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PARTITIONING OF ACID–BASE REGULATION BETWEEN RENAL AND EXTRARENAL SITES IN THE ADULT, TERRESTRIAL STAGE OF THE SALAMANDER *AMBYSTOMA TIGRINUM* DURING RESPIRATORY ACIDOSIS

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Summary

Adult *Ambystoma tigrinum* were cannulated non-occlusively in the truncus arteriosus and subjected to 24 h of hypercapnia in 3 % CO_2. Adults showed the typical compensatory pattern, shared by larvae and many other amphibians, of partial compensation (44 %) for the induced respiratory acidosis. Adults whose urinary bladders were ligated to allow the urine to bypass the bladder compensated as well as shams, indicating that the urinary bladder of this species is not necessary for compensation. Radioisotopic measurements of net and unidirectional fluxes of Na^+ or Cl^- in whole animals showed no effects of hypercapnia. Partitioning of acid–base responses showed that 75–80 % of the regulation takes place across the skin. The rest is accomplished by the kidneys. This did not change during hypercapnia and there was no evidence of renal involvement in the compensation. Total ammonia ($NH_3+NH_4^+$) comprised only about one-sixth of the total cutaneous acid excretion. The charge associated with the cutaneous excretion of H^+ equivalents was balanced by both Na^+ uptake and Cl^- loss. In contrast to larvae, whose cutaneous electrical potential difference (PD) increases during hypercapnia, adults decrease their PD. This could mean that acidosis stimulates an electrogenic H^+ secretion and/or that cutaneous Na^+ and Cl^- permeabilities change. Both possibilities are consistent with the data.

Introduction

The salamander *Ambystoma tigrinum* provides a good model for the study of the evolution of acid–base regulatory mechanisms during the transition of vertebrates from the aquatic to the terrestrial environment. The species has an aquatic larval form and a terrestrial adult form. Both stages utilize their skin (Alvarado and Kirschner, 1963) and kidneys (Stiffler and Alvarado, 1974; Hartenstein and Stiffler, 1990) for ion exchanges, and skin exchanges have been implicated in acid–base balance in the larval form (Stiffler *et al.* 1987; Stiffler,

Key words: cutaneous ion transport, Na^+ transport, Cl^- transport, H^+ transport, ammonia, *Ambystoma tigrinum*.

1988, 1989). The role of the kidneys in amphibian acid–base balance has not been thoroughly investigated. Although mammals make extensive use of renal mechanisms in acid–base balance (Gonzalez, 1986; Sullivan, 1986), it is clear that these mechanisms do not extend to all vertebrates. In most fish, the ionic fluxes related to acid–base balance are confined to their gill surfaces, with little, if any, participation by renal tubules (Heisler, 1982, 1986). It is not known at what stage in vertebrate evolution the kidney tubules became involved in acid–base regulation; however, one logical place to look is in amphibians. A number of isolated nephron studies have clearly demonstrated the presence of net fluxes of acid or base equivalents across segments of amphibian kidney tubules (Oberleithner *et al.* 1984; Yucha and Stoner, 1986, 1987; Stanton *et al.* 1987; Stanton, 1988). However, whole-animal studies in frogs and toads (Yoshimura *et al.* 1961; Tufts and Toews, 1985) suggest that the kidneys of these anurans do not respond to respiratory acidosis.

The objectives of these experiments are (1) to assess the ability of the terrestrial, adult stage of the salamander *Ambystoma tigrinum* to regulate extracellular pH during a respiratory acidosis and (2) to determine the partitioning of the sites of regulation between cutaneous and renal components.

Materials and methods

Animals

Adult *Ambystoma tigrinum* were purchased from Charles Sullivan, Amphibians of North America, Nashville, TN, USA. The average body mass was approximately 60 g. The salamanders were maintained in damp aquaria with access to water at room temperature. The ionic composition of the water in which the animals were housed was: pH, 7.6; $[Na^+]$, $1.5\,mmol\,l^{-1}$, $[K^+]$, $0.1\,mmol\,l^{-1}$; $[Ca^{2+}]$, $3.5\,mmol\,l^{-1}$; $[Mg^{2+}]$, $1.4\,mmol\,l^{-1}$; $[Cl^-]$, $1.2\,mmol\,l^{-1}$; $[HCO_3^-]$, $3.2\,mmol\,l^{-1}$. All experiments were performed at room temperature (approx. 25 °C).

Surgery

Animals were anesthetized in 0.1 % tricaine methanesulfonate buffered to pH 7 with 0.1 % $NaHCO_3$. Anesthesia required approximately 10 min and the animals recovered after approximately 30 min. Arterial cannulation was accomplished by placing a non-occlusive PE 50 cannula into the truncus arteriosus (Stiffler *et al.* 1983). To assess the possible role of the urinary bladder in the acid–base balance responses to hypercapnia, a separate group of adults received flank incisions and had their urinary bladders ligated prior to experimentation. This is possible in amphibians because the ureters empty directly into the cloaca with the urinary bladder arising separately. Sham surgery was performed on a third group as a control.

A fourth group of adults was studied for renal contributions to acid–base balance. The cloaca of each was cannulated with a piece of PE 240 tubing, bent at

90° and flared at one end. After the flared end of this cannula had been anchored in the cloaca with a purse-string suture, a tared urine collection bag, tied to a short segment of Tygon tubing, was attached. The inside diameter of the Tygon tubing was drawn down to fit snugly over the PE 240 cloacal cannula. A flank incision was made in each animal to ligate the colon and prevent fecal contamination of the urine. All animals were allowed 24 h to recover from surgery before the start of experiments.

Blood gas analysis

Blood gases and pH were measured with Radiometer electrodes. The CO_2 electrode was connected to a Radiometer PHM 84 pH meter and P_{CO_2} was transformed from the $\log P_{CO_2}$/pH regression. The P_{O_2} electrode was connected to a Cameron OM 100 oxygen meter. The electrodes were calibrated using gas mixtures prepared with a Wösthoff Digamix pump. The pH electrode was attached to another Radiometer PHM 84 pH meter and was calibrated using Radiometer precision buffers. Bicarbonate concentration was calculated using the Henderson–Hasselbalch equation with a pK′ of 6.05 and a CO_2 solubility of 0.248 $mmol\,l^{-1}\,kPa^{-1}$ (Boutilier *et al.* 1979*a*).

Experimental protocol for hypercapnia responses

After 24 h a control blood sample (0.5–0.7 ml) was taken and pH and blood gases were measured. The plasma and cells were separated by centrifugation and the plasma was stored frozen for later analysis. The blood cells were resuspended in Ringer's solution containing 3 % bovine serum albumin (BSA) and reinfused back into the animal. After the control sample had been taken, hypercapnia was commenced by gassing the chamber with 3 % CO_2 prepared with Cameron GF-series gas-mixing flowmeters. After 2 h of hypercapnia, a second sample was taken to establish the acute response to the hypercapnia. A third sample was taken after 24 h to assess the extent of compensation. At this point the CO_2 was turned off and normocapnic conditions were re-established. After 24 h of recovery a fourth sample was taken. This protocol was followed on intact, sham and bladder-ligated individuals.

Cutaneous ionic fluxes

Net and unidirectional fluxes were measured using Kirschner's (1970) method, as previously described (Stiffler *et al.* 1986). Briefly, animals were placed in measured volumes (100–250 ml) of 1 $mmol\,l^{-1}$ NaCl containing 1 $\mu Ci\,l^{-1}$ of either ^{22}Na or ^{36}Cl (New England Nuclear). Samples were taken at 0, 4, 8, 12 and 24 h and influx was calculated from the disappearance of radioactivity from the bath and the specific activity of the bath. Net flux was calculated from the change in the total amount of Na^+ in the bath and efflux was calculated as the difference between net flux and influx.

Experimental protocol for urine collection experiments

After 24 h of recovery from surgery, each animal was equipped with a tared urine collection bag, which was slipped over the cloacal cannula, and urine collection was carried out for 4–5 h under normocapnic conditions. A blood sample was taken at the midpoint of each urine collection period. Control pH and blood gases were measured on this sample and the plasma was removed and stored frozen for later chemical analysis. The blood cells were resuspended in Ringer's solution containing 3 % bovine serum albumin and reinfused into the animals. Following collection of the urine sample, the animals were gassed with 3 % CO_2 prepared with a Cameron GF-series flowmeter and allowed 24 h to compensate. At the end of this period a second tared balloon urine collection bag was attached to the cloacal cannula and urine collected as before with midpoint blood sampling and chemical analysis as described above. These measurements were used to assess the response to the hypercapnic challenge.

Renal clearance measurements

Glomerular filtration rate was measured using the clearance of inulin (C_{in}):

$$C_{in} = \frac{U_{in}}{P_{in}} \times V, \tag{1}$$

where U_{in} is urine inulin concentration, P_{in} is plasma inulin concentration and V is urine flow. Inulin (15 %, Sigma Chemical Co.) was injected subcutaneously at least 18 h before the experiments began. This has been shown to be sufficient for the inulin to distribute itself uniformly throughout the extracellular space in *Ambystoma* (Stiffler and Alvarado, 1974). Plasma samples were taken at the midpoint of the 4-h clearance periods. Since *Ambystoma* plasma inulin concentration, when inulin is administered as it was in these experiments, decreases by only about 2.5 % h^{-1} (Stiffler *et al.* 1980), the midpoint plasma inulin concentration is a reliable indicator of mean plasma inulin concentration during the clearance period. Fractional reabsorption of ions ($\% T_X$) was calculated as:

$$\% T_X = \frac{(P_X \mathrm{GFR}) - (U_X V)}{(P_X \mathrm{GFR})} \times 100, \tag{2}$$

where P_X is plasma concentration of X, GFR is glomerular filtration rate and U_X is urine concentration of X. Fractional reabsorption of water ($\% T_{H_2O}$) was calculated as:

$$\% T_{H_2O} = \frac{\mathrm{GFR} - V}{\mathrm{GFR}} \times 100. \tag{3}$$

Measurement of pulmonary ventilation rates

Pulmonary ventilation rate was measured in a separate group of larvae by placing subdermal electrodes above the maxilla, below the mandible and on the tail (ground) of each animal. These electrodes were attached to an impedance

pneumograph which was, in turn, attached to a Narco Physiograph which recorded ventilation movements.

Chemical analyses

Sodium and potassium concentrations were measured by flame photometry (Coleman model 51). Calcium and magnesium concentrations were measured with a Perkin–Elmer model 4000 atomic absorption spectrometer. Chloride concentration was measured with a Buchler chloridometer. Total ammonia ($NH_3+NH_4^+$) concentration was measured by a modification (D. G. McDonald, personal communication) of the method of Verdouw *et al.* (1978). Inulin was measured by the method of Nakamura (1968). Urinary titratable acidity (TA) was measured as $TA-HCO_3^-$ (Hills, 1973). The acidity of the bath was measured by the method used by McDonald and Wood (1981). Bath samples (3 ml) were gassed with a stream of air for 1 h to remove CO_2. The samples were then titrated to a pH of 4.0 with $0.02\,mol\,l^{-1}$ HCl to determine apparent alkalinity.

The decrease in alkalinity was taken to be equal to the acidification in the bath between two sampling times. The method does not distinguish between acid excretion and base absorption. Therefore, when H^+ excretion is discussed it is with the implied understanding that it is apparent H^+ excretion; it could also be base uptake that is being observed. The concentration of urinary HCO_3^- was estimated as total CO_2 using a Cameron Capni-Con 3a.

Transepithelial potential differences

The skin potential (PD) was measured by placing one Ringer–agar bridge made with PE 50 tubing under the skin of an animal and placing another such bridge in the bath. The two bridges were then placed in calomel electrodes connected to a Tektronix DM 502 digital multimeter. The details have been published previously (Stiffler *et al.* 1986, 1987).

Results

Responses of adults to hypercapnia

The response of adult *A. tigrinum* to hypercapnia in 3 % CO_2 was very similar to that of the larvae (Stiffler *et al.* 1983, 1987; Fig. 1). Gassing the animal's chamber with 3 % CO_2 elevated bath P_{CO_2} to approximately 2.7 kPa after 2 h and this remained stable for 24 h. Bath P_{O_2} was 12–13 kPa and bath pH was 6.9–7.0. This elevated blood P_{CO_2} to 2.8±0.2 kPa and produced a typical respiratory acidosis. After another 22 h, pH had increased by 0.1 unit as a result of an increase in [HCO_3^-]. The increase in bicarbonate concentration represents a 44.8±6.9 % compensation (Siesjo, 1971). After 24 h of recovery, pH returned to control levels, but [HCO_3^-] and P_{CO_2} remained elevated. Measurements of ventilation rate over a 24-h period showed that breathing rate doubled after 2.5 h. This returned to control levels after 5 h and remained fairly stable throughout the remainder of the 24 h (Fig. 2). The hyperventilation was matched by a significant increase in P_{O_2}

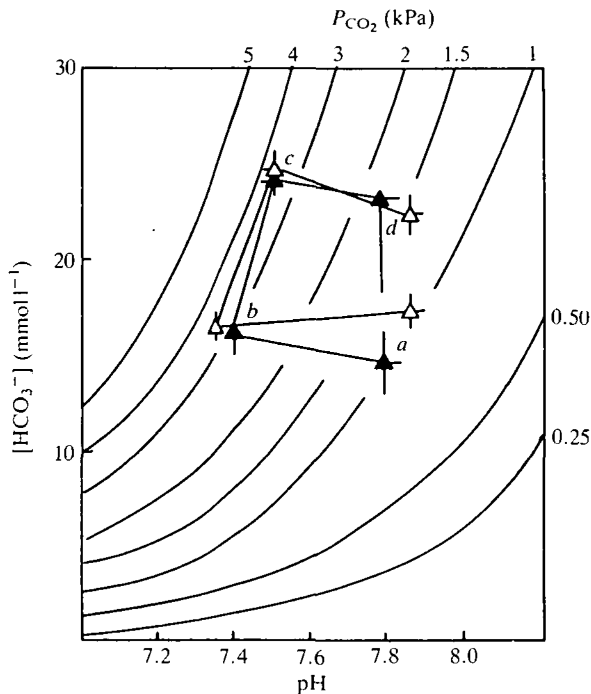

Fig. 1. Acid–base balance responses of *Ambystoma tigrinum* to hypercapnia. Closed triangles refer to adult salamanders in the present study (N=7). Open triangles are data from larvae taken from Stiffler *et al.* (1983, 1987) (N=15). *a*, Control values; *b*, 2 h of hypercapnia; *c*, 24 h of hypercapnia; *d*, 24 h of normocapnic recovery. Values are mean±S.E.M.

and a significant decrease in the difference between arterial P_{CO_2} and inspired P_{CO_2} ($Pa_{CO_2}-PI_{CO_2}$), also shown in Fig. 3. These had reversed towards control levels after 24 h.

The role of the urinary bladder

A second group of adult *A. tigrinum* was subdivided into a series of animals whose urinary bladders were ligated and a sham series to determine the contribution of this organ to the compensatory response. The responses of the shams and the bladder-ligated animals were very similar to those of the control adults, with compensations of 34.9±4.7 and 36.2±6.1 %, respectively. These values were not significantly different from the compensation seen in untreated controls (Fig. 4).

Responses of unidirectional ion fluxes in whole animals

A third group of adults was subdivided into two series of animals that were evaluated for responses of unidirectional and net sodium and chloride fluxes and net $NH_3+NH_4^+$ fluxes. There were no significant differences in influx, efflux or

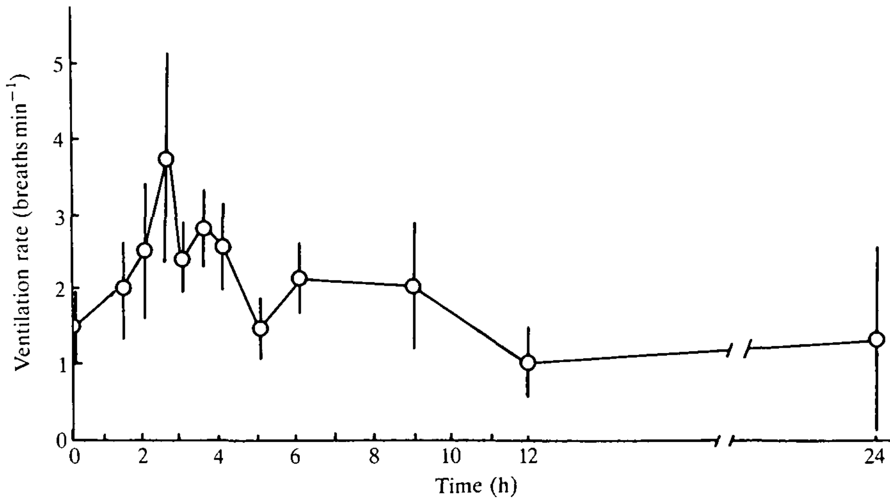

Fig. 2. Pulmonary ventilation in adult *Ambystoma tigrinum*. The control value at time zero is the breathing rate prior to hypercapnia. Hypercapnia commenced at time zero. Values are mean±S.E.M. (N=5).

net flux of sodium or chloride during hypercapnia or recovery in adults (Table 1). There was a significant increase in net loss of NH_3+NH_4^+ during hypercapnia, however.

Partitioning of acid–base ion exchanges between skin and kidneys

The fourth group of adult *A. tigrinum* was prepared with both arterial and cloacal cannulae to separate urinary and cutaneous exchanges of acid equivalents before, during and after hypercapnia. The blood gas and acid–base data during the control period and after 24 h of hypercapnia were very similar to those of the

Table 1. *Sodium, chloride and total ammonium fluxes in adult* Ambystoma tigrinum

		Flux (μequiv 100 g^{-1} h^{-1})		
	N	Control	Hypercapnia	Recovery
Na^+ influx	13	6.0±1.0	7.3±1.1	13.4±4.9
Na^+ efflux	13	−13.5±2.8	−10.3±2.1	−15.8±5.2
Na^+ net flux	13	−7.5±2.6	−3.0±1.7	−2.6±1.6
NH_3+NH_4^+ net flux	15	−5.5±1.1	−23.3±2.8*	1.7±2.8
Cl^- influx	8	6.6±1.2	7.3±0.9	5.7±0.8
Cl^- efflux	8	−10.4±2.6	−13.8±2.3	−9.6±1.2
Cl^- net flux	8	−3.8±1.8	−6.5±2.3	−3.9±1.5

Fluxes are from whole animals without partitioning of renal and cutaneous components.
* Significantly different from control (P<0.01). A paired t-test was used.
Values are mean±S.E.M.

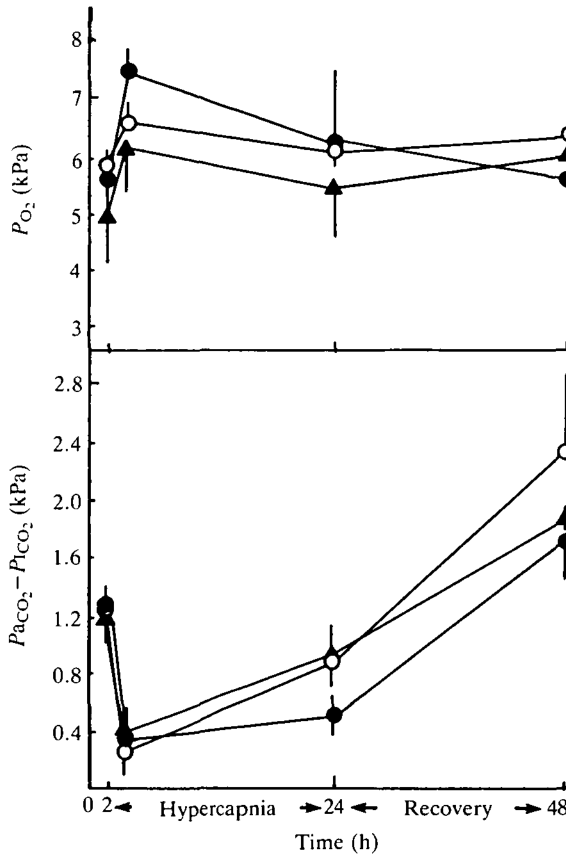

Fig. 3. Arterial P_{O_2} and $Pa_{CO_2} - PI_{CO_2}$ (difference between arterial and inspired P_{CO_2}) in intact (▲; N=7), bladder-ligated (●; N=12) and sham-operated (O; N=10) adult *Ambystoma tigrinum*. Values are mean±S.E.M.

first series of animals, which did not have urinary cannulae, suggesting that these cannulae did not produce additional stress (Table 2). There were no significant changes in plasma ion concentrations (Table 2). Urine flow and glomerular filtration rate did not change significantly during the compensated hypercapnic period (Table 3). There was an increased fractional tubular water reabsorption, as indicated by the increased U_{in}/P_{in} ratio. This may mean that the slight apparent increase in GFR and the slight apparent decrease in urine flow are real, in spite of our inability to detect them statistically. Urine [Na^+], [K^+] and [Cl^-] all increased during hypercapnia, but the corresponding fractional reabsorptions of the ions did not change significantly. Most of the urinary acid–base variables were also unaltered by hypercapnia. Urine pH, [HCO_3^-], NH_3+NH_4^+ excretion rate and titratable acid excretion remained constant (Table 3). Urine [NH_3+NH_4^+] increased significantly, however.

There were large changes in extrarenal acid flux, which increased more than fivefold during hypercapnia. During control and hypercapnic periods the extra-

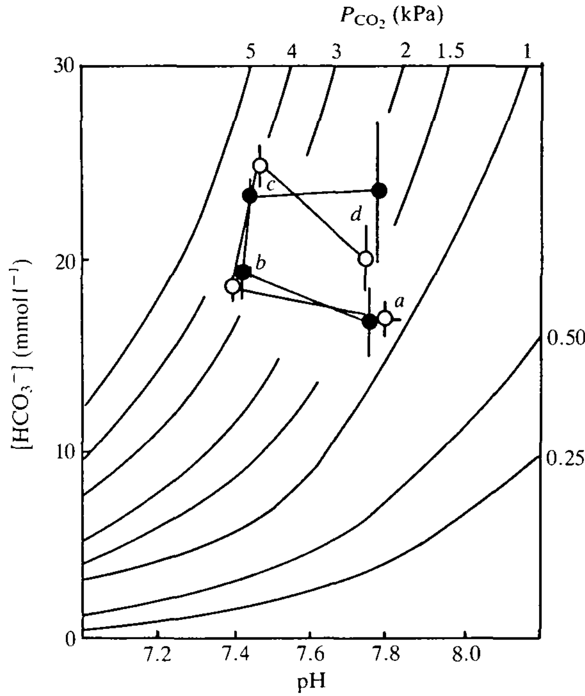

Fig. 4. Acid–base balance responses of adult *Ambystoma tigrinum* to hypercapnia. Closed circles are for animals whose urinary bladders were ligated (N=12). Open circles are for sham-operated animals (N=10). *a*, Control values; *b*, 2 h of hypercapnia; *c*, 24 h of hypercapnia; *d*, recovery. Values are mean±S.E.M.

Table 2. *Blood gas and ionic composition of animals used in renal clearance experiments*

Blood gases and ions	N	Control	Hypercapnia	P
pH	18	7.723±0.021	7.470±0.020	<0.001
P_{CO_2} (kPa)	18	1.4±0.1	4.0±0.5	<0.001
[HCO_3^-] (mmol l^{-1})	18	16.8±1.3	25.5±2.5	<0.001
[Na^+] (mmol l^{-1})	15	108.5±2.5	107.9±3.1	NS
[K^+] (mmol l^{-1})	15	4.0±0.6	5.4±0.9	NS
[Ca^{2+}] (mmol l^{-1})	19	1.0±0.1	0.9±0.1	NS
[Mg^{2+}] (mmol l^{-1})	19	0.4±0.0	0.4±0.0	NS
[Cl^-]$_p$ (mmol l^{-1})	18	78.1±2.4	78.1±2.6	NS

Control data from animals 24 h after surgical implantation of the cannulae, hypercapnic data from animals 24 h after the start of hypercapnia.

Statistical analysis using paired *t*-test.

Values are mean±S.E.M., NS, not significant.

Table 3. *Partitioning of acid–base regulation between renal and cutaneous transport sites in adult* Ambystoma tigrinum

Variable	N	Control	Hypercapnia	P
Renal function				
V (ml 100 g^{-1} h^{-1})	11	1.12±0.17	0.83±0.18	NS
GFR (ml 100 g^{-1} h^{-1})	9	1.97±0.45	2.33±0.76	NS
U_{in}/P_{in}	9	1.54±0.28	2.67±0.65	<0.050
pHu	10	7.08±0.69	6.79±0.61	NS
$[Na^+]_u$ (mmol l^{-1})	11	5.2±0.6	9.1±0.8	<0.025
$[K^+]_u$ (mmol l^{-1})	11	0.4±0.1	0.8±0.1	<0.010
$[Cl^-]_u$ (mmol l^{-1})	11	1.5±0.2	6.5±1.1	<0.010
$[NH_3+NH_4^+]_u$ (mmol l^{-1})	10	3.7±1.3	14.5±5.0	<0.050
$[HCO_3^-]_u$ (mmol l^{-1})	7	2.3±0.7	1.3±0.4	NS
$\% T_{Na^+}$	8	95.4±0.8	90.8±3.0	NS
$\% T_{K^+}$	8	90.5±1.6	90.8±3.3	NS
$\% T_{Cl^-}$	8	98.0±0.5	94.4±2.5	NS
$\% T_{HCO_3^-}$	4	93.9±2.1	98.6±0.7	<0.050
HCO_3^- excretion (μequiv 100 g^{-1} h^{-1})	4	−1.2±0.4	−1.8±1.2	NS
$NH_3+NH_4^+$ excretion (μequiv 100 g^{-1} h^{-1})	10	−5.2±2.3	−10.6±4.0	NS
Titratable acid excretion (μequiv 100 g^{-1} h^{-1})	10	−1.4±0.8	−1.1±0.8	NS
Total acid excretion (μequiv 100 g^{-1} h^{-1})	7	−5.1±1.6	−13.6±8.9	NS
Percentage of total*		24.3	18.1	
Cutaneous function				
Na^+ excretion (μequiv 100 g^{-1} h^{-1})	14	−14.4±6.1	7.4±8.4	<0.05
Cl^- excretion (μequiv 100 g^{-1} h^{-1})	18	−8.9±8.2	−43.1±9.0	<0.025
Na^+-Cl^- excretion (μequiv 100 g^{-1} h^{-1})	13	−5.6±9.6	52.6±12.8	<0.010
$NH_3+NH_4^+$ excretion (μequiv 100 g^{-1} h^{-1})	18	−7.3±1.7	−10.5±2.6	NS
H^+ excretion (μequiv 100 g^{-1} h^{-1})	13	−8.6±3.9	−50.5±16.2	<0.05
Total acid (μequiv 100 g^{-1} h^{-1})	13	−15.9±42.0	−61.6±16.0	<0.001
Percentage of total*		75.7	81.9	

Control data from animals 24 h after surgical implantation of the cannulae, hypercapnic data from animals 24 h after the start of hypercapnia.

* Percentage of total refers to the percentage of the total animal's total acid flux that occurs across the skin or *via* the kidney.

U_{in} is urine inulin concentration; P_{in} is plasma inulin concentration; GFR is glomerular filtration rate; V is urine flow; $[X]_u$ is urine concentration of X; $\% T_X$ is fractional reabsorption of X.

A negative flux refers to loss by the animal; positive is uptake.

Values are mean±S.E.M., NS, not significant. Statistical analysis using t-test.

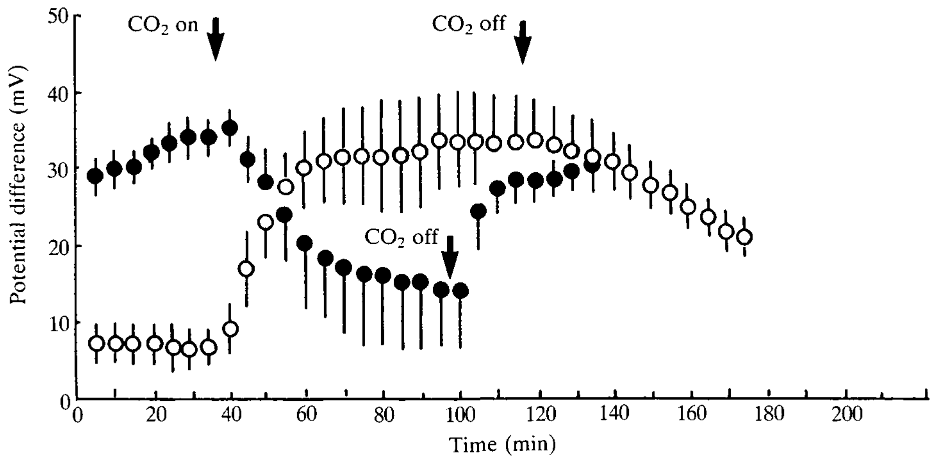

Fig. 5. Cutaneous potential difference in *Ambystoma tigrinum*. Closed circles are adults (N=5); open circles are larvae (N=5; data from Stiffler *et al.* 1987). CO_2 on signifies the start of hypercapnia, CO_2 off signifies the end of hypercapnia. Values are mean±S.E.M.

renal excretion of acid equivalents was 75–80% of total excretion, with the kidneys accounting for the other 20–25%. Although there was no significant increase in $NH_3+NH_4^+$ excretion by either skin or kidneys of partitioned animals the total excretion of this nitrogenous compound appeared approximately to double. The excretion rate of $NH_3+NH_4^+$ was also measured from nonpartitioned animals (Table 1) and here there was a significant increase from 5.5 to 23.3 μequiv 100 g^{-1} h^{-1}. Although it appears that total ammonium excretion contributes to acid excretion, it plays a relatively small role in total acid excretion.

Responses of transcutaneous electrical potential differences

A final group of animals was used to measure changes in cutaneous trans-epithelial potential difference in response to hypercapnia. The control potential difference across the skin (PD) of adults was higher than that found in larval *A. tigrinum* (Stiffler *et al.* 1987). The response to hypercapnia was also different from that in larvae, the PD decreasing instead of increasing (Fig. 5).

Discussion

The extracellular pH of adult *A. tigrinum* is significantly lower than that of larvae by 0.084 units. This is similar to the observation of Burggren and Wood (1981) in the same species. When subjected to hypercapnia, the adults follow a pattern of compensation similar to that of the larvae (Fig. 1; Stiffler *et al.* 1983, 1987) with a slightly higher percentage compensation (44% *versus* 26%). There

were also similar responses by blood gases. Both larvae (Stiffler *et al.* 1983) and adults initially decrease the gradient between arterial and inspired P_{CO_2} and increase P_{O_2} (Fig. 3). We previously interpreted this as being due to increased pulmonary ventilation in the larvae (Stiffler *et al.* 1983) and the same is true for adults during the first 2–3 h (Fig. 2).

To assess the contribution of the urinary bladder to the compensatory process, I ligated bladders of a group of adults and compared them to sham-operated animals. The isolated urinary bladder of *Bufo marinus* can acidify the mucosal bathing medium (Frazier and Vanatta, 1971; Ludens and Fanestil, 1972) and when these toads are fitted with ureteral cannulae, so that the bladder is bypassed by the urine, they are unable to compensate for a respiratory acidosis (Tufts and Toews, 1985). The results of urinary bladder bypass in adult *A. tigrinum* differed from those in the toad, as I observed compensation similar to that seen in shams (Fig. 4).

Mobilization of bone and other calcareous deposits has been suggested as a possible source of base in the compensation for respiratory acidosis in *Rana temporaria* (Simkiss, 1968) and *Bufo marinus* (Tufts and Toews, 1985). This does not occur in larval *Ambystoma tigrinum* (Stiffler *et al.* 1987; Rohrbach and Stiffler, 1987), which have a cartilaginous skeleton. The adults used in the present studies have a calcareous skeleton like the ranid and bufonid anurans described above and potentially could mobilize calcium and magnesium carbonates, bicarbonates and phosphates to act as buffers. Measurements of plasma [Ca^{2+}] and [Mg^{2+}] do not suggest that this is happening, however.

The responses of cutaneous ion exchanges to hypercapnia in non-partitioned adults were quite different from those previously observed in larvae. Hypercapnia in larvae leads to an increase in Na^+ influx and net uptake, a decrease in Cl^- influx, and an increase in net Cl^- loss (Stiffler *et al.* 1987). Similarly, exercise-induced mixed acidoses stimulate Na^+ influx (Rohrbach and Stiffler, 1987), whereas NH_4Cl-induced acidosis increases Na^+ influx and inhibits Cl^- influx (Talbot and Stiffler, 1988). In the present study with adults, I have seen no significant changes in the fluxes of these ions (Table 1). There were, however, similarities in the responses of total ammonia exchange. Although larvae, being aquatic and primarily ammoniotelic, have higher control NH_3+NH_4^+ excretion rates than the terrestrial, ureotelic adults, both are able to increase this nitrogen excretion during hypercapnia (Stiffler *et al.* 1990; Table 1). The increased total (renal plus cutaneous) ammonia excretion is, however, only about one-third of the total acid excretion. Although the increases in NH_3+NH_4^+ excretion appear to be divided between the skin and kidneys (Table 3), I cannot confirm this as there was too much variability in the data from partitioned animals for statistical verification.

The renal clearance data indicate that the kidney function of these animals is in the range we have previously observed for this species (Table 3; Stiffler *et al.* 1980). There were no statistically significant changes in urine flow or GFR. Water reabsorption between control and hypercapnic periods increased from 43 to 64 %.

The kidneys showed little, if any, ionic response to the respiratory acidosis. There were no significant changes in urine pH, $NH_3+NH_4^+$ excretion rate or excretion rate of titratable acidity. There were significant increases in urine Na^+, K^+, Cl^- and $NH_3+NH_4^+$ concentrations and fractional HCO_3^- reabsorption. There were no significant changes in fractional Na^+, K^+ or Cl^- reabsorption to coincide with the increased urinary concentration of these ions. The increased urinary concentrations of these ions, therefore, must have been due to the increased water reabsorption. Yucha and Stoner (1987) report increased HCO_3^- reabsorption in isolated segments of *A. tigrinum* nephron following acidosis. In similar experiments on the toad *B. marinus*, Tufts and Toews (1985) saw very small changes in urine pH and $[HCO_3^-]$ but no changes in titratable acid or $NH_3+NH_4^+$ excretion. Similarly, Yoshimura *et al.* (1961) saw no evidence of a renal response to hypercapnia in the bullfrog. The increased fractional HCO_3^- reabsorption that I observed is quite small and of questionable significance to the animal. There was no significant decrease in HCO_3^- excretion rate, in spite of the increased fractional reabsorption. This was due to the increase in filtered HCO_3^- that was secondary to the increased plasma $[HCO_3^-]$. The responses of the adult and larval kidneys appear to be similar in this species. During a metabolic alkalosis, aquatic larval *A. tigrinum* excrete over 90 % of the HCO_3^- across their skin (Stiffler and Bachoura, 1991) and during an ammonium-induced metabolic acidosis the cutaneous fraction is about the same (C. R. Talbot and D. F. Stiffler, unpublished results).

The experiments performed here were on animals placed in shallow water. This was necessary in order to obtain urine volumes large enough to allow the several chemical analyses that needed to be performed, since removing amphibians from water causes them to decrease urine flow by an order of magnitude to reduce dehydration. It is possible that during dehydration the picture changes. It has been shown that the toad *Bufo marinus* compensates for a respiratory acidosis to approximately the same extent as *Ambystoma tigrinum* when hydrated (Boutilier *et al.* 1979*a*). If, however, these toads are dehydrated, they compensate to a much greater extent (Boutilier *et al.* 1979*b*).

In contrast to the minor role played by the kidneys of adult *A. tigrinum*, the skin appears to play a major role in hypercapnic compensation. The total excretion rate of acid equivalents increased from 16 to 62 μequiv 100 g^{-1} h^{-1} and 82 % of this was across the skin. There were also changes in net cutaneous Na^+ and Cl^- fluxes when the contribution of the kidneys was partitioned from the bath. The increased net Na^+ uptake and increased net Cl^- loss combine to produce a rather large increase in net Na^+ flux minus net Cl^- flux. The net charge flux produced by the difference between Na^+ influx and Cl^- efflux balances the charge flux produced by the efflux of H^+. The mechanism for these acid/ion exchanges is unclear. One possibility is that of electrogenic secretion of H^+ across the skin. Electrogenic H^+ secretion occurs in the skin of the frog *Rana esculenta* (Ehrenfeld *et al.* 1985), and Na^+ influx is coupled indirectly through the charge distribution to this secretion. Such electrogenic H^+ secretion would be consistent with the decrease in cutaneous

PD that occurs in adults during hypercapnia. Changes in the potential difference alone cannot be used to distinguish which of the several possible factors are involved in the alterations in acid–base ion fluxes we have observed. The transepithelial electrical potential difference is a complex product of ionic concentration gradients across the epithelium, passive permeabilities (ionic conductances) of the epithelium and electrogenic ion transport systems (when they are present). It is clear that ionic concentration gradients did not change, since plasma ion concentrations remained stable and I did not alter the ionic composition of the bathing medium. Two other possibilities exist. Changes in the ratios of passive permeability to Na^+ and Cl^- (or other cations and anions) would cause changes in diffusion of these ions down their electrochemical gradients. When I separated net cutaneous fluxes from renal fluxes there was an increased net uptake of Na^+ and an increased net loss of Cl^-. This suggests that passive Na^+ permeability is decreasing and passive Cl^- permeability is increasing, because the isotopic fluxes show that influx of neither ion is altered by the acidosis. This means that changes in net cutaneous flux may be mediated by changes in passive loss across the skin, i.e. changes in permeability. A second possibility is that a decrease in internal positivity, resulting from increased H^+ secretion, would reduce the electrochemical gradient favoring passive efflux of Na^+ across the skin and this could thus increase net uptake of this ion without stimulating influx. Similarly, such electrogenic H^+ secretion would favor passive loss of Cl^-. In all likelihood, the changes in the electrochemical gradients resulting from H^+ secretion would affect the distributions of all permeant ions and the sum of their shifts in distribution could balance the charge deficit created by the secretion of H^+. Therefore, electrogenic H^+ secretion could be working in concert with changing ionic permeabilities to mediate the response.

There are clearly differences between larval and adult cutaneous ion exchanges in this group of salamanders. The decrease in the adult cutaneous PD is in marked contrast to the increase in the larval cutaneous PD. In larval *Ambystoma gracile*, the transcutaneous electrical potential difference increases in proportion to the external $[Na^+]$, regardless of the counterion. In adults, the increase in the potential difference depends upon both Na^+ and Cl^- (Alvarado and Stiffler, 1970). Also, Na^+ and Cl^- can be transported independently in larval *A. tigrinum* (Alvarado and Kirschner, 1963; Dietz *et al.* 1967).

It is impossible to conclude which of these possibilities describes the mechanisms underlying the responses of these animals to acidosis. Although whole-animal studies can be used to partition exchanges between body fluids and the environment among the various organs involved, they can do little but suggest possibilities for the mechanisms employed. Studies on isolated skins, which can be more precisely controlled, will be needed for a complete understanding of the phenomena.

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