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Hyperosmolality alters the ventilatory response to acute hypercapnia and hypoxia

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Abstract. Acute hyperosmolality in the Pekin duck results in an extracellular acidosis and hypercarbia without any stimulation of ventilation. The development of the extracellular acidosis is accompanied by the concurrent development of an intracellular alkalosis systemically which has been hypothesized to depress ventilation (Kasserra *et al.*, *J. Appl. Physiol.*, 1993). In order to investigate this apparent suppression of ventilation, the ventilatory response to various respiratory challenges (CO_2, O_2, K^+) was studied both before (normosmotic) and after (hyperosmotic) hypertonic sucrose infusion. Increased plasma osmolality caused a significant drop in arterial pH of 0.06 ± 0.01 units and a 4 Torr increase in Pa_{CO_2}, yet did not stimulate any significant increase in ventilation despite a significant increase in oxygen consumption. Acute hyperosmolality increased the Pa_{CO_2} associated with resting ventilation, and decreased the magnitude of the ventilatory response to a given increase in Pa_{CO_2}, compared with the response to the same ventilatory challenge in normosmotic animals. Acute hyperosmolality increased the ventilatory response to hypoxia and K^+ compared with normosmotic animals. The opposite effect of hyperosmolality on the ventilatory responses to hypercapnia compared with hypoxia suggests that the mechanisms of chemoreception for hypercapnia and hypoxia are different. The depressed ventilatory response curve to increased Pa_{CO_2} and decreased arterial pH during hyperosmolality, both alone and during the hypercapnic challenge, suggests that the peripheral chemoreceptor response to pH and CO_2 is suppressed. It is hypothesized that the suppression results from the intracellular alkalosis occurring during acute hyperosmolality.

Acid-base balance; Birds, duck; Chemoreceptors, suppression; Control of breathing; Hyperosmolality, intracellular; Osmotic pressure

It has been previously reported that acute, intravenous infusion of hypertonic solutions of essentially non-penetrating solutes results in a systemic extracellular acidosis concurrent with the increasing hyperosmolality. The mechanisms leading to this unusual acidosis are unknown. We have recently reported that this 'dilution acidosis' does not stimulate respiratory compensation and is, in fact, exacerbated by a respiratory acidosis due to apparent CO_2 retention (Kasserra *et al.*, 1991). Furthermore, the acidosis is characterized by a relative increase in extracellular Cl^- concentration ($[Cl^-]$). Nuclear magnetic resonance spectroscopy studies have shown that a marked intracellular alkalosis develops in skeletal muscle (a so-called contraction alkalosis which is essen-

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tially the antithesis of dilution acidosis), but there is no consistent intracellular effect in the brain (Kasserra *et al.*, 1993). Cserr *et al.* (1991) have also shown that the brain intracellular milieu is not disrupted by acute systemic hyperosmolality. The suppression of the normal ventilatory response to dilution acidosis and increased Pa_{CO_2} implies some degree of depression of chemoreceptor activity, at least in the periphery, which could be caused by intracellular alkalosis of the chemosensors. To test this, we examined the ventilatory response to several known respiratory stimuli (hypercapnia, hypoxia and K^+) before and during a hyperosmotic challenge. An acute ventilatory response to a hypercapnic challenge is mediated by both peripheral and central receptors, although primarily by central receptors. Since the brain intracellular compartment appears unaffected by systemic hyperosmolality, a suppression of the normal ventilatory response to hypercarbia would imply that ventilation is being depressed peripherally. Since the response to CO_2 is primarily mediated through changes in pH, such a suppression could therefore be due to the intracellular alkalosis caused by acute hyperosmolality. In contrast, hypoxic and K^+ stimulation are restricted to peripheral chemoreceptors and neither stimulus is believed to act through pH reception. If the intracellular alkalosis is suppressing normal respiratory compensation, the ventilatory response to hypoxia and K^+ should not be affected by hyperosmolality. Therefore, these experiments were performed to test the hypothesis that the intracellular acid-base perturbation caused by acute hyperosmolality would depress the normal ventilatory response to a respiratory stimulus that acted through acidotic pH reception (CO_2), while the response to stimuli not known to involve pH changes (O_2, K^+) would be unaffected.

Materials and methods

Eleven Pekin ducks (mean body weight 3.0 ± 0.2 kg were purchased from the Animal Care Facility at the University of British Columbia, and housed in individual cages at least 2 days before surgery. Each animal was chronically cannulated under local anaesthesia (approximately 0.5 ml xylocaine 2%; Astra, Ont.) with an exteriorized polyvinylchloride loop (Bolab VIII; Bolab, AZ) connecting the brachial artery to the ulnar vein. The cannula was inserted 4 cm into the artery and 5 cm into the vein. Long-term patency was ensured by prior treatment of the cannula with TD-MAC (Polysciences, PA). Each animal was given at least 48 h to recover.

Each duck was exposed to one or more known respiratory stimuli: hypoxia (10% O_2, 90% N_2), hypercapnia (3.5% CO_2 in air) or an i.v. infusion (approx. 0.5 ml) of a K^+ load equivalent to 50% of the calculated extracellular $[K^+]$. Seven ducks were exposed to both hypoxia and hypercapnia, one duck to hypoxia only, and three ducks were given two different K^+ loads. All ducks were given at least 5 days between trials. The protocol was identical for each stimulus. Each duck was lightly restrained with filament tape and then placed in a whole body plethysmograph, with the neck extending out of the main chamber through a double layered dental dam collar into a separate

head chamber (Fig. 1). The head chamber was ventilated with room air at 5 $L \cdot min^{-1}$. An opening at the top of the body plethysmograph was fitted with a Fleisch #0 pneumotachometer for measurement of ventilation ($\dot{V}E$), and the cannulae were led out of an air-tight hole. The body compartment was surrounded by a circulating cold water bath which has been used previously to maintain a normal duck body temperature of approximately 40 °C.

The animal was allowed 20 min to adjust to its surroundings, and then ventilation was recorded for 1 min every 10 min until it was stable (usually 4 recordings). Three samples of 0.7 ml of arterial blood were taken, with 0.4 ml analyzed for blood gases and pH on a blood/gas analyzer maintained at 40 °C (IL813; Instrumentation Laboratories, MA), while 0.3 ml were centrifuged and the plasma decanted and immediately frozen. The inflowing air and the end tidal gases were monitored via polyethylene cannulae (PE-60) connected to a MGA 200 clinical mass spectrometer (Centronic, UK) and sampled concurrently with $\dot{V}E$. Arterial blood pressure was measured simultaneously with an Elcomatic 715A blood pressure transducer (Harvard Instruments, MA) via the arterial side of the cannula and heart rate was derived from the blood pressure trace. All variables were recorded on a Harvard Universal oscillograph. Immediately after the resting period, the animal was exposed to either the hypercapnic or the hypoxic gas mixture for 2 min by turning a stopcock connected to the head chamber, which allowed the premixed gas to flow through the chamber at 5 $L \cdot min^{-1}$. Three of the animals were maintained on room air but infused with a K^+ bolus (approx. volume 0.5 ml) over 30 sec. Ventilation, blood pressure, heart rate, end tidal gases and the inspired gases were continuously monitored. A blood sample was collected at 2 min. The animal was allowed 15 min to recover while breathing room air, with all variables being sampled at 10 and 15 min into recovery to confirm a return to resting, normosmotic levels. Each animal was then infused intravenously with 26.5 $mmol \cdot kg^{-1}$ body wt. sucrose (approx. 25 ml) over 40 min. All variables were monitored every 7.5 min until 22.5 min, at which time the respiratory challenges were repeated.A final recording was made 15 min after the end of this second respiratory challenge.

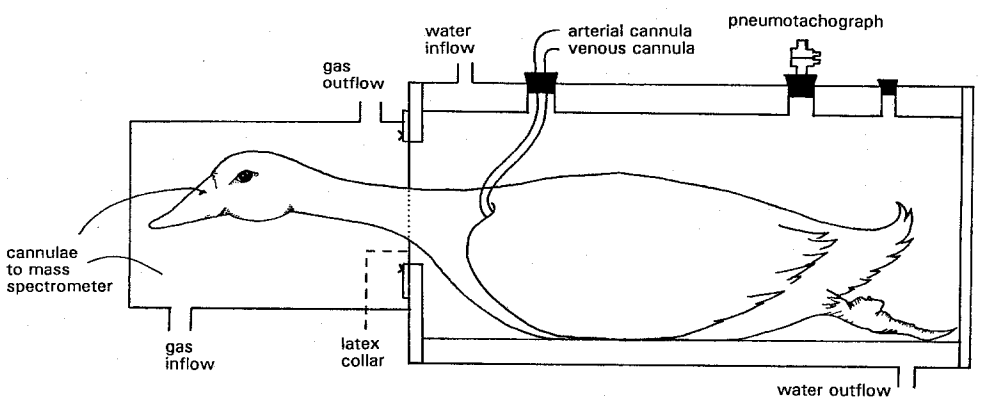

Fig. 1. Schematic of the experimental apparatus.

The 3 ducks were infused with K^+ once as a bolus, and once with the same K^+ load mixed in with the sucrose to assess the effect of rate of extracellular K^+ increase during dilution acidosis. These same animals were also given a bolus of 150 mM NaCl as an experimental control. These trials were given in random order approximately 5 days apart. The K^+ load was calculated to be 50% of extracellular K^+ assuming an extracellular space of 25% body weight and a mean plasma $[K^+]$ of 2.5 meq·kg^{-1} (Kasserra *et al.*, 1991). This load was empirically decided upon in preliminary experiments because it resulted in a clear, brief ventilatory increase in normosmotic animals without causing excessive stress (as measured by changes in cardiovascular variables). Changes in total extracellular K^+ caused by the sucrose infusion were taken into account by assuming an increase in extracellular fluid volume of 10% and a constant plasma $[K^+]$ of 2.5 meq·kg^{-1} (Kasserra *et al.*, 1991).

The mass spectrometer was calibrated with pure N_2 gas and a precision analyzed mixture of 14.00% O_2, 8.19% CO_2, 8.24% argon, with the balance N_2. Hypercapnic or hypoxic gas mixtures were mixed using a flow meter and the level of CO_2 or O_2 checked by the mass spectrometer. Ventilation was calibrated with known volumes of air delivered by a hand-driven syringe into the sealed, empty plethysmograph, and the airflow signal was integrated to yield tidal volume. $\dot{V}E$ was calculated as the product of tidal volume and respiratory frequency. The pressure transducer was calibrated using a mercury manometer. Plasma osmolality was measured with a vapor pressure osmometer (5500; Wescor, UT), plasma $[Na^+]$ and $[K^+]$ by flame photometry (IL943; Instrumentation Laboratories, MA) and plasma $[Cl^-]$ with a Buchler digital chloridometer (4-2500; Buchler Instruments, NJ). Plasma lactate was measured using the method of Noll (1974).

All results were analyzed by random block two-way ANOVA at a significance level of $P \leq 0.05$ using the statistical package Systat (Systat, Evanston, IL). With some variables, high inter-animal variability necessitated re-analysis by paired-comparison two-way ANOVA. Since the recovery measurements at 10 and 15 min after a respiratory challenge were not significantly different for any measured variable, the two values were averaged for statistical analysis. Results are reported as mean ± SE, or mean difference ± SE. Significant differences are from resting, normosmotic levels unless otherwise stated. Significant differences from hyperosmotic values always refer to the measured value at 22.5 min into sucrose infusion. O_2 consumption was calculated as $\dot{V}_{O_2} = \dot{V}E \cdot [1 - (FET_{O_2} + FET_{CO_2})/1 - (FI_{O_2} + FI_{CO_2})] \cdot FI_{O_2} - \dot{V}E \cdot FET_{O_2}$, where FET and FI represent the fraction of end tidal expired gas and the fraction of inspired gas, respectively.

Results

Hypercapnia. Inhalation of 3.5% CO_2 decreased arterial pH (pHa) from 7.48 ± 0.01 to 7.44 ± 0.01 in 2 min ($P < 0.01$). $\dot{V}E$ increased from 321 ± 36 to 458 ± 34 ml·(min·kg)$^{-1}$ in 1 min and to 656 ± 61 ml·(min·kg)$^{-1}$ in 2 min ($P < 0.001$) (Fig. 2). The increase in

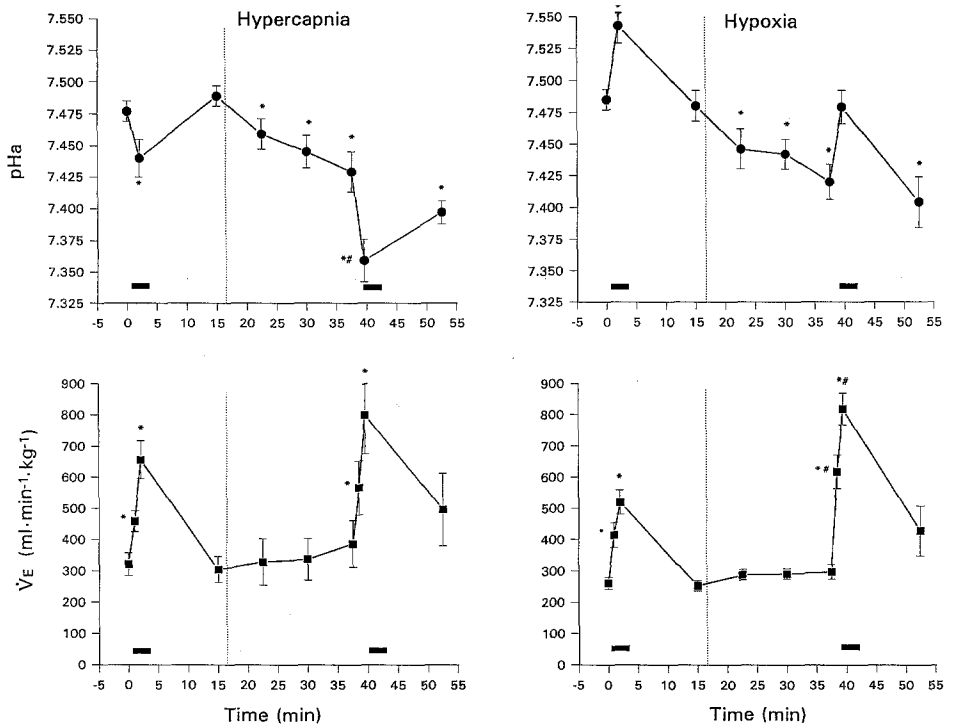

Fig. 2. pHa and ventilatory changes in response to a hypercapnic or hypoxic challenge before and during sucrose infusion. Black bars represent the period of hypoxic or hypercapnic challenge. The dotted line indicates the beginning of the sucrose infusion. * Significantly different from normosmotic value at time 0. # Significant difference between 1st and 2nd hypercapnic or hypoxic period.

$\dot{V}_E$ was due to an increase in tidal volume as respiratory frequency did not change (Fig. 3). Pa_{CO_2} increased from 31.5 ± 1.2 to 38.1 ± 1.2 Torr, while Pa_{O_2} increased from 94.7 ± 1.9 to 104.7 ± 1.6 Torr in 2 min ($P < 0.001$) (Fig. 4). While $F_{ET_{CO_2}}$ did not vary, $F_{ET_{O_2}}$ increased significantly from $15.3 \pm 0.2\%$ to $17.3 \pm 0.2\%$ (Fig. 5). Both $F_{ET_{CO_2}}$ and $F_{ET_{O_2}}$ closely reflected changes in blood gases throughout the experiment. $\dot{V}_{O_2}$ rose from 19.0 ± 2.5 to 27.8 ± 4.5 ml $O_2 \cdot (kg \cdot min)^{-1}$ ($P < 0.025$) (Fig. 6). None of these variables were significantly different from rest after 15 min after recovery. Heart rate, plasma ions, and plasma lactate were not significantly affected by the period of hypercapnia. Mean arterial blood pressure and plasma [K^+] did not vary at any time during the experiment.

Sucrose infusion decreased pHa 0.06 ± 0.01 units ($P < 0.01$), while Pa_{CO_2} increased 3.9 ± 0.8 Torr ($P < 0.001$) and $\dot{V}_E$ and Pa_{O_2} did not significantly change over the infusion period (Figs. 2 and 4). $\dot{V}_{O_2}$ increased to 25.2 ± 4.9 ml $O_2 \cdot (kg \cdot min)^{-1}$ ($P < 0.025$) (Fig. 6). $F_{ET_{CO_2}}$ rose slightly, while $F_{ET_{O_2}}$ decreased slightly during the infusion (both $P < 0.05$) (Fig. 5). Heart rate increased significantly from 129 ± 8 to 196 ± 19 beats min^{-1}. After 22.5 min of infusion, plasma osmolality increased from 284 ± 5 to

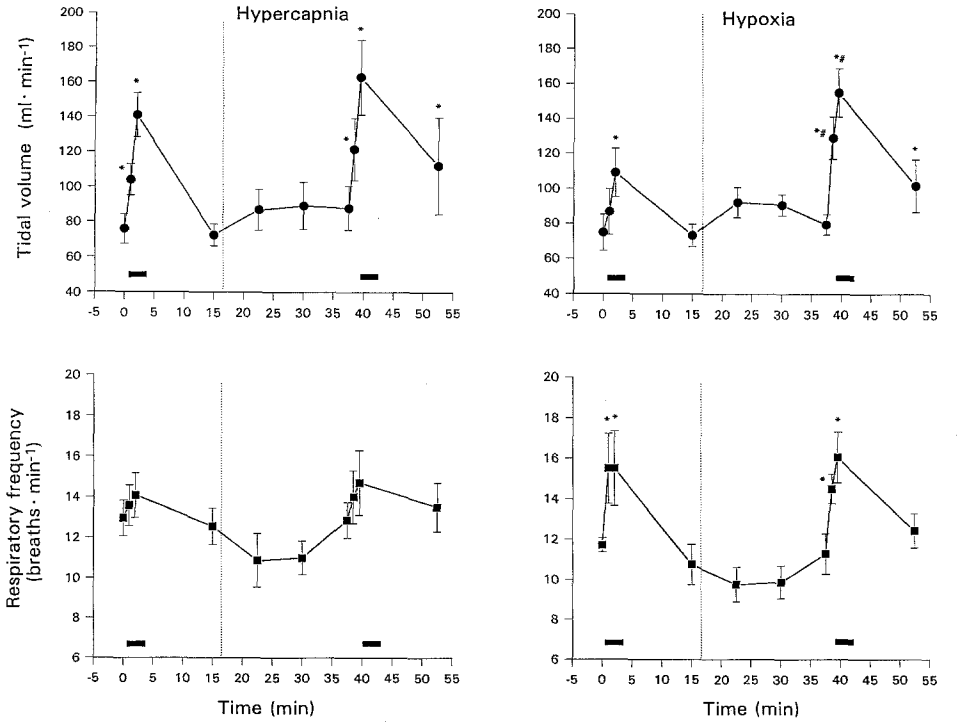

Fig. 3. Challenge in tidal volume and respiratory frequency in response to hypercapnia, hypoxia and hyperosmolality. Black bars represent the period of hypoxia or hypercapnia. The dotted line indicates the beginning of the sucrose infusion. * Significantly different from normosmotic value at time 0. # Significant difference between 1st and 2nd hypercapnic or hypoxic period.

319 ± 6 mosm·kg^{-1}, plasma [Na^+] decreased 19 ± 1 from a normosmotic value of 144 ± 2 meq·kg^{-1}, and [Cl^-] decreased 12 ± 1 from a normosmotic level of 108 ± 5 meq·kg^{-1} (all $P < 0.05$).

Hypercapnia during the sucrose infusion further decreased pHa from 7.42 ± 0.01 (the value after 22.5 min of sucrose infusion) to 7.36 ± 0.02 in 2 min ($P < 0.01$), while $\dot{V}E$ increased from 387 ± 75 to 566 ± 87 ml·(kg·min)$^{-1}$ in 1 min and to 799 ± 124 in 2 min ($P < 0.01$) (Fig. 2). The ventilatory responses to CO_2 in normosmotic and hyperosmotic ducks were not significantly different. However, there was an increase in the Pa_{CO_2} during hyperosmolality which was associated with the same $\dot{V}E$ as in normosmotic animals, and there was a corresponding decrease in the magnitude of the ventilatory response to any given increase in Pa_{CO_2} in hyperosmotic ducks. This is shown by the right-shifted curve of hyperosmotic animals (Fig. 7). There was, however, no change in sensitivity to Pa_{CO_2} as measured by the slopes of the lines (Fig. 7). Pa_{CO_2} increased from 35.5 ± 1.2 to 47.4 ± 2.5 Torr in 2 min ($P < 0.001$), a significantly greater change than the change in Pa_{CO_2} in the normosmotic hypercapnic animal. Pa_{O_2} increased from 94.3 ± 2.5 to 101.7 ± 1.7 ($P < 0.025$) (Fig. 4). FET_{CO_2} did not change with respect to the

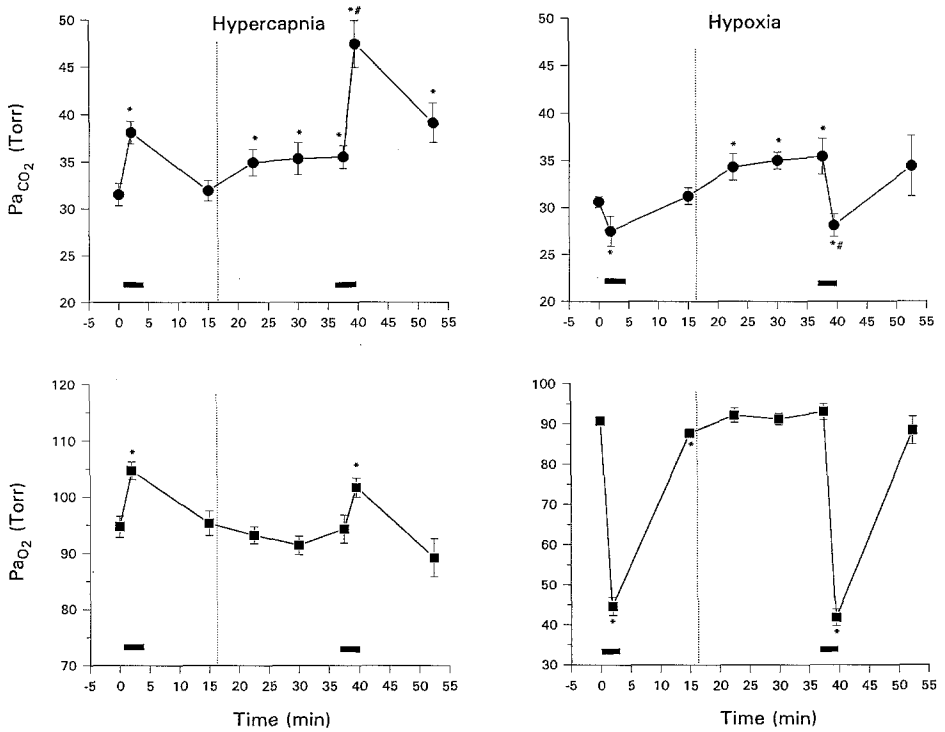

Fig. 4. Changes in blood gases in response to hypercapnia, hypoxia and sucrose infusion. Black bars represent the period of hypoxia or hypercapnia. The dotted line indicates the beginning of the sucrose infusion. * Significantly different from normosmotic value at time 0. # Significant difference between 1st and 2nd hypercapnic or hypoxic period.

FET_{CO_2} during the sucrose infusion, but was significantly increased over normosmotic FET_{CO_2} and FET_{O_2} increased significantly from 14.7 ± 0.3 to $16.6 \pm 0.3\%$ (Fig. 5). $\dot{V}_{O_2}$ was significantly greater then normosmotic levels but did not increase significantly from the hyperosmotic level (Fig. 6). Heart rate and plasma electrolytes did not change significantly over the 2 min test.

The pHa remained low (7.39 ± 0.01) 10 min post hypercapnia (Fig. 2), while $\dot{V}E$, Pa_{O_2} and FET_{O_2} returned to values not significantly different from normosmotic or hyperosmotic values. Pa_{CO_2}, FET_{CO_2}, and $\dot{V}_{O_2}$ remained significantly higher than normosmotic levels but not hyperosmotic levels (Figs. 4–6). Heart rate remained significantly elevated over normosmotic levels by 94 ± 30 beats·min^{-1} after 10 min recovery from hypercapnia. Plasma osmolality was further increased to 338 ± 6 mosm·kg^{-1}, a total increase of 54 mosm·kg^{-1} from normal osmolality. Plasma [Na^+] decreased to 122 ± 1 meq·kg^{-1}, a total of 21 ± 1 meq·kg^{-1} lower than the normosmotic level, and [Cl^-] decreased to 95 ± 4 meq·kg^{-1}, a total of 13 ± 2 meq·kg^{-1} below the normosmotic level (all $P < 0.05$). Plasma lactate, which did not increase significantly during the sucrose infusion (1.7 ± 0.4 to 3.0 ± 0.4 mmol·kg^{-1}), was significantly elevated 10 min post hypercapnia (3.9 ± 0.4 mmol·kg^{-1}).

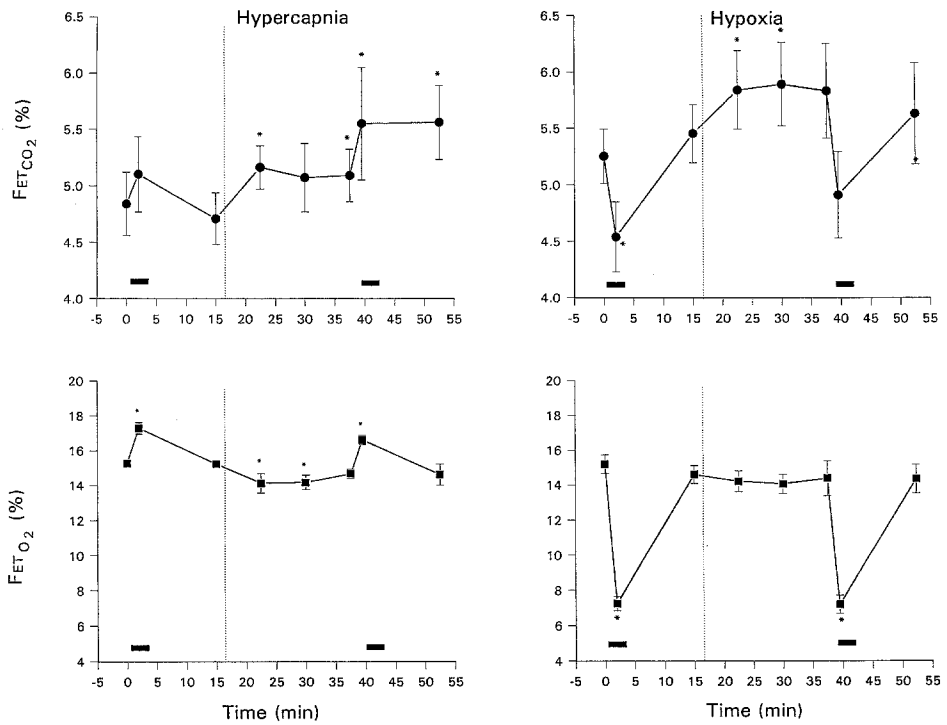

Fig. 5. Changes in $F_{ET_{CO_2}}$ and $F_{ET_{O_2}}$ in response to hypercapnia, hypoxia and hyperosmolality. Black bars represent the period of hypoxia or hypercapnia. The dotted line indicates the beginning of the sucrose infusion. * Significantly different from normosmotic value at time 0. # Significant difference between 1st and 2nd hypercapnic or hypoxic period.

Hypoxia. Arterial pH increased from 7.48 ± 0.01 to 7.54 ± 0.01 units ($P < 0.001$) after 2 min of breathing 10% O_2, but returned to resting, normosmotic levels after 15 min of recovery (Fig. 2). $\dot{V}E$ increased significantly from 261 ± 19 ml·(min·kg)$^{-1}$ to 414 ± 40 ml·(min·kg)$^{-1}$ in 1 min and to 519 ± 39 ml·(min·kg)$^{-1}$ in 2 min, and returned to normosmotic levels within 15 min (Fig. 2). The increase in $\dot{V}E$ was primarily due to a 33% increase in respiratory frequency during the first minute, which then remained stable, so that in the second minute, the increase in $\dot{V}E$ was mostly due to a 46% increase in tidal volume (Fig. 3). Pa_{CO_2} decreased from 30.6 ± 0.6 to 27.5 ± 1.6 Torr ($P < 0.05$), and Pa_{O_2} decreased from 90.7 ± 0.9 to 44.6 ± 2.3 Torr ($P < 0.001$) after 2 min of hypoxia (Fig. 4). $F_{ET_{CO_2}}$ declined from 5.3 ± 0.2 to 4.5 ± 3.1% ($P < 0.001$) after 2 min, while $F_{ET_{O_2}}$ decreased from 15.2 ± 0.5 to 7.2 ± 0.4% ($P < 0.001$) (Fig. 5). Heart rate increased by 16 ± 7 beats·min^{-1} from a normosmotic level of 124 ± 11 beats·min^{-1} ($P < 0.05$), but $\dot{V}_{O_2}$ plasma [ions], and plasma [lactate] were not affected by hypoxia. All variables returned to normosmotic levels within 15 min except Pa_{O_2}, which was depressed by 3.0 ± 1.1 Torr. Mean arterial blood pressure was not affected at any time during the experiment.

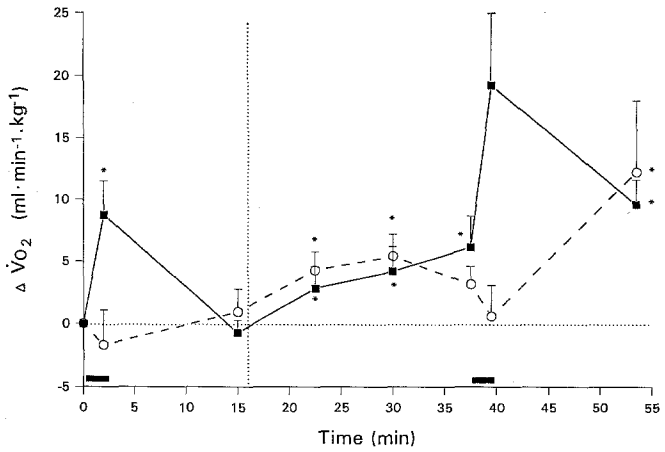

Fig. 6. Changes in $\dot{V}_{O_2}$ associated with hypercapnia, hypoxia and hyperosmolality plotted as difference from normosmotic $\dot{V}_{O_2}$. The dotted line indicates the beginning of the sucrose infusion. * Significantly different from normosmotic value at time 0. ■, hypercapnia; o, hypoxia.

Sucrose infusion caused a 0.06 ± 0.01 unit decrease in pHa ($P < 0.01$), with no significant increase in $\dot{V}E$ (Fig. 2) or Pa_{O_2}. Pa_{CO_2} increased from 31.2 ± 0.9 to 35.4 ± 1.9 Torr ($P < 0.001$) (Fig. 4). FET_{CO_2} rose slightly ($P < 0.05$) while FET_{O_2} decreased ($P < 0.025$) (Fig. 5). $\dot{V}_{O_2}$ significantly from a normosmotic level of 16.1 ± 1.2 ml $O_2 \cdot (kg \cdot min)^{-1}$ to a maximum of 20.6 ± 1.9 ml $O_2 \cdot (kg \cdot min)^{-1}$) (Fig. 6). Heart rate rose from 123 ± 11 to 169 ± 21 beats$\cdot$min^{-1} ($P < 0.05$). Plasma osmolality increased by 31 ± 3 mosm$\cdot$kg^{-1} after 22.5 min of infusion from a normosmotic level of 287 ± 3 mosm$\cdot$kg^{-1} ($P < 0.05$), [Na^+] decreased 18 ± 1 meq$\cdot$kg^{-1} from a value of 144 ± 1 meq$\cdot$kg^{-1} and [Cl^-] decreased 10 ± 1 meq$\cdot$kg^{-1} from a concentration of 106 ± 1 meq$\cdot$kg^{-1} in normosmotic animals ($P < 0.05$). Plasma [K^+] and [lactate] remained constant throughout the experiment.

In the second hypoxic period, pHa significantly increased back to normosmotic levels. This pH change was identical to that in the first hypoxic episode. The increase in $\dot{V}E$, through, was double that of the increase during the normosmotic hypoxic bout (Fig. 2). This is reflected in a 7.3 ± 1.4 Torr decrease in Pa_{CO_2} from the hyperosmotic level ($P < 0.001$), a significantly larger decrease compared to the decrease during the normosmotic hypoxia. However, the fall in Pa_{O_2} to 41.8 ± 2.1 Torr was not significantly different from that in normosmotic ducks during hypoxia (Fig. 4). Thus, there was an increase in the magnitude of the ventilatory response relative to the decrease in Pa_{O_2}, shown by the upward-shifted point representing hyperosmotic, hypoxic animals (Fig. 7). The increase in $\dot{V}E$ was due to a 63% increase in tidal volume in the first minute, which rose to 95% in the second minute of hypoxia, a significantly larger response than during normosmotic hypoxia. Respiratory frequency rose 28% in the first minute and 42% in the second minute, a response not significantly different from the hypoxic response in normosmotic ducks (Fig. 3). While FET_{O_2} decreased to exactly the same level as in the first hypoxic bout ($P < 0.001$), FET_{CO_2} and $\dot{V}_{O_2}$ were not affected (Figs. 5

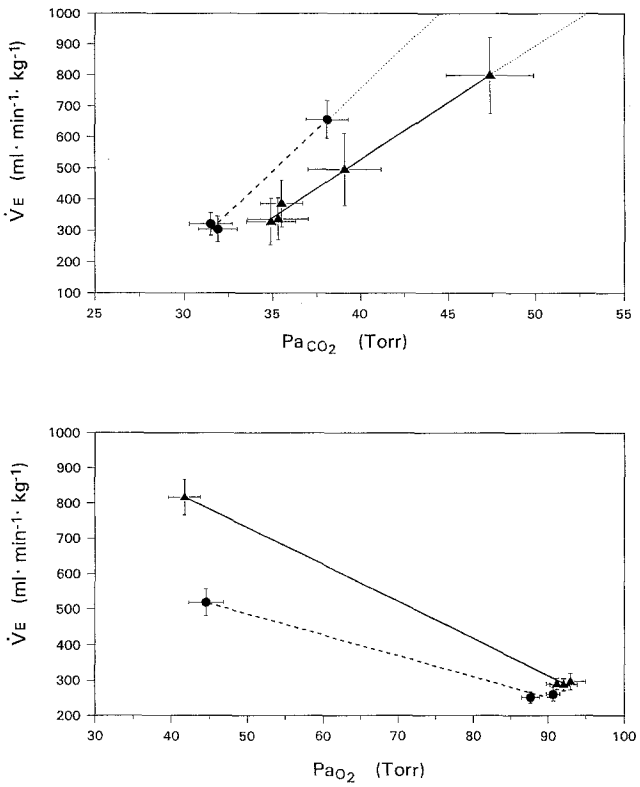

Fig. 7. CO_2 and O_2 ventilatory response curves. Dashed line represents the ventilatory response to the gases in normosmotic ducks (regression $Y = -1377.9 + 53.4X$), and the solid line represents the ventilatory response to the gases in hyperosmotic ducks (regression $Y = -950.5 + 36.9X$).

and 6). Heart rate increased significantly from 122 ± 9 to 215 ± 22 beats·min^{-1} after 2 min of hypoxia, and plasma lactate increased from 2.3 ± 0.3 mmol·kg^{-1} to 3.4 ± 0.4 mmol·kg^{-1} ($P < 0.05$).

At 10 min post hypoxia, $\dot{V}_E$, Pa_{CO_2}, Pa_{O_2}, FET_{CO_2}, FET_{O_2} and $\dot{V}_{O_2}$ had returned to levels not significantly different from either normosmotic or hyperosmotic levels, while heart rate and plasma lactate levels remained significantly higher than normosmotic but not the hyperosmotic values. pHa had further declined to 7.40 ± 0.02 ($P < 0.01$) (Fig. 2), osmotic pressure had increased by 55 ± 4 mosm·kg^{-1}, final plasma [Na^+] had decreased by 21 ± 1, and plasma [Cl^-] by 13 ± 1 meq·kg^{-1} (all $P < 0.01$).

Potassium. A K^+ bolus in normosmotic animals increased tidal volume significantly for 3 breaths, at which point it returned to a volume not significantly different from rest. The K^+ bolus after 22.5 min of sucrose infusion increased tidal volume for more than 15 breaths ($P < 0.01$) and did not return to hyperosmotic levels (which were not significantly different from rest) for over 2 min (Fig. 8). The K^+ bolus did not significantly affect inter-breath interval, heart rate or mean arterial blood pressure, while neither K^+

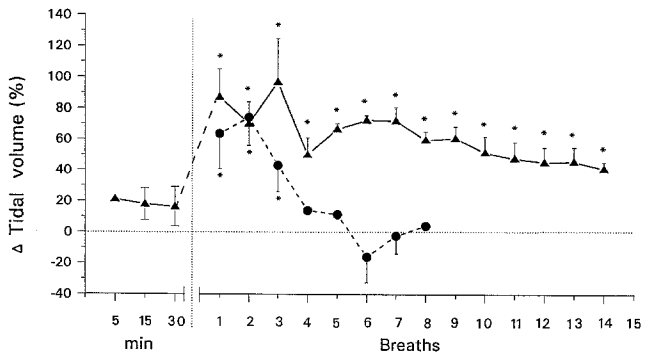

Fig. 8. The respiratory response to bolus K^+ infusion. The percent change in tidal volume from a resting level is shown breath-by-breath immediately post K^+ infusion in normosmotic ducks (dotted line). In hyperosmotic ducks, the percent change in tidal volume from a resting, normosmotic level is shown on the left of the figure during 30 min of sucrose infusion, and then the ventilatory response of hyperosmotic animals to a K^+ bolus is shown on a breath-by-breath basis. * Significantly different from normosmotic value at time 0.

added to the sucrose infusion nor the bolus of 150 mM NaCl had any significant effects on respiratory or cardiovascular variables at any time.

Discussion

As previous studies have shown, the extracellular acidosis induced by hyperosmolality did not stimulate an increase in ventilation (Kasserra *et al.*, 1991, 1993). This phenomenon has led to the hypothesis that the intracellular contraction alkalosis that develops systemically concurrent to the dilution acidosis suppresses the normal chemoreceptor response to the extracellular acidosis, thus resulting in no ventilatory change. A decrease in pHa of similar magnitude and time course is sufficient to double ventilation when the acidosis is caused by lactic acid infusion, which also decreases intracellular pH (Kasserra *et al.*, 1993). While it has not been fully resolved how systemic changes in osmolality affect the various fluid compartments of the brain (cerebrospinal, interstitial, and intracellular fluids), the available data suggest that there are minimal effects on the brain intracellular compartment (Cserr *et al.*, 1991; Kasserra *et al.*, 1993). This suggests that the primary effects of systemic hyperosmolality on ventilation take place via the peripheral chemoreceptors. The interpretation of the ventilatory data in this study must take into account that the response to hypercapnia is both peripherally and centrally mediated, while the response to hypoxia and K^+ is essentially solely under peripheral control.

A potential role for pHi in chemoreception: the hypercapnic response. The CO_2 response curve in Pekin ducks is linear over the range of interest in this study (30–50 Torr) (Dodd and Milsom, 1987; Bouverot *et al.*, 1974), so a 1st-order regression line was fitted to

the data in Fig. 7. The absolute $\dot{V}E$ values in the present study are somewhat lower than in the above studies, but the slopes of the CO_2 response curves in normosmotic ducks are similar.

Acute metabolic acidosis usually causes a displacement of the CO_2 response curve to significantly higher ventilatory levels (Saito *et al.*, 1960; Schuitmaker *et al.*, 1986). This does not occur during dilution acidosis. Hyperosmolality clearly caused a shift of the CO_2 ventilatory response curve to the right, implying an increase in the ventilatory threshold to CO_2 (ventilatory threshold being the level of Pa_{CO_2} at which there is a significant increase in $\dot{V}E$ from rest), and also slightly decreased the sensitivity to CO_2 (Fig. 7). Such a displacement of the CO_2 curve explains why there is no ventilatory response to the approximately 4 Torr increase in Pa_{CO_2} generated during the hyperosmotic infusion itself.

Compared to normosmotic hypercapnic animals, depressed chemoreceptor discharge has been observed in intrapulmonary chemoreceptors in ducks during saline or mannitol-induced hyperosmolality at all levels of Pa_{CO_2}, with the effect increasing as the level of inspired CO_2 increased (Adamson, 1984). While these changes in intrapulmonary chemoreceptor discharge would have little effect on $\dot{V}E$, being primarily involved in determining respiratory pattern (Milsom *et al.*, 1981), this effect could also be occurring in the carotid bodies, an interpretation which would support the ventilatory changes observed in this study. The 3.5% inspired CO_2 in this study would inhibit intrapulmonary chemoreceptor discharge only about 25% (Fedde *et al.*, 1974), therefore the $\dot{V}E$ changes seen are probably not due simply to an inability to maintain an adequate respiratory pattern. Depressed ventilatory responses to inspired CO_2 have also been observed in ducks after injection with acetazolamide (Powell *et al.*, 1978), although it is not clear how hyperosmolality could affect carbonic anhydrase.

Since acute hyperosmolality has not been shown to have any consistent, significant effect on the brain intracellular compartment (Cserr *et al.*, 1991; Kasserra *et al.*, 1993), the 2 min hypercapnic challenge administered in this study should have stimulated central chemoreceptors (Jones and Purves, 1970) to the same magnitude both before and during hyperosmolality. However, the ventilatory responses during hyperosmolality are occurring at significantly higher levels of Pa_{CO_2}. While much of the ventilatory response to CO_2 is centrally mediated (60–80%), it has been suggested that the arterial chemoreflex drive is essential for normal ventilatory responses to CO_2 since carotid body denervation shifts the ventilatory response curve to the right and decreases the sensitivity to CO_2 (Bouverot *et al.*, 1974; Lahiri *et al.*, 1978). The results of this study support that conclusion if indeed there is suppression or even significant depression of the peripheral drive during hyperosmolality. Actual depression of the peripheral chemoreflex drive would be hypothesized to occur if the central chemoreceptor threshold for CO_2 is lower than that of the peripheral chemoreceptors (Lahiri *et al.*, 1978). Suppression of any increase in, or even depression of, peripheral chemoreceptor discharge may be due to the systemic intracellular alkalosis that develops during acute hyperosmolality (Kasserra *et al.*, 1993). This would imply that both intra- and extracellular pH have a role in controlling ventilation, a concept that has been previously

suggested (Hanson *et al.*, 1981; Lassen, 1990) but for which there has never been any direct evidence. The opposite intra- and extracellular pH changes during hyperosmolality seem to offer a unique system to further study the contributions of pHi to chemoreception and ventilatory control.

Differences in chemoreceptive mechanisms: the hypoxic response. There was no apparent change in sensitivity to hypoxia after sucrose infusion, but the O_2 response curve appeared to be shifted upward compared with the response in normosmotic ducks (Fig. 7). However, the O_2 response curve for ducks is approximately exponential (Jones and Holeton, 1972; Bouverot and Sébert, 1979), rather than a straight line as shown in Fig. 7. Since the data generated in this experiment were insufficient to draw a curve, there is a possibility that the points between 40–45 Torr are merely on the steep region of the curve and that there is no upward shift. This is unlikely for the following four reasons. First, the O_2 response curve in ducks has never been shown to be so steep that, on average, a 2.8 ± 6.1 Torr difference in Pa_{O_2} would account for $\dot{V}E$ doubling (see Jones and Purves, 1970; Jones and Holeton, 1972). In addition, the control and the hyperosmotic Pa_{O_2} values after 2 min of hypoxia are not significantly different. Second, Jones and Holeton (1972) noted that the increase in $\dot{V}E$ in normosmotic animals terminated around a Pa_{O_2} of 47 Torr, at which point both in their study and in the current study (where Pa_{O_2} was actually 44.6 ± 2.3 Torr), $\dot{V}E$ was approximately double resting $\dot{V}E$. The same hypoxic challenge in hyperosmotic animals led to a similar drop in Pa_{O_2} (41.8 ± 2.1 Torr) but resulted in almost a tripling of $\dot{V}E$ in this study. In contrast, a further decrease in Pa_{O_2} in Jones and Holeton's ducks to 38 Torr actually decreased $\dot{V}E$. It seems clear that the response to hypoxia was significantly affected by hyperosmolality. This conclusion is further supported by the fact that carotid body discharge increases in hyperosmotic cats during hypoxia (Lahiri, 1977). Finally, the response to K^+ confirms the hypoxic data, particularly since K^+ is a stimulant which may be associated with the hypoxic response (López-López *et al.*, 1989) and, like hypoxia, is probably not dependent upon a pH change. The response to the K^+ bolus demonstrated that hyperosmolality significantly increased the ventilatory response to these stimuli (Fig. 8). It is not surprising that there was no ventilatory response to the slow K^+ infusion, since there was no measurable increase in plasma $[K^+]$ (the excess ion in the blood is quickly absorbed by muscle and bone), and a fast rate of arterial $[K^+]$ increase is crucial to initiate a ventilatory response. Increased ventilatory response to hypoxia with increased $[H^+]$ has long been established both *in vivo* (Natsui, 1970; Gabel and Weiskopf, 1975), and in carotid bodies *in vitro* (Biscoe and Duchen, 1990), while chemoreceptor discharge in response to hypoxia is abolished by alkalosis (Eyzaguirre and Koyano, 1965). It is unclear, however, whether intra- or extracellular pH is critical since intracellular pH is rarely measured. Since it known that there is an intracellular alkalosis during dilution acidosis (Kasserra *et al.*, 1993), the present results suggest that there is some interaction between the extracellular pH and hypoxia at the level of the carotid bodies. Alternatively, the transduction mechanisms in hypoxia may be affected by some other cellular perturbation caused by acute hyperosmolality.

As chemoreceptive mechanisms have not yet been elucidated, it is very difficult to speculate on the nature of any interaction.

The shifts in the two gas response curves (hypercapnic and hypoxic) in opposite directions suggests that the mechanisms of chemoreception of CO_2 versus O_2 are different, a concept which is generally supported by the literature but which has not yet been conclusively determined. Furthermore, stimulation of the chemoreceptors by K^+ and low O_2 appears to have some basic mechanism in common. This is interesting in light of the potential role of K^+ in the hypoxic stimulation of carotid bodies (López-López *et al.*, 1989).

Metabolism. There was a small but significant increase in metabolic rate during hyperosmolality in both experiments. Whole animal metabolic rate is known to increase during extracellular alkalosis and decrease during extracellular acidosis. It has been demonstrated that changes in $\dot{V}_{O_2}$ are dependent upon the extracellular pH and are inversely related to Pa_{CO_2} (see Patterson and Sullivan, 1978). However, Patterson and Sullivan (1978) point out that an intracellular site of action is likely and has, in fact, not been disproved. In the current study, the opposing changes in pH during hyperosmolality and the rise in $\dot{V}_{O_2}$ clearly support an intracellular pH influence. Alkalosis stimulates glycolysis, primarily through effects on the regulatory enzyme phosphofructokinase (Fidelman *et al.*, 1982), or the increased metabolic rate could reflect an increase in ion transport. The elevated metabolic rate may also have contributed to the increase in Pa_{CO_2} measured during the hyperosmotic challenge, especially since there was no modulating influence of increased ventilation.

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