Chronic hypercapnia resets CO₂ sensitivity of avian intrapulmonary chemoreceptors

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Bebout, D. E., and S. C. Hempleman. Chronic hypercapnia resets CO₂ sensitivity of avian intrapulmonary chemoreceptors. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R317–R322, 1999.—Avian intrapulmonary chemoreceptors (IPC) are vagal sensory neurons that participate in the control of breathing. IPC action potential frequency is inversely proportional to Pco₂, but it is unclear whether low Pco₂ or high pH is the immediate stimulus for signal transduction in IPC. To address this question, comparisons were made between single cell neural responses of 34 IPC recorded in 6 anesthetized ducks (Anas platyrhynchos) acclimatized 12 days to 7.5% inspired CO₂ and 22 IPC recorded in 9 normal anesthetized ducks. We hypothesized that if respiratory-linked pH changes determine IPC activity, action potential frequency as a function of inspiratory Pco₂ (PICO₂) should be greater after acclimatization due to metabolic-acid-base compensation and higher pH. Conversely, if Pco₂ alone determines IPC discharge, action potential frequency vs. Pco₂ should be unchanged by acclimatization. Results indicate that after acclimatization ventilation was depressed at 28 and 42 Torr PICO₂ (P < 0.05) and mean plasma pH at 40 Torr PICO₂ increased from 7.38 ± 0.03 to 7.56 ± 0.02 (P < 0.05), indicating significant metabolic-acid-base compensation and HCO₃⁻ retention. Mean IPC discharge rate was elevated by CO₂ acclimatization at all Pco₂ studied. In acclimatized vs. normal animals, regression analysis of IPC discharge as a function of lnPICO₂ showed increased mean intercepts of 81.1 ± 4.0 vs. 48.4 ± 3.6 impulses/s (P < 0.05) and increased mean slopes of −19.0 ± 1.0 vs. −12.0 ± 1.1 impulses/s⁻¹lnPICO₂⁻¹ (P < 0.05). Results indicate that IPC response to CO₂ is mediated by H⁺ from CO₂ hydration and not by CO₂ directly. intracerebral pH; CO₂ signal transduction; ventilatory control

AVIAN INTRAPULMONARY chemoreceptors (IPC) are CO₂-sensitive vagal afferents that innervate the lungs of birds. The rate of action potential generation by IPC is inversely proportional to intrapulmonary Pco₂, which varies during the breathing cycle (4, 9). IPC provide a sensory feedback path that has been implicated in the regulation of breathing pattern and arterial Pco₂ (2, 8, 21) and the adjustment of ventilation to metabolic demands (2, 10). Despite the phasic discharge of IPC with tidal breathing and its strong inverse dependence on lung Pco₂, the CO₂ transduction mechanisms of IPC are not well understood.

CO₂ transduction in IPC is dependent on the enzyme carbonic anhydrase (CA), which catalyzes the hydration-dehydration reaction between CO₂, H₂O, and H₂CO₃. Systemic CA blockade with the cell-permeable inhibitor acetazolamide increases IPC discharge to near maximal levels and makes IPC insensitive to increasing Pco₂ (16, 19). Although the complete mechanism of CO₂ transduction in IPC remains unclear, the action of acetazolamide on IPC suggests that H⁺ from the disso-
capnia (7). Therefore, to further test the hypothesis that IPC transduce H\(^+\), we acclimatized birds to 7.5% inspired CO\(_2\) for several weeks and compared their IPC responses to unacclimatized controls. We reasoned that bicarbonate production and retention in acclimatized birds should increase pH at all P\(_{\text{CO}_2}\) values relative to controls and allow a test for independent effects of H\(^+\) and CO\(_2\) on chemotransduction. If average IPC discharge at a given P\(_{\text{CO}_2}\) were unchanged by acclimatization, even though pH were more alkaline, this would suggest that IPC transduce CO\(_2\) directly. If average IPC discharge at a given P\(_{\text{CO}_2}\) was increased after acclimatization, this would suggest that IPC transduce H\(^+\) and are stimulated by the increased alkalinity. If average IPC discharge at a given P\(_{\text{CO}_2}\) were lower after acclimatization, this would not fit predictions of either the H\(^+\) or CO\(_2\) chemotransduction models and would indicate operation of other mechanisms.

**METHODS**

Animals and acclimatization. Fifteen adult Pekin ducks, Anas platyrhynchos, 4–8 mo old, of either sex were studied. Nine ducks [body mass 2.5 ± 0.1 kg (means ± SE)] were kept under normal vivarium conditions breathing room air [0.02% CO\(_2\), 0.1 Torr inspiratory P\(_{\text{CO}_2}\) (P(\text{I}CO\(_2\))) with food and water ad libitum. Six other ducks (body mass 2.3 ± 0.1 kg) were acclimatized for 12 ± 12 days in a 700-liter Plexiglas environmental chamber ventilated with 20 l/min of 7.5% CO\(_2\) in air. Chamber P\(_{\text{CO}_2}\) was monitored with a Beckman LB-2 CO\(_2\) analyzer and ranged from 45 to 55 Torr. Ducks were removed from the acclimatization chamber for 20 min daily to clean the bedding material and replenish food and water and were maintained in otherwise normal vivarium conditions.

Animal husbandry and experimental procedures were reviewed and approved by the local Institutional Animal Care and Use Committee.

Reflex ventilatory measurements. Awake ventilatory responses to inspired CO\(_2\) were studied before and after chronic CO\(_2\) exposure (n = 6 ducks) by whole body plethysmography. Animals were placed in a 14-liter sealed chamber that was ventilated through high-resistance inlet and outlet valves. Air with or without added CO\(_2\) was admitted at 20 l/min through the inlet and removed at the same rate through the outlet connection to a vacuum source. P\(_{\text{CO}_2}\) was measured with a Perkin Elmer MGA-1100 mass spectrometer. Mean chamber pressure was measured with a water manometer and set equal to atmospheric pressure by balancing the high-resistance valves. Tidal fluctuations in chamber pressure associated with heating and humidification of the inspired gas were measured with a Validyne MP-45 transducer and demodulator. Body and chamber temperatures were monitored with Yellow Springs Instruments thermistor probes, relative chamber humidity was monitored with an analog hygrometer, and temperature and pressure signals were recorded on a Gould pen writer using universal couplers.

Animals were allowed to relax in the darkened plethysmographic chamber until ventilation became regular and exploratory movements ceased. Inspired CO\(_2\) levels of 0, 2, 4, and 6% were then given in random order for ~5–10 min each, and ventilatory pressure traces, temperatures, and humidity were recorded. The chamber was calibrated after each measurement by injection of a known volume of air with a syringe. Tidal pressure traces were converted to tidal volume (12, 13), and minute ventilation was calculated from respiratory frequency and tidal volume.

Minute ventilatory response was analyzed using two-way ANOVA. Main effects were inspired CO\(_2\) test levels (0, 2, 4, and 6%) and acclimatization state (normal vs. acclimatized). Post hoc paired comparisons were made using the Scheffé method and least-squares means (SAS System GLM procedure), and P levels ≤0.05 were accepted as significant.

Neural recording. Animals were anesthetized by intravenous infusion of 35–40 mg/kg pentobarbital sodium. Supplemental doses were administered through an implanted brahial vein cannula to maintain deep surgical anesthesia (absent or minimal toe withdrawal response to strong pinch). Body temperature was monitored with a Yellow Springs Instruments colonic thermistor probe and regulated at 41 ± 1°C with a servocontrolled heat lamp and circulating hot water pad.

The thorax was opened with a sternal incision, and each lung was independently unidirectionally ventilated with 0.5 l/min of air mixed with 3% CO\(_2\). Ventilation was delivered through Foley catheters inserted into each primary bronchus to a point just caudal to the ostia of the mediastinal secondary bronchi. Gases for the right lung were mixed with a Matheson Rotameter. Gases for the left lung were mixed with a Cameron Instruments GF-1 mass flow controller. A loose umbilical tape snare was placed around the left pulmonary artery to allow reversible occlusion of blood flow.

The left vagus nerve was exposed in the neck and bathed in a mineral oil pool bounded by skin flaps. Single-cell recordings were made by dissecting the vagus and placing fine filaments on a 35-gauge platinum-iridium electrode. IPC electrical activity was referenced to an indifferent Ag-AgCl electrode on the nerve sheath through a high-impedance differential probe (Grass). Action potentials were amplified with a Grass P511K AC preamplifier and AM-5 audio amplifier, digitized with a Haer window discriminator, timed with a microcomputer, visualized on HP 130C and Tektronix 561A oscilloscopes, and recorded on a Vetter D FM tape system. A 60-Hz notch filter was engaged, and filters were set for bandpass between 100 and 3,000 Hz. IPCs were identified by their nearly immediate response to step changes in ventilatory gas P\(_{\text{CO}_2}\). Single cell IPC were identified by constancy of amplitude and shape of their action potentials.

When a single IPC was identified in the left vagus, right lung ventilation was adjusted to 2.5 l/min of 5% CO\(_2\) in air to maintain gas exchange in the animal. The snare was tightened around the left pulmonary artery to redirect cardiac output to the right lung, thereby stabilizing the left lung’s intrapulmonary P\(_{\text{CO}_2}\) at inspired levels (1, 2, 3, 5, 11, 15). Left lung ventilation was adjusted to 0.5 l/min of 50% O\(_2\) balanced with N\(_2\), and CO\(_2\) levels were set at steady levels ranging from 2 to 11% using the mass flow controller. IPC discharge rate was allowed to stabilize at each P\(_{\text{CO}_2}\) level tested (usually 1 min) and was then recorded by computer for 10–30 s. After measurements of IPC discharge at each P\(_{\text{CO}_2}\) value, duplicate measurements were made at several P\(_{\text{CO}_2}\) stimulus levels to verify repeatability. The pulmonary artery ligature was then loosened, ventilation was returned to premeasurement values, and the search for IPCs was resumed.

Stimulus response relationships for each IPC were quantified by simple linear regression of discharge frequency vs. the natural logarithm of intrapulmonary P\(_{\text{CO}_2}\) (1, 3, 11, 15, 19). Mean values and SEs of the resultant slope, intercept, and regression r values were calculated for control and acclimatized groups. Comparisons of slopes and intercepts were made by unpaired t-test and P levels ≤0.05 were accepted as significant.

Changes in blood pH buffering in acclimatized vs. normal birds were quantified from anaerobic blood samples drawn...
into heparinized 1-ml syringes from a cannula in the right carotid artery. \( P_{\text{CO}_2} \) and plasma pH were analyzed on an Instrumentation Laboratories IL 813 blood gas system, and plasma bicarbonate was calculated using the Henderson-Hasselbalch equation (7). Data were pooled separately for normal and acclimatized birds, and blood buffer curves were determined using simple linear regressions of pH vs. \( \log_{10} P_{\text{CO}_2} \). Regression coefficients for normal and acclimatized groups were compared with t-tests, and P levels \( \leq 0.05 \) were considered significant.

RESULTS

Chronic \( \text{CO}_2 \) acclimatization caused a right shift of the ventilatory response curve to inspired \( \text{CO}_2 \) (Fig. 1). Two-way ANOVA on minute ventilation revealed effects of both \( P_{\text{ICO}_2} \) (\( P < 0.05 \)) and acclimatization (\( P < 0.05 \)) and an interaction between \( P_{\text{ICO}_2} \) and acclimatization (\( P < 0.05 \)). Post hoc analysis showed that mean ventilations at 0 and 14 Torr \( P_{\text{ICO}_2} \) were unaffected by acclimatization (\( P > 0.3 \)), but mean ventilations at 28 and 42 Torr \( P_{\text{ICO}_2} \) were reduced by acclimatization (\( P < 0.05 \), \( n = 6 \) animals; Fig. 1).

Blood pH-\( \log_{10} \text{PCO}_2 \) relationships of \( \text{CO}_2 \)-acclimatized birds were elevated and right shifted compared with normal birds, indicating compensatory metabolic alkalosis and bicarbonate retention (Fig. 2). Linear regressions of the form pH = (slope)·\( \log_{10} \text{PCO}_2 \) + (intercept) were correlated with \( r \) values of 0.93 for normal birds (\( n = 21 \) blood samples) and 0.97 with acclimatization (\( n = 25 \) blood samples). Intercept values (\( \pm \)SE) were increased in acclimatized (8.760 ± 0.068 pH) compared with normal animals (8.413 ± 0.098 pH, \( P < 0.05 \)). Slope values (\( \pm \)SE) in acclimatized animals (0.751 ± 0.039 pH/\( \log_{10} \text{PCO}_2 \)) were not different from normal animals (0.645 ± 0.059 pH/\( \log_{10} \text{PCO}_2 \), \( P > 0.05 \)). Mean plasma pH at 40 Torr \( \text{PCO}_2 \) increased from 7.38 ± 0.03 (normal) to 7.56 ± 0.02 (acclimatized, \( P < 0.05 \)), and mean plasma bicarbonate increased from 22.9 ± 1.6 (normal) to 34.6 ± 1.5 mM (acclimatized, \( P < 0.05 \)).

Individual stimulus-response relationships for 22 normal and 34 \( \text{CO}_2 \)-acclimatized IPC are shown in Fig. 3. As a group, discharge of \( \text{CO}_2 \)-acclimatized IPC was elevated relative to normals. Note that the lowest \( \text{PCO}_2 \) administered to acclimatized IPC was 21 Torr, while the lowest administered to normal IPC was 14 Torr. Earlier studies have shown that normal IPC sometimes discharge erratically and appear impaired with sustained \( P_{\text{ICO}_2} \) values \( < 7 \)–14 Torr in nonperfused lungs (1, 11, 15). Similarly, we found that IPC acclimatized to high \( \text{PCO}_2 \) sometimes discharged erratically <14–21 Torr \( \text{PCO}_2 \), perhaps due to increased receptive site pH accompanying metabolic compensation. We therefore...
did not expose acclimatized animals to $P_{1}\text{CO}_2$ values $<21$ Torr.

Coefficients from the individual regressions of IPC discharge frequency vs. the natural logarithm of $P_{\text{CO}_2}$, $f_{\text{IPC}} = (\text{slope}) \cdot \ln P_{\text{CO}_2} + \text{intercept}$, were averaged for the 22 normal and 34 acclimatized IPC to estimate population responses. Mean slope values (± SE) were greater for acclimatized IPC $[-19.0 \pm 1.0 \text{ impulses/s}^{-1}]$ than for normal IPC $[-12.0 \pm 1.1 \text{ impulses/s}^{-1}]$, $P < 0.05$. Mean intercept values (± SE) were also greater for acclimatized IPC (81.1 ± 4.0 impulses/s) than for normal IPC (48.4 ± 3.6 impulses/s, $P < 0.05$). Average correlation coefficients indicated excellent fit to the logarithmic stimulus-response model for both normal ($r = -0.994 \pm 0.001$) and acclimatized ($r = -0.992 \pm 0.001$) IPC.

Regression relationships for each individual IPC were used to interpolate discharge frequency at four standard $P_{\text{CO}_2}$ levels: 14, 21, 35, and 56 Torr. Interpolated discharge rates were then averaged at each $P_{\text{CO}_2}$ level for normal and acclimatized groups, yielding the mean stimulus response curves shown in Fig. 4B. Mean IPC discharge at 14 Torr for acclimatized animals is shown with a different symbol and a dotted line to indicate it was extrapolated rather than interpolated. Two-way ANOVA revealed significant effects of intrapulmonary $P_{\text{CO}_2}$ ($P < 0.05$), acclimatization state ($P < 0.05$), and $P_{\text{CO}_2}$-acclimatization interaction ($P < 0.05$). Significant interaction is consistent with the increased slope of IPC responses to $P_{\text{CO}_2}$ after acclimatization as shown in regression analysis above. Post hoc analysis using methods of Scheffé and least-squares means showed that IPC discharge was higher in acclimatized vs. normal animals at all $P_{\text{CO}_2}$ levels ($P < 0.05$).

Figure 4A shows mean IPC discharge frequencies as a function of plasma pH associated with 14, 21, 35, and 56 Torr $P_{\text{CO}_2}$. Plasma pH was calculated from the regression relationships between pH and $\ln P_{\text{CO}_2}$ given above. Figure 4A reveals that normal and acclimatized IPC responses to pH were not different over the pH range of 7.55 to 7.67 ($P > 0.05$), in contrast to normal and acclimatized IPC responses to $P_{\text{CO}_2}$, which were different at all $P_{\text{CO}_2}$ studied (Fig. 4B, $P < 0.05$). Despite the overlap of IPC responses to pH, linear regression analysis of IPC discharge frequency vs. plasma pH, $f_{\text{IPC}} = (\text{slope}) \cdot \text{pH} + \text{intercept}$, revealed larger slope (57.7 ± 0.3 vs. 39.3 ± 2.1 impulses/s$^{-1}$, $P < 0.05$) and larger intercept ($-425 \pm 2$ vs. $-285 \pm 15$ impulses/s, $P < 0.05$) values in acclimatized IPC. Correlation between $f_{\text{IPC}}$ and plasma pH was high in both normal and acclimatized groups (0.997 vs. 0.999).

**DISCUSSION**

Acclimatization model. Birds breathing 7.5% inspired $\text{CO}_2$ for 12 days showed clear acclimatization. Their ventilatory responses had an increased threshold to $\text{CO}_2$ stimulation, as noted in other chronically hypercapnic species (22). Their blood $P_{\text{CO}_2}$-pH relationships were right shifted, suggesting metabolic compensation for respiratory acidosis (7) and mean IPC discharge rates were increased at all intrapulmonary $P_{\text{CO}_2}$ levels studied. Because the changes were chronic, the shifted pH-$\ln P_{\text{CO}_2}$ relationship in acclimatized animals provided a stable method for estimating the separate effects of pH and $P_{\text{CO}_2}$ on IPC chemotransduction.

H$^+$ vs. $\text{CO}_2$ signal transduction. The different IPC responses to $P_{\text{CO}_2}$ and $\text{CO}_2$-acclimatized IPC as a function of plasma pH predicted from $P_{\text{CO}_2}$ using the relationship in Fig. 2. Discharge rate vs. pH curves for normal and acclimatized IPC overlap each other between pH 7.55 and 7.67 ($P < 0.05$). Data suggest that IPC discharge rate may be a unique function of pH, not of $P_{\text{CO}_2}$. B: mean IPC discharge rates (means ± SE) as a function of intrapulmonary $P_{\text{CO}_2}$ in normal ducks (○) and $\text{CO}_2$-acclimatized ducks (■). The 14 Torr point for $\text{CO}_2$-acclimatized IPC was extrapolated from regression equations (see RESULTS). $\text{CO}_2$-acclimatized IPC (n = 34) had right-shifted and steeper responses to $\text{CO}_2$ compared with normal IPC (n = 22). *Differences between acclimatized and normal values, $P < 0.05$.**
after acclimatization. This may reflect a fundamentally curvilinear relationship between IPC membrane excitability and pH or perhaps a change in buffering, chemical environment, or gene expression that modifies chemotransduction after acclimatization.

Intracellular vs. extracellular pH. We could not measure intracellular pH directly because IPC endings are inaccessible in the lung parenchyma. However, changes in PCO2 are extremely effective in changing both intracellular and extracellular pH due to high permeability of CO2 and generation of H+ and HCO3− from CO2 hydration (7, 20). Furthermore, most cells in steady-state acid base balance have a 0.3–0.4 pH unit differential between the intracellular and extracellular spaces, with the intracellular space more acid (20). Because 2 wk of hypercapnia produced a steady-state compensatory metabolic acidosis, we reasoned that changes in intracellular pH should track changes in plasma pH but be ~0.3–0.4 pH units lower. Although our results indicate that IPC transduce mainly H+ rather than CO2, we cannot differentiate between intracellular or extracellular sites of H+ transduction using our data alone.

Comparisons with other studies. Chronic metabolic acidosis in chickens depresses IPC discharge at low PCO2 (14) and reduces the slope and intercept of IPC discharge vs. PCO2 (3). Our results showing increased IPC discharge and increased slope and intercept of IPC discharge vs. PCO2 are consistent with this earlier study. Because metabolic acidosis and metabolic alkalosis produce opposite changes in bicarbonate and pH levels, they should have opposite effects on IPC chemotransduction if pH rather than PCO2 is transduced by levels, they should have opposite effects on IPC chemotransduction. Because metabolic acidosis and metabolic alkalosis in chickens depresses IPC discharge at low PCO2 and reduces the slope and intercept of IPC discharge vs. PCO2 (3). Our results showing increased IPC discharge and increased slope and intercept of IPC discharge vs. PCO2 are consistent with this earlier study. Because metabolic acidosis and metabolic alkalosis produce opposite changes in bicarbonate and pH levels, they should have opposite effects on IPC chemotransduction if pH rather than PCO2 is transduced by IPC. The mechanism for changes in slope and intercept of IPC response to PCO2 during metabolic acid-base disturbances remains to be determined. However, increased slope may indicate weaker intracellular pH buffering of PCO2 changes in metabolic alkalosis compared with acidosis, and increased intercept may reflect chronically elevated intracellular bicarbonate and increased pH stimulation at low PCO2 in metabolic alkalosis compared with acidosis.

Similar to the chronic metabolic alkalosis studied here, acute metabolic alkalosis induced by sodium bicarbonate infusion in ducks also increases IPC discharge (1, 17). However, acute bicarbonate infusion causes smaller increases in IPC discharge rate, and unlike the effect of chronic metabolic alkalosis reported here, acute bicarbonate infusion causes a smaller decrease in slope of IPC responses to PCO2 (1). These somewhat different effects of acute and chronic metabolic alkalosis on IPC may reflect transient ionic imbalances related to infusion of 1.2 M sodium bicarbonate (3) or relatively smaller bicarbonate changes in intracellular vs. extracellular compartments with acute bicarbonate infusion. Although chronic metabolic alkalosis used here takes longer to produce than acute metabolic alkalosis, it has the advantage of minimizing transient ionic imbalances and producing stable acid-base changes in intracellular and extracellular compartments (3, 7).

Acclimatization vs. evolutionary adaptation to hypercapnia. An earlier study investigated ventilatory responses and IPC discharge in burrowing owls, animals that are genetically adapted to subterranean life and high inspired CO2 levels. Ventilatory CO2 responses of burrowing owls are blunted (14) like those of CO2-acclimatized ducks in this study. However, burrowing owls also have blunted IPC responses to PCO2 (14) relative to most other birds studied (mean slope = 6.9 ± 0.4 impulses·s−1·InPCO2−1), unlike the increased mean IPC-PCO2 response slope seen in acclimatized ducks in this study. These contrasting observations probably reflect fundamental differences between genetic adaptation and physiological acclimatization to hypercapnia. Whereas acclimatization involves the physiological plasticity available with an animal’s existing genetic makeup, adaptation results from generations of selection for traits particularly suited for a given lifestyle. The end results may be quite different.

Perspectives

The responses of avian IPC to PCO2 after interventions such as chronic metabolic alkalosis and acidosis, acute metabolic alkalosis and acidosis, CA inhibition, and SO2 insufflation, when taken together suggest that IPC transduce H+, not molecular CO2 (1, 3, 5, 6, 16, 17). Although current evidence points to an intracellular site for H+ chemotransduction, more experiments are needed to explicitly test this hypothesis. In particular, CA inhibitors with differing membrane solubilities can help test for extracellular vs. intracellular CA catalysis and H+ generation (19). If H+ is generated from CO2 intracellularly, various transmembrane exchangers including Na+/H+ and HCO3−/Cl− antiports may be involved in intracellular pH regulation (18). Also, transduction pathways that translate chemical changes into generator and action potentials have not been studied, and future experiments investigating ion channel function in IPC would be helpful.

Avian IPC are exquisitely sensitive CO2 chemoreceptors that are inhibited rather than excited by increased PCO2. Although IPC sensory endings have yet to be studied using intracellular techniques, their afferent activity is easily recorded from vagal filaments, and their in situ microenvironment is easily controlled with pulmonary ventilation and perfusion. Continued study of avian IPC may reveal fundamental aspects of CO2 chemoreception and should provide a useful comparative contrast to mammalian central and peripheral CO2 chemoreceptors.

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