INTERACTIONS BETWEEN ACID-BASE BALANCE AND CUTANEOUS ION TRANSPORT IN LARVAL AMBYSTOMA TIGRINUM (AMPHIBIA: CAUDATA) IN RESPONSE TO HYPERCAPNIA

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SUMMARY

Larval Ambystoma tigrinum (Green) were cannulated non-occlusively in the truncus arteriosus and allowed to recover for 20–24 h. They were then subjected to 24 h of hypercapnia in three groups: those adapted to tap water, 100 mmol l⁻¹ NaCl or running distilled water. The animals in tap water displayed a 32% compensation for the respiratory acidosis in that [HCO₃⁻] and strong ion difference (SID) were elevated to return pH back towards the control level. The animals in 100 mmol l⁻¹ NaCl behaved similarly. However, the animals in running distilled water were unable to compensate by increasing either [HCO₃⁻] or strong ion difference until NaCl was added to the medium. The clear requirement for NaCl in the medium of salamanders that are compensating for a respiratory acidosis led to measurements of Na⁺ and Cl⁻ fluxes. The influx of Na⁺ increased by 123% in animals immersed in NaCl and by 39% in animals in Na₂SO₄. The influx of Cl⁻ decreased by over 50% in animals immersed in NaCl and by over 30% in those in choline chloride. The transcutaneous potential difference increased rapidly and reversibly during hypercapnia and the increase was blocked by propranolol. These data suggest that the cutaneous active transport of Na⁺ into the extracellular space is stimulated and that the active transport of Cl⁻ is simultaneously inhibited during hypercapnia. Measurement of circulating interrenal steroids (RIA) revealed a clear increase in aldosterone concentration during hypercapnia and a possible accompanying increase in corticosterone. The results suggest that the compensatory response to hypercapnia in larval A. tigrinum involves alterations in cutaneous ion transport which increase the extracellular SID. These cutaneous responses may be under the short-term control of catecholamines and under the long-term control of interrenal steroids.

INTRODUCTION

Environmental hypercapnia has been used as a tool to study the regulation of body fluid pH in a variety of fish (Cameron & Randall, 1972; Heisler, 1980) and
amphibians (Boutilier, Randall, Shelton & Toews, 1979; Boutilier & Toews, 1981; Stiffler, Tufts & Toews, 1983). The generalized response is a respiratory acidosis, which occurs within 2 h and is followed by compensatory processes that elevate extracellular bicarbonate concentration to return pH back towards normal (Cameron & Randall, 1972; Boutilier et al. 1979).

Both fish and amphibians are capable of anion-independent transport of Na\(^+\) and cation-independent transport of Cl\(^-\) (Maetz & Garcia-Romeu, 1965; Alvarado, Dietz & Mullen, 1975; Garcia-Romeu & Ehrenfeld, 1975). This suggests a link between acid–base balance and electrolyte transport (Kirschner, 1983). Stewart (1981) has formalized this connection between acid–base and electrolyte balance by introducing the physicochemical concept of ‘strong ion difference’ (SID). The SID is the difference between the sum of the concentrations of strong cations (Na\(^+\), K\(^+\), etc.) and the sum of the concentrations of strong anions (Cl\(^-\)) and is one of the three independent variables that determine the pH of biological fluids. The other independent variables are the partial pressure of CO\(_2\) and the total weak acid concentration (A\(_{tot}\), primarily protein). All of the other variables that participate in acid–base reactions, including [H\(^+\)] and [HCO\(_3^-\)], are dependent upon these three. Thus changes in ion transport that alter the SID can play a regulatory role in acid–base balance. This role has been documented in only a few species of fish (Cameron, 1976; Cameron & Wood, 1978; Wood, Wheatley & Höbe, 1984).

Many fish and amphibians that cannot compensate for a respiratory acidosis have only very low rates of electrolyte transport across their exposed surfaces. For example, *Amphiuma means* and *Siren lacertina* have low rates of epithelial ion transport (Bentley, 1969) and are unable to compensate for hypercapnic acidosis (Heisler, Forcht, Ultsch & Anderson, 1982). Similarly, *Necturus maculosus* (Bentley & Yorio, 1977) and the teleost, *Synbranchus marmoratus* (Stiffler, Graham, Dickson & Stockmann, 1986b), transport ions only very slowly across their exposed surfaces and are unable to elevate [HCO\(_3^-\)] during respiratory acidosis (*N. maculosus*: Stiffler et al. 1983; *S. marmoratus*: Heisler, 1982). Other species, such as *Salmo gairdneri* (Wood et al. 1984), *Bufo marinus* (Crabbe, Fanestil, Pelletier & Porter, 1974) and *Ambystoma tigrinum* (Alvarado & Kirschner, 1963), which have high rates of ion transport across their skin, are quite capable of elevating [HCO\(_3^-\)] when exposed to external hypercapnia (*S. gairdneri*: Cameron & Randall, 1972; *B. marinus*: Boutilier et al. 1979; *A. tigrinum*: Stiffler et al. 1983).

Electrolyte transport in *A. tigrinum* is clearly supported by interrenal steroids. Aldosterone stimulates cutaneous transport (Alvarado & Kirschner, 1964) and renal reabsorption of Na\(^+\) (Heney & Stiffler, 1983). Hypernatraemia and hyponatraemia depress and elevate, respectively, circulating aldosterone concentrations in this species (Stiffler, DeRuyter, Hanson & Marshall, 1986a). Since there is a clear connection between electrolyte transport and acid–base balance and since hypercapnia potentiates steroid secretion in mammals (Raff, Shinsako, Keil & Dallman, 1983), it is possible that these hormones are involved in acid–base balance in amphibians as well.
Our objectives were to determine if there is a relationship between acid-base balance and cutaneous ion transport in larval *Ambystoma tigrinum* and to establish if interrenal steroids might be involved in the response to respiratory acidosis. A series of reciprocal experiments was designed to meet the first objective. Responses to environmental hypercapnia were monitored in arterially cannulated larvae adapted to distilled water, tap water and 100 mmol l\(^{-1}\) NaCl. Conversely, the effects of respiratory acidosis on electrolyte transport were assessed by measuring influx, efflux and net flux of Na\(^+\) and Cl\(^-\) before, during and after hypercapnia. Plasma concentrations of aldosterone, corticosterone and cortisol were also measured.

**MATERIALS AND METHODS**

Neotenic larval *Ambystoma tigrinum* (100–200 g) were purchased from St Croix Biological in Stillwater, MN and housed at 3°C in filtered water in an aquarium until used in experiments. Animals were allowed to acclimate to room temperature (25°C) for 48–72 h before initiating the experiments which were conducted at 25°C.

**Surgery**

The larvae were anaesthetized in 0·1 % tricaine methanesulphonate (TMS) buffered in 0·1 % (w/v) NaHCO\(_3\). When unconscious, the animals were draped with wet paper towels and placed on their backs for surgery. A 1-cm incision was made to expose the truncus arteriosus. A non-occlusive cannula constructed from PE 50 was then placed in the truncus as described previously (Stiffler et al. 1983). The cannula was filled with amphibian Ringer’s solution containing 125 units ml\(^{-1}\) heparin. The animals were then placed in stoppered jars containing 2·5 l of aerated tap water and allowed to recover overnight.

**Experimental design: responses of blood parameters to hypercapnia in various ionic environments**

Control larvae were prepared as above and subjected to 24 h of hypercapnia in tap water by gassing their chambers with 3 % CO\(_2\) prepared with Brooks rotameter flowmeters. Four blood samples (0·5–0·8 ml) were taken: prior to hypercapnia; 2 h after initiating gassing; at 24 h; and after 24 h of recovery following cessation of 3 % CO\(_2\) and resumption of aeration. The samples were treated with approx. 0·025 ml of heparinized Ringer’s solution. Following blood gas analysis (see below) the plasma was separated by centrifugation, removed, and stored frozen for later analysis. The red cells were resuspended in Ringer’s solution and reinjected into the larvae. A second group of larvae was adapted to 100 mmol l\(^{-1}\) NaCl. The NaCl was added following the control blood sampling. After 24 h a second sample was taken to establish the response to the external saline solution, and hypercapnia (3 % CO\(_2\)) was initiated. The sampling protocol and schedule described above were used for the remainder of the experiment. A third group of larvae was exposed to flowing distilled water to deprive them of an external electrolyte source. The animals were housed in jars which were equipped with side-drains. Following control blood sampling
distilled water, contained in reservoirs, was gravity fed into the chambers at 20 ml min$^{-1}$ to flush the chambers of ions continuously. The distilled water in the chambers had been previously equilibrated with 3% CO$_2$; the chambers containing the animals began receiving 3% CO$_2$ at the same time as the water began to flow. Blood samples were taken at 2 h and at 24 h after initiating hypercapnia. Bath samples were also taken for electrolyte analysis. After the first 24 h of hypercapnia, NaCl was added to the bath to bring the bath concentration to 0.4 mmol l$^{-1}$ NaCl and the distilled water was turned off. The CO$_2$ remained on for another 24 h before a final blood sample was taken.

**Blood analysis**

Blood gases were analysed with an Instrumentation Laboratories IL 113 blood gas analyser. The pH electrode was calibrated with IL precision buffers using temperature corrections supplied by the manufacturer. The CO$_2$ and O$_2$ electrodes were calibrated using certified gas mixtures of 1.00% CO$_2$, balance air and 1.96% CO$_2$, balance nitrogen. Plasma bicarbonate concentrations were calculated using the Henderson–Hasselbalch equation. The values used for pH ($6.05$) and solubility of CO$_2$ ($0.033$ mmol CO$_2$ l$^{-1}$ Torr$^{-1}$) were those determined by Boutilier et al. (1979).

Plasma [Na$^+$] and [K$^+$] were analysed using a Coleman model 51 flame photometer. Plasma [Ca$^{2+}$] and [Mg$^{2+}$] were analysed using a Perkin-Elmer Model 4000 Atomic Absorption Spectrometer. Plasma [Cl$^-$] was analysed using a Buchler chloridometer.

Circulating interrenal steroid concentrations were assayed by radioimmunoassay (RIA). Aldosterone and cortisol were assayd using the solid-state RIA procedures developed by Diagnostic Products (Los Angeles, CA), corticosterone was assayed by the procedure of Endocrine Sciences (Tarzana, CA) as previously reported (Stiffler et al. 1986a).

**Isotopic flux measurements**

Net and unidirectional fluxes of Na$^+$ and Cl$^-$ were measured using the isotopic influx methods described by Kirschner (1970). Larvae were placed in measured volumes containing approximately 2 mmol l$^{-1}$ Na$^+$ or Cl$^-$ and 1 $\mu$Ci l$^{-1}$ of either $^{22}$Na$^+$ (New England Nuclear) or $^{36}$Cl$^-$ (Oak Ridge National Laboratory). In this range of external NaCl concentrations the $K_m$ for transport of both Na$^+$ and Cl$^-$ is exceeded by an order of magnitude and the carriers are saturated so that changes in external [Na$^+$] or [Cl$^-$] that result from changes in net transport of the ions will not affect influx of the ions (Alvarado & Dietz, 1970). The bathing media of the larvae were sampled at measured intervals and counted on a Beckman 4000 gamma counter ($^{22}$Na$^+$) or a Packard Tricarb liquid scintillation counter ($^{36}$Cl$^-$). Ion concentrations were measured as described above. The influx, efflux and net fluxes of the ions were calculated as previously described (Stiffler et al. 1986b). The specific activity of the blood was less than 5% of the specific activity of the bath at the end of the experiments.
The experimental design for the flux measurements was to subject the animals to consecutive 24-h periods of normocapnia (control), hypercapnia (3% CO₂) and normocapnia (recovery) in NaCl, Na₂SO₄ and choline chloride. The latter two solutions were employed to study ion transport in the absence of a transported counterion and were changed after 12 h in each of the control and hypercapnic periods to prevent effluxing counterions from building up in the bath.

**Electrical measurements**

Transepithelial electrical potential difference (TEP) was measured between the extracellular fluid and the bathing medium using a Tektronix DM502 digital multimeter and calomel half-cells. Larvae were anaesthetized as above and small holes were made through the ventral skin. Ringer–agar salt bridges, made from PE50, connected the half-cells to the animal and bath. 1% urethane (ethyl carbamate) was placed in the bathing medium to maintain anaesthesia. Intraperitoneal injections of propranolol (Sigma Chemical Co., St Louis, MO, USA, 0·2 μg g⁻¹) were administered to test for beta-adrenergic mediation of the TEP response.

**RESULTS**

Larval *A. tigrinum* in tap water responded to 3% CO₂ as previously described (Stiffler et al. 1983). Equilibrating the bath with 3% CO₂ brought about an increase in arterial PₐCO₂ from 9·5 to 26·7 Torr in the first 2 h (Fig. 1). This resulted in a drop in pH from 7·85 to 7·35. After 24 h, PₐCO₂ had increased to 34 Torr and pH had increased to 7·41 due to a partial compensation through the accumulation of HCO₃⁻ which increased plasma [HCO₃⁻] from 18·4 to 28·8 mmol l⁻¹. This represents a 31·6 ± 8·7% compensation (mean ± s.e.m.) (Lai, Martin, Attebery & Brown, 1973) (ANOVA: P < 0·01). PₐO₂ remained relatively stable at 30–40 Torr. Analysis of plasma electrolytes demonstrated an increase in [K⁺] of 0·5 mmol l⁻¹ and a decrease in [Cl⁻] of 12·6 mmol l⁻¹ which were significant (P < 0·05). This, in turn, led to a 31% increase in SID after 24 h of hypercapnia (Table 1).

The animals subjected to respiratory acidosis while in distilled water responded to hypercapnia quite differently from the control animals in tap water (Fig. 1). While the initial control blood gases did not differ from the tap-water group, the blood gases at 2 h of hypercapnia in flowing distilled water demonstrated a mixed respiratory and metabolic acidosis slightly greater than that of the tap-water group. The biggest difference between the distilled-water-adapted larvae and the tap-water controls, however, occurred after 24 h. At this point the animals in the electrolyte-reduced medium (approx. 0·06 mmol l⁻¹ Na⁺) remained at essentially the same pH and [HCO₃⁻] as they had been after 2 h; in other words, there was no evidence of compensation. There were no significant changes in any of the plasma ion concentrations during the 24 h of hypercapnia in larvae adapted to distilled water. At the end of the 24-h period, the distilled water was turned off, a small amount of 1 mol l⁻¹ NaCl was added to the bath to bring the external [Na⁺] to 0·4 mmol l⁻¹,
which is above the $K_m$ for $\text{Na}^+$ transport in *Ambystoma* (Alvarado & Dietz, 1970), and the $\text{CO}_2$ was left on. After an additional 24 h of hypercapnia in the presence of external $\text{Na}^+$, the larvae had elevated their $[\text{HCO}_3^-]$ in the typical compensatory manner of the controls. During the experiment the mean $P_O_2$ increased from 23 to 42 Torr. No significant compensatory changes in plasma ionic composition were observed during the hypercapnic period; however, a decline in $[\text{NaCl}]$, probably due to depletion in distilled water (Heney & Stiffler, 1983), did occur over the course of the experiment.

The third group of larvae to be exposed to hypercapnia were first adapted to 100 mmol l$^{-1}$ $\text{NaCl}$ for 24 h (Fig. 1). Upon 2 h exposure to hypercapnia these larvae exhibited a mixed respiratory and metabolic acidosis: the pH decreased from 7.83 to 7.33; the $P_{\text{CO}_2}$ rose from 10.7 to 26.4 Torr; and $[\text{HCO}_3^-]$ dropped from 22.9 to

![Fig. 1. A pH-$P_{\text{CO}_2}$-$[\text{HCO}_3^-]$ diagram illustrating differences in responses to hypercapnia between groups of larvae immersed in tap water ($N = 13$, open circles), 100 mmol l$^{-1}$ $\text{NaCl}$ ($N = 5$, closed circles and dashed lines) and running distilled water ($N = 7$, open triangles). For larvae in tap water, point 1 refers to post-operative control values, point 2 refers to 2 h hypercapnia, point 3 refers to 24 h hypercapnia and point 4 refers to normocapnic recovery. For larvae immersed in 100 mmol l$^{-1}$ $\text{NaCl}$, point a refers to control in tap water, point b refers to 24 h in 100 mmol l$^{-1}$ $\text{NaCl}$, point c refers to 2 h of hypercapnia in 100 mmol l$^{-1}$ $\text{NaCl}$, point d refers to 24 h hypercapnia in 100 mmol l$^{-1}$ $\text{NaCl}$. For larvae subjected to running distilled water, point I refers to control in tap water, point II refers to 2 h hypercapnia in running distilled water, point III refers to 24 h hypercapnia in running distilled water, point IV refers to an additional 24 h hypercapnia after the distilled water flow had been terminated and $\text{NaCl}$ had been added to the bath.](image-url)
Table 1. Plasma electrolyte concentrations (in mmol l⁻¹) of larvae in tap water, distilled water and 100 mmol l⁻¹ NaCl before and during hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water (N = 13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>109.0 ± 2.5</td>
<td>110.3 ± 3.5</td>
<td>106.4 ± 5.4</td>
</tr>
<tr>
<td>K⁺</td>
<td>3.0 ± 0.1</td>
<td>3.3 ± 0.3</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>78.6 ± 2.2</td>
<td>79.0 ± 3.4</td>
<td>66.0 ± 3.8</td>
</tr>
<tr>
<td>SID</td>
<td>35.0 ± 2.0</td>
<td>36.0 ± 4.2</td>
<td>46.0 ± 4.8</td>
</tr>
</tbody>
</table>

Distilled water (N = 7)

|                      |                 |                 |                 |
| Na⁺                  | 118.4 ± 1.0     | 104.5 ± 4.0     | 109.9 ± 4.4     |
| K⁺                   | 3.2 ± 0.2       | 2.9 ± 0.3       | 3.5 ± 0.2       |
| Ca²⁺                 | 0.5 ± 0.1       | 1.0 ± 0.4       | 0.6 ± 0.1       |
| Mg²⁺                 | 0.2 ± 0.1       | 0.4 ± 0.1       | 0.2 ± 0.0       |
| Cl⁻                  | 79.6 ± 1.5      | 74.4 ± 3.3      | 72.3 ± 2.5      |
| SID                  | 43.4 ± 2.2      | 35.7 ± 3.8      | 42.8 ± 3.1      |

100 mmol l⁻¹ NaCl (N = 5)

|                      |                 |                 |                 |
| Na⁺                  | 122.5 ± 3.4     | 123.7 ± 6.8     | 126.2 ± 4.4     |
| K⁺                   | 2.7 ± 0.1       | 2.9 ± 0.2       | 2.8 ± 0.2       |
| Ca²⁺                 | 0.6 ± 0.1       | 0.6 ± 0.1       | 0.4 ± 0.0       |
| Mg²⁺                 | 0.3 ± 0.1       | 0.3 ± 0.1       | 0.3 ± 0.1       |
| Cl⁻                  | 83.7 ± 2.0      | 83.1 ± 3.1      | 72.2 ± 2.4     *|
| SID                  | 43.2 ± 1.8      | 45.5 ± 7.2      | 58.1 ± 3.4     *|

Values are means ± S.E.M.

* Significantly different from controls, P < 0.05.

SID, strong ion difference.

16.4 mmol l⁻¹. During the next 22 h the animals displayed a partial compensation of 15.8 ± 3.7 %, which was not significantly different from the compensation of the animals in tap water (P > 0.2; t-test). P O₂ increased from 31 to 37 Torr during hypercapnia. Analyses of plasma ion concentrations showed a significant decrease in [Cl⁻] and a significant increase in SID (Table 1).

Measurements of ionic fluxes yielded results which are consistent with the blood gas and electrolyte responses to hypercapnia. Larvae in 1.7 mmol l⁻¹ NaCl more than doubled Na⁺ influx rates (P < 0.05, ANOVA, paired t-test) during hypercapnia (P CO₂ = 30 Torr) and returned to below control rates (P < 0.05; Table 2) during recovery from the hypercapnia. Hypercapnia did not alter Na⁺ efflux: however, during recovery there was a significant increase in efflux (P < 0.01) of this ion. Net fluxes of Na⁺ also changed. A small net loss of Na⁺ was reversed (P < 0.01) during hypercapnia, and during recovery the net flux reverted to a net loss which exceeded the control value (P < 0.01).

Chloride fluxes of animals in 1.7 mmol l⁻¹ NaCl also showed some interesting changes during hypercapnia (Table 3). Exposure to CO₂ resulted in a decrease in Cl⁻ influx of over 50 % (P < 0.05; ANOVA, paired t-test). This was reversed during recovery. The efflux of Cl⁻ did not change significantly during the experiment.
Table 2. $Na^+$ fluxes of larvae in 1·7 mmol l$^{-1}$ NaCl before, during and after hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Na^+$ influx</td>
<td>10·5 ± 1·1</td>
<td>23·4 ± 4·6*</td>
<td>5·6 ± 1·0</td>
</tr>
<tr>
<td>$Na^+$ efflux</td>
<td>-14·1 ± 2·4</td>
<td>-14·0 ± 5·1</td>
<td>-24·2 ± 3·6</td>
</tr>
<tr>
<td>$Na^+$ net flux</td>
<td>-3·6 ± 2·0</td>
<td>9·3 ± 2·6*</td>
<td>-18·6 ± 3·5</td>
</tr>
</tbody>
</table>

+ Fluxes indicate uptake; − fluxes indicate loss of ions.
* Significantly greater than controls ($P<0·05$).

$N = 7$

There was, however, a significant ($P<0·05$) increase in net Cl$^-$ loss during hypercapnia which reverted to a small net uptake during recovery.

Net K$^+$ fluxes were also monitored in the animals used in the Cl$^-$ study reported above (Table 3). During the control period, there was a small net uptake of K$^+$ which changed to a net loss during hypercapnia ($P<0·01$). During recovery from hypercapnia, the net loss increased by more than five-fold.

Sodium fluxes were also measured in animals immersed in 2 mmol l$^{-1}$ Na$_2$SO$_4$ (Table 4). The $Na^+$ fluxes were higher in this group than in the group in NaCl; this may be related to the absence of the normal counterion. As was the case in NaCl, hypercapnia elevated the influx of $Na^+$ significantly ($P<0·01$, ANOVA, paired t-test). During the recovery period, $Na^+$ influx was less than it had been during hypercapnia ($P<0·05$) but greater than during control conditions ($P<0·05$). The larvae in sodium sulphate also demonstrated a reduced $Na^+$ efflux during hypercapnia which persisted throughout recovery ($P<0·05$). Changes in net flux also occurred in this group, which developed a three-fold increase in net uptake of $Na^+$ during hypercapnia. Net uptake remained at twice the control level during recovery ($P<0·01$). Chloride concentration remained below 0·1 mmol l$^{-1}$ in the bathing medium.

Chloride fluxes were measured in choline chloride (Table 5). Again the fluxes were higher than they had been in NaCl and this may also be due to the absence of the normal counterion. As occurred in NaCl, hypercapnia decreased the influx of Cl$^-$ in choline chloride. No significant differences were observed in efflux or net flux. The $[Na^+]$ in the bathing medium averaged 0·16 mmol l$^{-1}$ at the end of the flux periods.

Table 3. Cl$^-$ and K$^+$ fluxes of larvae in 1·7 mmol l$^{-1}$ NaCl before, during and after hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl$^-$ influx</td>
<td>12·0 ± 2·2</td>
<td>5·3 ± 0·9*</td>
<td>18·8 ± 4·0</td>
</tr>
<tr>
<td>Cl$^-$ efflux</td>
<td>-13·6 ± 3·0</td>
<td>-17·8 ± 2·6</td>
<td>-18·1 ± 2·4</td>
</tr>
<tr>
<td>Cl$^-$ net flux</td>
<td>-1·7 ± 1·7</td>
<td>-12·7 ± 2·8*</td>
<td>0·7 ± 4·0</td>
</tr>
<tr>
<td>K$^+$ net flux</td>
<td>0·8 ± 0·2</td>
<td>-1·2 ± 0·4*</td>
<td>-6·5 ± 0·6*</td>
</tr>
</tbody>
</table>

Sign convention as in Table 2, *$P<0·05$.

$N = 10$. 
Electrical transepithelial potential difference (TEP) measurements (Fig. 2) show a very rapid response to hypercapnia. In the first 20 min the TEP increased from approx. 10 mV to about 30 mV. This was followed by a small secondary increase in

Table 4. Na\(^+\) fluxes of larvae in 2 mmol l\(^{-1}\) Na\(_2\)SO\(_4\) before, during and after hypercapnia

<table>
<thead>
<tr>
<th>(μequiv 100 g(^{-1}) h(^{-1}))</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+) influx</td>
<td>28.0 ± 2.1</td>
<td>39.0 ± 1.9*</td>
<td>34.1 ± 2.0</td>
</tr>
<tr>
<td>Na(^+) efflux</td>
<td>−21.6 ± 3.5</td>
<td>−9.3 ± 1.8*</td>
<td>−13.4 ± 2.0</td>
</tr>
<tr>
<td>Na(^+) net flux</td>
<td>8.9 ± 2.8</td>
<td>29.6 ± 2.2*</td>
<td>20.8 ± 2.0</td>
</tr>
</tbody>
</table>

Sign convention as in Table 2, *P < 0.05. 
N = 10.

Table 5. Cl\(^−\) fluxes of larvae in 2 mmol l\(^{-1}\) choline chloride before, during and after hypercapnia

<table>
<thead>
<tr>
<th>(μequiv 100 g(^{-1}) h(^{-1}))</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(^−) influx</td>
<td>30.9 ± 5.4</td>
<td>20.6 ± 5.1*</td>
<td>30.8 ± 7.6</td>
</tr>
<tr>
<td>Cl(^−) efflux</td>
<td>−34.4 ± 11.3</td>
<td>−37.7 ± 9.5</td>
<td>−34.5 ± 1.7</td>
</tr>
<tr>
<td>Cl(^−) net flux</td>
<td>−3.2 ± 10.4</td>
<td>−17.1 ± 6.5</td>
<td>−3.8 ± 16.5</td>
</tr>
</tbody>
</table>

Sign convention as in Table 2, *P < 0.05. 
N = 12.

Fig. 2. Transcutaneous electrical potential difference (TEP) in response to hypercapnia. Five larvae (closed circles) responded with an almost instantaneous increase in potential difference, which showed a small, delayed secondary rise after approximately 1 h. A single control larva (open circles) showed a slow rise in potential difference over the same period and under the same conditions of anaesthesia; a response very different from the responses in the experimental group. Six larvae received propranolol injections (triangles) and these did not respond to the hypercapnia with an increased TEP. Bars indicate ±1 S.E.M.
TEP to about 35 mV after 1 h. The TEP was reversed during recovery. The response of a control animal which was anaesthetized in the same manner as the experimental animals but not exposed to hypercapnia was a very small increase in TEP which continued to increase slowly over 3 h. Intraperitoneal injections of propranolol (0.2 μg g⁻¹) blocked the response of the TEP to hypercapnia.

Measurements of interrenal steroid titres revealed some striking correlations. The concentration of aldosterone was negatively correlated with pH ($r = -0.68$, $P < 0.01$) and positively correlated with $P_{CO_2}$ ($r = 0.52$, $P < 0.01$). Corticosterone concentration was more weakly correlated with these parameters [$r = 0.37$, $P < 0.05$ ($P_{CO_2}$) and $r = -0.43$, $P < 0.05$ (pH)]. The cortisol concentration was very low and remained constant throughout the experiment. Aldosterone concentration increased more than five-fold after 2 h of hypercapnia and remained at almost three times the control level after 24 h (Fig. 3; $P < 0.01$, ANOVA). After 24 h of normocapnic recovery, aldosterone had returned to control levels. Corticosterone concentration appeared to increase during hypercapnia, and although there was a marginally significant correlation between pH and corticosterone concentration, analysis of variance failed to detect significant differences between groups.

**DISCUSSION**

*Extracellular acid–base and electrolyte balance in various ionic media*

The responses of larvae in tap water to hypercapnia were similar to those we have previously reported (Stiffler et al. 1983). The depression in pH was due primarily to a respiratory acidosis and secondarily to a slight metabolic acidosis, which we have previously attributed to an accumulation of fixed acids that results from swimming
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associated with increased air-breathing (Stiffler et al. 1983). Larvae immersed in 100 mmol l\(^{-1}\) NaCl displayed no significant changes in blood gas parameters between measurements taken when they were still in tap water and after 24 h in saline, and they were not significantly different from the control group in this regard (Fig. 1). Katz (1981) observed acidotic conditions in *Bufo viridis* exposed to hyperosmotic saline solutions; however, his toads were exposed for longer periods and to much higher concentrations. Our animals responded to hypercapnia with a typical acidosis but the metabolic component of the acidosis was more pronounced than that observed in tapwater-adapted animals. After 24 h of hypercapnia, the animals in 100 mmol l\(^{-1}\) NaCl appeared to compensate partially and were not significantly different from controls in this ability. Attempts to repeat this type of experiment in 200 mmol l\(^{-1}\) NaCl to approach more closely the conditions which produced acidosis in *B. viridis* (Katz, 1981) uniformly produced mortality in *A. tigrinum* during hypercapnia. Although this is indicative of the saline solutions’ ability to interfere with acid–base balance it is not entirely clear how the presence of high concentrations of external salts might act on the processes involved. The conventional interpretation is that salt-loading inhibits NaCl transport mechanisms which are needed for acid–base regulation. While it is true that salt-loading does inhibit Na\(^+\) transport in *A. tigrinum* (Kirschner, Kerstetter, Porter & Alvarado, 1971), the isosmotic–hyperosmotic conditions the animal is faced with also reduce glomerular filtration in the kidneys and might affect renal compensatory mechanisms (Stiffler & Alvarado, 1974). A third possibility is that adverse osmotic gradients might lead to reduced blood flow in the skin and this, in turn, might affect ion transport across it (D. F. Stiffler, in preparation). Osmotic water movement is influenced by cutaneous perfusion in frogs (Mahany & Parsons, 1978). To limit the number of variables but still be able to perturb ion transport in a way that might interfere with acid–base balance, we turned to removal of electrolytes from the bathing medium. By placing the animals in flowing distilled water, we were able to keep [NaCl] below the transport \(K_m\) for Na\(^+\) for these salamanders and follow their response to hypercapnia. The immediate response after 2 h of 3 % CO\(_2\) was a typical mixed acidosis with the largest metabolic component of the three groups. The most striking finding in this series of experiments was, however, that after 24 h of hypercapnia in the absence of external ions (hence the absence of ion transport) the larvae had elevated neither pH nor [HCO\(_3^-\)]. These larvae were very similar to *Necturus maculosus*, which shows very low rates of ion transport, in their response to hypercapnia (Stiffler et al. 1983). Following the 24 h of hypercapnia in distilled water, the water flow was stopped and NaCl was added to the bath; however, the CO\(_2\) was continued for another 24 h. At the end of this period a typical compensatory response was observed (Fig. 1). Examination of plasma ionic concentrations in these three groups of animals revealed changes in electrolyte concentrations which were consistent with the changes in blood gases and pH profiles. Those animals that were able to compensate for hypercapnia by [HCO\(_3^-\)] elevation (those in tap water and 100 mmol l\(^{-1}\) NaCl bathing media) displayed increases in strong ion difference which resulted primarily from Cl\(^-\) depletion. The elevation of Ca\(^{2+}\) and Mg\(^{2+}\) concentrations reported for
frogs (Simkis, 1968) and turtle (Jackson & Heisler, 1982) did not occur in our animals. This is not surprising, however, as larval ambystomatids have a cartilaginous skeleton and lack parathyroid glands (Baldwin, 1918). The catfish, *Ictalurus punctatus*, also fails to elevate extracellular \([\text{Ca}^{2+}]\) during hypercapnia (Cameron, 1985). The animals in distilled water which did not compensate for the respiratory acidosis by elevating \([\text{HCO}_3^-]\) also failed to elevate SID, which is again entirely consistent.

**Transcutaneous ion fluxes during hypercapnia**

In view of the apparent requirement for external NaCl for normal compensatory responses during hypercapnia, we have evaluated the responses of both \(\text{Na}^+\) and \(\text{Cl}^-\) transport mechanisms to 3% CO₂. Since ion exchange occurs almost exclusively across the skin of this species, with the gills playing only a minor role (Baldwin & Bentley, 1982), we consider that the electrolyte influx responses are cutaneous. The unidirectional influx of \(\text{Na}^+\) was increased during hypercapnia in larvae immersed in both NaCl and Na₂SO₄. This suggests that \(\text{Na}^+\) is transported independently (of \(\text{Cl}^-\)) into the animals, either in exchange for \(\text{H}^+\) (Kirschner, 1983) or alone (with consequent shifts in a number of external and internal chemical equilibria to preserve electroneutrality; Stewart, 1981). This elevates internal SID in the compensatory manner observed in larvae whose blood chemistry was followed throughout the 24 h of hypercapnia (Table 1). The fact that these larvae did not display elevated \([\text{Na}^+]\) in their plasma as a result of the increased influx may be due to shifts in water from the bath to the extracellular space. In 24 h, an additional 264 μequiv of \(\text{Na}^+\) would accumulate in the ECF of a 100-g animal as a result of the increased rate of ion transport. Such an animal has an extracellular fluid (ECF) volume of 32 ml (Stiffler & Alvarado, 1974) and a plasma \([\text{Na}^+]\) of 109 mmol l⁻¹ (Table 1); thus there would be about 3488 μequiv of \(\text{Na}^+\) in the ECF at the start of hypercapnia. The 264 μequiv accumulating in 24 h of increased \(\text{Na}^+\) influx would thus represent only a 7.5% increase and would require only a 7.5% volume expansion as a result of osmotic water uptake to maintain constant ECF concentration. Larval *A. tigrinum* easily tolerate volume expansion of this magnitude (Stiffler, Atkins, Burt & Roach, 1982). The isotopic flux method allows the simultaneous examination of efflux, which is the sum of diffusive loss across the skin and urinary loss through the kidneys. The fact that there were not consistent changes in \(\text{Na}^+\) efflux might mean that the kidneys are not involved in the compensatory mechanisms which result in bicarbonate accumulation in the blood. Simultaneous renal ionoregulatory responses may obscure the response. Direct assessments by renal clearance methods will have to be made before this question can be answered.

Chloride fluxes were also evaluated in animals in the presence (NaCl) and absence (choline chloride) of the physiological counterion. In both cases there was a significant depression in \(\text{Cl}^-\) influx during hypercapnia, which is consistent with the fact that the elevation of SID in the cannulated animals was, in part, due to a depression in blood \([\text{Cl}^-]\).
Although we did not measure unidirectional K\(^+\) fluxes we did follow net fluxes of this cation in one group of larvae (Table 3). A very small net uptake of K\(^+\) during control conditions was reversed to become a net loss during hypercapnia (\(P<0.01\)), which became even larger during recovery (\(P<0.01\)). This is consistent with the increased plasma [K\(^+\)] observed during hypercapnia in the cannulated animals in tap water. The toad *B. marinus* also increases K\(^+\) loss during hypercapnia (Tufts & Toews, 1985). The increased extracellular [K\(^+\)] might be the result of exchanges of extracellular H\(^+\) for intracellular K\(^+\) in order to buffer H\(^+\) on intracellular protein. The resultant accumulation of K\(^+\) in the extracellular fluid would require increased excretion of this ion. The avenue of excretion might be either renal (Stiffler *et al.* 1986a) or extrarenal across the skin (Nielson, 1984).

**Changes in circulating steroid hormone concentration during hypercapnia**

The dependence of acid–base regulatory responses on ionoregulatory processes documented here and elsewhere (Cameron & Wood, 1978; Wood *et al.* 1984) raises the question of how these changes in electrolyte transport might be mediated. Although many hormones are involved in the control of sodium transport, one group, the adrenal corticoids (interrenal steroids in amphibians), has also been strongly implicated in acid–base balance. Frazier & Vanatta (1971) and Ludens & Fanestil (1972) showed that toads from some populations of *Bufo marinus* are capable of acidifying the fluid contained in their bladders and that this acidification can be enhanced by aldosterone (Ludens & Fanestil, 1974; Frazier & Zachariah, 1979). Both aldosterone and corticosterone have been associated with sodium balance in *A. tigrinum* (Alvarado & Kirschner, 1964; Heney & Stiffler, 1983; Stiffler *et al.* 1986a). These findings prompted us to examine circulating concentrations of the three interrenal steroids which have been detected in amphibian plasma. As in previous studies (Krug, Honn, Battista & Nicoll, 1983; Stiffler *et al.* 1986a), cortisol concentrations were very low and did not change during the course of the hypercapnia. Corticosterone was higher in concentration and was weakly correlated with pH in our study; however, there were no significant differences between control and hypercapnic groups (ANOVA). By far the clearest implication of a role for steroid hormones in acid–base balance emerged from the analyses of aldosterone concentration. Within 2 h of the onset of hypercapnia, aldosterone concentration had increased dramatically. After 24 h, the aldosterone concentration had become reduced to levels below the 2-h value but was still greater than the control value.

**Changes in transcutaneous electrical potential difference during hypercapnia**

The changes in ion fluxes we have observed suggest that there might be changes in the electrochemical potential difference associated with ion transport in amphibian skin. This would provide an instantaneous measure of events associated with the responses and would allow us to evaluate the time course of the response with much greater resolution (see Fig. 2). The response is very rapid, occurring in a matter of minutes. This is much too rapid for steroid hormones, which require time for gene activation and protein synthesis. Possible mediators of this rapid response might be
catecholamines. Beta-adrenergic stimulation of Na\(^+\) transport has been reported for teleost gill (Girard & Payan, 1977), and the beta-blocker propranolol blocks the response of the TEP to hypercapnia in \textit{A. tigrinum} (Fig. 2). It may be that catecholamines produce the immediate response of ion transport to hypercapnia and are later supplanted by steroids after the time required for protein synthesis has elapsed. This is consistent with the transient increase in catecholamine concentrations proposed for \textit{S. gairdneri} in response to acidosis (Boutilier, Iwama & Randall, 1986).

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REFERENCES


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