Benzolamide, acetzolamide, and signal transduction in avian intrapulmonary chemoreceptors

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Hempleman, S. C., T. A. Rodriguez, Y. A. Bhagat, and R. S. Begay. Benzolamide, acetzolamide, and signal transduction in avian intrapulmonary chemoreceptors. Am J Physiol Regulatory Integrative Comp Physiol 279: R1988–R1995, 2000.—Intrapulmonary chemoreceptors (IPC) are CO₂-sensitive sensory neurons that innervate the lungs of birds, help control the rate and depth of breathing, and require carbonic anhydrase (CA) for normal function. We tested whether the CA enzyme is located intracellularly or extracellularly in IPC by comparing the effect of a CA inhibitor that is membrane permeable (iv benzolamide) with one that is relatively membrane impermeable (iv benzolamide). Single cell extracellular recordings were made from vagal filaments in 16 anesthetized, unidirectionally ventilated mallards (Anas platyrhynchos). Without CA inhibition, action potential discharge rate was inversely proportional to inspired PCO₂ (−9.0 ± 0.8 s⁻¹·lnTorr⁻¹; means ± SE, n = 16) and exhibited phasic responses to rapid PCO₂ changes. Benzolamide (25 mg/kg iv) raised the discharge rate but did not alter tonic IPC PCO₂ response (−9.8 ± 1.6 s⁻¹·lnTorr⁻¹, n = 8), and it modestly attenuated phasic responses. Acetzolamide (10 mg/kg iv) raised IPC discharge, significantly reduced tonic IPC PCO₂ response to −3.5 ± 3.6 s⁻¹·lnTorr⁻¹ (n = 6), and severely attenuated phasic responses. Results were consistent with an intracellular site for CA that is less accessible to benzolamide. A model of IPC CO₂ transduction is proposed.

Birds and reptiles have CO₂-sensitive intrapulmonary chemoreceptors (IPC) in their lungs (30). Afferents from IPC are carried centrally in the vagus and provide phasic and tonic sensory feedback important for the control of breathing. The CO₂ stimulus detected by IPC varies during the breathing cycle (25) under the influence of inspired PCO₂, venous P CO₂, pulmonary ventilation and perfusion, and metabolism (1, 9, 12, 30, 32). IPC are therefore in a good position to detect CO₂ changes that help to match breathing to environmental and metabolic demands. However, more than 30 years after the discovery of IPC, their mechanism of CO₂ transduction remains poorly understood (23). This is unfortunate, because IPC have unusual properties compared with most other CO₂ chemoreceptors that make them a valuable comparative model of cellular CO₂ signal transduction. The most notable difference with IPC is their strong “inverse” sensitivity to CO₂; action potential discharge rate decreases as PCO₂ increases, unlike the positive relationship between discharge rate and PCO₂ in carotid bodies (8, 14–16) and in CO₂-sensitive neurons in the mammalian medulla (7, 26). Some mammalian CO₂-sensitive laryngeal mechanoreceptors have an inverse CO₂ sensitivity, like that of avian IPC (5), as does a subset of mammalian medullary CO₂ chemoreceptors (26), and therefore these receptors may share some aspects of CO₂ signal transduction mechanisms with IPC.

It is clear that IPC signal transduction requires carbonic anhydrase (CA), an enzyme that catalyzes the reversible hydration of CO₂ to H⁺ and HCO₃⁻. Acetzolamide, a membrane-permeable CA inhibitor, increases IPC discharge and attenuates or abolishes IPC discharge response to CO₂ (28). A similar response is seen in mammalian CO₂-sensitive laryngeal mechanoreceptors (5) and reptilian IPC (29). One interpretation is that CA inhibition causes alkalosis that mimics low CO₂ and stimulates IPC discharge (9, 25). These observations and others (2, 4) suggest that H⁺ from hydrated CO₂, rather than CO₂ itself, is the signal transduced by IPC. However, because acetzolamide is freely permeable to cell membranes, it alone cannot be used to distinguish extracellular from intracellular sites of CA activity in IPC (24). The critical site of catalyzed CO₂ hydration and H⁺ chemosensitivity in or around the IPC sensory endings remains uncertain.

Our hypothesis is that an intracellular CA is required for CO₂ signal transduction in IPC. To test this hypothesis, we compared the effects of CA inhibitors with different membrane permeabilities. Specifically, benzolamide, a potent CA inhibitor (association constant (Ki) = 10⁻⁹ M) with limited membrane permeability, was compared with acetzolamide, a less potent CA inhibitor (Ki = 10⁻⁸ M) with high membrane permeability (10, 18, 20, 24). If CO₂ hydration and signal transduction occur intracellularly in IPC, benzolamide should be a less effective inhibitor of IPC function than acetzolamide. If CO₂ hydration and signal transduction occur extracellularly in IPC, benzolamide should be a more effective inhibitor than acetzolamide.

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METHODS

The following protocol was approved by the Institutional Animal Care and Use Committee at Northern Arizona University. Sixteen mallard ducks, *Anas platyrhynchos*, aged 4–6 mo, body mass 1.0–1.4 kg, of either sex, were anesthetized to a deep surgical level by intravenous infusion of 35–40 mg/kg pentobarbital sodium. Supplemental anesthetic doses were administered as needed through a brachial vein cannula. Birds were unidirectionally ventilated with a 2 l/min humidified gas stream from a Cameron GF-1 four-channel mass flow controller. Gas entered the respiratory tract through a pediatric cuffed endotracheal tube and exited through a surgical incision in the interclavicular air sac (2). Colonic temperature was monitored with a mercury thermometer and regulated at 40 ± 1°C with a warm water pad.

The left vagus nerve was exposed in the neck and bathed in a mineral oil pool. Single-unit recordings were made by dissecting fine filaments from the vagus nerve and placing them on a platinum-iridium electrode. Electrical activity in the filaments was referenced to an indifferent Ag-AgCl electrode on the nerve sheath through a high-impedance differential probe (Grass HIP). Action potentials were amplified with a Grass P511K AC preamplifier and AM-5 audio amplifier. Single units were identified by the constant amplitude and shape of their action potentials using a Haer slope/height window discriminator. Action potentials were timed with a microcomputer, visualized on an oscilloscope, and recorded on a Vetter four-channel VHS tape system. Signals were notch filtered at 60 Hz and bandpass filtered to preserve the frequencies between 30 and 3,000 Hz. IPCs were identified by their nearly immediate response to step changes in ventilatory gas PCO₂. (2, 14).

**Phasic and tonic IPC responses to CO₂: control treatment.** Fine vagal filaments were tested for IPC activity while stepping inspired CO₂ between 0 and 6% at 11-s intervals. When a single IPC was identified, phasic receptor responses were analyzed using stimulus cycle-triggered histograms of action potential discharge averaged over 5–10 CO₂ stimulus cycles. The CO₂ step was then turned off, inspired CO₂ was adjusted to a steady 2%, and IPC discharge rate was allowed to stabilize for ~1 min. Tonic IPC discharge at the constant PCO₂ was then recorded on tape and computer. Inspired CO₂ was increased by 1%, the receptor discharge was allowed to restabilize, and steady-state discharge was again recorded. This was repeated until tonic IPC responses to 2, 3, 4, 5, and 6% CO₂ had been recorded. Duplicate recordings of IPC discharge were then made by returning to one or two CO₂ levels and checking discharge rates for reproducibility. IPC with nonreproducible responses to steady CO₂ were not studied further. After normal IPC responses were established, animals were given either acetazolamide or benzolamide.

**Phasic and tonic IPC responses to CO₂ by the acetazolamide treatment.** Acetazolamide (Sigma) was dissolved in alkaline-distilled water to produce a 25 mg/ml stock solution. After recording was made of normal CO₂ responses of the IPC just described, the inspired CO₂ step was turned back on, and acetazolamide was infused intravenously. Acetazolamide usually increased IPC discharge within 30 s, and a steady-state increase was achieved in ~5–10 min. IPC responses to phasic and tonic CO₂ stimuli were then recorded on computer and tape, as we have described. The entire recording protocol was repeated at several cumulative acetazolamide dosages (10, 25, and 50 mg/kg) but not all IPC received all acetazolamide dosages (see Table 1). Some IPC were maximally affected at low dosages (i.e., no remaining CO₂ response), and higher dosages were not given. Some IPC were lost before all dosages could be given. The entire protocol was completed in ~45–60 min. Animals were euthanized with 100 mg/kg pentobarbital at the end of the experiment.

**Phasic and tonic IPC responses to CO₂ by the benzolamide treatment.** Figure 1 gives an example of raw recordings of an IPC responding to CO₂ and benzolamide. Benzolamide was kindly provided by Dr. Erik Swenson of the University of Washington (Seattle, WA). The compound was dissolved in alkaline distilled water, giving a 25 mg/ml stock solution, and responses to benzolamide were recorded in the same manner as described for acetazolamide. Because benzolamide proved to be a less effective inhibitor of IPC function than acetazol-
amidine, a wider range of dosages was given: 10, 25, 50, and 100 mg/kg. As with acetazolamide, not all animals received all dosages of benzolamide (see Table 1). Some animals were started at 25 mg/kg to avoid prolonged recording times, and some IPC were lost before the highest dosage could be given. Animals were euthanized at the end of the experiment with 100 mg/kg pentobarbital.

**Blood acid-base measurements.** Arterial blood was sampled to measure blood acid-base status before and after 25 mg/kg acetazolamide (2 animals) or 50 mg/kg benzolamide (1 animal). Birds were ventilated with 3, 6, and 6% inspired CO₂, and multiple samples were drawn at each CO₂ level over a period of ~1 h. Arterial blood samples (0.7 ml) were drawn anaerobically into 1-ml heparinized tuberculin syringes and analyzed immediately on a Cameron Instruments BGMS002 for Pco₂ and pH. pH and Pco₂ samples were plotted (pH vs. \( \ln(P\text{CO}_2) \)), semilogarithmic regressions were calculated, and slopes and intercepts of the blood buffer curves were compared among treatments by use of ANOVA (SAS, Cary, NC).

**Statistical analysis of IPC responses.** Tonic discharge frequencies of IPC to steady levels of Pco₂ were compared between control and acetazolamide or benzolamide treatments using two-way analysis of variance (ANOVA, SAS). Differences were accepted as significant at \( \alpha = 0.05 \). Post hoc analysis of treatment effects were done with least squares means with adjustment for multiple comparisons.

Linear regression of IPC discharge frequency \( f_{IPC} \) vs. the natural logarithm of Pco₂ was used to quantify the response of IPC to tonic Pco₂ levels. Previous studies have shown that tonic IPC stimulus response curves are well fit by the following semilogarithmic function (2, 12, 13, 22):

\[
f_{IPC} = \beta_0 + \beta_1 \cdot \ln(P\text{CO}_2)
\]

Means and standard errors of the intercepts \( (\beta_0) \), slopes \( (\beta_1) \), and regression correlation values \( (R) \) were determined for the control groups and for each acetazolamide and benzolamide dosage group. Values of regression parameters were compared with the appropriate control by use of \( t \)-tests. Differences were accepted as significant at \( \alpha = 0.05 \).

**RESULTS**

**Tonic responses of IPC before and after acetazolamide.** Figure 2 shows the seven IPC that were studied; Table 1 shows numbers of animals at each treatment dosage. Under control conditions, IPC neural discharge decreased significantly with increasing inspired Pco₂. With 10 mg/kg acetazolamide, average IPC discharge was increased relative to normal, especially at higher Pco₂, and IPC discharge no longer decreased significantly with increasing Pco₂. IPC responses to 25 mg/kg and 50 mg/kg acetazolamide were not different from those at 10 mg/kg (Fig. 2, and below), indicating that a dose of 10 mg/kg was sufficient to produce a maximal inhibitory effect on CA.

The statistical analysis ANOVA revealed a significant effect of CO₂ \( (P < 0.0001) \) and acetazolamide \( (P < 0.0001) \) on IPC discharge. Post hoc testing revealed that IPC response to CO₂ after 10, 25, and 50 mg/kg acetazolamide was significantly different from control \( (P < 0.0001 \text{ in each case}) \). No differences were seen among the 10, 25, and 50 mg/kg acetazolamide treatments \( (0.7069 < P < 0.998) \).

**Tonic responses of IPC to Pco₂ before and after benzolamide.** Figure 3 shows the nine IPC that were studied; Table 1 shows numbers of animals at each treatment dosage. Under control conditions, mean IPC neural discharge decreased significantly with increasing inspired Pco₂. With 10 and 25 mg/kg benzolamide, IPC discharge rates were elevated above control, but IPC discharge still decreased normally with increasing Pco₂. With 50 and 100 mg/kg benzolamide, IPC discharge rates were elevated further, and the IPC response to Pco₂ was attenuated. The largest dosage of benzolamide \( (100 \text{ mg/kg}) \) produced effects that were comparable to the smallest dosage of acetazolamide \( (10 \text{ mg/kg}) \).

The statistical analysis ANOVA revealed significant effects of CO₂ \( (P < 0.0001) \) and benzolamide \( (P < 0.0001) \) on IPC discharge rate. Post hoc testing indicated that benzolamide treatments of 10 mg/kg \( (P < 0.008) \), 25 mg/kg \( (P < 0.0008) \), 50 mg/kg \( (P < 0.0004) \), and 100 mg/kg \( (P < 0.0001) \) were significantly different

**Table 1. Logarithmic regressions of IPC discharge frequency vs. ln(PCO₂)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slope ( (\beta_1) ) ( (\ln(P\text{CO}_2) \text{s}^{-1}) )</th>
<th>Intercept ( (\beta_0) ) ( (\text{s}^{-1}) )</th>
<th>( R )</th>
<th>( N ) (IPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetazolamide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>(-9.1 \pm 1.1^\dagger )</td>
<td>(36.5 \pm 3.4)</td>
<td>(-0.98 \pm 0.01)</td>
<td>7</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>(-3.5 \pm 3.6)</td>
<td>(32.8 \pm 12.1)</td>
<td>(-0.62 \pm 0.27)</td>
<td>6</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>(-3.2 \pm 2.1^\ast)</td>
<td>(31.6 \pm 8.8)</td>
<td>(-0.49 \pm 0.23)</td>
<td>7</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>(-2.8 \pm 0.9^\ast)</td>
<td>(28.3 \pm 6.2)</td>
<td>(-0.95 \pm 0.01)</td>
<td>2</td>
</tr>
<tr>
<td>BENZOLAMIDE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>(-8.9 \pm 1.2^\dagger)</td>
<td>(33.7 \pm 4.8)</td>
<td>(-0.97 \pm 0.02)</td>
<td>9</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>(-10.4 \pm 4.4^\ast)</td>
<td>(44.3 \pm 14.6)</td>
<td>(-0.88 \pm 0.02)</td>
<td>2</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>(-9.8 \pm 1.6^\ast)</td>
<td>(41.5 \pm 6.6)</td>
<td>(-0.96 \pm 0.02)</td>
<td>8</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>(-6.7 \pm 2.1^\dagger)</td>
<td>(35.9 \pm 8.4)</td>
<td>(-0.82 \pm 0.08)</td>
<td>7</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>(-3.2 \pm 0.5^\ast)</td>
<td>(24.9 \pm 1.2)</td>
<td>(-0.88 \pm 0.06)</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are results according to model of intrapulmonary chemoreceptor (IPC) discharge frequency vs. \( \ln(P\text{CO}_2) \): \( f_{IPC} = \beta_1 \ln(P\text{CO}_2) + \beta_0 \). Parameters were averaged for each treatment and expressed as means ± SE. Acetazolamide or benzolamide was infused iv at indicated dosage. \^ Different from its respective control \( (P < 0.05) \). \^Significantly different from zero \( (P < 0.05) \).
from control but not significantly different from each other (0.06 < P < 0.84).

**Regression of IPC discharge rate with respect to PCO2.**

Individual regressions of IPC discharge frequency vs. the natural logarithm of PCO2 yielded slope, intercept, and correlation (R) values that were averaged for each treatment. These are summarized in Table 1, and the slope values reflecting IPC sensitivity to PCO2 are plotted in Fig. 4.

The slope of the IPC discharge vs. PCO2 relationship quantifies the response of IPC to tonic CO2 stimuli. The slope is normally negative because of the inverse relationship between IPC discharge and PCO2. Acetazolamide treatment caused a large reduction in the absolute value of mean slope relative to control at all dosages, significantly at 25 and 50 mg/kg. At 10, 25, and 50 mg/kg acetazolamide, the mean slopes were not different from zero (P > 0.05). Benzolamide treatment caused no significant change in mean slope relative to control until a dosage of 100 mg/kg, and slopes remained non-zero at all benzolamide dosages (P < 0.05).

**Cycle-triggered stimulus histograms.** The averaged responses of IPC to CO2 steps are shown in Figs. 5 and 6. Control IPC response included a rate-sensitive phasic overshoot during the CO2 down-step followed by partial adaptation. Note that with this brief CO2 step cycle, mean IPC discharge approaches but does not reach its peak response. After acetazolamide, mean response loses phasic overshoot, and tonic response to CO2 is elevated at high PCO2. Heavy lines, mean discharge rates; vertical lines, SE.

Mean intercept values after acetazolamide and benzolamide treatments were not different from their respective controls. There appeared to be a trend toward increased intercepts at low benzolamide dosages and a trend for a reduced intercept at all acetazolamide dosages and at the highest benzolamide dosages.

**Fig. 3.** Average responses of IPC to tonic PCO2 levels with and without benzolamide. Benzolamide was a less effective blocker of CO2 response than acetazolamide (Fig. 2). Values are means ± SE for nos. shown in Table 1.

**Fig. 4.** Mean slopes of the logarithmic IPC stimulus-response curves as a function of dosage of carbonic anhydrase blocker. Both acetazolamide and benzolamide attenuated the absolute value of the slopes at high dosages, but acetazolamide was more effective at low dosages. Values are means ± SE. *Slope different from its respective control at 0 mg/kg. IPC numbers in each treatment are shown in Table 1.

**Fig. 5.** Cycle-triggered stimulus histograms showing mean response of IPC to 0–6% inspired CO2 steps before and after administration of 25 mg/kg acetazolamide (n = 7). fIPC, IPC discharge frequency. Inspired CO2 cycle period was 11 s. Control response shows characteristic phasic overshoot in discharge rate during CO2 down-step and low tonic discharge rate at high PCO2. After acetazolamide, mean response loses phasic overshoot, and tonic response to CO2 is elevated at high PCO2. Heavy lines, mean discharge rates; vertical lines, SE.

**Fig. 6.** Cycle-triggered stimulus histograms showing mean response of IPC to 0–6% inspired CO2 steps before and after administration of 25 mg/kg benzolamide (n = 8). Inspired CO2 cycle period was 11 s. Control response shows characteristic phasic overshoot in discharge rate during CO2 down-step and low tonic discharge rate at high PCO2. After benzolamide, phasic overshoot is strongly attenuated, tonic response to CO2 is slightly elevated, but IPC discharge oscillation still shows a strong response to the CO2 step. Heavy lines, mean discharge rates; vertical lines, SE.
Arterial P CO2 values were higher at 1% inspired CO2 after both water to produce H+ and HCO3−. Mals) slows pulmonary CO2 elimination and produces a phasic overshoot to the PCO2 down-step and raised mean discharge rate, especially at high PCO2 periods of high PCO2, and nearly abolished the discharge oscillation during the PCO2 step (Fig. 5). Benzolamide reduced but did not abolish the rate-sensitive phasic overshoot to the PCO2 down-step and raised mean discharge frequency at both high and low PCO2 stimulus levels (Fig. 6). Unlike acetazolamide, benzolamide did not attenuate the mean discharge oscillation. The response to benzolamide was more variable than that to acetazolamide, as indicated by the larger SE bars, but the general effect of CA inhibition was to raise mean discharge rate, especially at high PCO2 values, and reduce dynamic responsiveness to abrupt CO2 steps.

**Blood acid-base balance.** There were no changes in the semilogarithmic blood-buffer curves relating arterial pH to arterial PCO2 with either 25 mg/kg acetazolamide or 50 mg/kg benzolamide (Fig. 7, P > 0.05). This indicates that no metabolic acidosis or metabolic alkalosis occurred in arterial blood during the course of CA inhibitor treatment. However, some other differences were noted. At a constant 1% inspired CO2, arterial PCO2 was normally 14 ± 1 Torr (4 samples), but after acetazolamide, arterial PCO2 (PaCO2) increased to 30 ± 4 Torr (2 samples, P < 0.05), and after benzolamide, PaCO2 increased to 24 ± 2 Torr (4 samples, P < 0.05). This suggests that CA inhibition in birds (as in mammals) slows pulmonary CO2 elimination and produces equilibrated PaCO2 values that are significantly higher than airway PCO2 (21).

**DISCUSSION**

CA catalyzes the reversible reaction of CO2 with water to produce H+ and HCO3−. Seven isoforms of CA (I through VII) have been identified. CA II (a soluble form) and CA IV (a membrane-bound form) have been localized to the nervous system (20). Interestingly, CA II has the highest catalysis rate of any known enzyme (106 s−1). When CA II is present, the rate of CO2 hydration is limited mainly by the rate of diffusion of substrates, not by enzyme kinetics (17).

Neubauer (20) recently reviewed the distribution of CA in sensory neurons. In the central nervous system, CA is more common in glia than in neurons. In peripheral sensory systems of birds and mammals, CA is most common in larger diameter sensory neurons of the dorsal root ganglia, and to a lesser extent neurons of the trigeminal ganglia (22%) and the nodose ganglia (2%). The avian IPC studied here have their cell bodies in the nodose ganglia (13, 22), and it is likely that the 2% of nodose ganglia neurons positive for CA includes the somata of IPC. This remains a question for future study, because no histochemical studies have been performed on IPC. IPC are known mainly by their physiological responses to CO2 as measured by single-unit vagal afferent recordings and ventilatory reflexes (30).

Powell (24) and Scheid et al. (28) were the first to show that intravenous acetazolamide strongly stimulated IPC discharge rate and effectively abolished the response to CO2. The excitatory effect of acetazolamide on IPC has been confirmed by others (this study and Refs. 9 and 29). However, acetazolamide is freely permeable to cell membranes, and it has been unclear from previous studies whether the catalyzed CO2 hydration occurred intracellularly or extracellularly in IPC (24). The site of catalysis is an important question, because H+ or HCO3−, not molecular CO2, appears to be the stimulus for IPC signal transduction (see review in Ref. 2). Acid-base sensing and control mechanisms should be different if the H+/HCO3− produced by CA were in a relatively closed intracellular space compared with the relatively open extracellular space (24).

To test the site of CA catalysis, the effect of benzolamide, a potent CA inhibitor (Ki = 10−8 M) that has limited ability to cross cell membranes, was compared with the effect of acetazolamide, a less potent CA inhibitor (Ki = 10−5 M) that freely crosses cell membranes (10, 18, 20, 24). Acetazolamide produced greater stimulation of IPC discharge rates and greater attenuation of both the phasic and tonic responses to PCO2, consistent with an intracellularly localized CA in IPC endings. Benzolamide also stimulated IPC discharge rate and attenuated IPC responses to CO2, but the effects were smaller and larger dosages were needed, presumably to overcome benzolamide’s limited membrane permeability. Hanson et al. (10) reported a similar, larger effect of acetazolamide compared with benzolamide on cat medullary chemoreceptor responses to CO2, an effect that they also attributed to differences in membrane permeabilities of the two inhibitors.

Major features of CA inhibition in IPC included elevation of IPC discharge to high levels and reduced or abolished response of IPC to tonic CO2 stimuli. Cycle-triggered stimulus histograms from CO2 steps showed that CA inhibition first attenuated the rapid phasic response of IPCs (Fig. 6), and then it elevated the tonic IPC activity at high PCO2 (Fig. 5).
Critique of method. Intravenous infusion of acetazolamide and benzolamide produces systemic as well as local effects. Clearly it would have been better to microinject the CA inhibitors around the IPC endings to localize effects to IPC but that was not technically feasible. Because CA is a widely distributed enzyme, many systemic effects are possible. For example, when CA inhibitors are administered chronically for many hours or days, renal reabsorption of filtered bicarbonate is impaired, and eventually systemic metabolic acidosis results. Chronic metabolic alkalosis or acidosis can affect IPC (2), so we investigated this further.

Experiments performed here were acute, not chronic, and blood analysis showed that the 1- to 1.5-h exposure time to CA inhibitors was too short to cause metabolic acidosis or alkalosis (all points lie on the same blood-buffer line, Fig. 7). CA inhibition did elevate PaCO₂, producing a respiratory acidosis despite unchanged inspired P CO₂. In mammals, the elevated P CO₂ after CA inhibition is ascribed to slow, uncatalyzed exchange of CO₂, HCO₃⁻, and H⁺ between erythrocytes and plasma occurring after the blood leaves the lungs (21).

Mammalian medullary CO₂ chemoreceptors are stimulated by acetazolamide, and this may reflect CO₂ accumulation and acidosis in brain tissue caused by slowed CO₂-bicarbonate-chloride exchange with erythrocytes (20, 21). Avian IPC are also stimulated by acetazolamide; however, IPC endings are located in the pulmonary gas exchange region, where CO₂ diffuses easily between air, blood, and tissue (13). Even though CA inhibition probably slows CO₂-bicarbonate-chloride exchange in avian erythrocytes (Fig. 7), it is unlikely that CO₂ could accumulate in well-ventilated lungs (21). Also, IPC discharge is inversely proportional to P CO₂, and therefore the strong stimulation of IPC discharge by acetazolamide suggests that IPC are detecting a decreased P CO₂ stimulus or alkalosis, not elevated P CO₂ and acidosis.

Like IPC, mammalian CO₂-sensitive airway receptors have an inverse response to P CO₂. However, although both avian IPC (shown in Refs. 24 and 28 and this study) and mammalian laryngeal receptors (4) are stimulated by systemically administered acetazolamide, only avian IPC (9) are stimulated by airway-administered acetazolamide. We wondered why this might occur, and we hypothesize that the different responses may be related to relative diffusion distances between blood and gas for each receptor type. For example, IPC endings may be very close to sites of acetazolamide delivery by both blood and gas (1, 9, 13), whereas mammalian airway receptor endings may be deeper in the airway tissue and influenced mainly by acetazolamide delivery by blood.

Physiological significance. Depending on the CA isoform present, the catalyzed hydration/dehydration rates for CO₂ are up to 1,000-fold faster than the uncatalyzed rates (17). However, because CA, like all enzymes, accelerates the forward and reverse reaction rates equally, the equilibrium expected for a simple CO₂ hydration/dehydration reaction is approximately the same whether or not CA is present; it just takes longer to achieve without CA. Therefore, it would be reasonable to predict that CA inhibition should slow phasic chemoreceptor responses to rapidly changing CO₂ signals. CA inhibition may also elevate P CO₂ and H⁺ around poorly perfused (or ventilated) receptors because of slowed CO₂-bicarbonate-chloride exchange between tissue and erythrocytes passing through blood capillaries. Nevertheless, tonic chemoreceptor responses to steady levels of CO₂ should persist if enough time is allowed for equilibration. This is generally what occurs with CA inhibition in mammalian carotid bodies, subesophageal ganglia cells of the pulmonate snail,
Proposed model of cellular CO₂ transduction in IPC.

The general model is shown in Fig. 8. As intrapulmonary P CO₂ increases, CO₂ diffuses across the IPC cell membrane and is rapidly hydrated by intracellular CA to produce H⁺ and HCO₃⁻; the opposite occurs when lung P CO₂ falls. Some of the H⁺ formed is buffered intracellularly, and some of the H⁻ and HCO₃⁻ is rapidly transported across the cell membrane by exchangers or pumps, yet to be identified. The intracellular H⁺ and HCO₃⁻ concentrations at any given P CO₂ level would reflect the simultaneous kinetic processes of CO₂ hydration, H⁺ and/or HCO₃⁻ transmembrane transport, and buffering. Inhibition of intracellular CA would slow CO₂ hydration, but H⁺ transport out of the cell should remain rapid. This upset of the normal kinetic balance may produce an intracellular alkalosis that would mimic low P CO₂ levels and increase IPC discharge (Fig. 2). Ion channels modulated by intracellular [H⁺] may be the final step coupling intracellular alkalosis to a generator potential and action potentials, but this also remains to be tested.

Tallman et al. (31) recently formulated a model of CO₂ transduction in IPC using a Wiener Cascade of linear differential equations combined with static nonlinearities. Their mathematical model accurately simulates tonic and phasic IPC responses to CO₂ but does not relate these processes to specific biological events. Our biological model may help to expand their model by suggesting the underlying cellular processes.

Perspectives

The results of this study show the importance of intracellular CA in IPC. Our model of CO₂ signal transduction in IPC integrates this with earlier experimental and theoretical studies and can be used to make testable predictions about the roles of intracellular CA, high-activity transmembrane H⁺ and/or HCO₃⁻ exchangers, intracellular buffering, and ion channels modulated by intracellular pH. The key element of the proposed model is a kinetic balance between high rates of intracellular CO₂ hydration and transmembrane acid-base transport. Supporting this hypothesis, we have preliminary evidence that di-methyl amiloride (an H⁺/Na⁺ exchange blocker) powerfully attenuates IPC discharge in micromolar dosages, presumably by allowing intracellular accumulation of H⁺ produced by CO₂ hydration (11). Interestingly, mammalian medullary chemoreceptors are relatively insensitive to amiloride blockade of Na⁺/H⁺ exchange (27), and mammalian carotid bodies are only minimally affected by blocking amiloride-sensitive Na⁺/H⁺ exchange or HCO₃⁻/Cl⁻ exchange (3, 8, 16). It has been suggested that CO₂ transduction in these mammalian chemoreceptors may be critically dependent on a lack of regulation of intracellular pH during CO₂ acidosis (7, 27). IPC therefore appear to be fundamentally different from most other CO₂ chemoreceptors: not only are they inhibited rather than excited by CO₂, but they appear to control intracellular pH more actively and dynamically than other CO₂ chemoreceptors. The continued study of avian IPC should help the general understanding of CO₂ chemotransduction and illustrate the diversity of solutions existing in nature for solving signal transduction problems.

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