

Dilution acidosis: evidence for a role of intracellular pH in the control of ventilation

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Kasserra, C. E., and D. R. Jones. Dilution acidosis: evidence for a role of intracellular pH in the control of ventilation. *J. Appl. Physiol.* 80(5): 1804–1810, 1996.—Acute hyperosmolality results in an extracellular dilution acidosis and hypercarbia that does not stimulate ventilatory compensation. The osmotic stress is also associated with shifts in water and electrolyte balance and an increase in intracellular pH. The alkaline intracellular pH was hypothesized to have a role in preventing a normal respiratory response to the extracellular acidosis and hypercarbia. Therefore, this study examined the effect of ion-exchange blockade on intra- and extracellular pH and ventilation during acute hyperosmolality in the Pekin duck (*Anas platyrhynchos*) by using ^{31}P -nuclear magnetic resonance spectroscopy. Both 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and amiloride inhibited the development of the intracellular alkalosis that normally develops in muscle during acute hyperosmolality. Instead, exposure to hyperosmotic stress during ion-exchange blockade resulted in a significant acidosis both intracellularly and extracellularly. Arterial pH decreased 0.10 ± 0.04 pH unit with a sucrose infusion after either blocker, and intracellular pH decreased 0.11 ± 0.06 and 0.16 ± 0.04 pH units with a sucrose infusion after DIDS and amiloride, respectively. Ventilation increased 79 ± 28 and $122 \pm 100\%$, respectively, during acute hyperosmolality after ion-exchange blockade with either DIDS or amiloride. The results suggest that intracellular pH may play a role in the ventilatory response to acid-base perturbations. The data also indicate that both $\text{Cl}^-/\text{HCO}_3^-$ and Na^+/H^+ exchanges are involved in the development of the intracellular alkalosis during hyperosmotically induced extracellular acidosis.

acid-base; hyperosmolality

ACUTE HYPEROSMOLALITY causes a prolonged extracellular acidosis termed dilution acidosis (12, 13, 17), an unusual effect because it occurs without the addition or depletion of either an acid or a base. The acid-base disturbance is also unique because it does not stimulate the expected respiratory compensation (12). The acidosis is associated with a relative increase in total extracellular Cl^- (12, 17) and is accompanied by the concomitant development of an intracellular alkalosis (termed contraction alkalosis) that has been measured in both muscle and red blood cells (11, 17). It has been speculated that the intracellular alkalosis may also occur in the peripheral chemoreceptors, resulting in suppression of chemoreceptor discharge and therefore suppression of the normal ventilatory increase associated with a decrease in extracellular pH (11).

The mechanism underlying these pH changes is not known. Studies of pH changes and ion exchange during hyperosmotic perturbations in vitro and in vivo suggest a predominant role for anion exchange (12, 17, 25).

These studies have measured a relative increase in extracellular Cl^- concentration ($[\text{Cl}^-]$), implying an outward flux of Cl^- . The predominant cation exchange involved in pH regulation is the Na^+/H^+ exchange, which has been shown to be activated during hyperosmotic exposure (22). We therefore investigated the effect of two ion-exchange blockers on the acid-base response to acute hyperosmolality. We hypothesized that ion-exchange blockade would prevent the intracellular alkalosis, thereby removing the inhibitory effects on ventilation. We used 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) to inhibit carrier-mediated anion exchange, including the $\text{Cl}^-/\text{HCO}_3^-$ exchange, and amiloride to inhibit the Na^+/H^+ antiporter. Intracellular pH changes were measured using ^{31}P -nuclear magnetic resonance (NMR), which allowed noninvasive repeated measurements over time. Changes in the acid-base status and ventilation in hyperosmotic animals were compared with lactacidotic animals.

METHODS

Surgical methods. Twenty-six female Pekin ducks (1.8–3.0 kg) were obtained from the Animal Care Facility of the University of British Columbia and housed in the Zoology Department in individual cages. Purina duck food and water were provided ad libitum. The brachial artery and ulnar vein in the wing of each animal were chronically cannulated under local anesthesia (2% Xylocaine; Astra) with a single piece of polyvinyl chloride tubing to form an exteriorized loop. One end of the cannula was inserted 4 cm into the artery and the other end 5 cm into the vein. Long-term patency was ensured by prior treatment of the cannula with TD-MAC (Polysciences), a heparin compound that binds to the wall of the tubing. The animals were allowed at least 2 days to recover before the start of any experiments.

Experimental protocol. In 15 animals, the wing cannula was severed and a second cannula section long enough to reach the outside of the NMR magnet was attached to each end. The animal was fitted with an endotracheal cannula under local anesthesia (2% Xylocaine) and then placed in a normal sitting position in a cradle and carefully restrained. Although not under general anesthesia, ducks are quiescent under restrained conditions and movement was not a significant problem. Once inside the magnet, the duck was allowed ~15 min to adjust to its surroundings, and then four to five resting spectra of the pectoral muscle were obtained as described in *^{31}P -NMR spectra*. During this period, three (0.8-ml) samples of arterial blood were taken; 0.4 ml was analyzed immediately for blood gases and pH on a blood gas analyzer (IL813, Instrumentation Laboratories) maintained at 40.5°C, and 0.4 ml was centrifuged and the plasma decanted and frozen for the subsequent measurement of ions. The endotracheal cannula was attached to a Fleisch no. 0 pneumotachometer connected to a differential pressure transducer (DP103-18, Validyne) for measurement of ventilation (\dot{V}_E). The total dead space was ~3 ml. \dot{V}_E was recorded for

1 min every 5 min until it was stable. Arterial blood pressure was measured simultaneously with an Elcomatic 715A blood pressure transducer (Harvard Instruments), and heart rate was derived from the arterial blood pressure trace.

Nine animals were then intravenously infused with physiological saline containing DIDS (30 $\mu\text{mol/kg}$ in 5 ml) over 10 min while six other ducks received 20 mg/kg of amiloride (0.075 $\mu\text{mol/kg}$) in the same manner. All solutions were freshly made immediately before use, and opaque cannulas were used to protect the light-sensitive stilbenes. Cardiovascular and respiratory variables were continuously monitored, and blood samples were taken 20 and 30 min after the end of infusion to ensure that the animal was stable. If the animal did not appear to be stable, variables were monitored until at least three samples indicated a new steady state had been achieved. This never took longer than 40 min. One final NMR spectrum was acquired over the last 6 min of the equilibration period to use as a control value. The 15 animals treated with ion-exchange blockers were then infused with 26.5 mmol/kg body wt of sucrose (~25 ml) over 36 min. All variables were monitored every 6 min during the infusion period and during the 12-min recovery period.

Eleven other animals experienced a similar protocol but were not placed in the magnet and did not receive ion-exchange blockers. Eight ducks were infused with 0.25 meq·kg⁻¹·min⁻¹ of lactic acid at 0.5 ml/min. The changes in intracellular pH (pH_i) during lactic acidosis have been measured previously (13), so this experiment was performed to serve as a control to the respiratory response. Three additional animals were infused only with sucrose, and blood samples were taken after 15 and 30 min of infusion for the measurement of total plasma CO_2 with a Carle AGC series 100 gas chromatograph. The purpose of this measurement was to confirm any directional changes in extracellular HCO_3^- concentration ($[\text{HCO}_3^-]$) that might correlate with changes in $[\text{Cl}^-]$. \dot{V}_E was not measured in these three animals because \dot{V}_E during sucrose-induced hyperosmolality has been previously studied in this laboratory (11, 12).

Instrument calibration. The pneumotachometer was calibrated with known volumes of air delivered over timed intervals, and the airflow signal was integrated to yield tidal volume. The blood pressure transducer was calibrated with a mercury manometer. Plasma osmolality was measured with a

vapor pressure osmometer (model 5500, Wescor), plasma Na^+ ($[\text{Na}^+]$) and K^+ concentrations ($[\text{K}^+]$) by atomic absorption spectroscopy (model 2380, Perkin-Elmer), and plasma $[\text{Cl}^-]$ with a Buchler Digital chloridometer (model 4-2500, Buchler Instruments). The gas chromatograph was calibrated with 25- μl samples of 20 mM NaHCO_3 standard.

³¹P-NMR spectra. The animals were placed in a custom-designed Perspex cradle and tilted slightly from the dorsoventral axis so that the thickest portion of the right pectoral muscle was directly over the surface coil. The cradle was then slid into a 1.89-T horizontal superconducting magnet (Oxford Instruments, Oxford, UK) connected to a Nicolet 1280 spectrometer. The surface coil used was a 3.75-cm inductively coupled loop-gap (or spiral-resonating) coil made from 0.005-cm-thick copper foil shielded by Teflon sheets. This type of coil was selected because it has a very high "Q," which resulted in a very high sensitivity compared with other capacitatively coupled surface coils that were tested under the conditions of the experiment. However, this coil was not double tuned, and, therefore, the magnetic field was shimmed on a phosphorus sample and not on the proton signal from the animal. The large (~7-cm³) homogeneous field of the magnet, combined with a cradle design that allowed repeated precise placement of the surface coil and animal in the magnet, resulted in well-resolved spectra with excellent signal-to-noise ratios (Fig. 1). Phosphorus spectra of 172 free induction decays were collected at 32.5 MHz by using a 35- μs pulse width and a 1.5-s pulse interval (total time 6 min). Data were zero filled, and the summed free induction decays were multiplied by an exponent corresponding to 6-Hz line broadening before Fourier transformation.

Statistical analysis. pH_i was calculated from the difference in chemical shift (ΔP_i) of the P_i and phosphocreatine peaks according to the formula $\text{pH}_i = \text{pK}' + \log [(\Delta\text{P}_i - \delta_A)/(\delta_B - \text{P}_i)]$, where δ_A and δ_B are the acidic and basic phosphate titration end points, respectively, and pK' is the apparent pK' for phosphates, all calculated from Kost (14) for a body temperature of 41°C. All results were analyzed by a paired-comparison analysis of variance. Results are expressed as means \pm SE or mean proportional changes \pm SE from resting levels, and statistical significance was assumed when $P < 0.05$. All data points on graphs are plotted at the 3-min mark

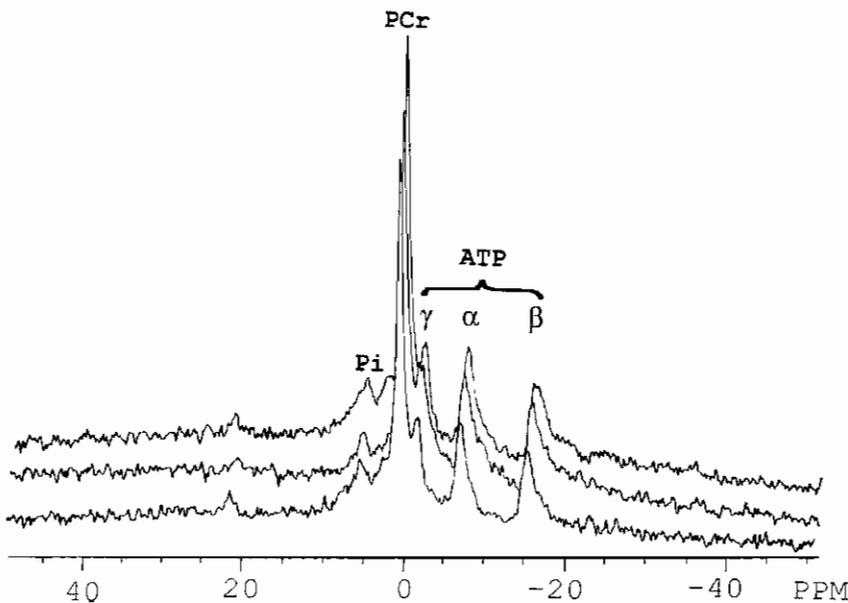


Fig. 1. Three representative spectra of duck pectoral muscle. *Bottom spectrum* represents a resting state, with following 2 spectra obtained at 22.5 (*middle spectrum*) and 37.5 min (*top spectrum*) into hypertonic sucrose infusion after treatment with 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). Each spectrum is sum of 172 free induction decays. Scale at *bottom*, chemical shift in parts per million (PPM) with respect to phosphocreatine (PCr) signal, which is set to 0. Each spectrum has been shifted 1 mm to the right of the spectrum below it.

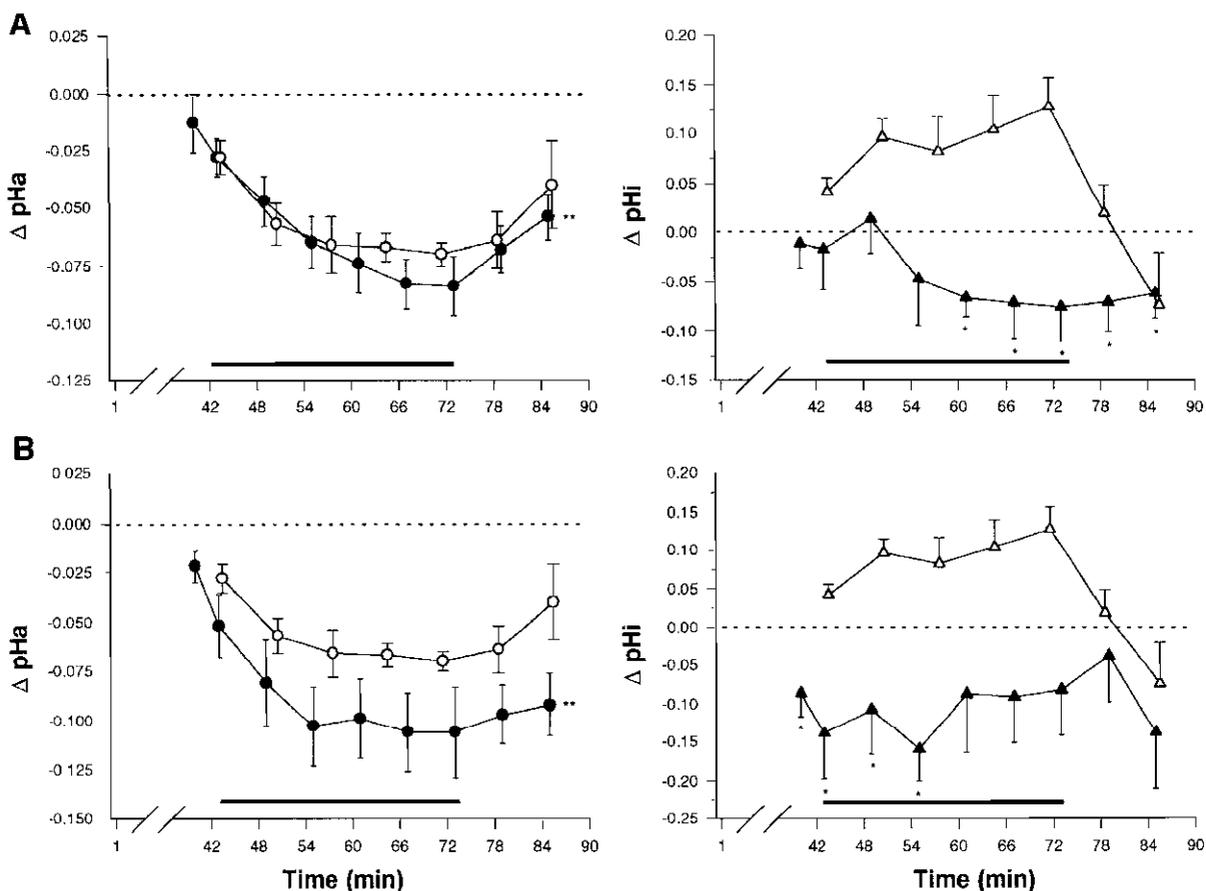


Fig. 2. Effect of DIDS (A) and amiloride (B) treatments on changes in arterial pH (ΔpH_a) and intracellular pH (ΔpH_i) responses to hyperosmolality. Open symbols, animals given a sucrose load only and are data from Kasserra et al. (13) shown for comparison purposes. Solid symbols, animals given an ion-exchanger blocker. First solid symbol, response to drug alone; last 2 solid symbols, recovery period; thick solid line, sucrose infusion. Values are means \pm SE. *Significantly different from resting, $P < 0.05$. **All points significantly different from resting, $P < 0.05$

of the 6-min acquisition periods required for the NMR spectra and described likewise in the text.

RESULTS

DIDS. Infusion of DIDS did not significantly affect arterial pH (pH_a) or pH_i (Fig. 2). Resting pH_a was 7.48 ± 0.01 pH units, and resting pH_i was 7.14 ± 0.01 pH units. However, \dot{V}_E increased slightly after DIDS infusion from a control level of 326 ± 57 ml \cdot kg $^{-1}$ \cdot min $^{-1}$ to 428 ± 73 ml \cdot kg $^{-1}$ \cdot min $^{-1}$ ($P < 0.05$; Fig. 3). Neither arterial PCO_2 (Pa_{CO_2}), arterial PO_2 (Pa_{O_2}), respiratory frequency, tidal volume, nor plasma $[\text{Na}^+]$ or $[\text{Cl}^-]$ changed significantly. Mean arterial blood pressure decreased 9 ± 4 mmHg from a resting level of 230 ± 5 mmHg, and heart rate increased 41 ± 8 beats/min from a resting rate of 135 ± 6 beats/min (both $P < 0.05$). All animals were stable for at least the last 10 min of the equilibration period.

Sucrose infusion caused a significant decrease in pH_a to 7.39 ± 0.02 pH units and decreased pH_i to 7.03 ± 0.06 pH units by the end of the infusion (Fig. 2). The decrease in pH_a began immediately on infusion, but pH_i was not significantly affected until 21 min into the infusion. \dot{V}_E increased significantly throughout the hyperosmotic infusion, rising to 712 ± 157 ml \cdot kg $^{-1}$ \cdot min $^{-1}$.

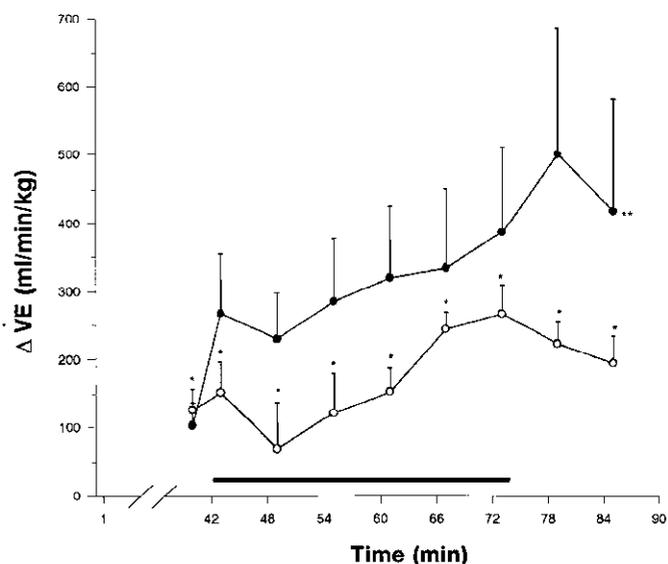


Fig. 3. Changes in ventilation ($\Delta\dot{V}_E$) in response to hyperosmolality during ion-exchange blockade. ●, DIDS; ○, amiloride. First symbols, responses to blocker alone; last 2 symbols, recovery period; thick solid line, sucrose infusion. Values are means \pm SE. *Significantly different from resting, $P < 0.05$. **All points significantly different from resting, $P < 0.05$.

min⁻¹ after 33 min of infusion (Fig. 3). Pa_{CO₂} did not change at any time, but Pa_{O₂} rose significantly, from 79 ± 2 to 91 ± 2 Torr after 21 min, and then remained stable (Fig. 4). Mean arterial blood pressure decreased to 212 ± 6 mmHg, whereas heart rate increased to 197 ± 14 beats/min within 15 min (both *P* < 0.05). Plasma [Cl⁻] decreased significantly, from 108 ± 2 to 98 ± 2 meq/kg, and plasma [Na⁺] fell from 125 ± 2 to 113 ± 4 meq/kg by the end of the infusion (Table 1). Plasma [K⁺] also significantly decreased during the infusion, from a resting level of 4.74 ± 0.22 to 3.96 ± 0.13 meq/kg. All variables except plasma [K⁺] remained stable and significantly different from the resting levels during the recovery period.

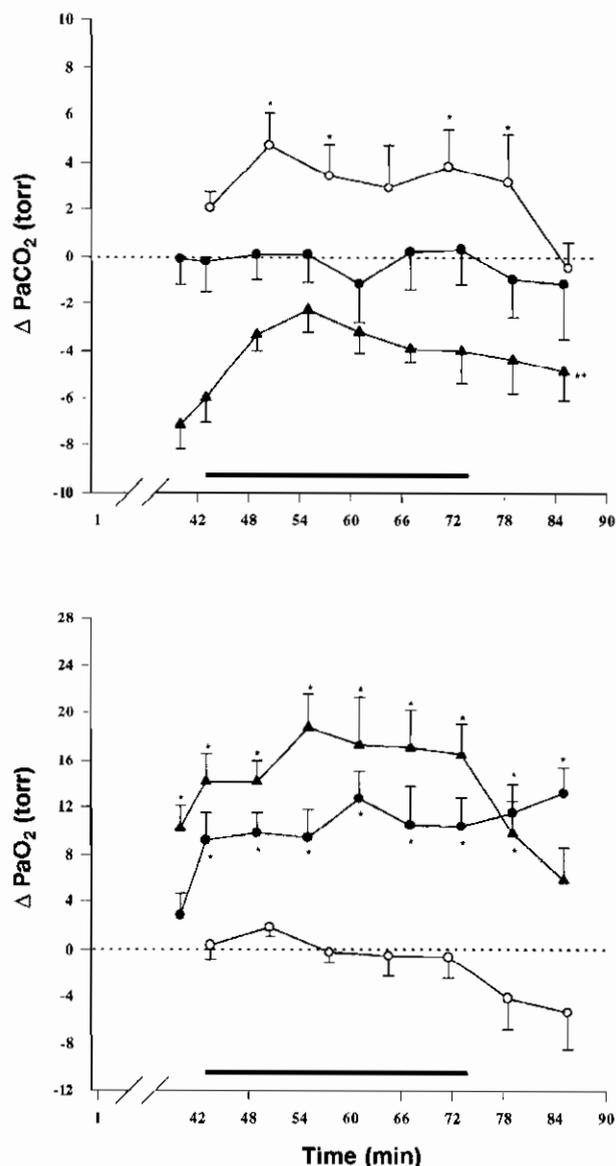


Fig. 4. Changes in blood gases [arterial PCO₂ (ΔPa_{CO₂}) and arterial PO₂ (ΔPa_{O₂})] in response to hyperosmolality during ion-exchange blockade. ○, Animals given a sucrose load only; data from Kasserra et al. (13) shown for comparison purposes; ●, DIDS; ▲, amiloride. First symbols, responses to blocker alone; last 2 symbols, recovery period; thick solid line, sucrose infusion. Values are means ± SE. *Significantly different from resting, *P* < 0.05. **All points significantly different from resting, *P* < 0.05.

Table 1. Decreases in electrolytes during acute hyperosmolality after ion-exchange blockade

	[Cl ⁻]	[Na ⁺]	[K ⁺]
Sucrose after amiloride	20.9 ± 2.9*	20.2 ± 12.2*	0.65 ± 0.32*
Sucrose after DIDS	9.9 ± 1.6*	11.7 ± 4.6*	0.64 ± 0.39*

Values are means ± SE in meq/kg from resting levels after 30 min of sucrose infusion. [Cl⁻], [Na⁺], and [K⁺], concentration of Cl⁻, Na⁺, and K⁺, respectively; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid. *Significantly different changes from rest. *P* < 0.05.

Amiloride. Infusion of amiloride significantly decreased pH_a (7.50 ± 0.02 to 7.48 ± 0.02 pH units) and pH_i (7.14 ± 0.02 to 7.05 ± 0.03 pH units) and increased \dot{V}_E (336 ± 19 to 462 ± 37 ml · kg⁻¹ · min⁻¹; Figs. 2 and 3). The initial resting levels of all three variables were not significantly different from those measured in the DIDS-treated animals. Pa_{CO₂} decreased 7.1 ± 1.1 Torr from a resting value of 28.5 ± 2.2 Torr, whereas Pa_{O₂} increased 10 ± 2 Torr from 87 ± 2 Torr (both *P* < 0.05; Fig. 4). Plasma [Cl⁻] increased from 113 ± 1 to 119 ± 3 meq/kg (*P* < 0.05), and although [Na⁺] also increased 6 ± 4 meq/kg, this change was not significant. There was no change in plasma [K⁺] (resting level 4.63 ± 0.21 meq/kg), blood pressure (205 ± 18 mmHg), or heart rate (121 ± 10 beats/min).

Sucrose infusion decreased pH_a further to 7.39 ± 0.03 pH units. Muscle pH_i decreased 0.16 ± 0.04 pH units below the resting level and remained low for 21 min of the sucrose infusion (*P* < 0.05) but then recovered to a value (0.09 ± 0.06 pH units below the resting level) not significantly different from the resting or amiloride-treated values (Fig. 2). \dot{V}_E increased during the sucrose infusion to 603 ± 53 ml · kg⁻¹ · min⁻¹ except for a transient return to resting levels 9 min into the infusion (Fig. 3). The increase in \dot{V}_E was primarily due to a 55% increase in tidal volume. Pa_{CO₂} increased from 21.4 ± 1.4 to 26.2 ± 2.1 Torr after 15 min of sucrose infusion and then stabilized but still remained significantly lower than the resting values (Fig. 4). Pa_{O₂} increased during the hyperosmotic infusion from 97 ± 2 to 106 ± 2 Torr (*P* < 0.05) in 15 min and then remained relatively stable. Plasma [Cl⁻] decreased 21 ± 3 meq/kg (*P* < 0.05), a significantly greater decrease than during DIDS treatment (Table 1). Plasma [Na⁺] fell a maximum of 24 ± 9 meq/kg (*P* < 0.05), and plasma [K⁺] also decreased significantly (by a maximum of 1.04 ± 0.08 meq/kg) during the sucrose infusion, but both ions recovered to levels not significantly different from the resting levels by the end of the infusion. The drop in [K⁺] was not significantly different from that during DIDS treatment. Mean arterial blood pressure decreased transiently by 24 ± 7 mmHg midway through the infusion, and heart rate increased slowly to reach 43 ± 15 beats/min over resting levels by the end of the infusion (both *P* < 0.05). \dot{V}_E , pH_a, Pa_{CO₂}, and plasma [Cl⁻] remained significantly different from the resting values at the end of the recovery period.

Lacticacidosis. Lactate infusion significantly decreased pH_a 0.08 ± 0.1 pH unit from resting levels, and the change in pH_a was not significantly different

from that caused by the hypertonic infusions. $\dot{V}E$ increased significantly (by $230 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; see Fig. 4), and this was reflected in an increase in Pa_{O_2} of $13.4 \pm 1.7 \text{ Torr}$ and a decrease in Pa_{CO_2} of $4.3 \pm 0.5 \text{ Torr}$ (all $P < 0.05$).

Total CO_2 . Total CO_2 decreased significantly during sucrose infusion, from a control normosmotic level of $24.5 \pm 0.1 \text{ mM}$ to $21.8 \pm 0.5 \text{ mM}$ after 30 min of infusion ($P < 0.025$).

DISCUSSION

Control of $\dot{V}E$. Both DIDS and amiloride prevented the intracellular alkalization of muscle and the suppression of ventilatory compensation caused by acute hyperosmolality. Instead, hyperosmotic infusion during ion-exchange blockade resulted in both an intra- and extracellular acidosis and a significant increase in $\dot{V}E$. Lactacidosis also resulted in both an intra- and extracellular acidosis and a significant increase in $\dot{V}E$ (13). In contrast, acute hyperosmolality without ion-exchange blockade generates an extracellular acidosis and an intracellular alkalosis and is associated with suppressed $\dot{V}E$ (12, 13). The relationship of pH_a to the ventilatory response is illustrated in Fig. 5. The graph shows that the ventilatory response during hyperosmolality is altered by ion-exchange blockade to a relationship similar to that seen during lactic acid infusion, whereas nonblocked hyperosmotic animals have a depressed ventilatory response. A major physiological difference between the animals in this study and animals that are only hyperosmotic is their pH_i (see Fig. 1). The former animals, in which $\dot{V}E$ increased, all showed an pH_i that was acidotic relative to the resting state. Animals that are solely hyperosmotic and do not exhibit a compensatory increase in $\dot{V}E$ have a pH_i that is alkalotic to the resting pH_i . Although such a correlative relationship is not proof of a causal association

between pH_i and $\dot{V}E$, it is suggestive. Therefore, the data suggest that both pH_a and pH_i may have a role in the control of $\dot{V}E$.

The major assumption made in this experiment was that any pH_i disturbance that occurred in the skeletal muscle would also be occurring in the carotid bodies. At present, accurate *in vivo* measurement of pH_i in carotid bodies would be technically very difficult. We considered this assumption to be reasonable because both tissues are well perfused (4) and would therefore be similarly exposed to any changes in tonicity and because carotid bodies, by the very nature of their function, are sensitive to blood chemistry changes. Additionally, the buffering capacity of muscle is very high, unlike that of carotid body tissue, which is very low (23), suggesting that any pH_i change measured in muscle might be even more pronounced in the carotid bodies. However, any extrapolation of the data from this study with respect to carotid bodies must remain speculative. A role of pH_i in the control of chemoreceptor function has been proposed before (2, 8, 15), but to date there is no direct evidence to either support or negate this hypothesis.

The ion-exchange blockers prevented the K^+ efflux that usually occurs during acute hyperosmolality (12, 17). Such a flux could change membrane potential in the direction of hyperpolarization. Two times the normal tonicity has been shown to cause an alkalization of $\sim 0.1 \text{ pH}$ unit (22) and a small hyperpolarization of 4–5 mV in isolated muscle (20, 22, 24). If a similar flux occurred in the carotid bodies, the opening of only a few K^+ channels could significantly affect membrane potential (16) because the cells are small and have a high membrane resistance (6). Because DIDS and amiloride prevented the K^+ efflux, any hyperpolarization would also have been prevented. This is a potential factor in the reversal of the normal ventilatory suppression during hyperosmolality by the ion-exchange blockers.

Amiloride-treated animals showed a significant drop in Pa_{CO_2} consistent with increased $\dot{V}E$, whereas Pa_{CO_2} did not decrease in DIDS-treated animals despite the larger increase in $\dot{V}E$. Pa_{CO_2} normally increases significantly during acute hyperosmolality by $\sim 4 \text{ Torr}$ (12). It is possible that the inhibition of $\text{Cl}^-/\text{HCO}_3^-$ exchange by DIDS would inhibit CO_2 hydration, leading to an increased Pa_{CO_2} .

Ion exchange. The altered electrolyte balance due to the ion-exchange blockers reflects the difficulty of interpreting the whole body effects of these drugs. DIDS was very effective in preventing intracellular alkalization due to sucrose infusion in this study. However, extracellular $[\text{Cl}^-]$ decreased less than has been previously reported during the same osmotic load (11), the opposite of what would be predicted. DIDS also affected Na^+ efflux because extracellular $[\text{Na}^+]$ was $\sim 8 \text{ meq}$ higher than predicted due to dilution (12). Amiloride had no measurable effect on changes in extracellular $[\text{Na}^+]$ due to sucrose infusion but caused an unusually large decrease in extracellular $[\text{Cl}^-]$. However, pH_i remained low in response to hyperosmotic stress after amiloride, suggesting a role for Na^+/H^+ exchange in the alkalization

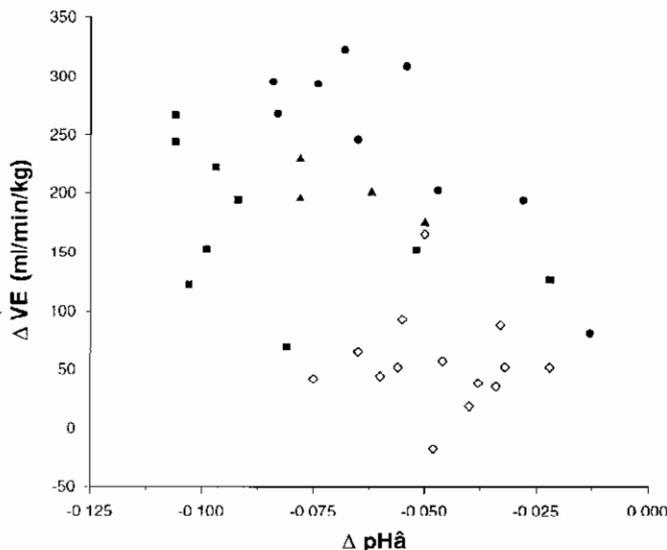


Fig. 5. Relationship between changes in ventilation ($\Delta\dot{V}E$) and changes in pH_a during various acid-base disturbances. ●, DIDS + hyperosmolality; ■, amiloride + hyperosmolality; ▲, lactacidosis; ◇, hyperosmolality alone [latter data from Kasserra et al. (12)].

tion process normally accompanying cell shrinkage (9). Even though both blockers inhibited contraction alkalosis, the ion-exchange mechanisms during hyperosmotic stress are extremely complicated and are difficult to predict in the whole animal or to compare with other studies because ion exchangers are both cell and species specific. It appears that Na^+ , Cl^- , and K^+ all play a prominent role in the hypertonic response, but the mechanisms are complicated and must be identified at an *in vitro* level. Red blood cells and myocytes undergo a net loss of Cl^- when exposed to a hypertonic medium (1), and in red blood cells, the loss was accompanied by an intracellular alkalization that was completely inhibited by 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (25). The significant decrease in total CO_2 *in vivo*, combined with the significant increase in Pa_{CO_2} that always accompanies acute hyperosmolality, indicates that the $[\text{HCO}_3^-]$ is decreasing during the osmotic challenge. This confirms that the acid-base disturbance associated with acute hyperosmolality is not simply a respiratory acidosis and again implies that $\text{Cl}^-/\text{HCO}_3^-$ exchange may play a primary role in this perturbation.

DIDS is a stilbene disulfonate that binds specifically and irreversibly to cell membrane band 3 protein to inhibit anion transport in a variety of cell types. If it is assumed that the distribution of DIDS is primarily extracellular and that the extracellular space of ducks is ~25% of the body weight (21), then the DIDS concentration in the extracellular fluid would have been ~1.2 mmol/l or 1,000 times the inhibition constant (concentration resulting in 50% inhibition of ion transport) for DIDS in red blood cells (1.2 $\mu\text{mol/l}$). This concentration is similar to that used in other systemic studies (5, 10). DIDS is irreversible and would have remained effective over the 85-min protocol.

The dosage of amiloride used is similar to that generally reported in the literature (3, 19). If the same assumptions about extracellular space and distribution described above are used, then the amiloride concentration in the extracellular fluid would have been ~0.15 mmol/l. Amiloride is an effective inhibitor of the Na^+/H^+ antiporter at a concentration of 0.1–1.0 mM when extracellular $[\text{Na}^+]$ is in the physiological range (7). The action of amiloride is reversible, but because pH_i decreased due to amiloride and did not recover during the experiment, this suggests that the amiloride was effectively present throughout the study.

Conclusions. The data suggest that pH_i plays a role in initiating ventilatory changes because normal respiratory compensation to the extracellular acidosis is prevented when the intracellular milieu is alkalotic but is initiated when the intracellular milieu is acidotic or homeostatic. The acid-base disturbance associated with acute hyperosmolality appears to offer a unique system to investigate chemoreceptor responses to changes in both intra- and extracellular pH .

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