10^{-2} \text{ cm} \cdot \text{s}^{-1}$, would, however, be required to account for the observed initial rate of fall in pH_i (~ 0.26 per minute). Moreover, depolarization should reduce the rate of internal acidification. Yet exposure to a depolarizing solution (0Cl^- , 2 mM Ba^{2+} ; Astion et al., 1989) had no effect on this rate. Therefore, although electrodiffusion cannot be ruled out, other mechanisms must be operative as well. (b) Changes in activity of the Na^+/H^+ exchanger. Its rate might be reduced in response to external acidification (reversal would seem unlikely at normal $[\text{Na}^+]_o$). However, the exchanger was found to be still functioning even after pH_o had been reduced to 6.80 (pH_i 6.60): when $[\text{Na}^+]_o$ was then removed, pH_i fell further by ~ 0.6 . This corresponds to about the same increase in intracellular H^+ ions as when Na^+ was removed at pH_o 7.40, taking buffering power into account. Therefore, a rate reduction of the exchanger under these conditions seems unlikely. Might the increase in pH_i upon raising pH_o have been due to stimulated Na^+/H^+ exchange? When pH_o was raised from 7.40 to 8.00 in the absence of Na^+ , pH_i increased from 6.50 ± 0.12 to 6.90 ± 0.10 ($n = 7$). This indicates a ratio, $\Delta\text{pH}_i/\Delta\text{pH}_o$, of 0.67, significantly less than that in the presence of Na^+ (0.85; Fig. 3). Thus, a contribution of Na^+/H^+ exchange to the observed rise in pH_i cannot be dismissed. (c) K^+/H^+ exchange. As will be shown below, the membrane of the glomus cell probably contains an electroneutral K^+/H^+ exchanger. Raising extracellular $[\text{H}^+]$ might increase exchanger-mediated H^+ influx (and K^+ efflux). It should be added that the low intracellular buffering power of the glomus cell (see Fig.

8) would enhance pH_i changes due to transmembrane H^+ movements regardless of mechanism.

K⁺/H⁺ Exchange

In the calibration procedure at the end of each experiment, the cells were exposed to a Na^+ -free solution containing 140 mM K^+ and 10 μM nigericin at pH_o 7.10. In nearly every case the eventually stable pH_i which, it is assumed, closely approaches pH_o , was preceded by a steep and transient rise; this is illustrated in Figs. 2 and 6. We know that removal of Na^+ by itself produces a *fall* in pH_i (Fig. 4). We suspected that the transient rise was caused by the elevated $[\text{K}^+]_o$. Indeed, simple substitution of 20, 50, or 140 mM Na^+ by equal amounts of K^+ produced a reversible rise in pH_i (see Fig. 6) which, in general, was greater the higher $[\text{K}^+]_o$. Lowering $[\text{K}^+]_o$ from 4.7 to 1.5 mM produced a reversible fall in pH_i . We have shown (see Results) that these effects are not due to changes in membrane voltage. They suggest the presence of a membrane-bound K^+/H^+ exchanger. Recently, Bonanno (1991) has provided evidence for the presence of such an exchanger in rabbit corneal epithelium. The exchanger is probably electroneutral: we found that the responsiveness of $[\text{H}^+]_i$ to changes in $[\text{K}^+]_o$ was not affected by depolarization. Under "normal" steady-state conditions ($[\text{K}^+]_i$ 140 mM, $[\text{K}^+]_o$ 4.7 mM, pH_i 7.2, pH_o 7.4) this mechanism would produce a net efflux of K^+ in exchange for net influx of H^+ and thus would acidify the cell. Cellular alkalization due to the Na^+/H^+ exchanger would balance this process. When $[\text{K}^+]_o$ is raised, unidirectional K^+ influx, and thus H^+ efflux which is coupled to it, should increase. The K^+/H^+ exchanger seems to be separate from the Na^+/H^+ exchanger: in the absence of Na^+ the rise in pH_i induced by 50 mM K^+ was unaffected by HMA (15 μM).

It is important to point out that in these Na^+ -free experiments the steady-state ratios of the H and K ions across the membrane are not the same, as would be expected if the K^+/H^+ exchanger were the sole regulator of pH_i . The ratio $[\text{H}^+]_i/[\text{H}^+]_o$ was always significantly less than $[\text{K}^+]_i/[\text{K}^+]_o$. Thus, Na^+ removal in one specific experiment lowered pH_i to 6.40 (pH_o 7.40), yielding an H^+ ratio of 10, whereas the K^+ ratio was $140/4.7 \approx 30$. Even gross deviations from the assumed $[\text{K}^+]_i$ cannot explain this discrepancy. One could raise the objection that at these low pH_i values the dye is an imprecise indicator. However, when Na^+ was removed at an elevated pH_o of 7.70 (average pH_i 6.83, $n = 4$), the discrepancy in the ratios remained. Additional insertion of K^+/H^+ exchangers by adding nigericin further reduced pH_i , and the H^+ and K^+ ratios were now 20 and 30, respectively; closer than without the drug but still not identical. It is apparent that even in the absence of Na^+/H^+ exchange alkalizing factors must have been present. One such factor might be outward electrodiffusion of H ions from the acidified cytosol. In the absence of membrane voltage measurements, the contribution of this factor cannot be evaluated.

We have no information about the kinetic properties of the K^+/H^+ exchanger other than that the initial rate of alkalization continued to increase over the entire range of $[\text{K}^+]_o$ through which it could be studied (7–140 mM).

We compared the pH_i effect of raising $[\text{K}^+]_o$ with the effect of Rb^+ or Tl^+ in the continued presence of 4.7 mM K^+ . In each cell K^+ was first transiently increased, and

then the same amount of Rb^+ or Tl^+ was added. 50 mM Rb^+ had no effect on pH_i . On the other hand, the initial rates of alkalization due to 5–40 mM Tl^+ were three to six times those produced by K^+ . Apparently the exchanger's affinity for Tl^+ greatly exceeds that for K^+ . The similarity in sequence of effectiveness of the three ions to that observed by Hille (1973) in K^+ channel permeability of myelinated frog nerve fiber might point to some common feature of molecular structure of carrier and channel.

Effect of Na^+ on Recovery

When, at pH_o 7.40, the pH_i was lowered either by Na^+ removal or by an NH_4Cl prepulse, the rate of the subsequent recovery due to Na^+/H^+ exchange was dependent on $[\text{Na}^+]_o$. Without Na^+ there was no recovery, as was also found by Buckler et al. (1991a). This Na^+ dependence agrees with what has been found in many other cell types (see, for instance, Thomas, 1977). The relationship between $[\text{Na}^+]_o$ and rate of pH_i recovery could be expressed by simple Michaelis-Menten kinetics, with an apparent K_m of 58 mM (Fig. 5). This represents a rather low affinity of the exchanger: K_m in rat mesangial cells is 27 mM (Boyarsky et al., 1988) and K_m in frog muscle (exposed to 50 mM K^+) is 12 mM (Putnam, Roos, and Wilding, 1986).

Cl^- Removal

The absence of chloride had no effect on resting pH_i , pH_i sensitivity to pH_o changes (from 7.40 to 6.80 or 7.80), or pH_i response to a pulse of CO_2 . If, however, chloride was removed in the midst of CO_2 exposure, there was a distinct rise in pH_i ; this was previously observed by Buckler et al. (1991a). They found this rise to be Na^+ independent and ascribed it to exchange of external HCO_3^- for internal Cl^- . We confirmed that disulfonic stilbenes (DIDS and SITS) inhibit the rise.

Buffering Power

Between pH_i 7.70 and 6.60 the intrinsic buffering power, β , of glomus cells fell with increasing pH_i ; but below 6.60, β did not appear to change. A curvilinear regression representing the presence of one "equivalent" intracellular buffer fits our data better than a linear regression. Other observers working either with glomus cells or with other cell types have fitted their buffering data to a straight line with negative slope (glomus cells: Buckler et al., 1991a; barnacle muscle fibers: Boron, McCormick, and Roos, 1979; rat renal mesangial cells: Boyarsky et al., 1988; frog muscle: Amorena, Wilding, Manchester, and Roos, 1990; sheep Purkinje fibers: Vaughan-Jones and Wu, 1990). We found that Na^+ removal did not significantly affect the pH_i -buffering power relationship, nor did lowering temperature to 22°C.

Hypoxia

Finally, we found that hypoxia (2% O_2) had no observable effect on pH_i in either the presence or absence of CO_2 . This agrees with the lack of effect of 2 mM cyanide (Biscoe et al., 1989), and seems to indicate that in the chain of events linking extracellular hypoxia to nerve discharge, pH_i does not play a role.

Some years ago, Hornbein and Roos (1963) presented evidence that the excitatory effect of blood (extracellular) acidity on carotid body nerve activity is produced through changes in pH_o rather than through changes in PCO_2 or bicarbonate concentration per se. The exceptional sensitivity of the pH_i of the isolated glomus cell to pH_o , which is nearly the same in the presence and absence of CO_2 , makes the role of pH_i as a link between extracellular acidity and chemoreceptor activity an attractive possibility.

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REFERENCES

- Aickin, C. C. 1984. Direct measurement of intracellular pH and buffering power in smooth muscle cells of guinea-pig vas deferens. *Journal of Physiology*. 349:571–585.
- Aickin, C. C., and R. C. Thomas. 1977. Micro-electrode measurement of the intracellular pH and buffering power of mouse soleus muscle fibres. *Journal of Physiology*. 267:791–810.
- Amorena, C. E., T. J. Wilding, J. K. Manchester, and A. Roos. 1990. Changes in intracellular pH caused by high K in normal and acidified frog muscle. Relation to metabolic changes. *Journal of General Physiology*. 96:959–972.
- Astion, M. L., A. L. Obaid, and R. K. Orkand. 1989. Effects of barium and bicarbonate on glial cells of *Necturus* optic nerve. *Journal of General Physiology*. 93:731–744.
- Biscoe, T. J., and M. R. Duchon. 1990. Responses of type I cells dissociated from the rabbit carotid body to hypoxia. *Journal of Physiology*. 428:39–59.
- Biscoe, T. J., M. R. Duchon, D. A. Eisner, S. C. O'Neill, and M. Valdeolillos. 1989. Measurements of intracellular Ca^{2+} in dissociated type I cells of the rabbit carotid body. *Journal of Physiology*. 416:421–434.
- Bonanno, J. A. 1991. K^+ - H^+ exchange, a fundamental cell acidifier in corneal epithelium. *American Journal of Physiology*. 260:C618–C625.
- Boron, W. F., W. C. McCormick, and A. Roos. 1979. pH regulation in barnacle muscle fibers: dependence on intracellular and extracellular pH. *American Journal of Physiology*. 237:C185–C193.
- Boyersky, G., M. B. Ganz, R. B. Sterzel, and W. F. Boron. 1988. Intracellular pH regulation in single glomerular mesangial cells. I. Acid extrusion in the absence and presence of HCO_3^- . *American Journal of Physiology*. 255:C844–C856.
- Buckler, K. J., and R. D. Vaughan-Jones. 1990. Application of a new pH-sensitive fluoroprobe (carboxy-SNARF-1) for intracellular pH measurement in small, isolated cells. *Pflügers Archiv*. 417:234–239.
- Buckler, K. J., R. D. Vaughan-Jones, C. Peers, and P. C. G. Nye. 1991a. Intracellular pH and its regulation in type I carotid body cells of the neonatal rat. *Journal of Physiology*. 436:107–129.
- Buckler, K. J., R. D. Vaughan-Jones, C. Peers, D. Lagadic-Gossmann, and P. C. G. Nye. 1991b. Effects of extracellular pH, PCO_2 and HCO_3^- on intracellular pH in isolated type-I cells of the neonatal rat carotid body. *Journal of Physiology*. 444:703–721.
- De Castro, F. 1928. Sur la structure et l'innervation du sinus carotidien de l'homme et des mammifères. Nouveaux faits sur l'innervation et la fonction du glomus caroticum. Études anatomiques et physiologiques. *Trabajos del Laboratorio de Investigaciones Biológicas de la Universidad de Madrid*. 25:331–380.
- Duchon, M. R., K. W. T. Caddy, G. C. Kirby, D. L. Patterson, J. Ponte, and T. J. Biscoe. 1988. Biophysical studies of the cellular elements of the rabbit carotid body. *Neuroscience*. 26:291–311.
- Ellis, D., and R. C. Thomas. 1976. Direct measurement of the intracellular pH of mammalian cardiac muscle. *Journal of Physiology*. 262:755–771.

- Fidone, S. J., and C. González. 1986. Initiation and control of chemoreceptor activity in the carotid body. In *Handbook of Physiology: The Respiratory System II*. A. P. Fishman, N. S. Cherniack, J. G. Widdicombe, and S. R. Geiger, Editors. American Physiological Society, Bethesda, MD. 247–312.
- Funder, J., and J. O. Wieth. 1966. Chloride and hydrogen ion distribution between human red cells and plasma. *Acta Physiologica Scandinavica*. 68:234–245.
- Hille, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *Journal of General Physiology*. 61:669–686.
- Hornbein, T. F., and A. Roos. 1963. Specificity of H ion concentration as a carotid chemoreceptor stimulus. *Journal of Applied Physiology*. 18:580–584.
- Koppel, M., and K. Spiro. 1914. Über die Wirkung von Moderatoren (Puffern) bei der Verschiebung des Säure-Basengleichgewichtes in biologischen Flüssigkeiten. *Biochemische Zeitschrift*. 65:409–439.
- López-López, J., C. González, J. Ureña, and J. López-Barneo. 1989. Low pO₂ selectively inhibits K channel activity in chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology*. 93:1001–1015.
- Peers, C., and F. K. Green. 1991. Inhibition of Ca²⁺-activated K⁺ currents by intracellular acidosis in isolated type I cells of the neonatal rat carotid body. *Journal of Physiology*. 437:589–602.
- Podleski, T., and J.-P. Changeux. 1969. Effects associated with permeability changes caused by gramicidin A in electroplax membrane. *Nature*. 221:541–545.
- Putnam, R. W., A. Roos, and T. J. Wilding. 1986. Properties of the intracellular pH-regulating systems of frog skeletal muscle. *Journal of Physiology* 381:205–219.
- Rink, T. J., R. Y. Tsien, and T. Pozzan. 1982. Cytoplasmic pH and free Mg²⁺ in lymphocytes. *Journal of Cell Biology*. 95:189–196.
- Roos, A., and W. F. Boron. 1980. The buffer value of weak acids and bases: origin of the concept, and first mathematical derivation and application to physico-chemical systems. The work of M. Koppel and K. Spiro (1914). *Respiration Physiology*. 40:1–32.
- Roos, A., and W. F. Boron. 1981. Intracellular pH. *Physiological Reviews*. 61:296–434.
- Silman, H. I., and A. Karlin. 1968. Action of antibiotics affecting membrane permeability on the electroplax. *Proceedings of the National Academy of Sciences, USA*. 61:674–679.
- Simchowicz, L., and E. J. Cragoe, Jr. 1986. Inhibition of chemotactic factor-activated Na⁺/H⁺ exchange in human neutrophils by analogues of amiloride: structure-activity relationships in the amiloride series. *Molecular Pharmacology*. 30:112–120.
- Simchowicz, L., and A. Roos. 1985. Regulation of intracellular pH in human neutrophils. *Journal of General Physiology*. 85:443–470.
- Thomas, J. A. 1986. Intracellularly trapped pH indicators. In *Optical Methods in Cell Physiology*. P. De Weer and B. M. Salzberg, editors. Wiley-Interscience, New York. 311–325.
- Thomas, J. A., R. N. Buchsbaum, A. Zimniak, and E. Racker. 1979. Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated in situ. *Biochemistry*. 18:2210–2218.
- Thomas, R. C. 1977. The role of bicarbonate, chloride, and sodium ions in the regulation of intracellular pH in snail neurones. *Journal of Physiology*. 273:317–338.
- Ureña, J., J. López-López, C. González, and J. López-Barneo. 1989. Ionic currents in dispersed chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology*. 93:979–999.
- Vaughan-Jones, R. D., and M. Wu. 1990. pH dependence of intrinsic H⁺ buffering power in the sheep cardiac Purkinje fibre. *Journal of Physiology*. 425:429–448.
- Verna, A. 1979. Ultrastructure of the carotid body in mammals. In *International Review of Cytology*. G. W. Bourne and J. F. Danielli, editors. Academic Press, New York. 60:271–330.
- Wilding, T. J., B. Cheng, and A. Roos. 1991. The relationship between extracellular pH (pH_o) and intracellular pH (pH_i) in adult rat carotid body glomus cells. *Biophysical Journal*. 59:184a. (Abstr.)