DILUTION ACIDOSIS: THE EFFECTS OF HYPEROSMOLALITY ON ACID-BASE BALANCE AND VENTILATION

by

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ABSTRACT

The effects of acute osmotic changes on acid-base balance and respiratory control were studied in the Pekin duck, *Anas platyrhynchos*. Acute hyperosmolality following intravascular injection of essentially non-penetrating solutes such as NaCl or sucrose caused an increase in extracellular fluid volume, a prolonged extracellular acidosis (so-called dilution acidosis), and a relative increase in extracellular Cl^- concentration. In contrast, hyposmolality did not cause complementary changes in these variables. Studies in acute hyperosmotic stress were therefore undertaken to investigate both the nature of the acid-base disturbance and the implications for ventilatory control. ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR) on duck pectoral muscle showed that the dilution acidosis caused by acute hyperosmolality was accompanied by an intracellular contraction alkalosis, implying the uncoupling of intra- and extracellular pH. The increase in extracellular [Cl^-] and the pH changes suggested a primary role for Cl^-/HCO_3^- exchange during this perturbation. However, both the anion-exchange blocker DIDS and the Na^+/H^+ exchange blocker amiloride reversed the intracellular pH change from alkalosis to acidosis, although they did not affect the extracellular acidosis caused by acute hyperosmolality. These results indicated that hyperosmolality altered both Cl^-/HCO_3^- and Na^+/H^+ exchange and also suggested the involvement of one or more additional exchange mechanisms.

Despite the pronounced extracellular acidosis during hyperosmolality, there was no compensatory stimulation of ventilation. An extracellular pH decrease of similar magnitude and time course when caused by lactic acid infusion stimulated a 100% increase in ventilation, and decreased both extracellular and intracellular pH in contrast to the intracellular alkalosis during hyperosmolality. Although intracellular pH was not directly measured in chemoreceptor tissue, it is reasonable to assume that such well-perfused tissue would be exposed to any osmotic stress. Reversal of the intracellular alkalosis during

hyperosmolality by DIDS and amiloride, so that both extra- and intracellular pH were acidotic, resulted in a significant increase in ventilation. These data are unique, since it is the first piece of clear, although indirect, evidence that intracellular pH plays a role in initiating ventilatory changes to acid-base disturbances at the peripheral chemoreceptors. Since brain intracellular pH as measured by ^{31}P NMR during systemic hyperosmolality showed only a consistent trend towards an alkalosis, then central chemoreception, if based on intracellular pH, is unlikely to be affected by hyperosmolality. Acute hyperosmolality also increased the ventilatory threshold to acute hypercapnia, reaffirming a depression of peripheral chemoreception. However, the ventilatory threshold to hypoxia was decreased and sensitivity to K^+ was increased, indicating that the chemoreceptive mechanisms for CO_2 and O_2 are different, and that both intra- and extracellular pH are crucial to ventilatory control.

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INTRODUCTION

Changes in extracellular osmolality are often accompanied by changes in extracellular and intracellular water volumes, electrolyte concentrations, and acid-base parameters. Acute hyperosmolality due to essentially non-penetrating solutes such as sucrose or NaCl leads to a prolonged extracellular acidosis, which has been termed dilution acidosis (Shires and Holman, 1948; Sotos *et al.*, 1962; Makoff *et al.*, 1970). This is an unusual effect because it occurs without the addition or depletion of either an acid or a base. Dilution acidosis has been examined cursorily in mammals, and a similar plasma acidosis has been demonstrated in teleosts and amphibians in response to a hypertonic environment (Wilkes and McMahon, 1986; Walker *et al.*, 1990). Although large, acute changes in osmolality are not normally experienced by many vertebrates except humans and certain fish, acute osmotic increases in humans are a serious pathophysiological condition experienced during illness such as diabetes and in treatments involving hemodialysis or altered electrolyte retention. Despite the clinical implications, little work has been done on this phenomenon. Much more work has been done on the antithesis of dilution acidosis, namely contraction (or Cl^- deficiency) alkalosis (Galla and Luke, 1988; Wesson, 1990; Galla *et al.*, 1984). The conventional explanation of dilution acidosis is based upon the dilution of plasma by the movement of intracellular water to the extracellular space in response to the osmotic gradient created by hypertonic infusion. Due to the continuous tissue production of CO_2 and its ready diffusion across cell membranes (therefore $PaCO_2$ would not change), the dilution would only measurably reduce bicarbonate concentration, resulting in a drop in extracellular pH (pHe). Bicarbonate generation and equilibration may be inhibited during extracellular hyperosmolality because of perturbation of intermediary cell metabolism (Chang *et al.*, 1975; Makoff *et al.*, 1970), although this is controversial. It has also been suggested that bicarbonate transfer out of cells is instead augmented (Winters *et al.*, 1964).

The development of the extracellular acidosis is apparently accompanied by concomitant development of an intracellular alkalosis, which implies the uncoupling of intra- and extracellular pH. There have been numerous demonstrations of various tissues and single cells *in vitro* becoming alkalotic upon exposure to a hypertonic medium, including skeletal muscle (Adler *et al*, 1975; Abercrombie and Roos, 1983), cardiac muscle (Whalley *et al.*, 1991), glial cells (Jean *et al.*, 1986), and osteoblasts (Green *et al.*, 1988), but there is a paucity of information obtained *in vivo*. Makoff *et al.* (1970) reported a significant increase in intracellular pH (pHi) of erythrocytes in anesthetized dogs (pump ventilated, nephrectomized, splenectomized) 2 hr after infusion with hypertonic NaCl or mannitol, but it is not known whether this response occurs in any other tissue. There are likely to be significant differences in the response to osmotic stress between cells which are excellent cell volume regulators, such as erythrocytes, and those that are not, such as skeletal muscle.

Dilution of the extracellular space by infusion of isotonic or hypotonic fluids generally does not result in a measurable acidemia (see Garella *et al.*, 1975), although rapid infusion of massive volumes of isotonic fluid can transiently increase the hydrogen ion concentration ($[H^+]$; Shires and Holman, 1948; Rosenbaum *et al.*, 1969). Infusion of freely diffusible solutes, such as urea, which do not cause a shift of water from the intracellular compartment or affect pHi (Adler *et al.*, 1975), also have no effect on pHe (Winters *et al.*, 1964). This suggests that the crenation, or the increase in intracellular osmolality or possibly pH, during exposure to hypertonic solutions somehow alters membrane transport. While most of the studies in single cells implicate Na^+/H^+ exchange in the response to a hypertonic medium (Green and Muallem, 1989; Whalley *et al.*, 1990), *in vivo* studies suggest a predominant role for anion exchange (Makoff *et al.*, 1970; Sotos *et al.*, 1962). These *in vivo* studies show a relative increase in extracellular $[Cl^-]$, implying an outward flux of Cl^-. If the Cl^- were exchanged for HCO_3^-, this could result in an extracellular acidosis and intracellular alkalosis. Use of selective ion-exchange

blockers *in vivo* would help to resolve which ion-exchange perturbations occur in response to hyperosmolality.

Normally, extracellular acid-base disturbances elicit compensatory changes in respiration. The degree of respiratory compensation has never been measured during dilution acidosis, but the literature shows anomalous changes in blood gases. In studies with rabbits and dogs, which were allowed to breathe normally during dilution acidosis, $PaCO_2$ either decreased only very slightly or actually increased, even after a fall in arterial pH (pHa) of 0.3 pH units, suggesting a lack of ventilatory compensation (Sotos *et al.*, 1962; Asano *et al.*, 1966). The same decrease in pHa caused by infusion of acids approximately doubled ventilation ($\dot{V}_E$) in rabbits (Maskrey and Trenchard, 1989; Nattie, 1983) and decreased $PaCO_2$ 8 Torr (Nattie, 1983). It has long been hypothesized that intracellular pH changes may be involved in chemoreception (in addition to extracellular changes) (Hanson *et al.*, 1981; Adler *et al.*, 1990; Lassen, 1990), but there is no conclusive evidence regarding this hypothesis to date. Suppression of the normal chemoreceptor response to acidosis could be explained if an intracellular contraction alkalosis developed concomitantly, and the pH sensitive component of the chemoreceptor response was at least partially dependent upon intracellular conditions. The testing of such a hypothesis would involve ascertaining the response of both peripheral and central tissues to hyperosmotic stress. There is currently very little information on the response of the brain to acute, systemic, hyperosmotic stress. The brain is a tissue which cell volume regulates to some degree (see Macknight *et al.*, 1992) and is partially protected by the blood-brain barrier.

Chemoreceptors in birds are located both peripherally and centrally, but knowledge of them is not nearly as extensive as in mammals. Peripheral receptors are located primarily in the carotid bodies (Jones and Purves, 1970a) and the intrapulmonary chemoreceptors (Fedde *et al.*, 1974), the afferents of which travel in the carotid sinus and vagus nerves, respectively, while central receptors exist somewhere caudal to the

mesencephalon (see Scheid and Piiper, 1986). Carotid chemoreceptors are sensitive to O_2, and all three receptor groups are sensitive to pH and CO_2 (see Scheid and Piiper, 1986 for review). Transduction in the chemoreceptors has not yet been conclusively determined. The majority of the work has been done on the carotid bodies, on which this brief review will be based (for a recent, more complete review, see Gonzalez *et al.*, 1992). The most accepted proposed model for transduction of acidic stimuli (Rocher *et al.*, 1991) suggests that an increase in $[H^+]i$ stimulates operation of Na^+-coupled H^+-extruding systems that increase $[Na^+]i$. The increase in intracellular Na^+ drives the entry of Ca^{2+} by the Na^+/Ca^{2+} exchanger, which activates exocytosis and release of dopamine. Dopamine is the neurotransmitter that then activates the apposed afferent nerve terminals (Rigual *et al.*, 1991). However, the kinetics of this model have not been worked out, nor has the relationship between intra- and extracellular pH been established. The chemotransduction of hypoxia is less clear and has been proposed in two models. The plasma membrane model suggests that low PO_2 inhibits K^+ channels, producing an initial depolarization that activates voltage-dependent Ca^{2+} channels and allows entry of Ca^{2+}. Simultaneous activation of Na^+ channels allows faster recruitment of Ca^{2+} channels, thereby increasing the entry of Ca^{2+} and subsequent release of neurotransmitters (Lopez-Lopez *et al.*, 1989; Urena *et al.*, 1989). The nature of the O_2 sensor in this model is unknown, and there is considerable controversy over the relationship between carotid body PO_2 and the sensitivity of the K^+ channels. The second model that has been proposed is the metabolic model, in which hypoxia results in a decrease in carotid body phosphorylation potential, which somehow is a signal to decrease neurotransmitter release (originally proposed by Anichkov and Belenkii, 1963). A recent version of this model suggests that the actual signal for chemoreception in hypoxia is the slowing of the electron transfer in the respiratory chain of chemoreceptor cells, resulting in a decrease in the mitochondrial H^+ electrochemical gradient. This causes release of Ca^{2+} from the mitochondria, a rise in $[Ca^{2+}]i$ and release of the neurotransmitter dopamine (Biscoe and

Duchen, 1990a). However, there are numerous criticisms of the metabolic model. Cytochrome oxidase has a very high affinity for O_2 while the carotid bodies have a very high tissue PO_2. This would require the presence of a low-affinity cytochrome oxidase, the existence of which has been questioned (Acker and Eyzaguirre, 1987). Secondly, it is difficult to reconcile a decrease in the phosphate potential being the signal for hypoxic chemoreception since that is the time of maximal function of the carotid body (Acker *et al.*, 1989). Finally, the ability of mitochondria to sufficiently elevate cytosolic Ca^{2+} is questionable (McCormack *et al.*, 1990).

Intracellular pH is traditionally a difficult measurement to make repetitively with both accuracy and precision. The use of classical techniques, such as freeze fracture or the distribution of weak acids like dimethadione, all have a number of serious shortcomings. All of these methods are highly invasive, do not easily lend themselves to repeated measurements, and may contain large error since the final calculation of pHi depends upon the measurement of several other variables, each associated with some error. Intracellular pH, however, can presently be measured in many tissues, including muscle and brain, by 31-phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR), which has several advantages as an analytical tool. It is non-invasive, allows repeated measurements over time in a single animal, and follows cellular energy homeostasis by monitoring changes in high energy phosphate metabolites and consequently, pHi. ^{31}P NMR has a resolution of pHi measurement at least equal to conventional biochemical techniques.

The experiments in this thesis were done with the domestic Pekin duck, *Anas platyrhynchos*, because of its tolerance to large osmotic changes. The purpose of this thesis was to identify the ionic changes leading to acid-base disturbance during acute plasma osmolality changes, and to relate respiratory changes to specific compartmental pH changes. It was hypothesized that acute hyperosmolality would result in both an extracellular dilution acidosis and an intracellular contraction alkalosis, and that the

intracellular alkalosis would suppress the increase in ventilation normally associated with acidosis. Furthermore, the pH perturbations were hypothesized to be primarily a result of altered Cl^-/HCO_3^- exchange. The study first characterized the ionic, cardiovascular, respiratory and acid-base response to acute hypo- and hyperosmolality, and compared the response to a more conventional acidosis (lactacidosis). The investigation continued by determining the intracellular response of both brain and systemic muscle to the extracellular acidosis generated by either acute hyperosmolality or lactic acid infusion in the conscious animal, thus establishing a relationship between different compartmental changes in pH to changes in ventilation. Since it is not feasible to monitor pHi within a carotid body *in vivo* by NMR (the primary location of the peripheral chemoreceptors), we measured pHi in skeletal muscle. The assumption that the effect of hypertonicity is similar in the pectoral muscle and carotid body tissue is reasonable, since both tissues are well perfused (De Castro and Rubio, 1968) and would therefore be similarly exposed to any changes in tonicity, and these tissues do not volume regulate extensively under acute hyperosmotic conditions (see Macknight *et al.*, 1992). Specific ion-exchange blockade was used during hyperosmotic stress in an attempt to prevent the pH perturbations, and to define the ion-exchange mechanisms involved. We used 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) to inhibit carrier-mediated anion exchange, including Cl^-/HCO_3^- exchange, and amiloride to inhibit the Na^+/H^+ antiporter. Since a suppression of the normal ventilatory response to dilution acidosis would imply some degree of depression of chemoreceptor discharge at least in the periphery, this was tested by examining the ventilatory response to several known respiratory stimuli (hypercapnia, hypoxia and K^+) during a hyperosmotic challenge. The hypercapnic response should be mediated by both the peripheral and central receptors, while the hypoxia and K^+ stimulation should involve only peripheral receptors, which would allow further characterization of hyperosmotic effects on the different chemoreceptor groups.

MATERIALS AND METHODS

Surgical methods

Adult, female Pekin ducks (*Anas platyrhynchos*), weighing 1.5-3.7 kg, were obtained from the Animal Care Facility of the University of British Columbia and housed indoors in individual wire cages with free access to food and water. The brachial artery and the ulnar vein in the wing of each animal were chronically cannulated under local anaesthesia (Xylocaine 2%; Astra, Ont.) with a single piece of polyvinylchloride tubing (Bolab VIII; Bolab, AZ) 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, PA), a heparin compound which binds to the wall of the tubing. The animals were allowed at least 48 hr to recover before the start of any experiments. All experiments were performed in accordance with Canadian Animal Care guidelines, and approved by the UBC Animal Care Committee.

Physiological responses to acute changes in osmolality

These experiments were designed to characterize the respiratory, cardiovascular and acid-base effects of osmotic stress.

Hyperosmolality

Experimental protocol Sixteen ducks were used in these experiments. Each duck was weighed and placed in a water-cooled, whole body plethysmograph (Fig. 1). Wings and legs were lightly restrained with filament tape. The head of the animal extended out of the plethysmograph through a dental dam collar and the venous and arterial cannulas were led out of an air-tight hole. The animals were then left undisturbed for 45 min to allow them to adjust to their surroundings. A 1 hr control period followed in which three arterial blood samples (0.8 ml) were drawn anaerobically for resting blood gas and pH analysis, using a blood/gas analyzer (IL813; Instrumentation Laboratory, MA) maintained

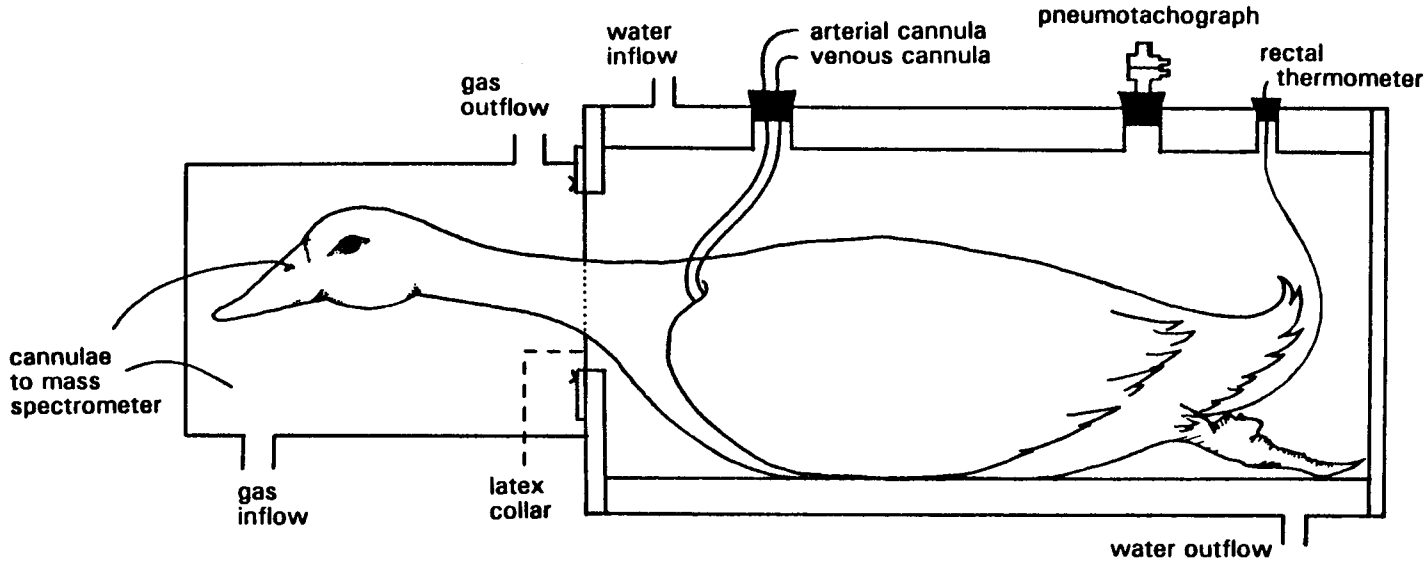


Fig 1. Schematic diagram of the experimental setup for all studies involving plethysmography. The head compartment was only used when the animal was exposed to gases other than room air. See METHODS for details.

at duck body temperature (41°C). The IL813 was calibrated before each sample using commercially prepared gas mixtures and pH standards. The remainder of the blood sample (0.4 ml) was centrifuged and the plasma decanted and immediately frozen. Measurements of resting ventilation and blood pressure (Elcomatic 715A blood pressure transducer; Harvard Instruments, MA) were taken every 10 min. Heart rate was derived from the blood pressure trace. Eight ducks were each infused with three different solutions (4 M NaCl, 2.4 M sucrose, or 0.15 M NaCl) in random order (as outlined below), while the other eight ducks were infused with lactic acid. Every animal was given at least 48 hr to recover between infusions.

Each animal received 7.5 $mmol \cdot kg^{-1}$ body wt of 4 M sodium chloride (NaCl) infused over 15 min (total volume 5-6 ml). Since this infusion always stimulated salt gland secretion, plasma osmotic pressure was maintained for the duration of the experiment by subsequent infusion of a 540 mM NaCl solution at a rate of 0.1 $ml \cdot min^{-1}$ to approximately match salt gland secretion. Blood samples were taken at 5, 10 and 15 min, then every 15 min for the remainder of the first hour, and every 30 min in the following hour. This is termed the infusion period. The total volume of blood removed during each experiment was 8.8 ml. Cardiovascular and respiratory variables were measured every 5 min for the first hour and every 10 min during the second hour. Each animal also received two other infusions; an equivalent osmotic load of 2.4 M sucrose over approximately 25 min (total volume 18-22 ml), and 5-6 ml of isotonic saline (isotonic control infusion). Ducks infused with lactic acid received 0.25 $meq \cdot (kg \cdot min)^{-1}$ at 0.5 $ml \cdot min^{-1}$. A maintenance infusion was not required during any of these infusions since salt gland secretion was insignificant.

Three ducks were infused with the same hyperosmotic NaCl load to measure the resultant change in extracellular fluid volume and hematocrit. Extracellular fluid volume was measured by injecting 5 μCi of ^{36}Cl in two animals and ^{22}Na in the third animal. After 30 min equilibration at rest, 3 (0.5 ml) arterial blood samples were taken over 15

min and the 4 M NaCl was then infused. Blood samples were taken at 10 min intervals for 130 min. The blood was centrifuged and duplicate 100 μl plasma samples were transferred to 4 ml of Aquasol scintillation cocktail for measurement of ^{36}Cl on a liquid scintillation counter (LS9000, Beckman, CA), while samples were measured for ^{22}Na in a Minaxi 5000 gamma counter (Canberra-Packard, Ont.).

Plasma Na^+ and K^+ concentrations ($[Na^+]$, $[K^+]$) were determined by flame photometry (IL943; Instrumentation Laboratory, MA), plasma Cl^- concentration ($[Cl^-]$) using a Buchler digital chloridometer (4-2500; Buchler Instruments, NJ) and plasma osmolality was measured with a vapor pressure osmometer (5500; Wescor, UT). Plasma lactate concentration was measured in two ducks during each of the three treatments in the hyperosmolality series using the method of Noll (1974). The pressure transducer was calibrated against a mercury manometer. Temperature was monitored in several ducks by rectal thermometer (Physitemp BAT-12; Sensortek, NJ) and did not change throughout the experiment. Ventilation was measured by whole body plethysmography. Ventilatory changes in body volume were measured as changes in pressure due to air flow through a Fleisch #0 pneumotachograph connected to a port in the plethysmograph. The airflow signal was measured with a gas pressure transducer (Hewett-Packard 275A) and integrated to yield tidal volume. The system was calibrated with known volumes of air delivered by hand-driven syringe. $\dot{V}_E$ was calculated as the product of tidal volume and respiratory frequency. All variables were recorded on a Harvard Universal oscillograph writing on rectilinear coordinates.

Hyposmolality

Experimental protocol The purpose of this experiment was to determine whether dilution without hyperosmolality or extracellular fluid volume increases would affect acid-base balance. Plasma hyposmolality was induced in five ducks by withdrawing arterial blood with a pulsatile infusion pump which was concurrently replaced with an equal

volume of sterile, deionized water maintained in a water bath at 40°C. Each 10 ml aliquot of blood was immediately centrifuged, the plasma decanted and the red blood cells reinfused. The control period for measurement of resting variables was identical to that described for the hyperosmotic experiment above but, during the experimental period, measurements were made and samples taken after the first and each subsequent exchange of 40 ml of blood. Respiratory, pH and ion measurements were taken as described above, except blood gases were not analyzed. Red cell water content was measured by drying packed red blood cells at 105°C to a constant weight.

Hyper- and hyposmotic calculations and data analysis

Data were analysed as the difference from the mean resting value by analysis of variance (ANOVA) and Tukeys post-hoc test (Zar, 1984) at a significance level of $P \leq 0.05$ using the statistical package Systat (Systat, Evanston, IL). pHa was converted to $[H^+]$ for statistical analysis. The bicarbonate concentration was calculated using the Henderson-Hasselbalch equation, assuming a solubility coefficient for CO_2 of 0.0282 $mmol \cdot (L \cdot mm\ Hg)^{-1}$, and a pK_1 of 6.090 (Helbacka *et al.*, 1964). [SID] was calculated as $[SID] = ([Na^+] + [K^+]) - ([Cl^-] + [lactate^-])$. All data are shown as differences (mean $\pm$ SEM) from the mean resting value established in the 1 hr control period before each infusion. Data from the NaCl and sucrose infusion experiments are compared to the isotonic control infusion experiment.

^{31}P Nuclear Magnetic Resonance Spectroscopy

^{31}P NMR was performed on twenty-one Pekin ducks in order to quantify intracellular pH and metabolic changes both systemically and centrally during hyperosmotic disturbance, and to establish a relationship between pHi and ventilation.

Experimental protocol The wing cannula was severed and PVC tubing, long enough to reach the outside of the NMR magnet, was attached to each end. The animal

was placed in a normal sitting position in a cradle and carefully restrained with the aid of shaped foam pieces and tape. Once inside the magnet, the duck was given 10-15 minutes to relax, and then 3-5 resting spectra were obtained as described in the following sections. During this period, 3 (0.8 ml) arterial blood samples were taken for immediate analysis of blood gases and pH on a blood/gas analyzer maintained at 41°C. Each duck in which pectoral muscle was examined was then given an infusion of either hypertonic NaCl (20 $meq \cdot kg^{-1}$), hypertonic sucrose (25.5 $meq \cdot kg^{-1}$), or lactate (9.5 $meq \cdot kg^{-1}$), over 35 min. In four of the animals, body temperature was monitored via a thermistor inserted into the trachea. Five spectra were obtained over this period, and at the end of the infusion, recovery was monitored with a further 1-2 spectra. A blood sample was taken in the middle of each spectrum. Each duck underwent 2-3 random trials spaced at least two days apart, with no duck receiving the same infusate twice. Ducks that were used for brain study were infused only with hypertonic sucrose over 55 min. All solutions were warmed to approximately 40°C just before the start of infusion.

^{31}P NMR spectra of pectoral muscle

These experiments were performed in the Department of Chemistry at the University of British Columbia. Each animal (n=16) was placed in a custom-fitted perspex cradle at a slight angle such that the thickest portion of the right pectoral muscle lay directly over the surface coil, thus avoiding the sternum. The cradle was then slid into a 1.89 Tesla horizontal superconducting magnet (Oxford Instruments, Oxford, UK). The ^{31}P-NMR spectra were acquired in the Fourier transform mode on a Nicolet 1280 spectrometer. The surface coil used was a 3.75 cm, inductively coupled, loop gap (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' (approx. 600 unloaded), which resulted in a very high sensitivity compared to other, capacitatively-coupled, surface coils that were tested. However, this coil was not double-tuned, and therefore the magnetic

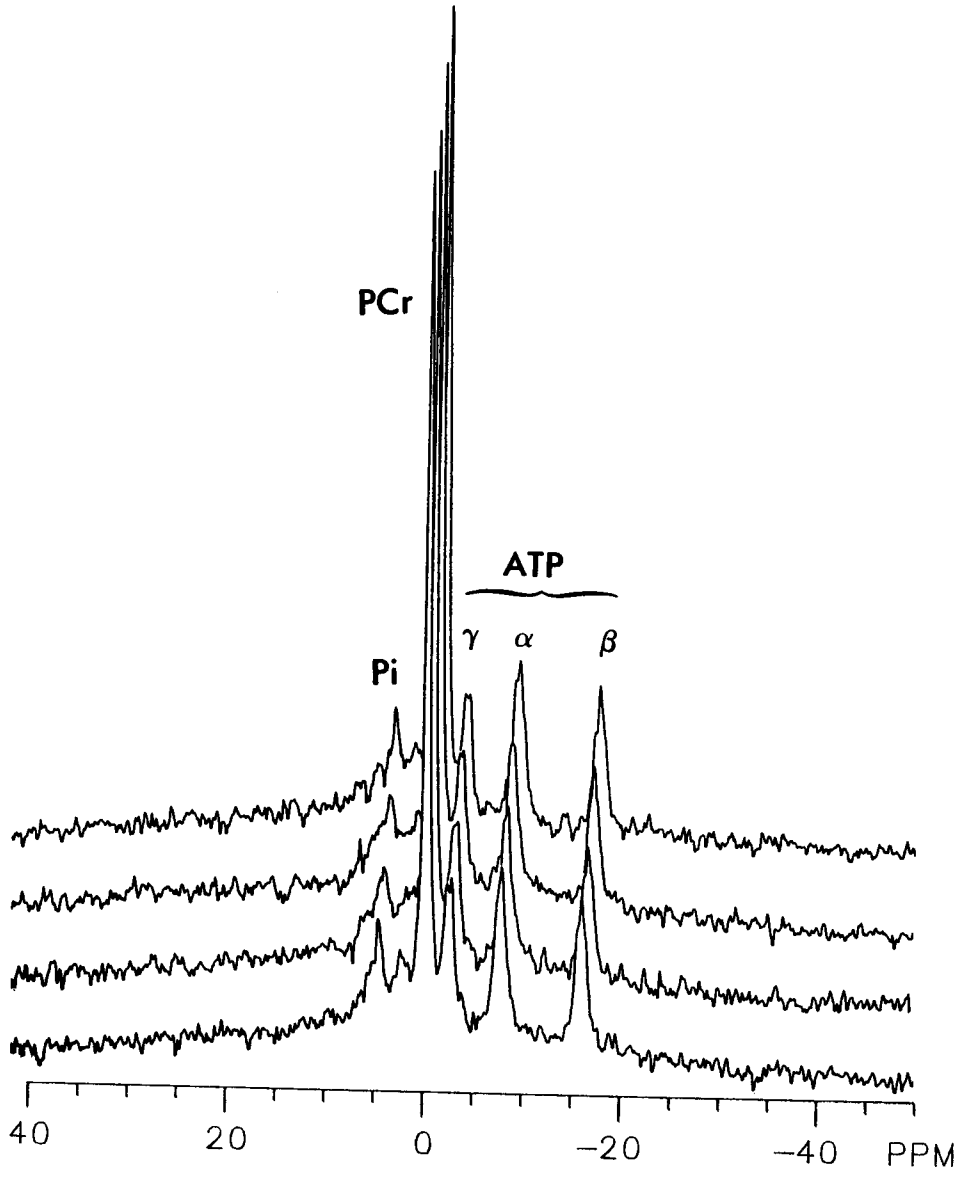
field was shimmed on a phosphorus sample and not on the proton signal from the animal. The large (approximately 7 cm^3), homogeneous field of the magnet, combined with a cradle design that allowed repeated, precise placement of the surface coil and animal in the magnet, resulted in well resolved spectra with an excellent signal-to-noise ratio (Fig. 2). The phosphorus spectra were obtained at 32.5 Mhz, using a 35 μsec pulse width, $\pm$2000 Hz sweep width with quadrature detection and 2,048 points per scan. A total of 240 free induction decays were collected with a 1.5 sec pulse interval (total time = 7 min). Data were zero filled to 4,096 points and the summed free induction decays multiplied by an exponential corresponding to 6 Hz line broadening before Fourier transformation.

^{31}P NMR spectra of brain

This study was done in the Department of Radiology and Physiology at the University of Washington, Seattle. The experiments were performed in accordance with the U.S. Animal Care guidelines and approved by the UW Animal Care Committee. The protocol was similar to that used during the pectoral muscle study, except that eleven spectra were obtained over the infusion period, and recovery was monitored with a further 6 spectra. The blood/gas analyzer at UW was maintained at 37°C and the data later corrected to 41°C using a correction factor of $-0.015 \cdot °C^{-1}$. Ducks (n=5) were sacrificed at the end of the experiment with an overdose of sodium pentobarbitol.

After placement in the cradle and immobilization of the head, the duck was placed in a 2 Tesla, horizontal, superconducting magnet (Oxford Instruments, Oxford, UK). The ^{31}P-NMR spectra were obtained in the Fourier transform mode on a GE CSI II spectrometer with a Nicolet 1280 computer at a frequency of 34.6 Mhz. A 2-turn, 2.5 cm coil of Teflon-coated copper wire was mounted directly over the head. Since there is no muscle tissue directly over the cranium in Pekin ducks, the signals monitored by the coil were from brain tissue up to 1.25 cm deep. Magnetic field homogeneity was shimmed

Fig. 2. Four successive spectra of duck pectoral muscle. Bottom spectrum represents a resting state, with the following 3 spectra obtained 7, 14 and 21 minutes into hypertonic sucrose infusion. Each spectrum is the sum of 240 free induction decays. The scale at the bottom refers to the chemical shift in parts per million with respect to the PCr signal, which is set to 0. Each spectrum has been shifted 1 mm to the right of the spectrum below it.


PCr
ATP
γ
α
β
Pi
40
20
0
−20
−40
PPM

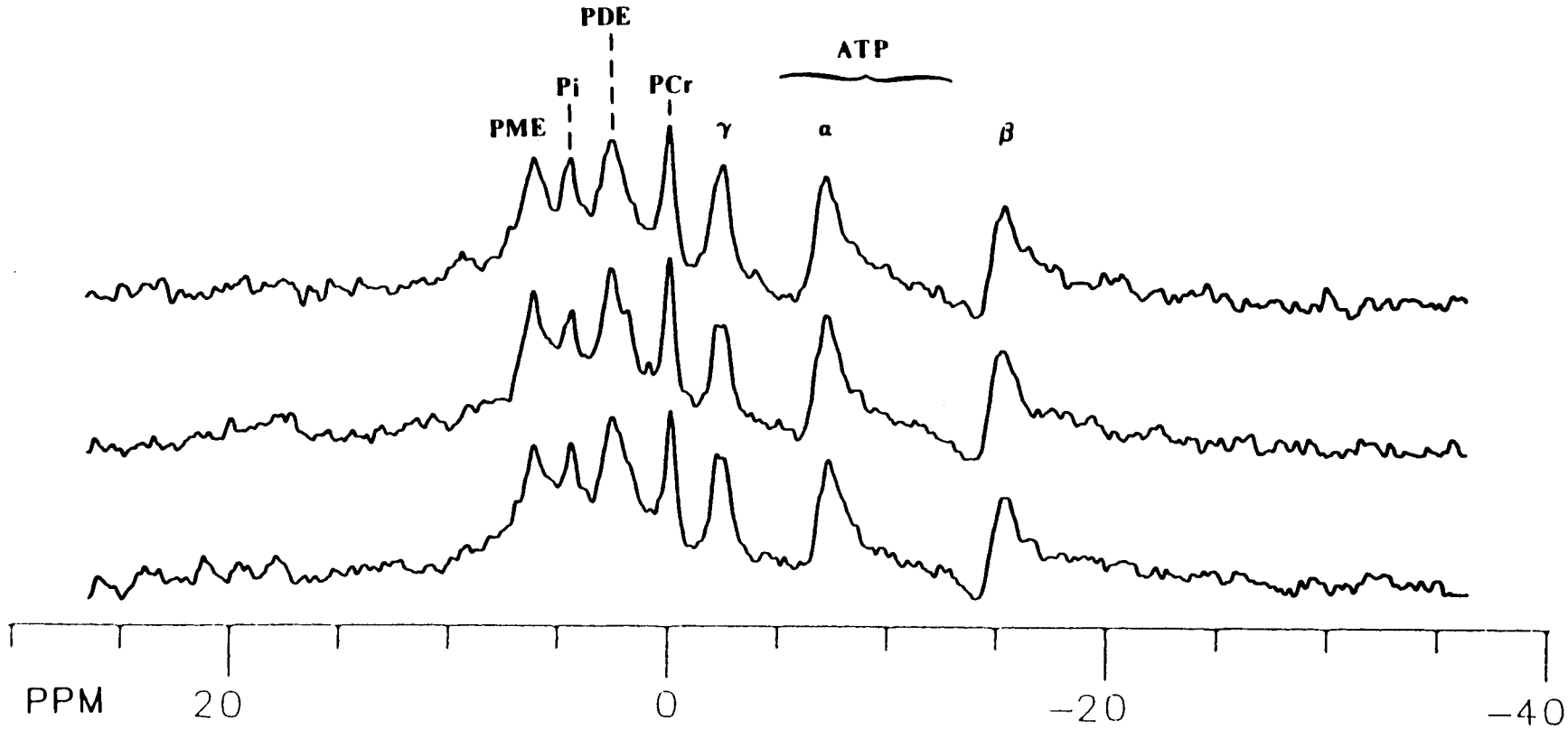
with the animal in place using the proton signal from the brain water, so that proton line width was in the range of 30-40 Hz. Phosphorus spectra were obtained with a 20 μsec pulse width, $\pm$1500 Hz sweep width, and 2048 data points per scan. A total of 300 free induction decays were collected with a 320 msec pulse interval (total time = 5 min), and the summed free induction decays multiplied by an exponential corresponding to 15 Hz line broadening before Fourier transformation (Fig. 3).

^{31}P NMR calculations and data analysis

Intracellular pH was calculated from the difference in chemical shifts (Δ) of the Pi and the PCr peaks according to the formula $pHi = pK' + \log[(\Delta Pi - \delta_A)/(\delta_B - \Delta Pi)]$. The parameters δ_A and δ_B (the acidic and basic phosphate titration end-points), and pK' (the apparent pK for phosphates), were calculated from Kost (1990) for a body temperature of 41°C. The peak area for each metabolite was integrated and corrected for saturation. The saturation factor was calculated as the ratio of the individual peak areas obtained with a pulse interval of 1.5 sec to the areas obtained with a pulse interval of 18 sec. The latter pulse interval corresponds to approximately five times the longest spin-lattice relaxation time of the metabolites, the interval required for assumption of complete relaxation between scans.

To reduce normal inter-animal variation, all data were analyzed using the difference between the individual's resting value and each experimental value. Data from the pectoral muscle experiment were analyzed by ANOVA and Dunnetts post-hoc test, to determine significant differences from a control value. Control data against which the experimental data were compared were obtained from three animals that were treated in a manner similar to the experimental animals, except that they were not given an infusion. Data from the brain experiment were analyzed by t-test for paired comparisons. All results are presented as mean differences ($\pm$ SEM) from resting values, and absolute values are indicated where appropriate.

Fig. 3. Representative spectra of duck brain with skull and overlying skin intact. Each spectrum is the sum of 300 free induction decays. The scale at the bottom refers to the chemical shift in parts per million with respect to the PCr signal, which is set to 0. PME, phospomonoesters; PDE, phosphodiesters.


PDE
ATP
Pi
PCr
PME
γ
α
β
PPM
20
0
−20
−40

^{31}P NMR of pectoral muscle during ion-exchange blockade

The purpose of these experiments was to identify some of the ion-exchange mechanisms involved in the pH perturbation during hyperosmolality and to confirm any relationship between pHi and ventilation by manipulation of pHi.

Experimental protocol Eighteen female Pekin ducks were used in these experiments. Opaque cannulae were used in this experiment to protect the light-sensitive ion-exchange blockers. The animal was fitted with a endotracheal cannula, and then placed in a normal sitting position in the cradle and carefully restrained as described above. Once inside the magnet, the duck was allowed approximately 15 min to adjust to its surroundings, and then 4-5 resting spectra of the pectoral muscle were obtained. The spectra were obtained as previously described, except that 172 free induction decays/spectra were collected at 4096 points per scan, requiring 6 min per spectra. During this period, 3 (0.8 ml) samples of arterial blood were taken; 0.4 ml were analyzed immediately for blood gases and pH on a blood/gas analyzer maintained at 41°C, and 0.4 ml were centrifuged, the plasma decanted and frozen for subsequent measurement of ions. Plasma Na^+ and K^+ were measured by atomic absorption spectroscopy (2380; Perkin-Elmer, CT). The endotracheal cannula was attached to a Fleisch #0 pneumotach (total dead space approximately 3 ml) connected to a differential pressure transducer (DP103-18; Validyne, CA) for measurement of $\dot{V}_E$ for 1 min every 5 min until it was stable. Arterial blood pressure was measured simultaneously.

Nine animals were then intravenously infused with physiological saline containing DIDS, 30 μmol·kg^{-1} in 5 ml, over 10 min. Six other ducks received 20 mg·kg^{-1} amiloride (0.075 μmol·kg^{-1}) in the same manner. Blood samples were taken and $\dot{V}_E$ and blood pressure monitored 20 and 30 min after the end of infusion to ensure that most variables were stable. One NMR spectrum was acquired over the last 6 min of the equilibration period. The animals were then infused with 26.5 mmol·kg^{-1} body wt. sucrose (approx. 25

ml) over 36 min. All variables were monitored every 6 min during the infusion period and during the 12 min recovery period.

Three animals were infused only with the sucrose, and blood samples were taken after 15 and 30 min of infusion for measurement of total CO_2 using a Carle AGC series 100 gas chromatograph. Calibration was performed using 25 μl of 20 mM $NaHCO_3$ standards.

Calculations and data analysis

Calculations were performed as previously described. All results were analyzed using paired-comparison ANOVA.

Responses to respiratory stimuli

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.

Experimental protocol Eleven Pekin ducks were 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 allowed at least five days between trials. Each duck was 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 pneumotach for measurement of $\dot{V}_E$. The body compartment was surrounded by a circulating cold water bath which maintained duck body temperature at 41°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 41°C, while 0.3 ml were centrifuged, the plasma decanted and immediately frozen. The inflowing air and the end tidal gases (F_{ET}) were monitored via polyethylene 60 cannulae connected to a MGA 200 clinical mass spectrometer (Centronic, U.K.) and sampled concurrently with $\dot{V}_E$. Arterial blood pressure was also recorded and heart rate was derived from the blood pressure trace. Immediately after the resting control 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 post stimulus to confirm a return to resting levels. Each animal was then infused with 26.5 $mmol \cdot kg$ body $wt.^{-1}$ sucrose (approx. 25 ml) over 40 min. All variables were monitored every 7.5 min until 22.5 min, at which time the respiratory stimulus was repeated. A final recording was made 15 min after the end of this second respiratory challenge.

Three ducks were twice 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 (Ruch and Hughes, 1975) and a mean plasma $[K^+]$ of 2.5 $meq \cdot kg^{-1}$. 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 $meq.kg^{-1}$.

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.

Calculations and data analysis

All results were analyzed by random block two-way ANOVA. 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 post stimulus were not significantly different for any measured variable, the two values were averaged for statistical analysis. Results are reported as mean $\pm$ SEM, or mean difference $\pm$ SEM. Significant differences are from resting, normosmotic levels unless otherwise stated. Significant differences from hyperosmotic values always refers to the measured value at 22.5 min into sucrose infusion. O_2 consumption was calculated as $\dot{V}O_2 = \dot{V}E \cdot [1 - (FEO_2 + FECO_2) / 1 - (FIO_2 + FICO_2)] \cdot FIO_2 - \dot{V}E \cdot FEO_2$, where FE and FI represent the fractions of end tidal expired and inspired gas, respectively.

RESULTS

Physiological responses to acute changes in osmolality

Hyperosmolality versus lactate

Infusion of 4 M NaCl caused a significant increase in mean extracellular fluid volume of 10±5%. The expansion was complete 30 min after the start of infusion and was maintained for the rest of the sampling period. This extracellular fluid volume expansion must have been due to the movement of intracellular water since the volume of the NaCl infusate never exceeded 1% of extracellular fluid volume. Resting hematocrit was 39±4% and decreased 4±2% with the infusion.

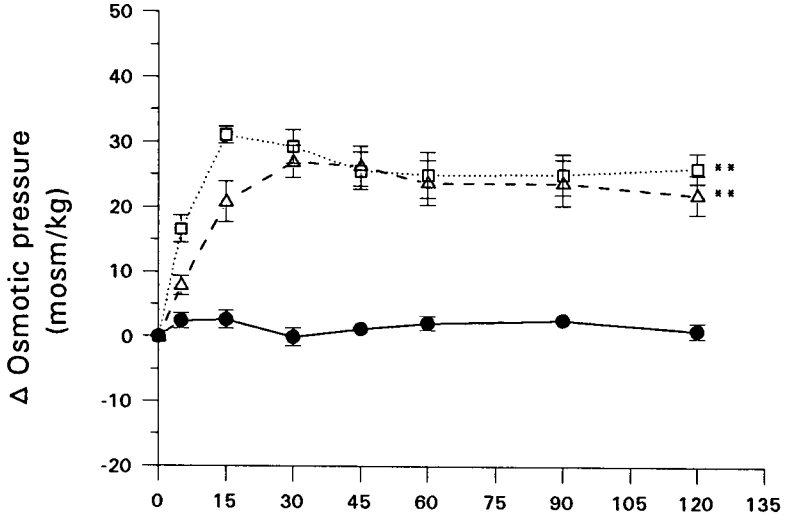
There were no significant changes in plasma osmolality, $[Na^+]$ or $[Cl^-]$ during the isotonic control infusion (Fig. 4). Plasma ions were not measured during lactate infusions. Plasma osmolality increased by a maximum of 31±1 and 27±2 $mosm \cdot kg^{-1}$ above resting during the NaCl and sucrose infusions and remained significantly higher throughout the infusion period (Fig. 4). Plasma $[Na^+]$ increased and decreased a maximum of 15±4 and 16±2 $meq \cdot kg^{-1}$ with the NaCl and sucrose infusions, respectively ($P < 0.05$). However, plasma $[Cl^-]$ increased a maximum of 21±1 $meq \cdot kg^{-1}$ and decreased a maximum of 11±2 $meq \cdot kg^{-1}$ with the NaCl and sucrose infusions, respectively ($P < 0.05$). The increase in plasma $[Cl^-]$ remained significantly higher (5-6 $meq \cdot kg^{-1}$) than the increase in plasma $[Na^+]$ throughout the NaCl infusion period and during the first hour of the sucrose infusion period. [SID] decreased significantly during the hypertonic infusions, primarily because of the relative increase in the $[Cl^-]$ (Table I). Both plasma $[Na^+]$ and $[Cl^-]$ returned to resting levels by the end of the sucrose infusion period. Plasma $[K^+]$ remained constant for the first 90 min and then increased slowly but significantly over the last 30 min during all three infusion periods (Fig. 4). Plasma $[lactate^-]$ only changed significantly during the lactate infusion, increasing by 7.8±0.4 $mmol \cdot kg^{-1}$ (Table I).

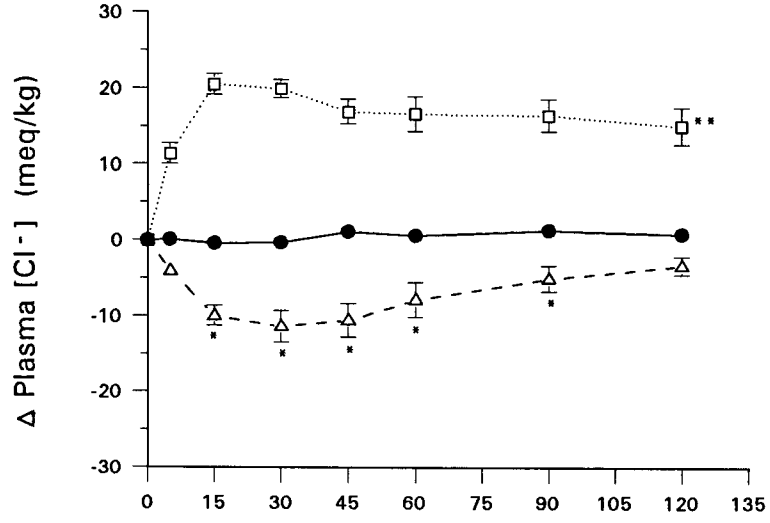
Fig. 4. Changes observed in osmotic pressure, plasma [Na^+], [Cl^-], and [K^+] during control (isotonic saline), 4 M NaCl and 2.4 M sucrose infusions. Mean ($\pm$SEM) differences from resting values are shown. n=8.

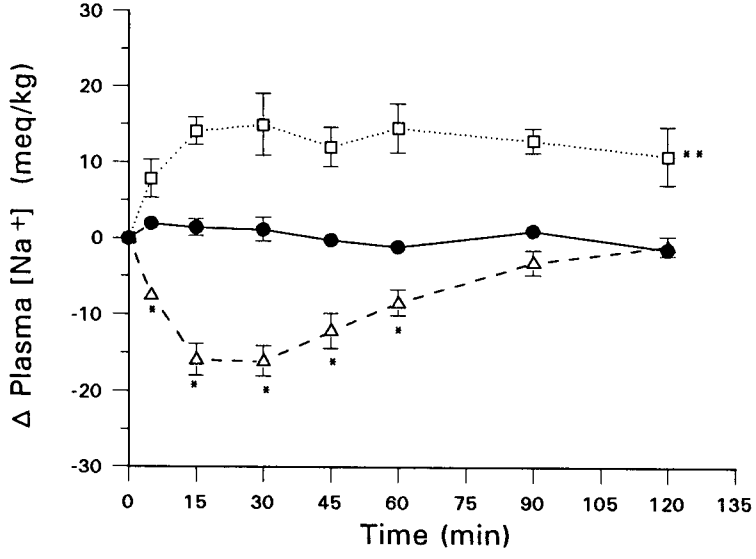
● =control, □ =NaCl, Δ =sucrose infusion.

* indicated point significantly different from rest ($P < 0.05$)

** significantly different from rest throughout infusion


Δ Osmotic pressure (mosm/kg)
50
40
30
20
10
0
-10
-20
0
15
30
45
60
75
90
105
120
135
**
**


Δ Plasma [Cl⁻] (meq/kg)
30
20
10
0
-10
-20
-30
0
15
30
45
60
75
90
105
120
135
**
*
*
*
*
*
*


Δ Plasma [Na⁺] (meq/kg)
30
20
10
0
-10
-20
-30
0
15
30
45
60
75
90
105
120
135
Time (min)
**
*
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*
*
*

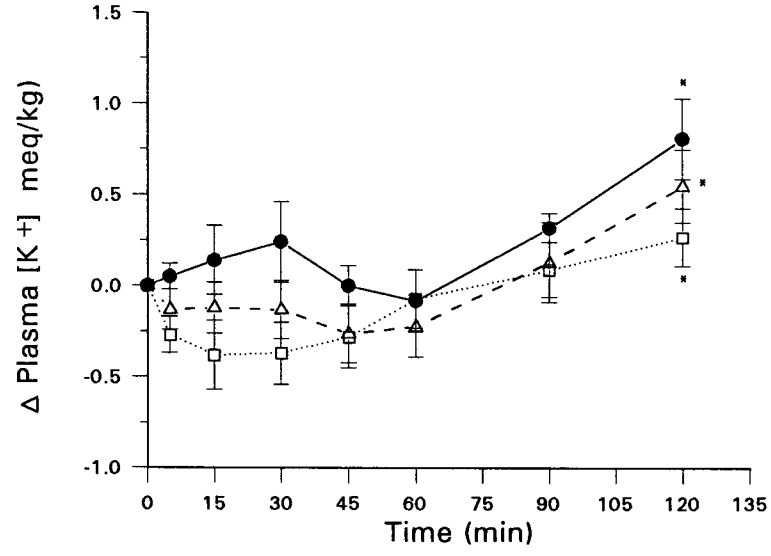

Δ Plasma [K⁺] meq/kg)
1.5
1.0
0.5
0.0
-0.5
-1.0
0
15
30
45
60
75
90
105
120
135
Time (min)
*
*
*

Table I. Ionic, pHa and blood gas changes 30 min after the start of hyperosmotic infusion. Data are shown as the difference from resting values (i.e. when t=0). Absolute resting values are in parentheses; mean±SEM.

	Isotonic saline	Hypertonic NaCl	Hypertonic sucrose	Lactate
OP	0.0±1.4	29.5±2.6*	27.0±2.5*	
(mosm·kg^{-1})	(280±6)	(282±4)	(283±5)	
Na^+	1.0±1.6	15.0±4.1*	-16.0±2.0*	
(meq·kg^{-1})	(147±2)	(148±5)	(148±4)	
K^+	0.2±0.2	-0.4±0.2	-0.1±0.2	
(meq·kg^{-1})	(2.4±0.4)	(2.6±0.6)	(2.5±0.4)	
Cl^-	0.0±0.5	20.0±1.2*	-11.0±2.1*	
(meq·kg^{-1})	(108±2)	(108±4)	(111±3)	
Lactate$^-$	-0.1[a]	0.8[a]	0.2[a]	7.8±0.4*
(mmol·kg^{-1})	(2.0)	(2.5)	(2.0)	(2.1±0.8)
SID	1.9±4.1	-6.5±7.4*	-5.1±2.7*	
	(43.0±4.0)	(42.0±10.0)	(41.0±5.0)	
pHa	0.00±0.00	-0.06±0.01*	-0.05±0.01*	-0.08±0.01*
	(7.48±0.03)	(7.48±0.03)	(7.49±0.03)	(7.48±0.02)
$PaCO_2$	0.5±0.7	2.0±2.0	3.0±1.6	-3.9±0.6*
(torr)	(29±4)	(31±3)	(31±3)	(29.7±0.8)
PaO_2	0.0±1.4	0.9±1.0	-2.3±1.6	11.6±1.8*
(torr)	(84.1±4.4)	(87.3±7.9)	(86.1±2.4)	(89.6±3.3)
HCO_3^-	-0.4±0.5	1.5±1.1	0.5±0.6	5.5±0.4
(mmol·kg^{-1})	(20.8±2.4)	(22.3±2.0)	(21.9±2.3)	(20.5±1.0)

n=8; [a] n=2

* significantly different from isotonic saline (control)

With both hypertonic infusions, only minor changes in respiratory variables were observed despite significant decreases in pHa (Fig. 5). During the NaCl and sucrose infusion experiments, $\dot{V}_E$ did not change significantly from resting $\dot{V}_E$ except for a transient 53% increase 15 min after the start of the NaCl infusion, due to small increases in both respiratory frequency and tidal volume. However, pHa decreased significantly within 5 min after the start of both the NaCl and sucrose infusions. In one animal, $\dot{V}_E$ decreased 45% by the end of the sucrose infusion and remained low for 90 min, despite a fall in pHa of 0.15 pH units and a rise in $PaCO_2$ from 34 to 50 torr (Fig. 6). The reduction in $\dot{V}_E$ was almost entirely due to a decrease in respiratory frequency. Similar but less dramatic changes also occurred in two other sucrose infused animals. The pHa continued to decline throughout the NaCl experiment, but pHa began to recover 60 min after the start of the sucrose infusion (Fig. 5). Neither $\dot{V}_E$ or pHa changed significantly over the isotonic control infusion period. With the exception of the three sucrose infused animals mentioned above, there was no change in the pattern of respiration, i.e. in tidal volume or respiratory frequency, during any of the infusions. There were no significant differences in $PaCO_2$ or PaO_2 among the three treatments at any time (Fig. 5). $PaCO_2$ increased significantly an average of 3.5 ± 1.5 torr and PaO_2 decreased significantly an average of 4 ± 2 torr during all three infusion periods. Calculated bicarbonate concentration did not change significantly over the infusion periods. There were no significant changes in either mean arterial blood pressure or heart rate during any of the infusion.

Lactate infusion significantly decreased pHa from resting levels, and the change in pHa was not significantly different from that due to the hypertonic infusions. $\dot{V}_E$ increased significantly, and this was reflected in an increase in PaO_2 of 13.4 ± 1.7 torr and a decrease in $PaCO_2$ of 4.3 ± 0.5 torr (all $P < 0.05$) (Table I).

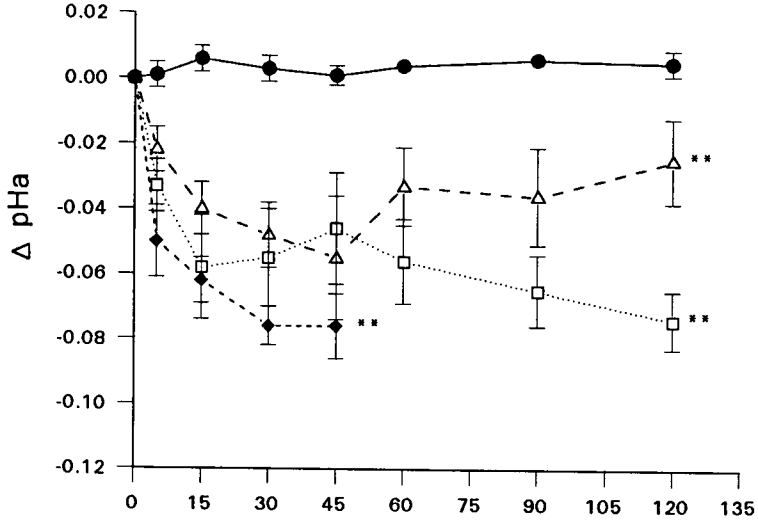
Fig. 5. The changes observed in pHa, $\dot{V}_E$, $PaCO_2$ and PaO_2 during control (isotonic saline), 4M NaCl, 2.4M sucrose and lactate infusions. Mean ($\pm$SEM) differences from resting values are shown. n=8.

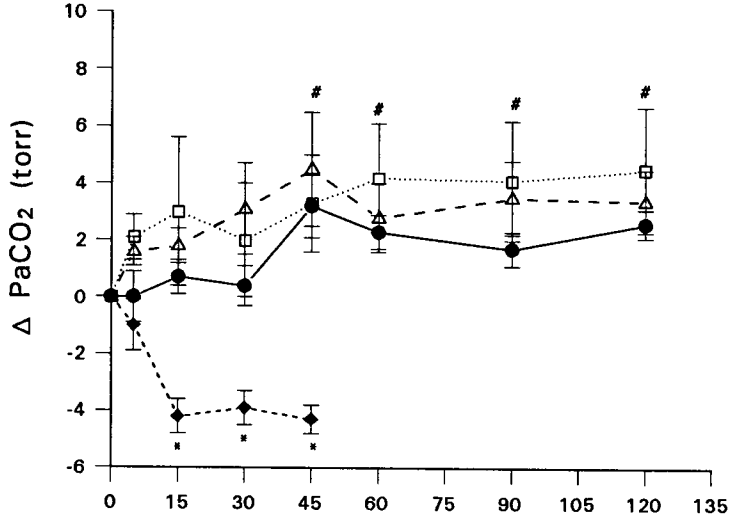
● =control, □ =NaCl, Δ =sucrose, ♦ =lactate infusion.

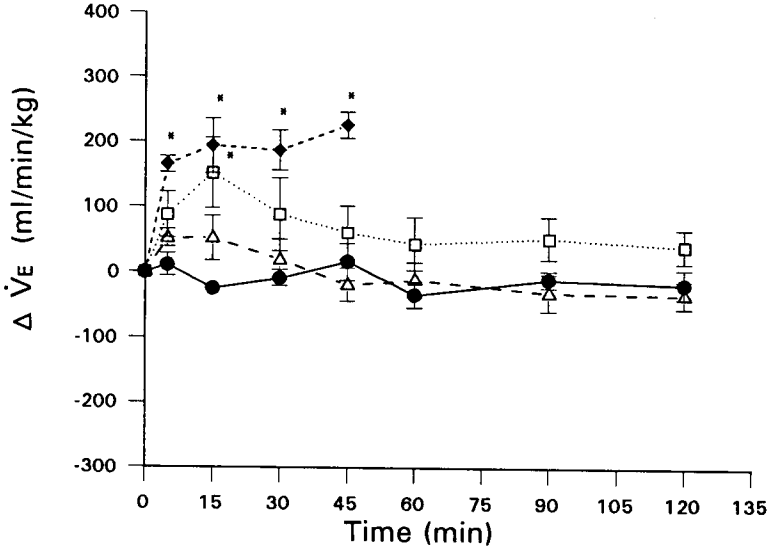
* indicated point significantly different from rest ($P < 0.05$)

** significantly different from rest throughout infusion

significantly different from 0 for all three treatments


Δ pHa
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
0
15
30
45
60
75
90
105
120
135
**
**
**


Δ PaCO2 (torr)
10
8
6
4
2
0
-2
-4
-6
0
15
30
45
60
75
90
105
120
135
#
#
#
#
*
*
*


Δ V̇E (ml/min/kg)
400
300
200
100
0
-100
-200
-300
0
15
30
45
60
75
90
105
120
135
Time (min)

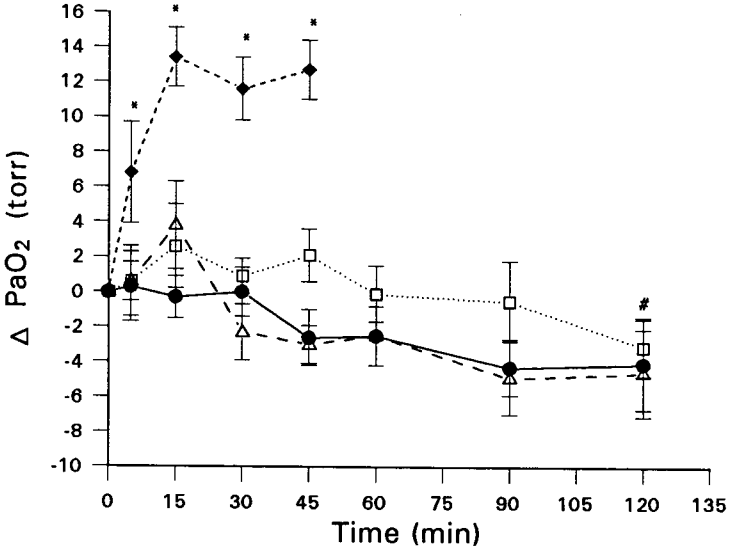
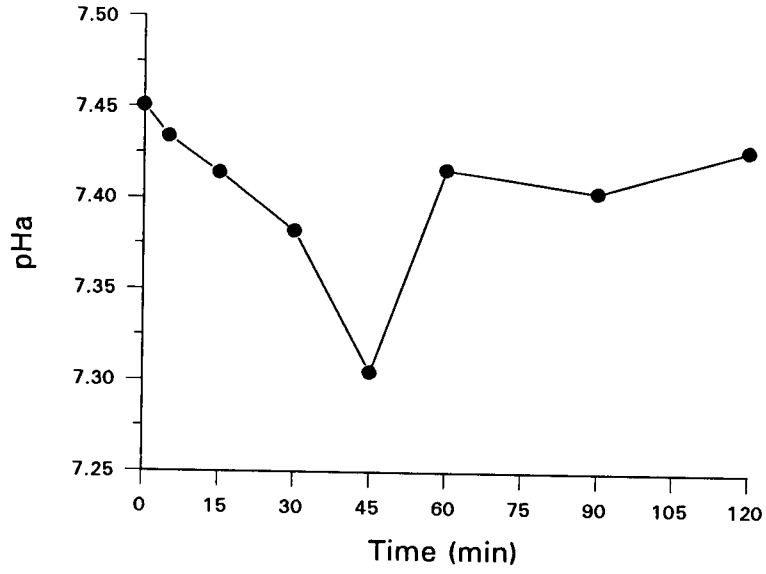
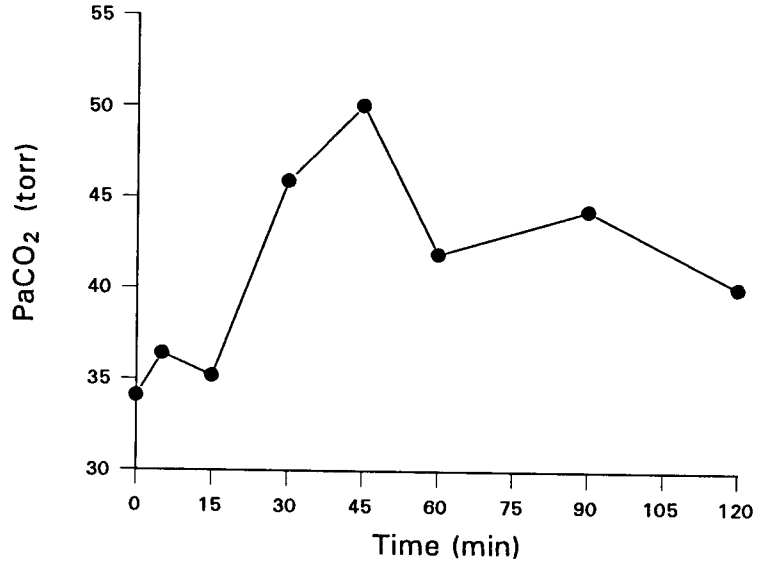
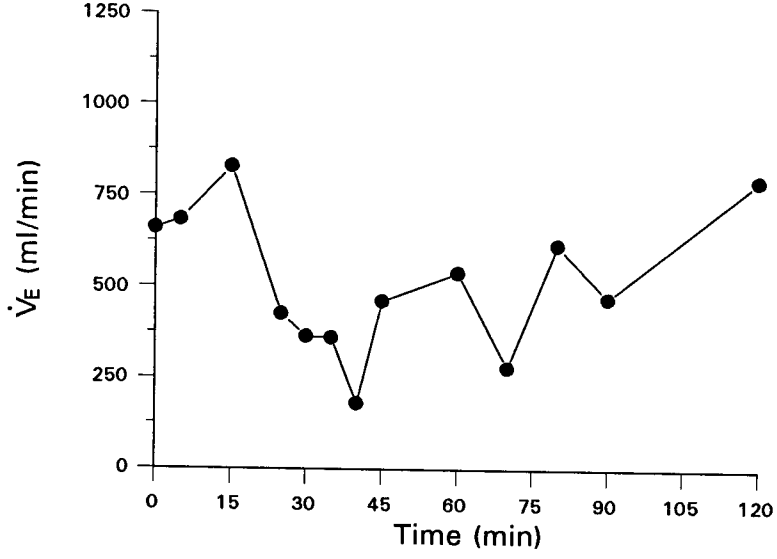

Δ PaO2 (torr)
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
0
15
30
45
60
75
90
105
120
135
Time (min)
#

Fig. 6. The changes in pHa, $\dot{V}_E$, and $PaCO_2$ measured in a single animal during infusion of 2.4 M sucrose (7.5 $mmol \cdot kg^{-1}$).


pHa
7.50
7.45
7.40
7.35
7.30
7.25
0
15
30
45
60
75
90
105
120
Time (min)


PaCO2 (torr)
55
50
45
40
35
30
0
15
30
45
60
75
90
105
120
Time (min)


V̇E (ml/min)
1250
1000
750
500
250
0
0
15
30
45
60
75
90
105
120
Time (min)

Hyposmolality

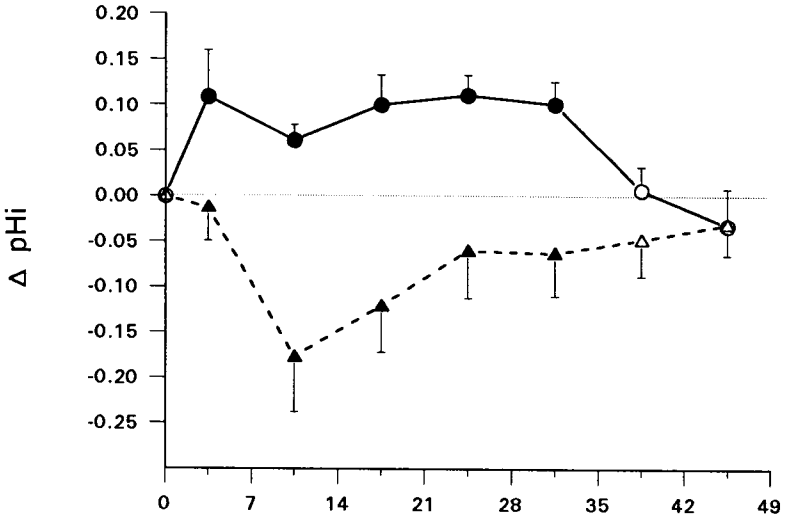
Replacement of plasma with deionized water did not elicit the changes in acid-base status caused by hyperosmolality. There was a significant decrease in plasma osmolality of 21 ± 1 mosm·kg^{-1} from the average resting osmolality of 282 ± 3 mosm·kg^{-1}. Plasma [Na^+] decreased 8 ± 2 meq·kg^{-1} while plasma [Cl^-] decreased 6 ± 2 meq·kg^{-1} ($P<0.05$). There was no significant difference between the changes in plasma [Na^+] and [Cl^-] at the end of the experiment. There was no significant effect of hyposmolality on pHa, $\dot{V}_E$, hematocrit, [K^+], red cell water content, or mean arterial blood pressure. Heart rate increased 60% by the end of the experimental period, but this change was not significant.

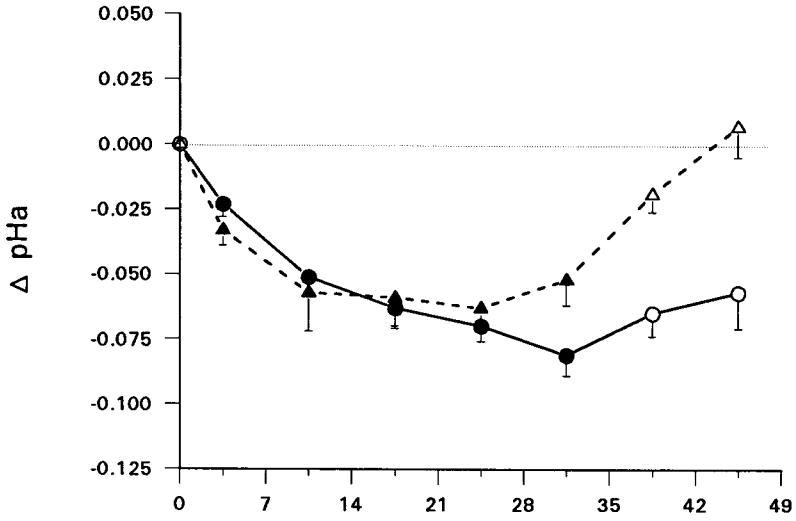
^{31}P nuclear magnetic resonance spectroscopy

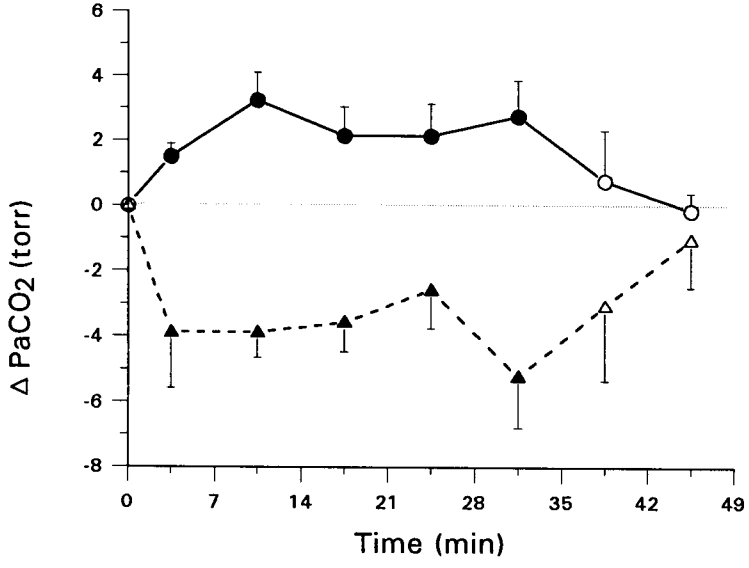
Pectoral muscle

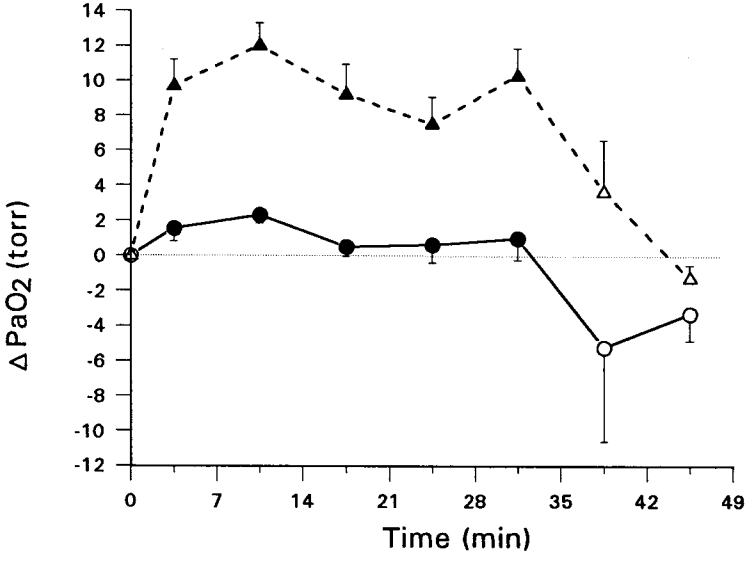
Resting, mean body temperature was 41.0 ± 0.5°C, and it did not vary by more than 0.2°C during any of the experiments. There was no significant difference in any measured variable between the sucrose or NaCl infused animals, therefore the data for the two groups were combined and analyzed together (results shown in Fig. 7). pHa decreased 0.08 ± 0.01 units from a resting value of 7.52 ± 0.04 over 35 min of hypertonic infusion ($P<0.05$), and remained significantly different from rest 15 min post-infusion. $PaCO_2$ rose 3.3 ± 0.8 torr during the infusions ($P<0.05$), and returned to resting values after 15 min of recovery. PaO_2 did not change significantly over the infusion period, but decreased 5 ± 5 torr upon cessation of the infusion and remained low during the recovery period ($P<0.05$). The large decrease in PaO_2 at 7 min post-infusion was largely due to one animal. Mean resting muscle pHi was 7.13 ± 0.04 and it increased significantly by 0.11 ± 0.02 units within the first 7 min of perfusion and remained relatively stable over the infusion period. The maximum increase in muscle pHi in individual animals ranged from 0.059 to 0.316 pH units. Post-infusion recovery was very fast, with pHi back at resting values within 7 min, and slightly overcompensated at 15 min. There was no significant

Fig. 7. Changes in pH and blood gases during hypertonic infusions (solid line) and lactic acid infusion (dotted line). Filled symbols represent the infusion period.


Δ pHi
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
0
7
14
21
28
35
42
49


Δ pHa
0.050
0.025
0.000
-0.025
-0.050
-0.075
-0.100
-0.125
0
7
14
21
28
35
42
49


Δ PaCO2 (torr)
6
4
2
0
-2
-4
-6
-8
0
7
14
21
28
35
42
49
Time (min)


Δ PaO2 (torr)
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
0
7
14
21
28
35
42
49
Time (min)

change in the relative concentrations of any of the high energy phosphate metabolites measured. There was a trend toward a relative increase in PCr, and a trend toward a relative decrease in Pi during infusion, neither of which were significant.

The time course and total decrease in pHa resulting from lactic acid infusion were not significantly different from the acidosis measured during the hypertonic infusions (Fig. 7), except that recovery from the lactacidosis following the cessation of infusion was more rapid, and was complete in 15 min (Fig. 7). $PaCO_2$ decreased 5.2 ± 1.6 torr during the infusion period ($P < 0.05$), but returned to resting levels 15 min post-infusion. PaO_2 rose 12 ± 1 torr during the infusion ($P < 0.05$) and recovered in 15 min, slightly overshooting resting values. Muscle pHi decreased significantly by 0.18 ± 0.06 pH units within 15 min of infusion and then partially recovered by 30 min and remained approximately 0.06 pH units below resting values for the duration of the infusion. The maximum decrease in muscle pHi in individual animals over the course of the infusion ranged from 0.114 to 0.370 pH units. Recovery was essentially complete by 15 min post-infusion. The resting muscle pHi, 7.16 ± 0.03, was not significantly different from the resting pHi during the hypertonic experiments. There was no significant change in any of the measured phosphate metabolites during the infusion.

Brain

Infusion of hypertonic sucrose decreased pHa 0.186 ± 0.07 units after 47.5 min of infusion ($p < 0.05$) from a resting pHa of 7.47 ± 0.05 (Fig. 8). During the recovery period, pHa decreased even further to 0.32 ± 0.07 units below the resting level. There was no significant change in $PaCO_2$ except for an increase at 17 min into the infusion. Considerable inter-animal variation was observed, with the $PaCO_2$ in one animal rising from 33.5 to 59.4 torr by the end of the infusion, peaking at 66.4 torr midway through the recovery period and then declining (data not shown). PaO_2 remained unchanged during these experiments. Mean resting brain pHi was 7.08 ± 0.02. Brain pHi did not show a

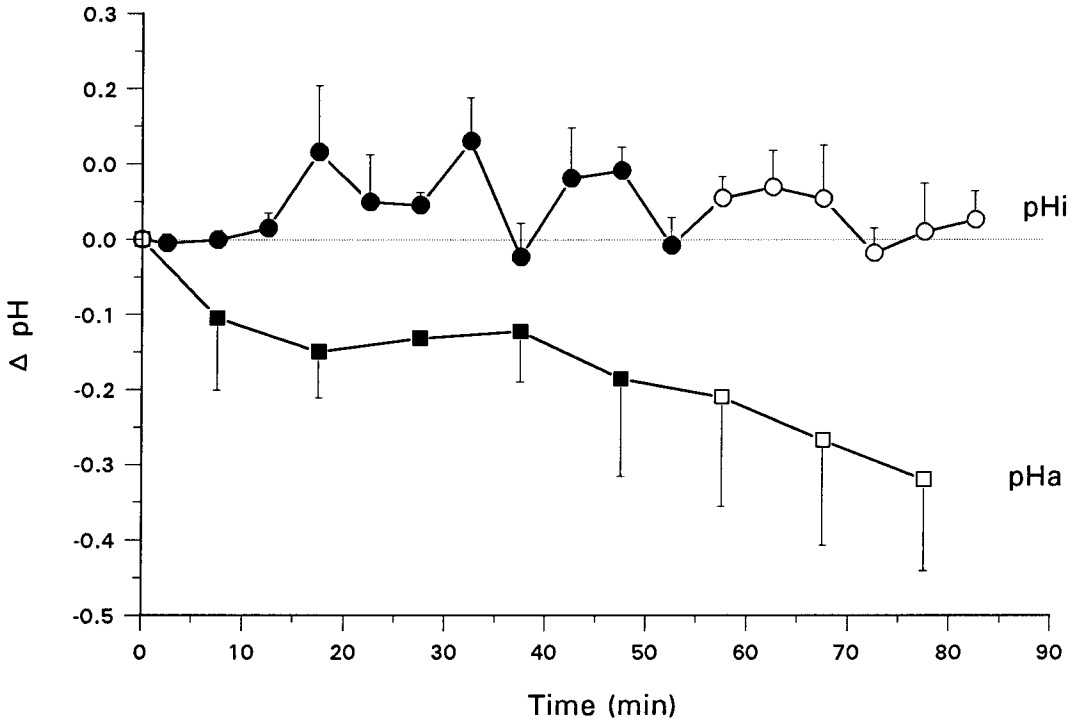


Fig. 8. Changes in arterial pH and brain pH during hypertonic sucrose infusion. Filled symbols represent the infusion period.

consistently significant change during the experiment, and was significantly increased only at 30, 35, and 50 min into the infusion period (Fig. 8). Mean brain pHi during the infusion was 7.11±0.01.

Ion-exchange blockade

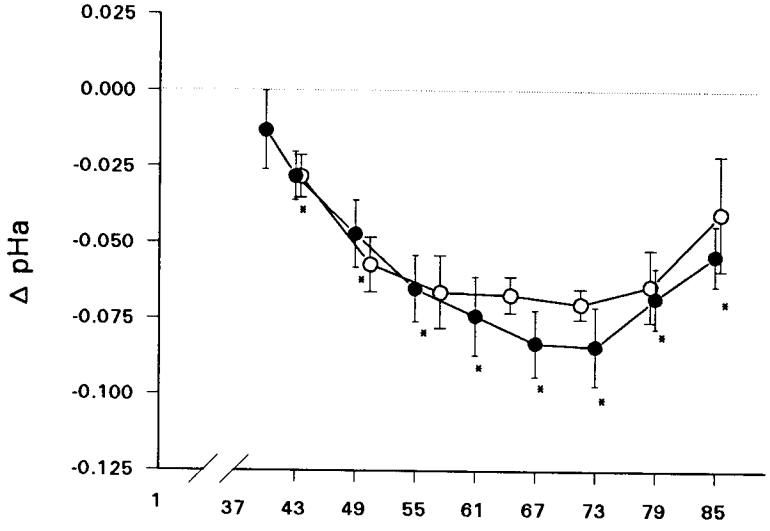
DIDS Infusion of DIDS did not significantly affect pHa or muscle pHi (Fig. 9). Resting pHa was 7.48±0.01 and resting muscle pHi was 7.14±0.01. However, $\dot{V}_E$ increased slightly upon DIDS infusion from a resting level of 326±57 to 428±73 ml·(kg·min)$^{-1}$ ($P<0.05$) (Fig. 10). Neither $PaCO_2$, PaO_2, respiratory frequency, tidal volume, plasma [Na^+], [Cl^-] nor [K^+] 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^{-1} ($P<0.05$).

Sucrose infusion caused a significant decrease in pHa to 7.39±0.02, and decreased muscle pHi to 7.06±0.03 ($P<0.05$) (Fig. 9). The decrease in pHa began immediately upon infusion, but muscle pHi was not significantly affected until 21 min into the infusion. $\dot{V}_E$ continued to increase throughout the infusion ($P<0.05$), rising to 712±157 ml·(kg·min)$^{-1}$ (Fig. 10). $PaCO_2$ did not change at any time, but PaO_2 rose significantly from 79±2 to 91±2 torr (Fig. 9). Mean arterial blood pressure continued to decrease to 212±6 mmHg, and heart rate increased a total of 62±19 beats·min^{-1} ($P<0.05$). Plasma [Cl^-] decreased significantly from 108±2 to 98±2, and plasma [Na^+] fell from 125±2 to 113±4 meq·kg^{-1} by the end of the infusion (Fig. 11). Plasma [K^+] also significantly decreased during the infusion. All variables except plasma [K^+] remained significantly different from resting levels during the recovery period.

Amiloride Infusion of amiloride significantly decreased pHa and muscle pHi (Fig. 12), and increased $\dot{V}_E$ (Fig. 10) ($P<0.05$). The resting levels of all three variables were not significantly different from those measured in the DIDS-treated animals. $PaCO_2$ decreased 7.1±1.1 torr from a resting value of 28.5±2 torr, while PaO_2 increased 10±2

Fig. 9. The effect of DIDS treatment on the pH and blood gas response to hyperosmolality. Open symbols represent animals given a sucrose load only, and are data from the ^{31}P NMR experiment on pectoral muscle shown for comparison purposes. Closed symbols represent animals given an ion-exchange blocker. The first closed symbol represents the response to the drug alone, and the last 2 closed symbols represent the recovery period.

* significantly different from resting ($P < 0.05$).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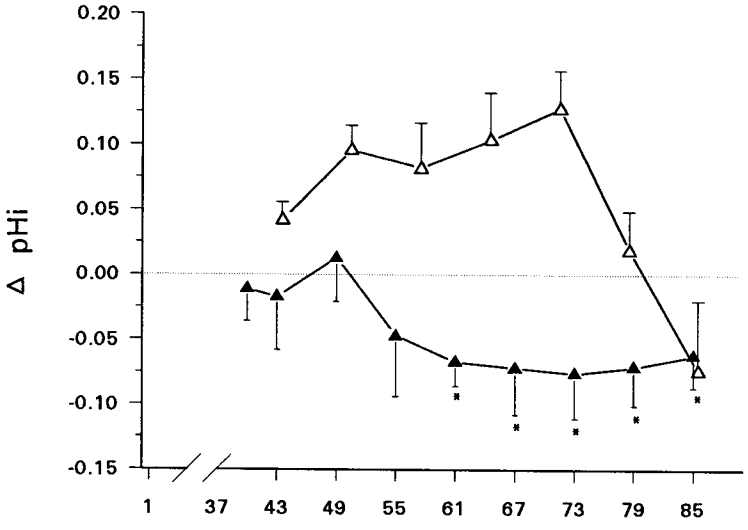
0.000
-0.025
-0.050
-0.075
-0.100
-0.125
1
37
43
49
55
61
67
73
79
85


Δ pHi
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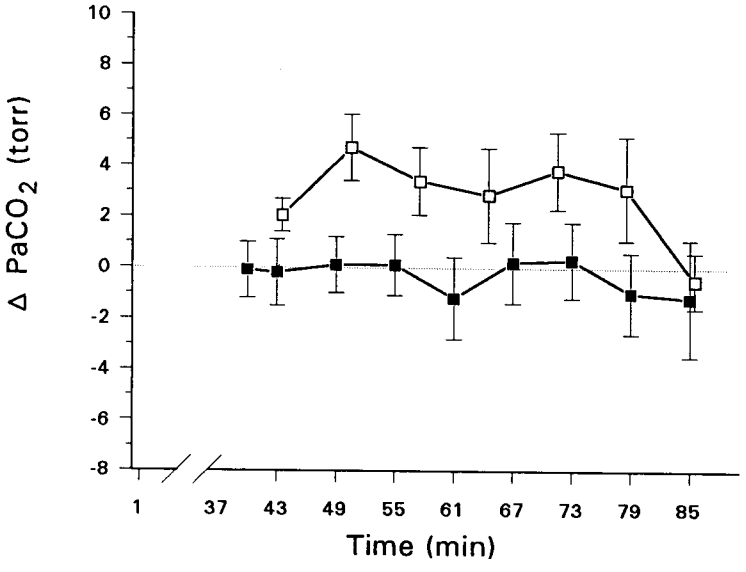
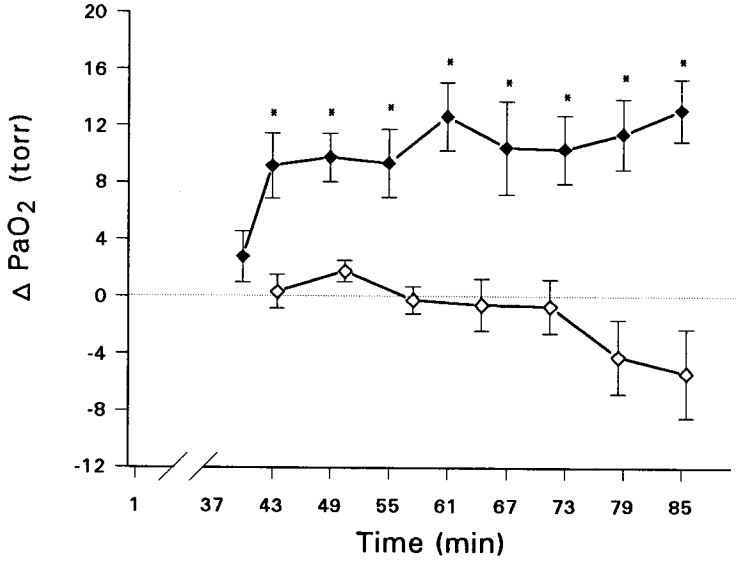
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
1
37
43
49
55
61
67
73
79
85


Δ PaCO2 (torr)
10
8
6
4
2
0
-2
-4
-6
-8
1
37
43
49
55
61
67
73
79
85
Time (min)


Δ PaO2 (torr)
20
16
12
8
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0
-4
-8
-12
1
37
43
49
55
61
67
73
79
85
Time (min)

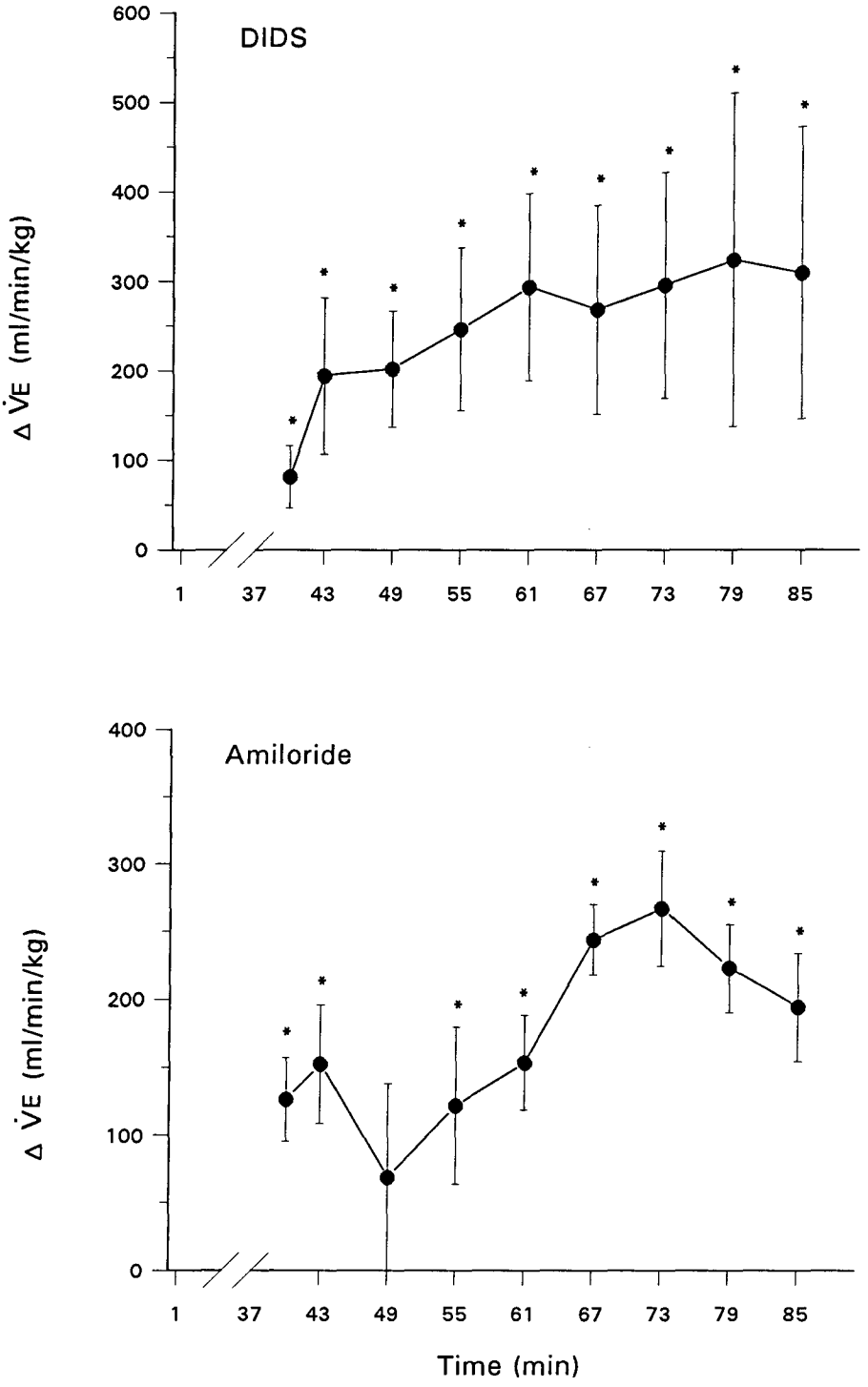


Fig. 10. Changes in ventilation in response to hyperosmolality during ion exchange blockade. The first symbol represents the response to the blocker alone, and the last two symbols represent the recovery period. * significantly different from resting.

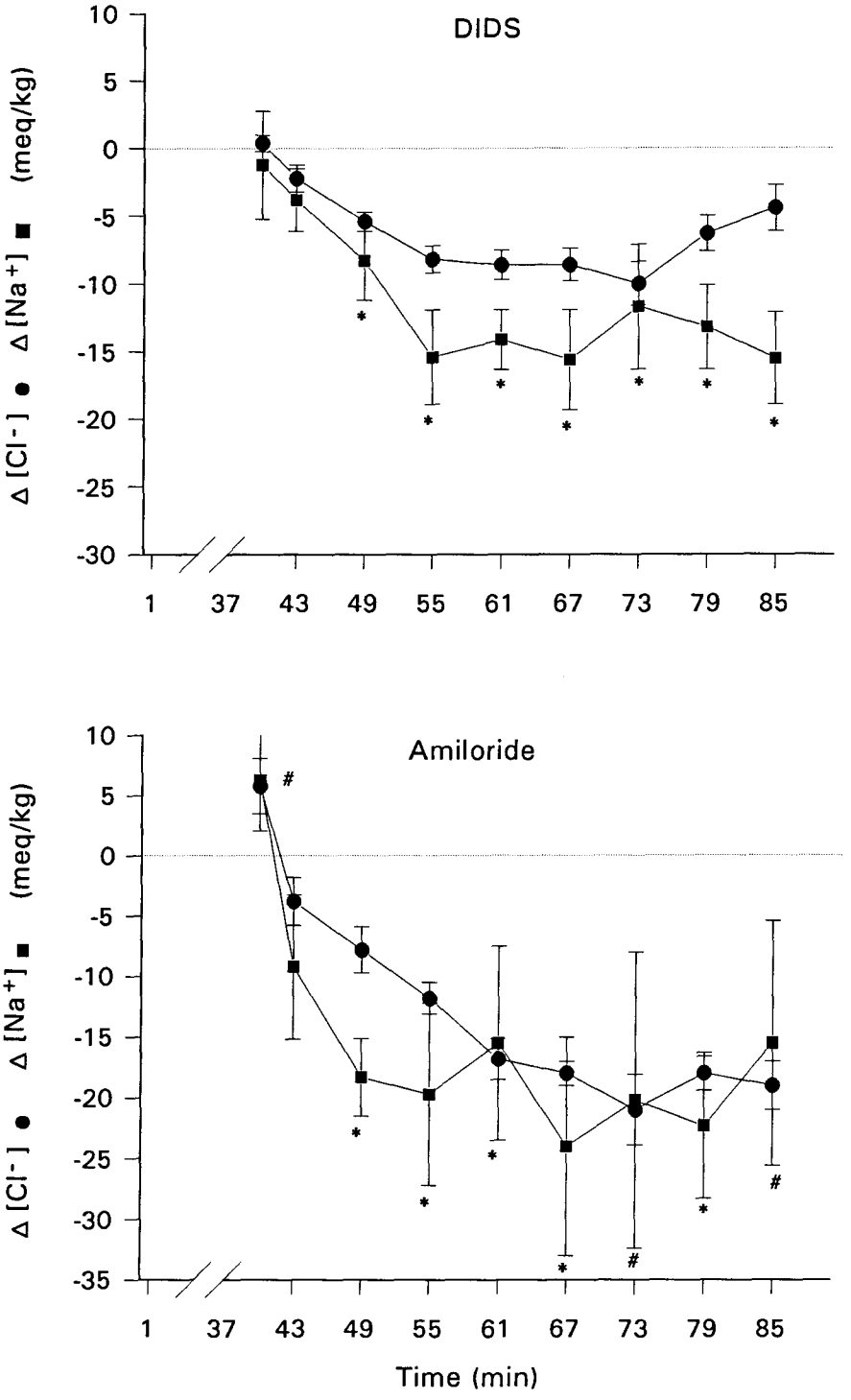
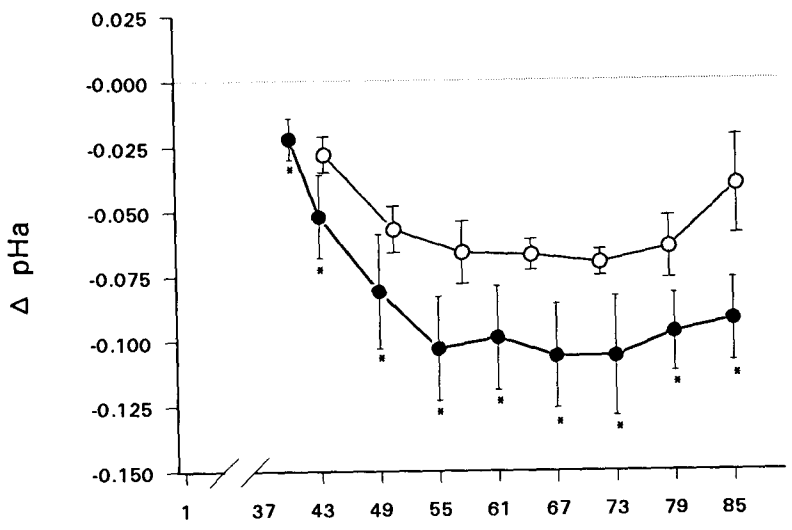


Fig. 12. Changes in plasma [Na^+] and [Cl^-] during hyperosmolality and ion exchange blockade. The first symbol represents the response to the blocker alone, and the last two symbols represent the recovery period. * points from both lines significantly different from resting. # amiloride only significantly different from resting.

Fig. 12. The effect of amiloride treatment on the pH and blood gas response to hyperosmolality. Open symbols represent animals given a sucrose load only, and are data from the ^{31}P NMR experiment on pectoral muscle shown for comparison purposes. Closed symbols represent animals given an ion-exchange blocker. The first closed symbol represents the response to the drug alone, and the last 2 closed symbols represent the recovery period.

* significantly different from resting ($P < 0.05$).


Δ pHa
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-0.000
-0.025
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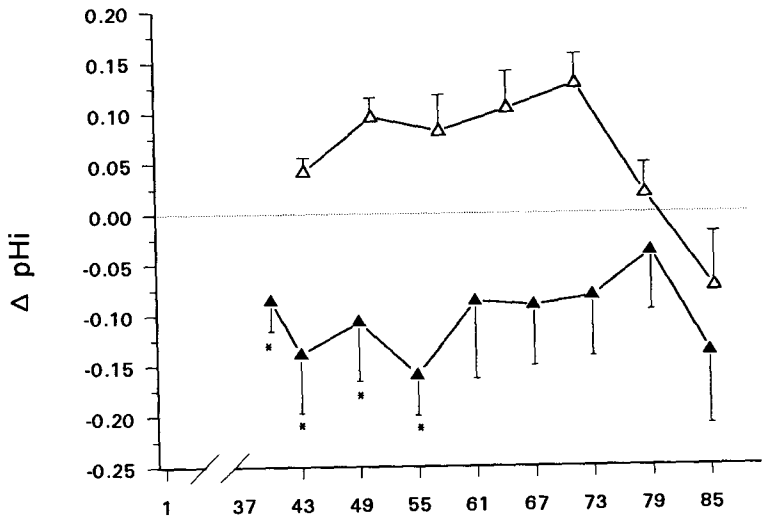
-0.150
1
37
43
49
55
61
67
73
79
85


Δ pHi
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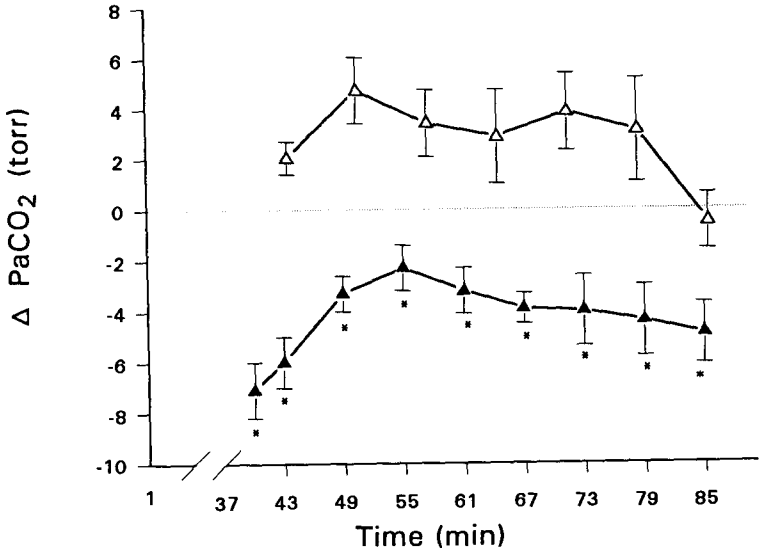
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
1
37
43
49
55
61
67
73
79
85


Δ PaCO2 (torr)
8
6
4
2
0
-2
-4
-6
-8
-10
1
37
43
49
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61
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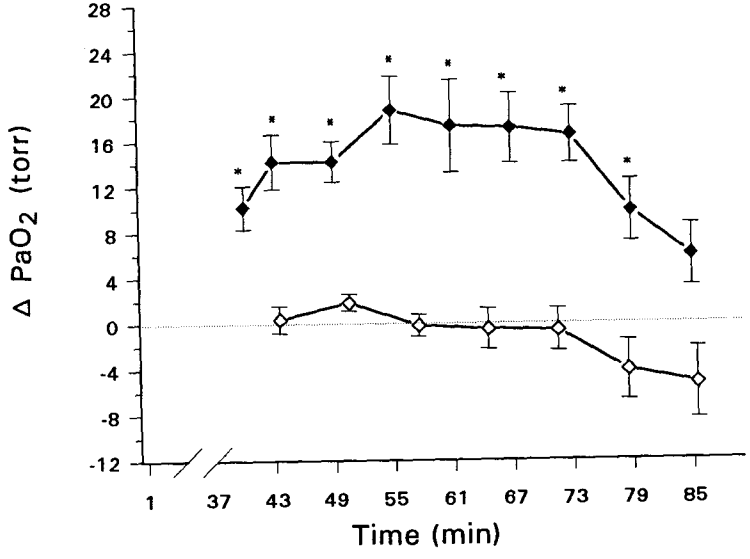
73
79
85
Time (min)


Δ PaO2 (torr)
28
24
20
16
12
8
4
0
-4
-8
-12
1
37
43
49
55
61
67
73
79
85
Time (min)

torr from 87±2 torr (Fig. 12) ($P<0.05$). Plasma [Cl^-] increased 6±2 meq·kg^{-1} ($P<0.05$), while [Na^+] also increased 6±4 meq·kg^{-1}, although this change was not significant (Fig. 11). There was no change in plasma [K^+], blood pressure or heart rate.

Sucrose infusion decreased pHa further by 0.11±0.02 pH units (Fig. 12). Muscle pHi remained low (0.16±0.04 below resting) for 21 min of sucrose infusion ($P<0.05$), but then recovered to a value (0.09±0.06 pH units below resting) not significantly different from the resting value. $\dot{V}_E$ remained high ($P<0.05$) and continued to increase during the sucrose infusion to 603±53 ml·(kg·min)$^{-1}$, except for a transient return to resting levels 9 min into the infusion (Fig. 10). The increase in $\dot{V}_E$ was primarily due to a 55% increase in tidal volume. $PaCO_2$ increased during the infusion to a maximum of 26±2 torr, but remained significantly lower than resting values (Fig. 12). PaO_2 increased further during the infusion to 106±2 torr ($P<0.05$). Plasma [Cl^-] decreased 21±3 meq·kg^{-1} ($P<0.05$) (Fig. 11), a significantly greater decrease than during DIDS treatment. Plasma [Na^+] fell 24±9 meq·kg^{-1} and plasma [K^+] also decreased significantly during the sucrose infusion, but both ions recovered to levels not significantly different from resting levels by the end of the infusion. The drop in [K^+] was not significantly different from that during DIDS treatment, and averaged 0.7 mEq. 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^{-1} over resting levels by the end of the infusion ($P<0.05$). $\dot{V}_E$, pHa, $PaCO_2$, PaO_2, and plasma [Cl^-] remained significantly different from resting values at the end of the recovery period.

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

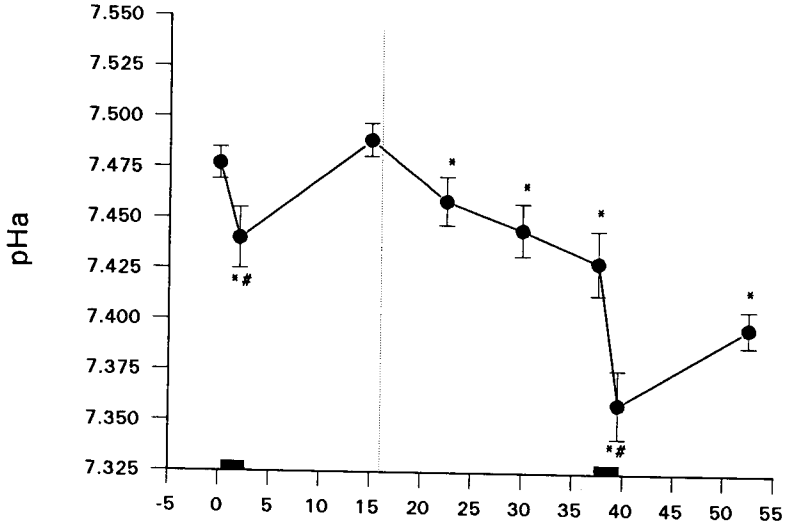
Responses to respiratory stimuli

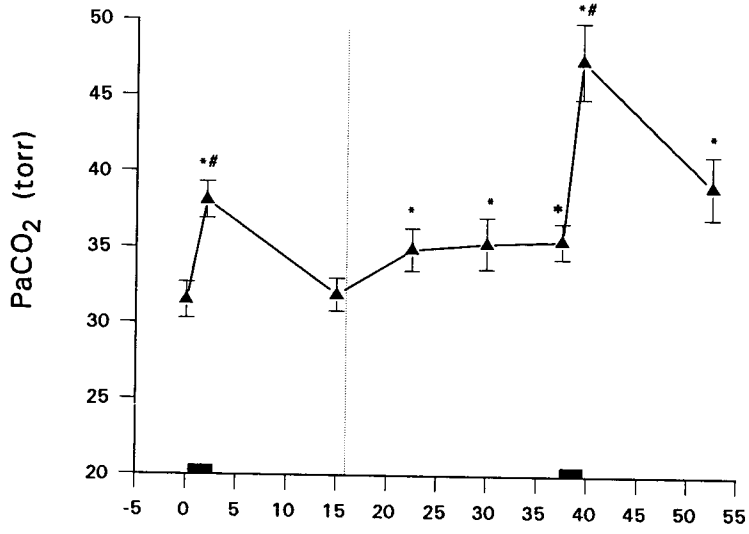
Hypercapnia Inhalation of 3.5% CO_2 decreased pHa from 7.48±0.01 to 7.44±0.01 in 2 min ($P<0.01$) (Fig. 13). $\dot{V}_E$ increased from 321±36 to 458±34 ml·(min·kg)$^{-1}$ in 1 min and to 656±61 ml·(min·kg)$^{-1}$ in 2 min ($P<0.001$). The increase in $\dot{V}_E$ was due to an increase in tidal volume as respiratory frequency did not change. $PaCO_2$ increased from 31.5±1.2 to 38.1±1.2 torr, while PaO_2 increased from 95±2 to 105±2 torr in 2 min ($P<0.001$) (Fig. 13). While F_ETCO_2 did not vary, F_ETO_2 increased significantly from 15.3±0.2% to 17.3±0.2%. Both F_ETCO_2 and F_ETO_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·(kg·min)$^{-1}$ ($P<0.025$) (Fig. 14). None of these variables were significantly different from resting values after 15 min of 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 over the experiment.

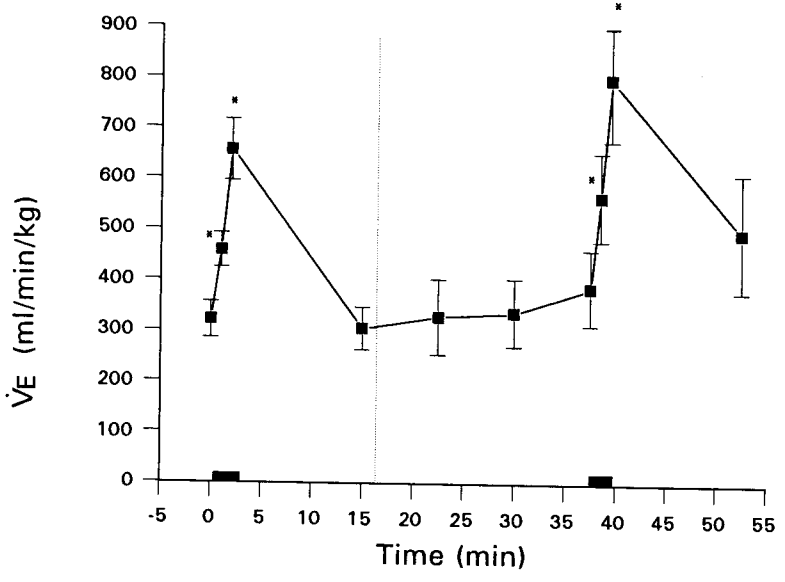
Sucrose infusion decreased pHa 0.06±0.01 units ($P<0.01$), while $PaCO_2$ increased 3.9±0.8 torr ($P<0.001$) and $\dot{V}_E$ and PaO_2 did not change over the infusion period (Fig. 13). $\dot{V}O_2$ increased to 25.2±4.9 ml O_2·(kg·min)$^{-1}$ ($P<0.025$) (Fig. 14). F_ETCO_2 rose slightly, while F_ETO_2 decreased slightly during the infusion ($P<0.05$). 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 319±6 mosm·kg^{-1}, plasma [Na^+] decreased 19±1 meq·kg^{-1} from a normosmotic value of 144±2 meq·kg^{-1}, and [Cl^-] decreased 12±1 meq·kg^{-1} from a normosmotic level of 108±5 meq·kg^{-1} (all $P<0.05$).

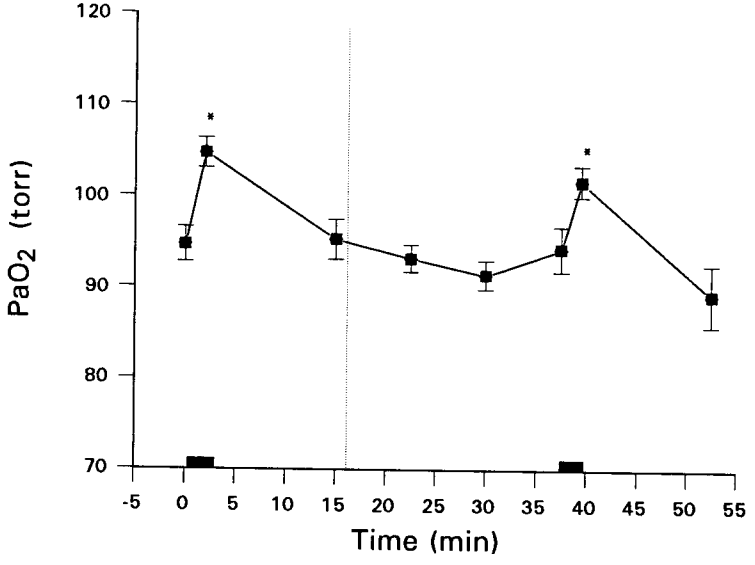
Hypercapnia during the sucrose infusion further decreased pHa from 7.42±0.01 (the value after 22.5 min of sucrose infusion) to 7.36±0.02 in 2 min ($P<0.01$), while $\dot{V}_E$ increased from 387±75 to 566±87 ml·(kg·min)$^{-1}$ in 1 min and to 799±124 in 2 min ($P<0.01$) (Fig. 13). The ventilatory responses to CO_2 in normosmotic and hyperosmotic ducks were not significantly different. However, there was an increase in the $PaCO_2$

Fig. 13. pHa, blood gas and ventilatory changes in response to hypercapnia and sucrose. Black bars represent the period of hypercapnia. The dotted line indicates the beginning of the sucrose infusion. * significantly different from resting value. # significant difference between 1st and 2nd hypercapnic period.


pHa
7.550
7.525
7.500
7.475
7.450
7.425
7.400
7.375
7.350
7.325
-5 0 5 10 15 20 25 30 35 40 45 50 55


PaCO2 (torr)
50
45
40
35
30
25
20
-5 0 5 10 15 20 25 30 35 40 45 50 55


V̇E (ml/min/kg)
900
800
700
600
500
400
300
200
100
0
-5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)


PaO2 (torr)
120
110
100
90
80
70
-5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)

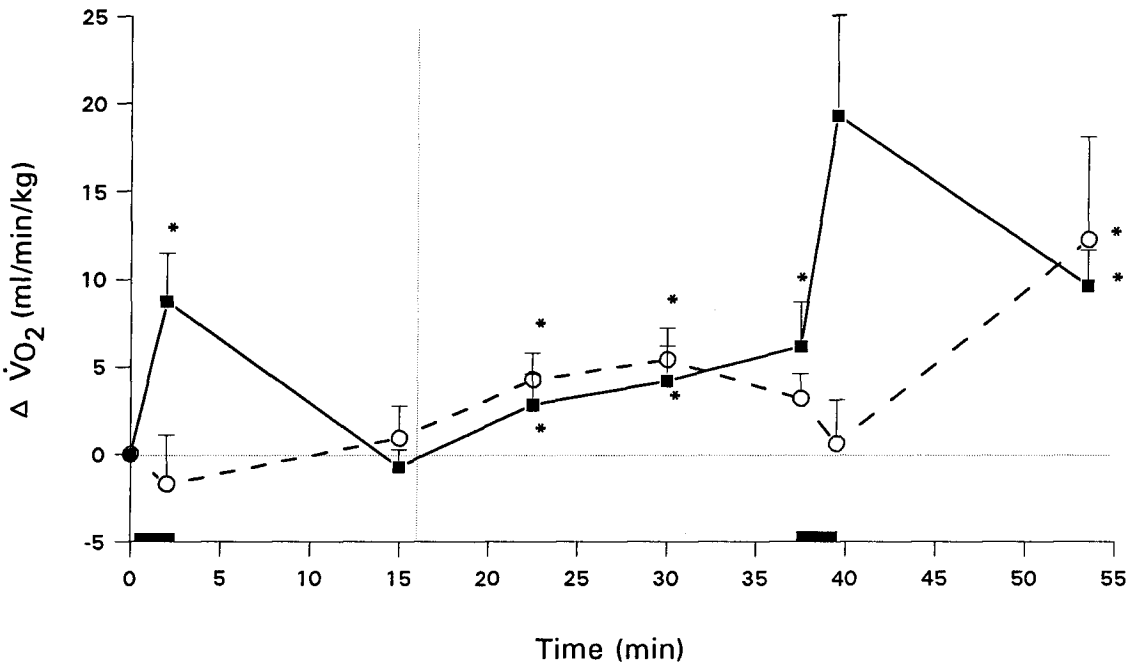


Fig. 14. Changes in $\dot{V}O_2$ associated with hypercapnia ■ and hypoxia ○ before and during hyperosmotic stress. Black bars represent the period of hypercapnia or hypoxia. The dotted line indicates the beginning of the sucrose infusion. * significantly different from rest.

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 $PaCO_2$ in hyperosmotic ducks. This is shown by the right-shifted curve of hyperosmotic animals (Fig. 15). There was, however, no change in sensitivity to $PaCO_2$ as measured by the slopes of the lines (Fig. 15). $PaCO_2$ 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 $PaCO_2$ during the normosmotic hypercapnia (Fig. 13). PaO_2 increased from 94 ± 3 to 102 ± 2 ($P < 0.025$). F_ETCO_2 did not change with respect to the F_ETCO_2 during the sucrose infusion, but was significantly increased over normosmotic F_ETCO_2 , and F_ETO_2 increased significantly from 14.7 ± 0.3 to $16.6 \pm 0.3\%$. $\dot{V}O_2$ was significantly greater than resting, normosmotic levels, but did not increase significantly from the hyperosmotic level (Fig. 14). 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, while $\dot{V}_E$, PaO_2 (Fig. 13), and F_ETO_2 returned to values not significantly different from normosmotic or hyperosmotic values. $PaCO_2$, F_ETCO_2, and $\dot{V}O_2$ remained significantly higher than normosmotic levels but not hyperosmotic levels. Heart rate remained significantly elevated over resting normosmotic levels by 94 ± 30 beats$\cdot$min^{-1} after 10 min recovery from hypercapnia. Plasma osmolality was further increased to 338 ± 6 mosm$\cdot$kg^{-1}, a total increase of 54 mosm$\cdot$kg^{-1} from resting osmolality. Plasma [Na^+] decreased to 122 ± 1 meq$\cdot$kg^{-1}, a total of 21 ± 1 meq$\cdot$kg^{-1} lower than the normosmotic level, and [Cl^-] decreased to 95 ± 4 meq$\cdot$kg^{-1}, a total of 13 ± 2 meq$\cdot$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$\cdot$kg^{-1}), was significantly elevated 10 min post hypercapnia (3.9 ± 0.4 mmol$\cdot$kg^{-1}).

Hypoxia Arterial pH was 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 levels after 15 min of

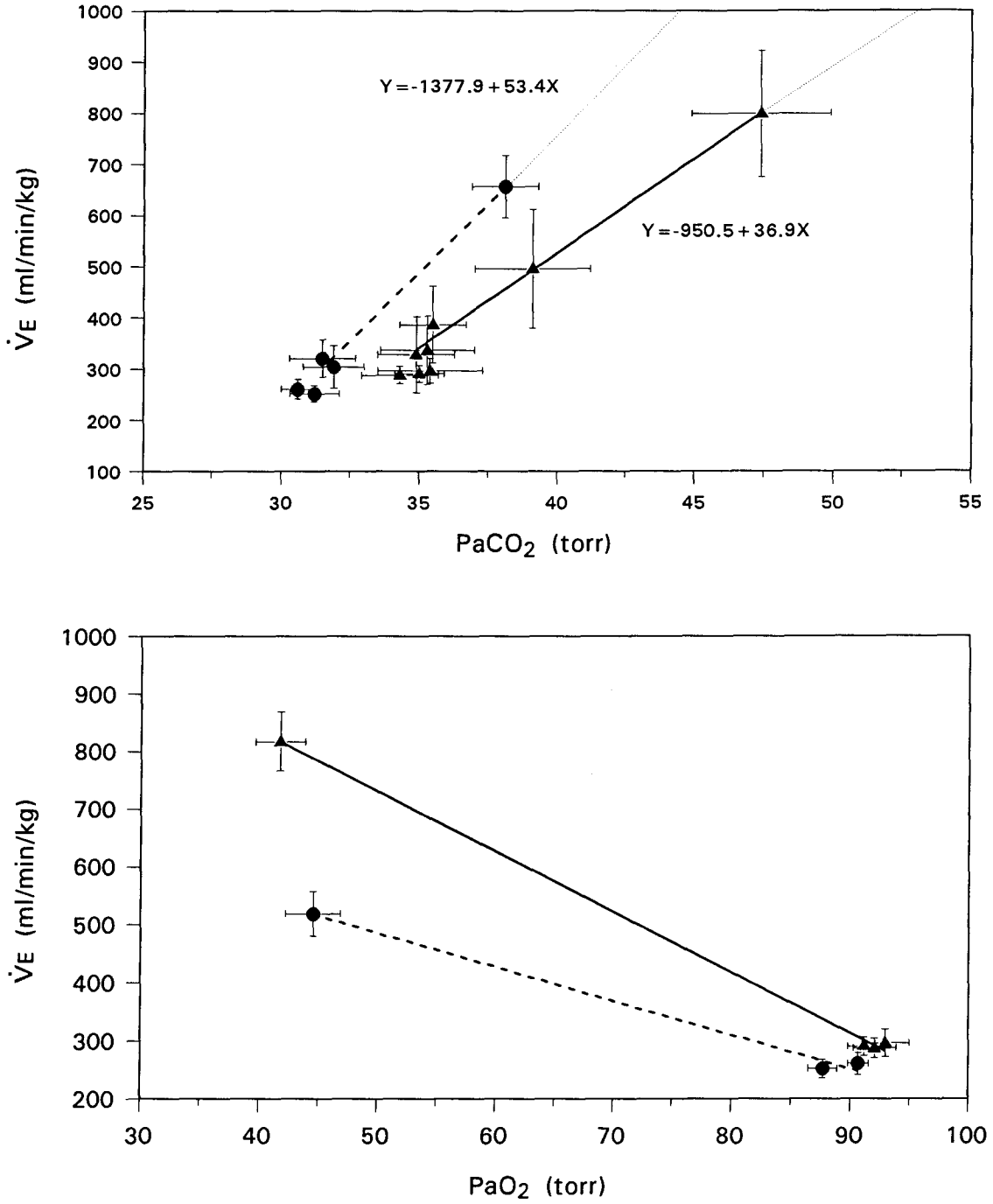


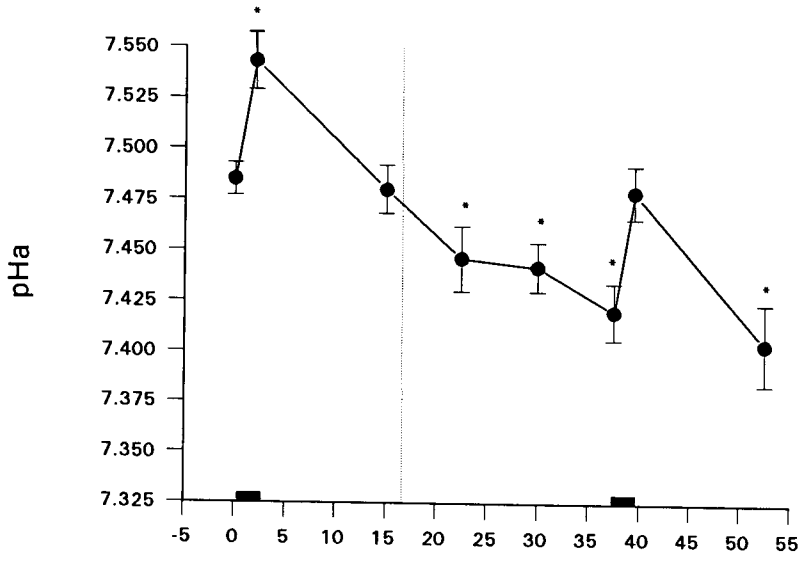
Fig. 15. CO_2 and O_2 ventilatory response curves. Dashed line represents the control ventilatory response to the gases, and the solid line represents the ventilatory response to the gases during hyperosmolality.

recovery (Fig. 16). $\dot{V}_E$ 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 resting levels within 15 min (Fig. 16). 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. $PaCO_2$ decreased from 30.6±0.6 to 27.5±1.6 torr ($P<0.05$), and PaO_2 decreased from 91±1 to 45±2 torr ($P<0.001$) after 2 min of hypoxia (Fig. 16). F_ETCO_2 declined from 5.3±0.2 to 4.5±3.1 % ($P<0.001$) after 2 min, while F_ETO_2 decreased from 15.2±0.5 to 7.2±0.4% ($P<0.001$). Heart rate increased by 16±7 beats·min^{-1} from a resting normosmotic level of 124±11 beats·min^{-1} ($P<0.05$), but $\dot{V}O_2$ (Fig. 14), plasma ions, and plasma [lactate$^-$] were not affected by hypoxia. All variables returned to resting levels within 15 min except PaO_2, which was depressed by 3.0±1.1 torr. Mean arterial blood pressure was not affected at any time during the experiment.

Sucrose infusion caused a 0.06±0.01 unit decrease in pHa ($P<0.01$), with no significant increase in $\dot{V}_E$ or PaO_2 (Fig. 16). $PaCO_2$ increased from 31.2±0.9 to 35.4±1.9 torr ($P<0.001$). F_ETCO_2 rose slightly ($P<0.05$) while F_ETO_2 decreased ($P<0.025$). $\dot{V}O_2$ increased significantly from a resting normosmotic level of 16.1±1.2 ml O_2·(kg·min)$^{-1}$ to a maximum of 20.6±1.9 ml O_2·(kg·min)$^{-1}$ (Fig. 14). Heart rate rose from 123±11 to 169±21 beats·min^{-1} ($P<0.05$). Plasma osmolality increased 31±3 mosm·kg^{-1} after 22.5 min of infusion from a normosmotic level of 287±3 mosm·kg^{-1}, [Na^+] decreased 18±1 meq·kg^{-1} from a value of 144±1 meq·kg^{-1}, and [Cl^-] decreased 10±1 meq·kg^{-1} from a normosmotic concentration of 106±1 meq·kg^{-1} (all $P<0.05$). Plasma [K^+] and [lactate$^-$] remained constant throughout the experiment.

In the second hypoxic period, pHa significantly increased back to resting normosmotic levels. This pH change was identical to that in the first hypoxic episode. The increase in $\dot{V}_E$, though, was double that of the increase during the control hypoxia (Fig. 16). This is reflected in a 7.3±1.4 torr decrease in $PaCO_2$ from the hyperosmotic

Fig. 16. pHa, blood gas and ventilatory changes in response to hypoxia and sucrose. Black bars represent the period of hypoxia. The dotted line indicates the beginning of the sucrose infusion. * significantly different from resting value. # significant difference between 1st and 2nd hypoxic period.


pHa
7.550
7.525
7.500
7.475
7.450
7.425
7.400
7.375
7.350
7.325
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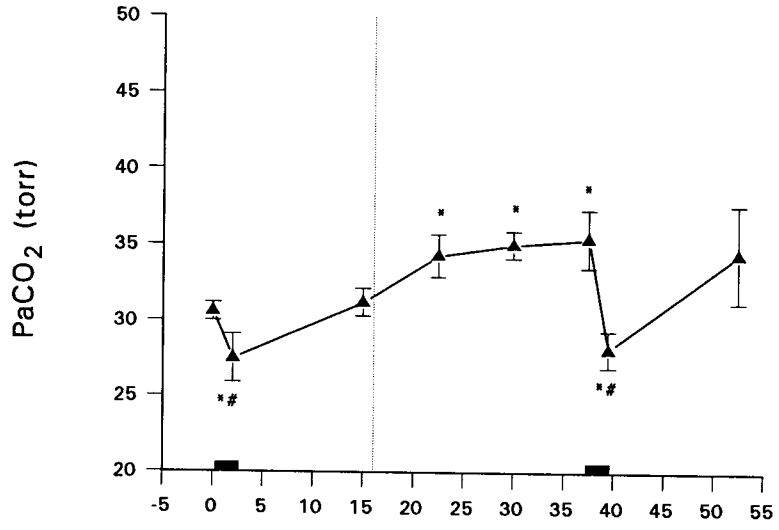
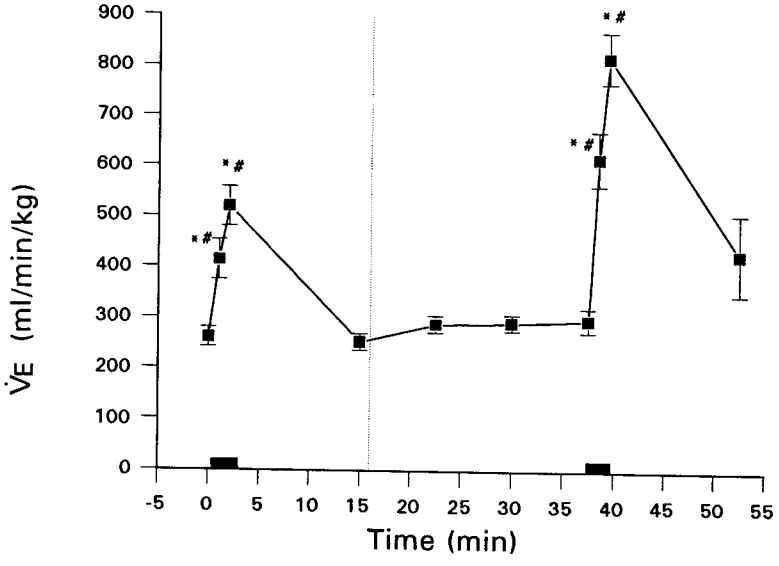
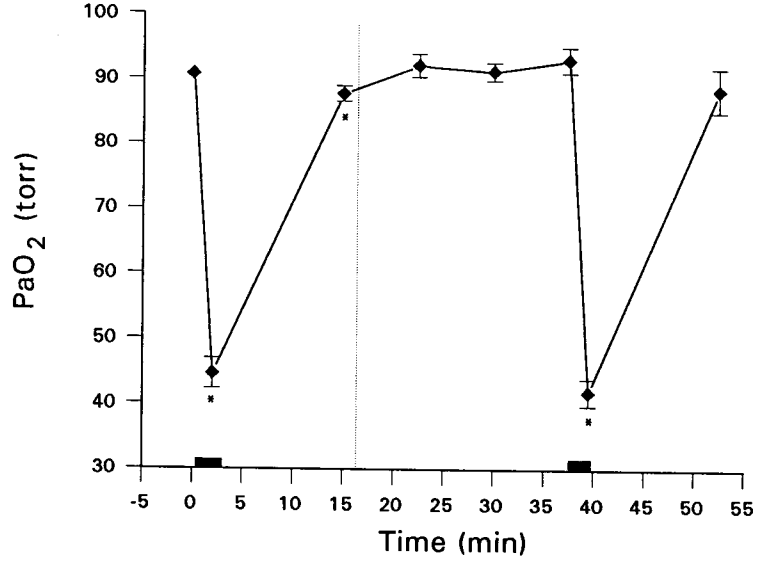

PaCO2 (torr)
50
45
40
35
30
25
20
-5 0 5 10 15 20 25 30 35 40 45 50 55


V̇E (ml/min/kg)
900
800
700
600
500
400
300
200
100
0
-5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)


PaO2 (torr)
100
90
80
70
60
50
40
30
-5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)

level ($P<0.001$), a significantly larger decrease compared to the decrease during the normosmotic hypoxia. However, the fall in PaO_2 to 41.8 ± 2.1 torr) was not significantly different from that in normosmotic ducks during hypoxia. Thus, there was an increase in the magnitude of the ventilatory response relative to the decrease in PaO_2, shown by the upward-shifted point representing hyperosmotic hypoxic animals (Fig. 15). 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, a significantly larger response than during the normosmotic hypoxia. Respiratory frequency rose 28% the first minute and 42% the second minute, a response not significantly different from the hypoxic response in normosmotic ducks. While $F_{ET}O_2$ decreased to exactly the same level as in the first hypoxic bout ($P<0.001$), $F_{ET}CO_2$ and $\dot{V}O_2$ were not affected. 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, $\dot{V}_E$, $PaCO_2$, PaO_2, $F_{ET}CO_2$, $F_{ET}O_2$ and $\dot{V}O_2$ had returned to levels not significantly different from either resting normosmotic or hyperosmotic levels, while heart rate and plasma lactate levels remained significantly higher than resting normosmotic values but not the hyperosmotic values. pHa had further declined to 7.40 ± 0.02 ($P<0.01$), osmotic pressure had increased 55 ± 4 mosm·kg^{-1}, final plasma [Na^+] had decreased 21 ± 1, and plasma [Cl^-] 13 ± 1 meq·kg^{-1} (all $P<0.05$).

Potassium A K^+ bolus in resting, normosmotic animals increased tidal volume significantly for 3 breaths, at which point it returned to a volume not significantly different from resting levels (Fig. 17). 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. 17). The K^+ bolus did not significantly affect inter-breath interval, heart rate or mean arterial blood pressure, while neither K^+ added to the sucrose infusion nor the bolus of

150 mM NaCl had any significant effects on respiratory or cardiovascular variables at any time.

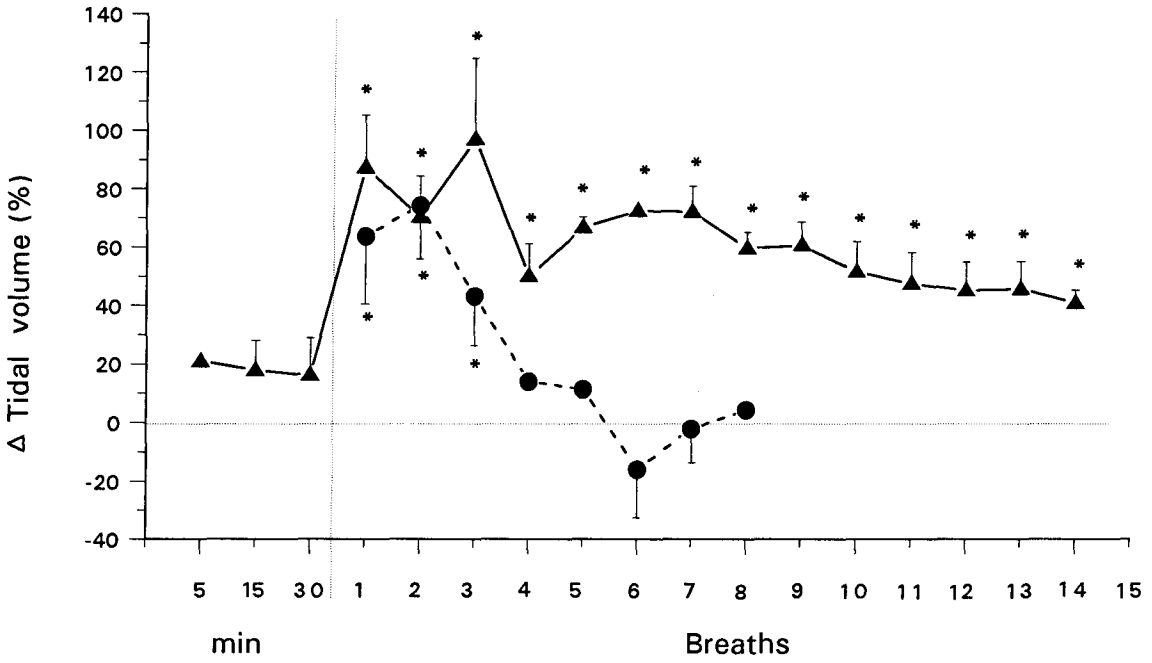


Fig. 17. The respiratory response to bolus K^+infusion. The percent change in tidal volume from a resting level is shown breath-by-breath immediately post K^+ infusion in control animals (dotted line). In hyperosmotic animals, the percent change in tidal volume from a resting level is shown first during 30 min of sucrose infusion, and then the ventilatory response to a K^+ bolus is shown on a breath-by-breath basis.
* significantly different from resting value.

DISCUSSION

Physiological responses to acute changes in osmolality

Analysis of acid-base status Calculated plasma bicarbonate apparently did not change significantly during the hypertonic infusions, but since the pK of carbonic acid is altered with ionic strength, these calculated bicarbonate values may be incorrect. Unfortunately, it is not possible to calculate with any accuracy the changes in pK during the hypertonic infusions with the available data. However, the pK should increase with NaCl infusion and decrease with sucrose infusion, which would cause opposing changes in calculated bicarbonate in spite of very similar changes in pHa and $PaCO_2$ during the two protocols. It was therefore felt that Stewart's approach to acid-base balance (Stewart, 1983) could provide a useful analysis of the available data and insight into the cause of the extracellular acidosis. According to Stewart, in salt solutions such as blood and cerebrospinal fluid (CSF), $[H^+]$, $[OH^-]$, and $[HCO_3^-]$ are dependent variables whose concentrations are determined by the three independent variables of the solution, which are the strong ion difference ([SID]), total weak acid concentration, and PCO_2. Despite inherent technical and conceptual problems, this analysis of acid-base balance can offer an informative approach to acid-base disturbances associated with large changes in electrolyte concentrations. The only significant change in the measured independent variables in this study in the first 45 min was a mean decrease of 5.8 $meq \cdot kg^{-1}$ in estimated [SID] during hyperosmolality (Table I). This decrease was almost entirely due to excess Cl^- in the extracellular fluid, as has been measured in mammals (Sotos *et al.*, 1962, Makoff *et al.*, 1970; Anderson and Jennings, 1988). The decrease in [SID] was independent of whether plasma $[Na^+]$ and $[Cl^-]$ were increased (NaCl infusion) or decreased (sucrose infusion). There is no doubt that this estimate of [SID] contains error, but the change in [SID] is larger than the possible error, and it does account for most of the measured pH change. Total weak acid concentration (protein) was not measured, but since plasma protein

concentration in ducks is very low, any change in protein concentration would be minor compared to the change in [SID]. There was a significant increase in $PaCO_2$ which occurred during all of the treatments. The rise in $PaCO_2$ in the control animals was smaller and occurred 45 min later than in the other animals, and did not measurably affect pHa. It is possible that the increase in $PaCO_2$ was due to a decrease in parabronchial ventilation during the relatively long restraint period.

The cellular mechanism underlying the hyperchloremia during dilution acidosis is not known. Hyperosmolality presumably causes ion transport alterations which decrease the cation:anion ratio, resulting in acidemia. The acidosis is independent of whether the hyperosmolarity is produced with ionic or non-ionic substances. Based on the most recent studies listed below, the term dilution acidosis is an inappropriate description of the acidosis caused by hyperosmolarity since neither dilution nor expansion of the extracellular space alone accounts for the observed acidosis. Hyposmolality with or without volume expansion has no measurable effect on pHa (Chang *et al.*, 1975; see RESULTS, *Physiological responses to acute changes in osmolality*), and volume contraction alkalosis occurring in association with chloride deficiency can be corrected by replacing Cl^- without restoring volume (Luke and Galla, 1983). Further studies of the ion transport effects of hyperosmolality will be required in order to understand the nature of the acidosis.

Constancy of plasma [K^+] Plasma [K^+] did not decrease significantly when extracellular fluid volume was expanded with either hypertonic solution, indicating that total K^+ increased slightly extracellularly (see Table II). The outward K^+ shift is simultaneous with the decrease in pHa (Makoff *et al.*, 1970; see RESULTS, *Physiological responses to acute changes in osmolality*), and may be a passive readjustment to maintain a normal [K^+]i/[K^+]e ratio. Other studies (Makoff *et al.*, 1970; Wathen *et al.*, 1982) have reported very large increases in extracellular [K^+] with hypertonic saline and mannitol infusions, although the loads infused were significantly greater than in this study.

Such a K^+ flux could change membrane potential in the direction of hyperpolarization, although the small magnitude of this efflux would probably render any membrane potential change physiologically insignificant. Two times normal tonicity leads to an alkalinization of about 0.1 pH units (Whalley *et al.*, 1991) and generally causes a small hyperpolarization of 4-5 mV in muscle (Parker and Zhu, 1987; Whalley *et al.*, 1991; Yamada, 1970). Hypertonicity does in fact reduce skeletal muscle contraction, although it is hypothesized to be the result of osmotic distortion of cell structure and not hyperpolarization (Parker and Zhu, 1987; Bruton, 1991). Such evidence suggests that membrane potential changes due to K^+ flux are not significant during hyperosmotic stress. However, if this flux occurred in the carotid bodies, because the cells are small and have a high membrane resistance (Duchen *et al.*, 1988), the opening of only a few K^+ channels could significantly affect membrane potential (Lynch and Barry, 1989). This remains to be investigated.

Respiratory compensation When evaluating the ventilatory response to an acidosis, the acid-base status of the extracellular fluid, intracellular fluid and the CSF (including brain interstitial fluid) must all be considered for their effect on chemoreceptor activity. In respiratory acidosis, arterial, CSF and intracellular pH all decrease (see Roos and Boron, 1981) and $\dot{V}_E$ increases significantly. During systemic metabolic acidosis, the resultant hyperventilation of spontaneously breathing animals can change CSF pH paradoxically because of the high permeability of the blood-CSF barrier to CO_2 and its relatively low permeability to ions (Robin *et al.*, 1958). Systemic pHe and pHi are decreased, but CSF pH and brain pHi may not be, resulting in some attenuation of the ventilatory response. During dilution acidosis, a concomitant, systemic, intracellular alkalosis may develop (see Fig. 7; Adler *et al.*, 1975; Makoff *et al.*, 1970). Potential changes to CSF pH and brain pHi are unknown, but there is clearly no accompanying, compensatory increase in ventilation. This is a novel observation, and while I am unable

to determine the reason for the lack of respiratory compensation from this first study, two possibilities are worthy of discussion.

The first possible explanation is that stimulation of respiration may be due solely to central stimulation of chemoreceptors, and there simply may have been a lack of CSF pH change and, therefore, no central chemoreceptor stimulation during the dilution acidosis. However, in ducks, as in mammals, peripheral chemoreceptor contribution to acute ventilatory changes is approximately 20-40% (Milsom *et al.*, 1981). Infusion of lactic acid in Pekin ducks, causing a systemic acidosis comparable to that seen in the present study, immediately increased $\dot{V}_E$ 225% (Jones and Shimizu, unpubl.). Thus, the significant systemic acidosis caused by hyperosmolality should also have increased ventilation, even if central chemoreceptors were not stimulated.

A second possibility is that both peripheral and central chemoreceptors were stimulated by pHe changes, but there was concurrent development of a condition that inhibited normal chemoreceptor stimulation. This possibility would be valid even if only peripheral receptors were affected. A significant difference between respiratory/metabolic acidosis and dilution acidosis in mammals, is the potential development of intracellular alkalosis during the latter perturbation. Evidence for a supression of ventilation by hyperosmolality is best supported by the response of three ducks to the infusion of sucrose, as reported in the results of the present study. In the most extreme case, neither a decrease in pHa of 0.15 units nor a 16 torr increase in $PaCO_2$ was capable of stimulating respiration either peripherally or centrally under the hyperosmolal conditions, and $\dot{V}_E$ actually decreased (Fig. 6). In ducks subjected to a period of submergence that resulted in a decrease in pHa of 0.2 pH units and an increase in $PaCO_2$ from 32-57 Torr, $\dot{V}_E$ increased 350% upon emergence (Shimizu and Jones, 1987). In this case, both systemic and central pHe and pHi would be decreased. Manipulation of a potentially concurrent extracellular acidosis and intracellular alkalosis with respect to ventilation is done in this study.

The effect of osmotic changes on chemoreceptor activity has been investigated only briefly. In cats, carotid body chemoreceptor activity increased when perfused *in vivo* with hyposmotic blood and decreased when perfused with blood made hyperosmotic with sucrose or NaCl (Gallego and Belmonte, 1979). The minimal osmolality variation necessary to obtain a detectable frequency change was 3-8% of the control. A 10% increase in osmolality decreased frequency about 20%, with generally greater reductions obtained with sucrose. However, superfused carotid bodies *in vitro* responded in exactly the opposite manner, with the authors concluding that the modifications in chemoreceptor activity *in vivo* were produced by changes in carotid body blood flow due to a direct effect of hypo- and hyperosmotic solutions on vascular muscle tone. However, this does not explain the *in vitro* results, unless presumably, the proposed cardiovascular effects *in vivo* were much greater than the direct effects on the receptors. In the present study, plasma osmolality was increased 9-11% and decreased 7% of resting values. Ventilatory depression also tended to be slightly greater during hyperosmotic periods due to sucrose than to NaCl in this study. I did not observe any change in $\dot{V}_E$ during hyposmolality, and a depression in $\dot{V}_E$ in only a few animals during hyperosmolarity. It is possible that the stimulation of ventilation by the extracellular acidosis is approximately offset by a depression of ventilation due to an intracellular alkalosis during hyperosmolality, resulting in no ventilatory change. Gallego and Belmonte's protocol could easily have allowed development of an intracellular alkalosis without extracellular acidosis, since the carotid bodies were continuously flushed with fresh, hyperosmotic solution. Further work is required to fully explain these results. Increases in blood osmolality in ducks by 10 mOsm (less than one-fifth of the osmotic change in this study) by either NaCl or mannitol also caused a decrease in intrapulmonary chemoreceptor discharge frequencies at all levels of $PaCO_2$ (Adamson, 1984). The depressive effect became much larger at higher CO_2 levels. Fedde *et al.* (1982) have shown that intrapulmonary chemoreceptors in ducks are sensitive to venous blood changes, so consequently, these receptors could respond to

changes in osmolality of the blood. Hypertonic infusions have also been shown to inhibit thermal panting in mammals (Baker and Dawson, 1985), which again points to inhibitory effects of hyperosmolality on ventilation.

Summary Analysis of the acid-base changes during hyperosmolality indicates that while there is no significant change in calculated [HCO_3^-], extracellular [Cl^-] is greater than predicted. The significance of this with respect to development of the acidosis is as yet unknown. The lack of respiratory compensation to the acidosis suggests a suppression of the predicted increase in chemoreceptor activity that normally occurs in response to an extracellular acidosis. It is hypothesized that an intracellular alkalosis develops at the same time as the extracellular acidosis which may affect the ventilatory response.

^{31}P NMR of muscle and brain

The mean resting pectoral muscle pHi measured by ^{31}P NMR (7.14 ± 0.04; $n=19$) is in excellent agreement with the pHi measured previously in ducks in the same muscle (7.17; Stephenson and Jones, 1993), and within the range of mammalian skeletal muscle pHi. The mean resting brain pHi (7.08 ± 0.02) is also similar to values measured by ^{31}P NMR in rats (Adler *et al.*, 1990; Barrere *et al.*, 1990). In calculating pHi, no attempt was made to correct for any possible changes in intracellular ionic strength. Roberts *et al.* (1981) have pointed out that changes in intracellular ion concentrations within usual physiological limits can significantly shift the Pi titration curve. Therefore, chemical shift could be altered by changes in intracellular conditions other than pH. Intracellular ionic strength may have been altered in response to the protocol because of the shift of intracellular water to the extracellular space and/or because of the selective addition of membrane permeable ions to the extracellular space. Osmolality and ion concentrations were not measured in this study, but identical doses have been used in other experiments in this thesis, and result in an increase in osmolality of 55 mOsm. Changes in pHi in response to hypertonicity measured with ^{14}C-labeled 5,5-dimethyl-2,4-oxazolidinedione in

rat diaphragm muscle *in vitro* (Adler *et al.*, 1975), and in erythrocytes of anesthetized dogs *in vivo* (Makoff *et al.*, 1970), were very similar during increases in osmolality comparable with those used in this experiment. The alkalinization in tissues *in vitro* appears to plateau at 0.1-0.15 pH units well before tonicity is doubled (Whalley *et al.*, 1991). Therefore, it seems likely that any changes in ionic strength induced by the protocol had a negligible effect on Pi chemical shift.

Peripheral chemoreception The unique aspect of dilution acidosis is the lack of respiratory compensation. While ventilation was not directly measured in the NMR experiments, the previous study in this thesis (see RESULTS, *Physiological responses to acute changes in osmolality*) showed that blood gases are reliable indicators of ventilatory alterations under these experimental conditions. The changes in PaO_2 and $PaCO_2$ measured in this study (see RESULTS, *^{31}P nuclear magnetic resonance spectroscopy*) are comparable to those measured in the previous study (see RESULTS, *Physiological responses to acute changes in osmolality*). Lactacidosis, typified by a decrease in both pHa and pHi, stimulated ventilation, whereas dilution acidosis, characterized by a comparable decrease in pHa but an increase in pHi, did not stimulate a ventilatory increase. The data suggest that a decrease in pHi is required for an increase in ventilation in response to a change in pHa or $PaCO_2$, therefore supporting a hypothesis that intracellular changes are necessary for chemoreception. The pHi changes measured here were not directly measured in the carotid bodies, as mentioned previously, but since these organs are well perfused and, by nature of their function, sensitive to changes in blood chemistry, it seems reasonable to make the assumption that the carotid body cells also responded to the osmotic changes and became alkalotic. In addition, the buffering capacity of muscle is very high while the buffering capacity of carotid body tissue is very low (Wilding *et al.*, 1992), meaning that any pHi change would be even more pronounced in the carotid body than in muscle.

As discussed previously (pp. 61-62), the few investigations that have been published do support the suppression of chemoreceptor discharge and $\dot{V}_E$ during

hyperosmolality. How hyperosmotic stress could affect chemoreceptor discharge is not clear. As many sensory receptors behave like mechanoreceptors, it is possible that the carotid chemoreceptors could be responding simply to a distortion due to the movement of water. However, Lahiri (1977) has measured changes in carotid chemoreceptor discharge *in vivo* during intravenous hyperosmotic challenges, and concluded that because of the lag time, they were not due to a mechanoreceptor response, but rather to some unidentified, intermediate step. Several studies have shown that increasing the osmolality of the medium bathing isolated muscle or nervous tissue causes intracellular alkalization, hyperpolarization and decreased electrical discharge (Adler *et al.*, 1975; Abercrombie and Roos, 1983; Whalley *et al.*, 1991), but, as previously discussed (pp. 58-59), the hyperpolarization is small and the significance is unclear. Depolarization and increased discharge during hyperosmolality have been measured in superfused, whole carotid body *in vitro* (Gallego *et al.*, 1979; Gallego and Eyzaguirre, 1976). When the acid-base disturbance is due to addition of an acid or base, intracellular alkalization in general is associated with hyperpolarization, decreased neurotransmitter release and depressed nervous discharge, including in carotid body glomus (Type 1) cells (Rigual *et al.*, 1984; Eyzaguirre *et al.*, 1989; He *et al.*, 1991). There is at present no explanation for the disparity in results with superfused *in vitro* carotid bodies. It is interesting to note that during recovery, pHi returned to resting levels while pHa was still significantly acidotic. However, ventilation as indicated by blood gases did not increase then in response to the arterial acidosis, suggesting that if internal alkalinization did inhibit chemotransduction, it did so in a non-reversible or slowly reversible manner. Since hyperosmolality has so many known (and probably unknown) effects on cells, it is difficult to hypothesize which effect is responsible.

Central chemoreception. Ventilation is controlled by both peripheral and central chemoreceptors, and in ducks, as in mammals, central receptors control 60-80% of an acute ventilatory response to changes in $PaCO_2$ (Milsom *et al.*, 1981). There is still

considerable controversy over whether systemic, metabolic acid-base disturbances cross the blood-brain barrier to any meaningful degree, although little work has been done on pH disturbances due to osmotic imbalance. Cserr *et al.* (1991) have shown that acute, systemic, hyperosmotic stress affects rat brain compartments selectively, decreasing brain extracellular volume and ion content, while the brain intracellular compartment maintains its water content and gains electrolytes, indicating a degree of volume regulation. The osmolality change and the time course of the experiment were comparable to that followed in this study. Although 3 of the 11 scans taken during the hyperosmotic infusion showed a significant alkalosis, there was no sustained significant increase in brain pHi found in this study. The mean brain pHi during the sucrose infusion was 7.11 ± 0.02, alkalotic compared with the resting pHi. Although controversial, there may be, as Cserr's work suggests, sufficient cell volume regulation to prevent any significant intracellular acid-base disturbance (see Macknight *et al.*, 1992), or, alternatively, pHi changes may be masked. The brain pHi monitored during this experiment was by necessity a global one since the size of the coil resulted in sampling from most of the brain tissue. The brain is heterogeneous in cell type, and it is likely that cell pH is altered differently in different brain regions or cell groups.

While $PaCO_2$ usually increases about 4-5 torr during an hyperosmotic episode, it has been noted that about 25% of animals subjected to hyperosmotic stress experience extremely depressed ventilation and large pHa and blood gas changes, as observed in the study of the physiological responses to acute changes in osmolality in this thesis. There was also considerable inter-animal variation in $PaCO_2$ in the brain study, with one animal having an increase in $PaCO_2$ of 26.5 torr near the end of the infusion. Since CO_2 is freely permeable across the blood-brain barrier, this would normally stimulate central receptors to initiate a ventilatory increase. A ventilatory increase did not occur since PaO_2 at this time was 27 torr lower than resting levels. If central receptors were unaffected by the hyperosmolality (as the brain study suggests), and were responding to

this increase in CO_2, there must have been an opposing ventilatory depression arising from some other source, presumably the peripheral receptors. Although I could not measure brain pH during lactic acid infusion, recent work has shown that a systemic lactacidosis of 0.36 pH units over 54 min did not alter brain pHi as measured by ^{31}P NMR (Adler *et al.*, 1990), indicating that the primary ventilatory drive during lactacidosis in this thesis was peripheral.

Metabolism Normally, the intracellular compartment is well buffered against extracellular pH variations, with pHi changes being only a fraction of the extracellular change. In lactacidosis, the large drop in muscle pHi was transitory, with the acidosis thereafter being relatively small. This was not the case during hyperosmotic stress. Zeidler and Kim (1977) have provided evidence that during osmotic perturbation, the intracellular alkalinization causes structural instability of the cellular membrane band 3 protein, which is associated with anion transport. Such a transport disturbance might inhibit recovery in the short term.

Perturbations in pHi of the magnitude measured in muscle in this study, particularly during hyperosmolality, could have a significant effect on metabolism (see Somero, 1986). Although there were no changes in the relative concentrations of Pi, PCr or ATP, proton concentration significantly decreased. Assuming that the creatine kinase reaction is in equilibrium, then the relative concentration of ADP presumably increased, and since ADP is considered to be a regulator of metabolism, this suggests that cellular respiration may have increased during hyperosmolality. This conclusion is supported by the increase in metabolism as measured by heat production reported in hyperosmotically stressed muscle in aerobic conditions (Yamada, 1970). However, the metabolic response to pHi perturbations are not always consistent (Chance and Conrad, 1959; Nioka *et al.*, 1987).

Summary It appears that brain pHi is little affected by acute systemic hyperosmolality, but that the peripheral intracellular compartment undergoes a significant

alkalosis during extracellular dilution acidosis. Exogenously produced lactacidosis results in a decrease in systemic pHi. The data suggest that intracellular pH may play a role in initiating ventilatory changes since normal respiratory compensation to the extracellular acidosis is prevented during hyperosmolality but not during lactacidosis. It is suggested that there is a ventilatory depression generated peripherally during acute systemic hyperosmotic stress.

^{31}P NMR of pectoral muscle during ion-exchange blockade

Dosage 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. Assuming that the distribution of DIDS is primarily extracellular and that the extracellular space of ducks is about 25% body weight (Ruch and Hughes, 1975), then the DIDS concentration in the extracellular fluid would have been about 1.2 $mmol \cdot l^{-1}$, or 1000 times the Ki (concentration resulting in 50% inhibition of ion transport) for DIDS in erythrocytes (1.2 $umol \cdot l^{-1}$). This concentration is similar to that used in some other studies (Deng and Johanson, 1989; Javaheri *et al.*, 1984), but is generally higher than that used in most CSF studies (Nattie and Adams, 1988; Nishimura *et al.*, 1988). DIDS is irreversible and would have remained effective over the 85 min protocol.

The dosage of amiloride is similar to that used by other investigators (Altenberg *et al.*, 1989; Obika, 1989). Using the same assumptions about extracellular space and distribution described above, then the amiloride concentration in the extracellular fluid would have been about 0.15 $mmol \cdot l^{-1}$. Amiloride is an effective inhibitor of the Na^+/H^+ antiporter at a concentration of 0.1-1 mM when extracellular $[Na^+]$ is in the physiological range (Fitzgerald *et al.*, 1990). The action of amiloride is immediate but reversible, and it is possible that the slight diuresis caused by sucrose infusion may have resulted in an effective reduction in the amiloride concentration. While this may have occurred, it would be somewhat offset by the improved effectiveness of amiloride due to the reduction

in extracellular [Na^+] (Mahnensmith and Aronson, 1985) caused by the sucrose infusion. Since pHi decreased due to amiloride and did not recover during the experiment, this suggests that the amiloride was effectively present throughout the study.

Control of ventilation Infusion of either DIDS or amiloride caused a decrease in both pHa and pHi, and an increase in $\dot{V}_E$. It has previously been reported that the ventilatory response to CO_2 after intracerebroventricular DIDS infusion is significantly enhanced (Adams and Johnson, 1988; Nattie and Adams, 1988). Extracellular pH regulation (and therefore presumably intracellular as well) is considered to be less effective after DIDS treatment (Nattie and Adams, 1988; Ahmad and Loeschcke, 1983), although no study has directly shown that the enhanced ventilatory responsiveness is due to the altered pH regulation. The results of this study strongly support that latter conclusion. The change in pHi, which normally becomes alkalotic during extracellular hyperosmolality, was reversed after DIDS treatment, while $\dot{V}_E$, which normally does not increase in response to the extracellular acidosis during hyperosmolality, increased significantly after DIDS treatment. Essentially the same is true for amiloride treatment under these conditions, although Fitzgerald *et al.* (1990) have shown that amiloride has no effect on the response of chemoreceptor discharge to hypercapnia (but significantly depressed the response to hypoxia). This may indicate that Cl^-/HCO_3^- exchange is of primary importance in pH regulation during hypercarbia.

The contribution of pHi to the ventilatory response is illustrated in Fig. 18. The slope of the ventilatory response during hyperosmolality is clearly different from the response when pHa and pHi are coupled. The results of this study however, suggest that a decrease in pHi is not essential for chemoreceptor stimulation, since $\dot{V}_E$ significantly increased in DIDS-treated animals when pHa was acidotic but pHi had not yet changed (Figs. 9 and 10). In amiloride-treated animals, pHi began to recover while pHa continued to decrease, yet $\dot{V}_E$ continued to increase. It is also interesting that drugs that inhibit the Na^+-dependent H^+-extruding systems involved in cell pH recovery, also inhibit dopamine

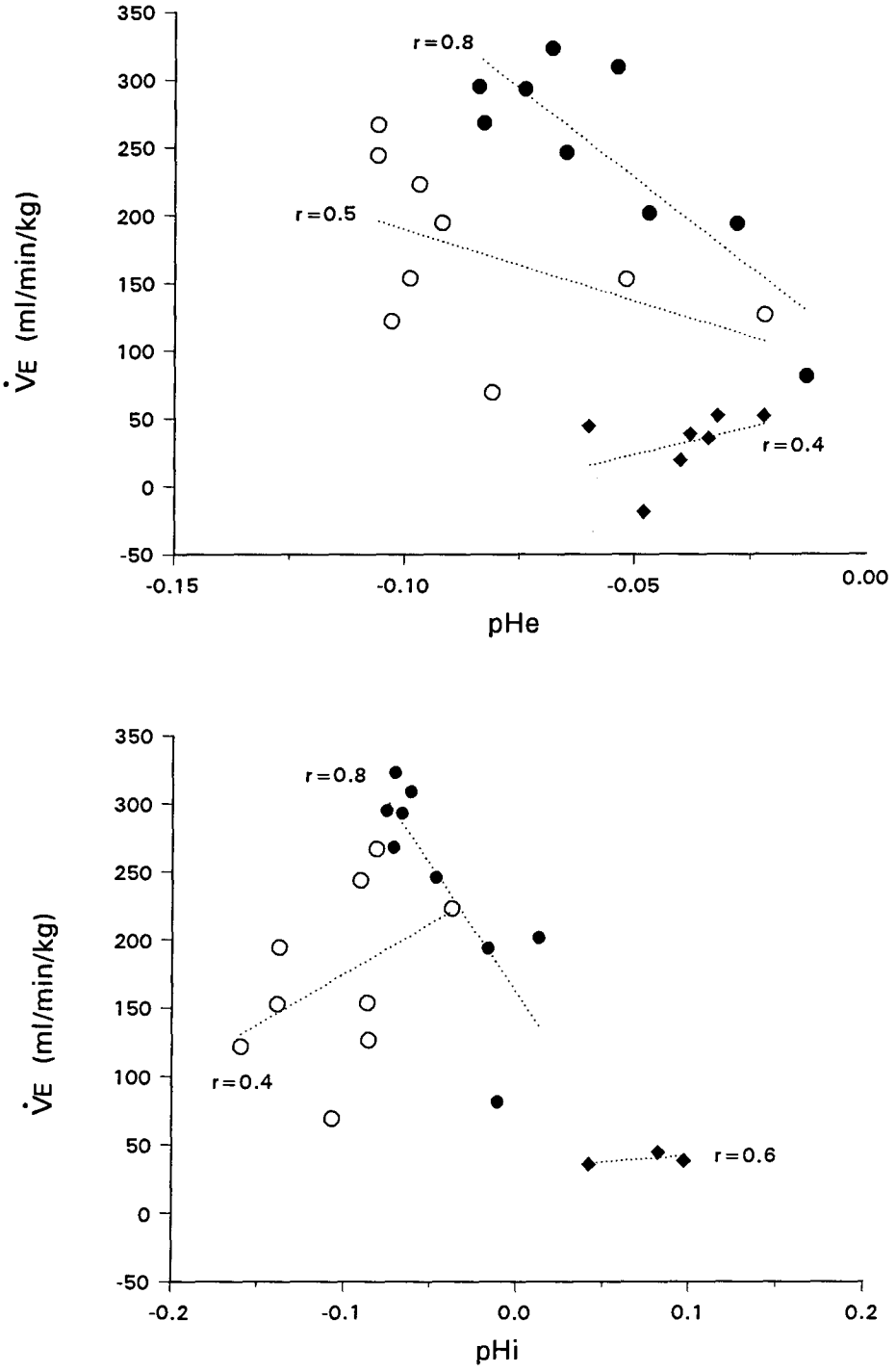


Fig. 18. The relationship between ventilation and intra- and extracellular pH during hyperosmolality. Filled circles represent DIDS-treated animals; open circles represent amiloride-treated animals; diamonds represent hyperosmolality alone. Lines are regressions through the points.

release (Rocher *et al.*, 1991), which should suppress nervous discharge and ventilatory increases. Since there was a substantial ventilatory increase with sucrose infusion in the amiloride-treated animals in this study, dopamine release might posibly have been augmented by hyperosmolality under these conditions despite the recovering pHi. Therefore, a drop in extracellular pH alone is apparently sufficient to stimulate chemoreceptors to increase ventilation. However, chemoreceptors do appear to respond to pHi, since an intracellular alkalosis under otherwise similar conditions suppresses the ventilatory increase. A role of pHi in control of chemoreceptor function has been proposed before (Hanson *et al.*, 1981; Adler *et al.*, 1975; Lassen, 1990), but to date there has been no evidence to either support or negate this hypothesis. There was an increase in $\dot{V}_E$ with DIDS infusion alone which occurred without any significant change in pHa or pHi in this study (Figs. 9 and 10). This has also been observed by Adams and Johnson (1988) and is somewhat confusing. Presumably this response is mediated by the chemoreceptors, but the mechanism is unknown. The concentration of DIDS alone in this protocol did not disrupt pH regulation enough under resting control conditions to cause a measurable change in pH, but was sufficient to alter regulation during an acid-base/respiratory challenge.

It is interesting to note the changes in blood gases during both treatments and during hyperosmolality alone. $PaCO_2$ always increased significantly during hyperosmolality by about 4 torr (Figs. 5 and 15). This could occur if reverse Cl^-/HCO_3^- exchange (Cl^- efflux, HCO_3^- influx) was augmented, which would inhibit CO_2 hydration intracellularly, thus increasing $PaCO_2$, or it may be due to the increased metabolic rate during hyperosmolality. Amiloride-treated animals showed a significant drop in $PaCO_2$ consistent with increased ventilation. $PaCO_2$ did not decrease in DIDS-treated animals despite the large increase in ventilation because the inhibition of Cl^-/HCO_3^- exchange would inhibit CO_2 hydration.

Ion exchange in single cells During *in vivo* hyperosmotic stress, calculations showed that extracellular [Na^+] is not decreased beyond what is accounted for by dilution due to the sucrose infusion and intracellular water flux, while extracellular [Cl^-] was significantly higher than expected (Makoff *et al.*, 1970; Anderson and Jennings, 1988; see RESULTS, *Physiological responses to acute changes in osmolality*). This resulted in a decrease in [SID] and suggested the stimulation of the Cl^-/HCO_3^- exchanger following hyperosmotic shrinkage. However, much of the *in vitro* work done on osmotic activation of ion exchange implicates the stimulation of Na^+/H^+ exchange by hyperosmotic stress as the primary ion regulating mechanism through increased intracellular proton affinity and decreased intracellular Na^+ affinity (Green *et al.*, 1988; Green and Muallem, 1989; Whalley *et al.*, 1991). Furthermore, most studies in volume regulating cells indicate that osmotic shrinkage generally leads to net Na^+ and Cl^- uptake (see Hoffmann and Simonsen, 1989), the latter being completely blockable by DIDS or SITS (4-acetamido-4'-isothiocynao-2,2'-stilbene disulfonic acid). In contrast, Abercrombie & Roos (1983) measured significantly lower Cl^- activity in hypertonic frog muscle, implying a net Cl^- efflux. Zeidler and Kim (1977) have also shown that calf and cow red cells exposed to hypertonic media undergo a net loss of Cl^- accompanied by an intracellular alkalinization which was completely inhibited by SITS. DIDS was very effective in preventing intracellular alkalinization due to sucrose infusion in this study, although there was a prominent time lag, and it also equalized the dilution of Na^+ and Cl^- (Table II). However, the decrease in extracellular [Cl^-] was even less than during the same osmotic load alone, the opposite of what would be predicted. This suggests that Cl^- was still coming out of the cell by the remaining functional Cl^-/HCO_3^- exchange plus some other transport mechanism(s). There was also some Na^+ efflux (extracellular [Na^+] is about 8 mEq higher than predicted), but no K^+ efflux. The extracellular dilution of K^+ suggests that the small intracellular hyperpolarization that normally occurs during hyperosmolality due to K^+ efflux would not have developed in the hyperosmotic, DIDS-treated animals.

This could be a factor in the ventilatory increase observed in the latter animals, as well as in amiloride-treated animals.

The results of the pH regulation in response to hyperosmolality are made more difficult to interpret because of the significant fall in pHi due to amiloride (also observed by Whalley *et al.*, 1991). However, pHi remained low in response to hyperosmotic stress after amiloride, suggesting a role for Na^+/H^+ exchange in the alkalinization process normally accompanying cell shrinkage. It is curious, however, that extracellular [Na^+] is not higher than in animals treated only with sucrose (Table II). In addition, because pH_i returned to values not significantly different from resting levels after 21 min of sucrose infusion, it is apparent that one or more additional exchange mechanisms are also involved. Amiloride also had the surprising effect of reducing Cl^- efflux (or increasing Cl^- influx), so that the Na^+ and Cl^- were equally diluted.

While 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 to other studies since 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 too complicated to identify at this whole animal level. Further single cell experiments monitoring all the major ions are required.

Total CO_2 The significant decrease in total CO_2, combined with the significant increase in $PaCO_2$ that always accompanies acute hyperosmolality, indicates that the HCO_3^- concentration is decreasing during the osmotic challenge. This confirms without doubt that the acid-base disturbance associated with acute hyperosmolality is not simply a respiratory acidosis, and again implies that Cl^-/HCO_3^- exchange may play a primary role in this perturbation.

Summary Since both DIDS and amiloride had the same effect on the normal response to hyperosmolality (both drugs reversed the intracellular alkalosis to an intracellular acidosis, and did not prevent the extracellular acidosis), it indicates that both

Table II. Decreases in electrolytes ($meq \cdot kg^{-1}$) during acute hyperosmolality alone and hyperosmolality after various treatments. Data are shown as the changes from resting levels after approximately 30 min of sucrose infusion. Amount of sucrose infused per kg animal is identical in each study except the first, where the load infused was smaller. See text for details.

	Δ Cl^-	Δ Na^+	Δ K^+
Sucrose	11.4±2.1	16.1±2.0	0.13±0.16
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
Sucrose (after hypercapnia)	13.6±1.6	21.4±1.1	0.20±0.17
Sucrose (after hypoxia)	12.9±0.8	20.9±1.1	0.16±0.37

ion exchange mechanisms are crucial to the complete perturbatory effects of hyperosmolality. However, the unusual changes in the measured extracellular ion concentrations suggests that the ion exchange disruptions are complex. The data strongly suggest that intracellular pH plays a role in initiating ventilatory changes since normal respiratory compensation to the extracellular acidosis is prevented when the intracellular milieu is alkalotic but initiated when the intracellular milieu is acidotic or homeostatic.

Responses to respiratory stimuli

As the previous studies have shown, the extracellular acidosis induced by hyperosmolality did not stimulate an increase in ventilation. This phenomenon has led to the hypothesis that the intracellular contraction alkalosis that develops systemically concurrent to the dilution acidosis supresses 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. 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 suggests that there are minimal effects on the brain intracellular compartment (Cserr *et al.*, 1991; this thesis). 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 periperally and centrally mediated, while the response to hypoxia and K^+ is essentially solely under peripheral control.

Hypercapnia 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. 17. The absolute $\dot{V}_E$ values in the

present study are somewhat lower than in the above studies, but the slopes of the control (normosmotic) CO_2 response curves 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 $PaCO_2$ at which here is a significant increase in $\dot{V}E$ from rest), and also slightly decreased the sensitivity to CO_2 (Fig. 16). Such a displacement of the CO_2 curve explains why there is no ventilatory response to the approximately 4 torr increase in $PaCO_2$ generated during the hyperosmotic infusion itself.

Compared with normosmotic hypercapnic animals, depressed chemoreceptor discharge has been observed in intrapulmonary chemoreceptors in ducks during saline or mannitol-induced hyperosmolality at all levels of $PaCO_2$, with the effect increasing as the level of inspired CO_2 was raised (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 inabilty to maintain an adequate respiratory pattern. Depressed ventilatory responses to inspired CO_2 have also been observed in ducks after injection with acetazolamide (Andersen and Hustvedt, 1967; Powell *et al.*, 1978), although it is not clear how hyperosmolality could affect carbonic anhydrase.

Acute hyperosmolality has not been shown to have any consistent, significant effect on the brain intracellular compartment (Cserr *et al.*, 1991; this thesis). 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. 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 are consistent with this conclusion, although it can not account for the lack of ventilatory increase to the extreme elevations of $PaCO_2$ seen in some ducks (see RESULTS, *Physiological responses to acute changes in osmolality*, and *^{31}P nuclear magnetic resonance spectroscopy* (*Brain*)). These cases suggest that there is also some depression of the peripheral drive during hyperosmolality, particularly if the central chemoreceptor threshold for CO_2 is normally lower than that of the peripheral chemoreceptors (Lahiri *et al.*, 1978). Potentially, the systemic intracellular alkalosis that develops during acute hyperosmolality may suppress chemoreceptor discharge. If central chemoreceptors respond to CO_2 via changes in $[H^+]$, as is accepted both generally (see Milsom, 1990 for review) and specifically in peripheral receptors (see Gonzalez *et al.*, 1992 for review), then the trend toward an intracellular alkalosis centrally could also decrease the ventilatory threshold to CO_2, without affecting the sensitivity.

Hypoxia and K^+ There was no apparent change in sensitivity to hypoxia after sucrose infusion, but the O_2 response curve was shifted upward compared with the control, normosmotic response (Fig. 15). However, the O_2 response curve for ducks is actually an exponential-shaped curve (Jones and Holeton, 1972; Bouverot and Sebert, 1979), rather than a straight line as shown in Fig. 15. 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 is not so steep that, on average, a 2.8 ± 6.1 torr difference in PaO_2 would account for $\dot{V}_E$

increasing almost 200% over resting compared with a 100% increase in $\dot{V}_E$ in control animals (see Jones and Purves, 1970; Jones and Holeton, 1972). In addition, the normosmotic and the hyperosmotic PaO_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 control animals terminated around a PaO_2 of 47 torr, at which point both in their study and in this study (where PaO_2 was actually 44.6 ± 2.3 torr), $\dot{V}_E$ was approximately double resting $\dot{V}_E$. The same hypercapnic challenge in hyperosmotic animals led to a similar drop in PaO_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 PaO_2 in Jones and Holeton's ducks to 38 torr actually decreased $\dot{V}_E$. It seems clear that the ventilatory 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^+ may be associated with the hypoxic response (Lopez-Lopez *et al.*, 1989; Ganfornina and Lopez-Barneo, 1991) and, like hypoxia, is apparently not dependent upon a pH change. The response to the K^+ bolus demonstrated that hyperosmolality was significantly increasing the ventilatory response to these stimuli (Fig. 17). 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, 1990b), while chemoreceptor discharge in response to hypoxia during alkalosis is abolished (Eyzaguirre and Koyano, 1965). It is unclear, however, whether intra- or extracellular pH is critical since intracellular pH is rarely measured. Since it is known that there is a systemic intracellular alkalosis during dilution acidosis, the present results suggest that there is some interaction between the extracellular pH and hypoxia at the level

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

Metabolism There was a small but significant increase in metabolic rate during hyperosmolality during both ventilatory challenges (which supports the ^{31}P NMR data discussed above, pg. 66). 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 $PaCO_2$ (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 activity of 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 $PaCO_2$ measured during the hyperosmotic challenge, especially since there was no modulationg influence of increased ventilation.

Summary Hyperosmolality decreased the normal ventilatory response to hypercapnia, but increased the normal respiratory response to hypoxia and K^+. The shifts in the two gas response curves in opposite directions have several interesting implications. First, it indicates that the mechanisms of chemoreception for CO_2 and O_2 are different, a concept which is generally supported in the literature but which has not been conclusively demonstrated (see Gonzalez *et al.*, 1992). Furthermore, stimulation of the chemoreceptors by K^+ and low O_2 may have some basic mechanism in common, and it supports the involvement of K^+ in the hypoxic response (Lopez-Lopez *et al.*, 1989). Finally, the changes in the ventilatory responses to the stimuli, combined with the opposite intra- and extracellular pH changes, imply that both intra- and extracellular pH have a role in controlling ventilation.

Conclusions

The data indicate that intracellular pH may play a role in initiating ventilatory changes since normal respiratory compensation to extracellular acidosis is prevented during hyperosmolality but not during lactacidosis. This is a unique observation, and it is concluded that there is a ventilatory depression generated peripherally during acute systemic hyperosmotic stress. Since hyperosmolality can have a number of effects on cells, chemoreceptor suppression could be due to intracellular alkalosis, hyperpolarization, cell structure disruption, decreased dopamine release or other perturbations not yet understood. However, the acid-base disturbance associated with acute hyperosmolality appears to offer a unique system to further investigate the probability that chemoreceptors respond to changes in both intra- and extracellular pH.

REFERENCES

Abercrombie, R.F. and Roos, A. (1983). The intracellular pH of frog skeletal muscle: its regulation in hypertonic solutions. *J. Physiol. (Lond.)* 345: 189-204.

Acker, H. and C. Eyzaguirre (1987).Light absorbance changes in the mouse carotid body during hypoxia and cyanide poisoning. *Brain Res.* 409: 380-385.

Acker, H., E. Dufau, J. Huber and D. Sylvester (1989). Indications to an NADPH oxidase as a possible pO_2 sensor in the rat carotid body. *FEBS Lett.* 256: 75-78.

Adams, J.M., and Johnson, N.L. (1988). Intracisternal DIDS enhances ventilatory response to rebreathing CO_2 in rats. *J. Appl. Physiol.* 65: 1611-1616.

Adamson, T.P. (1984). Discharge characteristics of intrapulmonary chemoreceptors in the Pekin duck. Ph.D. thesis, Univ. of California Davis. 92 pp.

Adler, A., Anderson, B. and Zett, B. (1975). Effect of osmolarity on intracellular pH of rat diaphragm muscle. *Am. J. Physiol.* 228: 725-729.

Adler, S., V. Simplaceanu and C. Ho (1990). Brain pH in acute isocapnic metabolic acidosis and hypoxia: a ^{31}P-nuclear magnetic resonance study. *Am. J. Physiol.* 258: F34-F40.

Ahmad, H.R. and H.H. Loeschcke (1983). Evidence for a carrier mediated exchange diffusion of HCO_3^- against Cl^- at the interphases of the central nervous system. In: *Central Neurone Environment*, ed. by M.E. Schlafke, H.P. Kepchen and W.R. See. Springer-Verlag, Berlin. pp. 13-21.

Altenberg, G.A., P.C. Aristimuno, C.E. Amorena and A.C. Taquini (1989). Amiloride prevents the metabolic acidosis of a KCl load in nephrectomized rats. *Clin. Sci.* 76: 649-652.

Anichkov, S.V. and M.L. Belenkii (1963). *Pharmacology of the Carotid Body Chemoreceptors*. Pergamon Press.

Andersen, H.T. and B.-E. Hustvedt (1967). Carbon dioxide excretion and pH-variations in diving ducks after carbonic anhydrase inhibition. *Acta Physiol. Scand.* 69: 203-208.

Anderson, J.W. and Jennings, D.B. (1988). Osmolality, NaCl dietary intake, and regulation of ventilation by CO_2. *Am. J. Physiol.* 255, R105-R112.

Asano, S., E. Kato, M. Yamauchi, Y. Ozawa, M. Iwasa, T. Wada and H. Hasegawa (1966). The mechanism of the acidosis caused by infusion of saline solution. *Lancet* 1: 1245-1246.

Baker, M.A. and D.D. Dawson (1985). Inhibition of thermal panting by intracarotid infusion of hypertonic saline in dogs. *Am. J. Physiol.* 249: R787-R791.

Barrere, B., P. Meric, J. Borredon, G. Berenger, J.-C. Beloeil and J. Seylaz (1990). Cerebral intracellular pH regulation during hypercapnia in unanesthetized rats: a ^{31}P nuclear magnetic resonance spectroscopy study. *Brain Res.* 516: 215-221.

Biscoe, T.J. and M.R. Duchen (1990a). Cellular basis of transduction in carotid chemoreceptors. Am. J. Physiol. 258:

Biscoe, T.J. and M.R. Duchen (1990b). Responses of type I cells dissociated from the rabbit carotid body to hypoxia. *J. Physiol. (Lond.)* 428: 39-59.

Bruton, J.K. (1991). Contraction of rat soleus muscle and the effects of hypertonic treatments. *Jap. J. Physiol.* 41: 189-201.

Bouverot, P. and Ph. Sebert (1979). O_2-chemoreflex drive of ventilation in awake birds at rest. *Respir. Physiol.* 37: 201-218.

Bouverot, P., N. Hill and Y. Jammes (1974). Ventilatory responses to CO_2 in intact and chronically chemodenervated Peking ducks. *Respir. Physiol.* 22: 137-156.

Chance, B. and H. Conrad (1959). Acid-linked functions of intermediates in oxidative phosphorylation. II. Experimental studies of the effect of pH upon respiratory, phosphorylative, and transfer activities of liver and heart mitochondria. *J. Biol. Chem.* 234: 1568-1570.

Chang, B.S., S.I. Kahn and S. Garella (1975). Influence of tonicity on bicarbonate generation in volume expansion. *Clin. Res.* 23: 357A.

Cserr, H.F., M. DePasquale, C. Nicholson, C.S. Patlak, K.D. Pettigrew and M.E. Rice (1991). Extracellular volume decreases while cell volume is maintained by ion uptake in rat brain during acute hypernatremia. *J. Physiol. (Lond.)* 442: 277-295.

De Castro, F. and M. Rubio (1968). The anatomy and innervation of the blood vessels of the carotid body and the role of chemoreceptive reactions in the autoregulation of the blood flow. In: *Arterial Chemoreceptors*, ed. by R.W. Torrance. Blackwell Press, Oxford. pp. 267-277.

Deng, Q.-S. and Johanson, C.E. (1989). Stilbenes inhibit exchange of chloride between blood, choroid plexus and cerebrospinal fluid. *Brain Res.* 501, 183-187.

Dodd, G.A.A. and W.K. Milsom (1987). Effects of H^+ *versus* CO_2 on ventilation in the Pekin duck. *Respir. Physiol.* 68: 189-201.

Duchen, M.R., K.W.T. Caddy, G.C. Kirby, D.L. Patterson, J. Ponte and T.J. Biscoe (1988). Biophysical studies of the cellular elements of the rabbit carotid body. *Neurosci.* 26: 291-311.

Eyzaguirre, C. and H. Koyano (1965). Effects of hypoxia, hypercapnia, and pH on the chemoreceptor activity of the carotid body *in vitro*. *J. Physiol. (Lond.)* 178: 385-409.

Eyzaguirre, C., L. Monti-Bloch, M. Baron, Y. Hayashida, and J.W. Woodbury (1989). Changes in glomus cell membrane properties in response to stimulants and depressants of carotid nerve discharge. *Brain Res.* 477: 265-279.

Fedde, M.R., R.N. Gatz, H. Salama and P. Scheid (1974). Intrapulmonary CO_2 receptors in the duck: I. Stimulus specificity. *Respir. Physiol.* 22: 99-114.

Fedde, M.R., J.P. Kiley, F.L. Powell, and P. Scheid (1982). Intrapulmonary CO_2 receptors and control of breathing in ducks: effects of prolonged circulation time to carotid bodies and brain. *Respir. Physiol.* 47: 121-140.

Fidelman, M.L., S.H. Seeholzer, K.B. Walsh and R.D. Moore (1982). Intracellular pH mediates action of insulin on glycolysis in frog skeletal muscle. *Am. J. Physiol.* 242: C87-C93.

Fitzgerald, R.S., M. Shirahata and S. Lahiri (1990). Amiloride and carotid body chemoreception of hypercapnia and hypoxia. *Respir. Physiol.* 81, 337-348.

Gabel, R.A. and R.B. Weiskopf (1975). Ventilatory interaction between hypoxia and $[H^+]$ at chemoreceptors of man. *J. Appl. Physiol.* 39: 292-296.

Galla, J.H. and R.G. Luke (1988). Chloride transport and disorders of acid-base balance. *Ann. Rev. Physiol.* 50: 141-158.

Galla, J.H., D.N. Bonduris, P.W. Sanders and R.G. Luke (1984). Volume-independent reductions in glomerular filtration rate in acute chloride-depletion alkalosis in the rat. *J. Clin. Invest.* 74: 2002-2008.

Gallego, R. and C. Belmonte (1979). The effects of blood osmolality changes on cat carotid body chemoreceptors in vivo. *Pfleugers Arch.* 380: 53-58.

Gallego, R. and C. Eyzaguirre (1976). Effects of osmotic pressure changes on the carotid body of the cat *in vitro*. *Fed. Proc.* 35: 404.

Gallego, R., C. Eyzaguirre, and L. Monti-Bloch (1979). Thermal and osmotic responses of arterial receptors. *J. Neurophysiol.* 3: 665-680.

Ganfornina, M.D. and J. Lopez-Barneo (1991). Single K^+ channels in membrane patches of arterial chemoreceptor cells are modulated by O_2 tension. *Proc. Natl. Acad. Sci. USA* 88: 2927-2930.

Garella, S., B.S. Chang and S.I. Kahn (1975). Dilution acidosis and contraction alkalosis: review of a concept. *Kidney Int.* 8: 279-283.

Green, J. and S. Muallem (1989). A common mechanism for activation of the Na^+/H^+ exchanger by different types of stimuli. *FASEB J.* 3: 2408-2414.

Green, J., D.T. Yamaguchi, C.R. Kleeman and S. Muallem (1988). Selective modification of the kinetic properties of Na^+/H^+ exchanger by cell shrinkage and swelling. *J. Biol. Chem.* 263: 5012-5015.

Gonzalez, C., L. Almaraz, A. Obeso and R. Rigual (1992). Oxygen and acid chemoreception in the carotid body chemoreceptors. *Trends Neurosci.* 15: 146-153.

Hanson, M.A., P.C.G. Nye and R.W. Torrance (1981). The exodus of an extracellular bicarbonate theory of chemoreception and the genesis of an intercellular one. In: *Arterial Chemoreceptors, Proceedings of the VI International Meeting*, ed. by C. Belmonte, D.J. Pallot, H. Acker and S. Fidone. Leicester University Press, Oxford. pp. 403-416.

He, A.-F., J.-Y. Wei, and C. Eyzaguirre (1991). Intracellular pH and some membrane characteristics of cultured carotid body glomus cells. *Brain Res.* 57: 258-266.

Helbecka, N.V.L., J.L. Casterline, C.J. Smith and C.S. Shaffner (1964). Investigation of plasma carbonic acid pK of the chicken. *Poultry Sci.* 43: 138-144.

Hoffman, E.K. and L.O. Simonsen (1989). Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* 69: 315-382.

Javaheri, S., J. Weyne, G. Demeester and I. Leusen (1984). Effects of SITS, an anion transport blocker, on CSF ionic composition in metabolic alkalosis. *J. Appl. Physiol.* 57: 92-97.

Jean, T., C. Frelin, P. Vigne and M. Lazdunski (1986). The Na^+/H^+ exchange system in glial cell lines. Properties and activation by hyperosmotic shock. *Eur. J. Biochem.* 160: 211-219.

Jones, D.R. and G.F. Holeton (1972). Cardiovascular and respiratory responses of ducks to progressive hypocapnic hypoxia. *J. exp. Biol.* 56: 657-666.

Jones, D.R. and M.J. Purves (1970a). The carotid body in the duck and the consequences of its denervation upon the cardiac responses to immersion. *J. Physiol. (Lond.)* 211: 295-309.

Jones, D.R. and M.J. Purves (1970b). The effect of carotid body denervation upon the respiratory response to hypoxia and hypercapnia in the duck. *J. Physiol. (Lond.)* 211: 295-309.

Kost, G.J. (1990). pH standardization for phosphorus-31 magnetic resonance heart spectroscopy at different temperatures. *Mag. Res. Med.* 14: 496-506.

Lahiri, S. (1977). Introductory remarks: oxygen linked response of carotid chemoreceptors. *Adv. Exp. Med. Biol.* 78: 185-202.

Lahiri, S., A. Mokashi, R.G. Delaney and A.P. Fishman (1978). Arterial PO_2 and PCO_2 stimulus threshold for carotid chemoreceptors and breathing. *Respir. Physiol.* 34: 359-375.

Lassen, N.A. (1990). Is central chemoreceptor sensitive to intracellular rather than extracellular pH? *Clin. Physiol.* 10: 311-319.

Lopez-Lopez, J., C. Gonzalez, J. Urena and J. Lopez-Barneo (1989). Low pO_2 selectively inhibits K channel activity in chemoreceptor cells of the mammalian carotid body. *J. Gen. Physiol.* 93: 1001-1015.

Luke, R.G.and J.H. Galla (1983). Chloride-depletion alkalosis with a normal extracellular fluid volume. *Am. J. Physiol.* 245: F419-F424.

Lynch, J.W. and P.H. Barry (1989). Action potentials initiated by single channels opening in a small neuron (rat olfactory receptor). *Biophys. J.* 55: 755-768.

Macknight, A.D.C., J. Grantham and A. Leaf (1992). Physiologic and pathophysiologic responses to changes in extracellular osmolality. In: *The Kidney: Physiology and Pathophysiology*, 2nd ed., ed. by D.W. Seldin and G. Giebisch. Raven, New York. pp. 1779-1806.

Mahnensmith, R.L. and P.S. Aronson (1985). The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. *Circ. Res.* 56: 773-788.

Makoff, S.L., A. Da Silva, B.J. Rosenbaum, S.E. Levy, and M.H. Maxwell (1970). Hypertonic expansion: acid-base and electrolyte changes. *Am. J. Physiol.* 218: 1201-1207.

Maskrey, M. and D. Trenchard (1989). A vagally mediated response to metabolic acidosis in anesthetized rabbits. *J. Appl. Physiol.* 66: 1635-1641.

McCormack, J.G., A.P. Halestrap and R.M. Denton (1990). Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol. Rev.* 70: 391-425.

Milsom, W.K. (1990). Control and co-ordination of gas exchange in air breathers. In: *Advances in Comparative and Environmental Physiology*, Vol. 6., ed. by R.G. Boutilier. Springer-Verlag, Berlin. pp. 347-400.

Milsom, W.K., D.R. Jones and G.R.J. Gabbot (1981). On chemoreceptor control of ventilatoy responses to CO_2 in unanesthetized ducks. *J. Appl. Physiol.* 50: 1121-1128.

Natsui, T. (1970). Respiratory response to arterial H^+ at different levels of arterial PCO_2 during hyperoxia or hypoxia. *Pfleugers Arch.* 316: 34-50.

Nattie, E.E. (1983). Ventilation during acute HCl infusion in intact and chemodenervated conscious rabbits. *Respir. Physiol.* 54: 97-107.

Nattie, E.E. and J.M. Adams (1988). DIDS decreases CSF HCO_3^- and increases breathing in response to CO_2 in awake rabbits. *J. Appl. Physiol.* 64: 397-403.

Nioka, S., B. Chance, M. Hilberman, G.V. Subramanian, J.S. Leigh, Jr., R.L. Veech, and R.E. Forster (1987). Relationship between intracellular pH and energy metabolism in dog brain as measured by ^{31}P-NMR. *J. Appl. Physiol.* 62: 2094-2102.

Nishimura, M., D.C. Johnson, and H. Kazemi (1988). Effects of inhibitors on chloride outflux from cerebrospinal fluid. *J. Appl. Physiol.* 64: 2183-2189.

Noll, F. (1974). Determination with LDH, GPT and NAD. In: *Methods of Enzymatic Analysis*, Vol. 3, ed. by H.U. Bergmeyer. Academic Press, New York. pp. 1475-1477.

Obika, L.F.O. (1989). Urinary kallikrein excretion during potassium chloride infusion in potassium-adapted rats: effect of amiloride. *Clin. Sci.* 77: 21-27.

Parker, I. and P.H. Zhu (1987). Effects of hypertonic solutions on calcium transients in frog twitch muscle fibres. *J. Physiol. (Lond.)* 383: 615-627.

Patterson, R.W. and S.F. Sullivan (1978). Dererminants of oxygen uptake during sodium bicarbonate infusion. *J. Appl. Physiol.* 45: 399-402.

Powell, R.L., M.R. Fedde, R.K. Gratz and P. Scheid (1978). Ventilatory response to CO_2 in birds. I. Measurements in the unanesthetized duck. *Respir. Physiol.* 35: 349-359.

Rigual, R., J.R. Lopez-Lopez and C. Gonzalez (1991). Release of dopamine and chemorecepor discharge induced by low pH and high PCO_2 stimulation of the cat carotid body. *J. Physiol.* 433: 519-531.

Rigual, R., E. Gonzalez, S. Fidone and C. Gonzalez (1984). Effects of low pH on synthesis and release of catecholamines in the cat carotid body in vitro. *Brain Res.* 309: 178-181.

Roberts, J.K.M., N. Wade-Jardetzky and O. Jardetzky (1981). Intracellular pH measurements by ^{31}P nuclear magnetic resonance. Influence of factors other than pH on ^{31}P chemical shifts. *Biochem.* 20: 5394-5402.

Robin, E.D., R.D. Whaley, C.H. Crump, A.G. Bickelmann and D.M. Tracis (1958). Acid-base relations between spinal fluid and arterial blood with special reference to control of ventilation. *J. Appl. Physiol.* 13: 385-392.

Rocher, A., A. Obeso, C. Gonzalez and B. Herreros (1991). Ionic mechanisms for the transduction of acidic stimuli in rabbit carotid body glomus cells. *J. Physiol.* 433: 533-548.

Roos, A. and W.F. Boron (1981). Intracellular pH. *Physiol. Rev.* 61: 296-434.

Rosenbaum, B.J., D.L. Makoff and M.H. Maxwell (1969). Acid-base and electrolyte changes induced by acute isotonic saline infusion in the nephrectomized dog. *J. Lab. Clin. Med.* 74: 427-435.

Ruch, F.E. and M.R. Hughes (1975). The effects of hypertonic sodium chloride injection on body water distribution in ducks (*Anas platyrhynchos*), gulls (*Larus glaucescens*) and roosters (*Gallus domesticus*). *Comp. Biochem. Physiol.* 52: 21-28.

Saito, K., Honda, Y. and N. Hasumura (1960). Evaluation of respiratory response to changes in pCO_2 and hydrogen ion concentration of arterial blood in rabbits and dogs. *Jap. J. Physiol.* 10: 634-645.

Scheid, P. and J. Piiper (1986). Control of breathing in birds. In: *Handbook of Physiology, Sect. 3, The Respiratory System*, Vol. II, Part 2, ed. by N.S. Cherniack and J.G. Widdicombe. American Physiological Society, MD. pp. 815-832.

Schuitmaker, J.J., A. Berkengosch, J. De Goede and C.N. Olievier (1986). Effects of CO_2 and H^+ on the ventilatory response to peripheral chemoreceptor stimulation. *Respir. Physiol.* 64: 69-79.

Shimizu, M. and D.R. Jones (1987). Acid-base balance in ducks (*Anas platyrhynchos*) during involuntary submergence. *Am. J. Physiol.* 252: R348-R352.

Shires, G.T. and J. Holman (1948). Dilution acidosis. *Ann. Int. Med.* 28: 557-559.

Somero, G.N. (1986). Protons, osmolytes, and fitness of internal milieu for protein function. *Am. J. Physiol.* 251: R197-R213.

Sotos, J.F., P.R. Dodge and N.B. Talbot (1962). Studies in experimental hypertonicity. II. Hypertonicity of body fluids as a cause of acidosis. *Pediatrics* 30: 180-193.

Stephenson, R. and D.R. Jones (1993). Energy metabolism of the Pekin duck during prolonged head submergence using ^{31}P-MRS. *Am. J. Physiol.*, in press.

Stewart, P.A. (1983). Modern quantitative acid-base chemistry. *Can. J. Physiol. Pharmacol.* 61: 1444-1461.

Urena, J., J. Lopez-Lopez, C. Gonzalez and J. Lopez-Barneo (1989). Ionic currents in dispersed chemoreceptor cells of the mammalian carotid body. *J. Gen. Physiol.* 93: 979-999.

Walker, R.L., A.E. Buie and N.L. Armstrong (1990). The effects of saline exposure on the electrolyte, acid-base and blood oxygen status of the toad, *Bufo marinus*. *Bulletin Can. Soc. Zool.* 21: 95.

Wathen, R.L., R.A. Ward, G.B. Harding and L.C. Meyer (1982). Acid-base and metabolic responses to anion infusion in the anesthetized dog. *Kidney Int.* 21: 592-599.

Wesson, D.E. (1990). Depressed distal tubule acidification corrects chloride-deplete alkalosis in rats. *Am. J. Physiol.* 259: F636-F644.

Whalley, D.W., P.D. Hemsworth and H.H. Rasmussen (1991). Sodium-hydrogen exchange in guinea-pig ventricular muscle during exposure to hyperosmolar solutions. *J. Physiol. (Lond.)* 444: 193-212.

Wilding, T.J., B.Cheng and A. Roos (1992). pH regulation in adult rat carotid body glomus cells. *J. Gen. Physiol.* 100: 593-608.

Wilkes, P.R.H. and B.R. McMahon (1986). Responses of a stenohaline freshwater teleost (*Catostomus commersoni*) to hypersaline exposure. I. The dependence of plasma pH and bicarbonate concentration on electrolyte regulation. *J. exp. Biol.* 121: 77-94.

Winters, R.W., P.R. Scaglione, G.G. Nahas and M. Verosky (1964). The mechanism of acidosis produced by hyperosmotic infusions. *J. Clin. Invest.* 43: 647-658.

Yamada, K. (1970). The increase in the rate of heat production of frog's skeletal muscle caused by hypertonic solutions. *J. Physiol. (Lond.)* 208: 49-64.

Zar, J.H. (1984). *Biostatistical Analysis.* Prentice-Hall, NJ.

Zeidler, R. and H.D. Kim (1977). Preferential hemolysis of postnatal calf red cells induced by internal alkalinization. *J. Gen. Physiol.* 70: 385-401.