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AN ANALYSIS OF BRANCHIAL AMMONIA EXCRETION IN THE FRESHWATER RAINBOW TROUT: EFFECTS OF ENVIRONMENTAL pH CHANGE AND SODIUM UPTAKE BLOCKADE

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SUMMARY

Short-term treatments (3 h) designed to change the relative NH_3 (ΔP_{NH_3}) and NH_4^+ (ΔNH_4^+) gradients and sodium transport (J^{Na}) across the gills were employed to analyse the normal mechanism(s) of branchial ammonia excretion (J_{net}^{Amm}) in trout acclimated to fresh water of pH ≃ 8·0. Control J_{net}^{Amm} occurred in the absence of, or against, an apparent ΔP_{NH_3} gradient, while ΔNH_4^+ was positive. Severe acid exposure (pH = 4·06) raised ΔP_{NH_3} and ΔNH_4^+, abolished J_{in}^{Na}, and reduced J_{net}^{Amm} by 28 %, while moderate acidity (pH = 6·64), which also elevated ΔP_{NH_3}, had no significant influence on J_{in}^{Na} and J_{net}^{Amm}. Severe alkaline exposure (pH = 9·54) raised ΔNH_4^+, reduced ΔP_{NH_3} to a very negative value, and decreased J_{in}^{Na} and J_{net}^{Amm} by equimolar amounts, representing 55 % and 80 % of control levels respectively. Moderate alkalinity (pH = 8·69) had similar effects on ΔP_{NH_3} and ΔNH_4^+, but reduced J_{in}^{Na} and J_{net}^{Amm} by only ~25 %. The sodium transport inhibitor amiloride (10^{-4} mol l^{-1} in the external water, pH ≃ 8·0) had very similar effects to pH = 4·06 on both J_{in}^{Na} and J_{net}^{Amm}, but did not alter ΔP_{NH_3} or ΔNH_4^+. The results discount the quantitative importance of NH_4^+ diffusion and favour a flexible combination of NH_3 diffusion and Na^+/NH_4^+ exchange as the major mechanisms of J_{net}^{Amm}, with the latter dominating under the particular control conditions of the present study.

INTRODUCTION

Of the three major respiratory gases of aquatic animals, ammonia is probably the least understood. Metabolic processes are generally thought to produce ammonia in the NH_3 form, but with a $pK' \simeq 9{\cdot}5$, the great majority exists in the NH_4^+ form at physiological pH. Early studies by Smith (1929) established that the gills were the principal site of ammonia excretion. While some ammonia may arise *de novo* in the gill tissue (Goldstein & Forster, 1961), it is now clear that the major part of

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branchial ammonia excretion represents clearance from the blood (Pequin & Serfaty, 1963; Goldstein, Forster & Fanelli, 1964; Payan & Matty, 1975; Payan & Pic, 1977; Cameron & Heisler, 1983). However there is some question as to the actual mechanism(s) by which ammonia moves across the branchial epithelium (see Kormanik & Cameron, 1981*a* for review). It is well documented that ammonia can passively diffuse across the gills as NH_3 (de Vooys, 1968; Kerstetter, Kirschner & Rafuse, 1970; Hillaby & Randall, 1979; Kormanik & Cameron, 1981*b*; Cameron & Kormanik, 1982; Cameron & Heisler, 1983; Holeton, Neumann & Heisler, 1983). There is also considerable evidence for a carrier-mediated Na^+/NH_4^+ exchange (Maetz & García-Romeu, 1964; Payan & Matty, 1975; Payan, Matty & Maetz, 1975; Kerstetter & Keeler, 1976; Payan, 1978; Pressley, Graves & Krall, 1981) as well as some support for simple NH_4^+ diffusion (Goldstein, Claiborne & Evans, 1982). It remains unclear whether one of these processes predominates, or whether a variable combination of two or more of these processes commonly occurs (e.g. Maetz, 1972, 1973; Maetz, Payan & De Renzis, 1976; Evans, 1977, 1982; Claiborne, Evans & Goldstein, 1982; Wood, Wheatley & Hōbe, 1984).

Until recently, analysis of ammonia movements was limited by the lack of reliable physical constants (pK′, solubility) for ammonia in water and fish plasma at typical fish temperatures (cf. Kormanik & Cameron, 1981*a*). These parameters have now been measured for the rainbow trout (Cameron & Heisler, 1983). A further limitation in the past has been the absence of studies relating internal ammonia levels under resting conditions, as sampled by chronic cannulation, to external levels, ammonia excretion and unidirectional sodium fluxes at the gills. The present study employs this approach and the physical constants of Cameron & Heisler (1983) to reinvestigate the mechanism(s) of branchial ammonia efflux in the rainbow trout. Our aim has been to assess the mechanism(s) under normal laboratory conditions, rather than how they might change during long-term exposure to abnormal conditions. Thus we have examined the short-term effects, and their reversibility, of various treatments (environmental pH changes, amiloride) calculated to alter the relative NH_3 and NH_4^+ gradients and sodium transport across the gills.

MATERIALS AND METHODS

Experimental animals

Experiments were performed on 77 rainbow trout (*Salmo gairdneri*; mean weight, 430 g; range 180–786 g), obtained from Spring Valley Trout Farm, Petersburg, Ontario. The trout were initially held in flowing, dechlorinated Hamilton tapwater at seasonal temperatures and fed regularly with Silver Cup 3/16″ Trout Chow. One week prior to experimentation, the fish were transferred in batches of 10–15 to a 350-l closed-circuit, temperature controlled (15± 2 °C) fibreglass tank containing the same tap water ($[Na^+] \simeq 0{\cdot}6$, $[Cl^-] \simeq 0{\cdot}8$, $[Ca^{2+}] \simeq 1{\cdot}6$, $[Mg^{2+}] \simeq 0{\cdot}3$, $[K^+] = 0{\cdot}05$ mequiv l^{-1}; titration alkalinity $\simeq 2{\cdot}0$ mequiv l^{-1}; total hardness $\simeq 140$ mg l^{-1} as $CaCO_3$; pH $\simeq 8{\cdot}0$). The water was changed regularly to keep ammonia levels generally below 200 μmol l^{-1}. During this acclimation period,

the fish were not fed in order to eliminate the influence of feeding history on ammonia excretion (Fromm, 1963).

Trout were fitted with chronic indwelling dorsal aortic cannulae (methods of Smith & Bell, 1964 or Soivio, Westman & Nyholm, 1972) and urinary bladder catheters (Wood & Randall, 1973) while under anaesthesia in a 1:10000 dilution of MS-222 (Sigma). The fish were allowed to recover at least 48 h in individual flux boxes (see diagram and description in McDonald, 1983*b*). For measurements of branchial ammonia and sodium exchange, the flux boxes could be operated as closed, low-volume (3–6 l) recirculating systems at 15 ± 1 °C. As urine was collected externally, changes in water composition in the closed system were assumed to represent branchial fluxes, for skin and intestinal fluxes are considered negligible in fresh water (Fromm, 1968; Morii, Nishikata & Tamura, 1978; Cameron, 1978).

Experimental protocols

In each series, a 3-h control period (I) was followed by a 3-h experimental period and then a second control period (II; i.e. recovery) of equal length. At the start of each 3-h flux period, the box was flushed with fresh acclimation water and the volume reduced to a minimum (3–6 l depending on the fish and box size) so as to maximize the sensitivity with which changes in water ammonia and sodium concentration could be measured. This caused no apparent disturbance to the fish. At each time, new ^{22}Na (New England Nuclear; 1 μCi l^{-1}) was added to the water for measurement of unidirectional sodium fluxes at the gills. Water samples were drawn at the start of each 3-h period and every subsequent hour. Water pH was continually monitored and adjusted as necessary with 1 mol l^{-1} HCl or 1 mol l^{-1} KOH. Water samples were immediately frozen and later analysed for total ammonia (T_{Amm}), total sodium and ^{22}Na radioactivity. Arterial blood samples (300 μl) were drawn anaerobically from the dorsal aortic cannula and replaced by an equal volume of heparinized Cortland saline [Wolf, 1963; sodium heparin (Sigma); 100 i.u. ml^{-1}]. Blood samples were taken at 0·5 h and 2·5 h of each 3-h period, and analysed for pH (pH_a), total plasma CO_2 content (C_T) and total plasma ammonia content (T_{Amm}).

Six series of experiments were performed.

(i) A control in which fish were subjected to unaltered acclimation water (pH = 8·07 ± 0·02, $N = 8$) in the experimental period to check for any effects of the protocol itself.

(ii) Exposure to severely acidic water (pH = 4·06 ± 0·03, $N = 10$) obtained by titration with 1 mol l^{-1} HCl; the water was vigorously aerated for several hours before use to avoid any complicating effects of elevated P_{CO_2}.

(iii) Exposure to moderately acidic water (pH = 6·64 ± 0·04, $N = 17$) prepared in the same manner as the severely acidic.

(iv) Exposure to severely alkaline water (pH = 9·54 ± 0·02, $N = 9$) obtained by titration with 1 mol l^{-1} KOH.

(v) Exposure to moderately alkaline water (pH = 8·69 ± 0·04, $N = 17$) prepared in the same manner as the severely alkaline.

(vi) Exposure to the drug amiloride ($C_6H_8ClN_7O$; Merck, Sharp & Dome

Laboratories), a specific sodium transport blocker (Benos, 1982) at 10^{-4} mol l^{-1} in water of unchanged pH (8·12 ± 0·05; $N = 9$).

A further seven trout were fitted with both dorsal aortic and ventral aortic cannulae, the latter by the method of Holeton & Randall (1967*a*). These fish were employed to assess the relative concentrations of ammonia in pre- and post-gill blood plasma and were sampled only under control conditions.

Analytical techniques

Water pH was monitored with a Canlab polymer body, sealed reference, combination pH electrode (H5503-20) coupled to a Radiometer pH 71 MK2 acid-base analyser or Fisher 119 digital pH meter. Total water ammonia was measured by a micro-modification of the salicylate-hypochlorite assay (Verdouw, van Echted & Dekkers, 1978). Total water sodium was determined by flame photometry (Eel Mark II), and ^{22}Na radioactivity by counting 5 ml of water in 10 ml ACS fluor (Amersham) on a Beckman LS 250 liquid scintillation counter. Arterial pH_a and plasma C_T were measured immediately upon collection using Radiometer microelectrodes and methodology outlined by Wood & Jackson (1980). Plasma was obtained by centrifugation at 9000 ***g*** for 2 min and frozen for later analysis of T_{Amm} by micro-modification of a commercial diagnostic kit (L-glutamic dehydrogenase/NAD method; Sigma, 1982). Different ammonia assays were used for plasma and water because the simpler salicylate-hypochlorite method occasionally gave spurious values for plasma. The two assays were cross-validated on the same water- and saline-based ammonia standards.

Calculations

Free (NH_3) and ionized (NH_4^+) ammonia concentrations in plasma and water were calculated from total ammonia concentrations (T_{Amm}) and pH by the Henderson-Hasselbalch equation, using values of pK′ appropriate for trout plasma and fresh water at experimental temperature from Cameron & Heisler (1983):

$$NH_4^+ = \frac{T_{Amm}}{1 + \text{antilog}(pH - pK')}. \quad (1)$$

Therefore:

$$NH_3 = T_{Amm} - NH_4^+. \quad (2)$$

The partial pressure of free ammonia (P_{NH_3} in μTorr: 1 Torr ≈ 133·322387 Pa) was then calculated using the appropriate solubility coefficient (αNH_3) from Cameron & Heisler (1983):

$$P_{NH_3} = \frac{NH_3}{\alpha NH_3}. \quad (3)$$

An analogous series of equations based on the Henderson-Hasselbalch relationship was used to calculate plasma HCO_3^- and Pa_{CO_2} from pH_a and C_T, using appropriate values of pK_1' and αCO_2 (Severinghaus, 1965; Albers, 1970).

Since ammonia excreted by the gills is cleared from the blood (see Introduction), it is the mean blood plasma level [(arterial T_{Amm}+venous T_{Amm})÷2] which best defines blood to water gradients. However, the venous cannulation procedure is more difficult, more interventive and undoubtedly more stressful than the arterial. Therefore, we chose to predict the mean level from the arterial, rather than directly measuring it in all cases. Twelve simultaneous arterial and venous measurements under control conditions in the seven fish implanted with both cannulae yielded a mean venous T_{Amm} ($241 \pm 31\,\mu mol\,l^{-1}$) which was 1·66-fold mean arterial T_{Amm} ($145 \pm 28\,\mu mol\,l^{-1}$). As this was very similar to the ratio (1·81) reported by Cameron & Heisler (1983) for *Salmo gairdneri* and there is essentially no difference between pH_a and pH_v, mean blood plasma T_{Amm} was routinely calculated as 1·33× arterial T_{Amm} for use in equations (1) and (2).

Diffusion gradients for free and ionized ammonia across the gill were estimated as:

$$\Delta P_{NH_3} = \text{mean plasma } P_{NH_3} - \text{water } P_{NH_3} \quad (4)$$

and

$$\Delta NH_4^+ = \text{mean plasma } NH_4^+ - \text{water } NH_4^+, \quad (5)$$

recognizing that the latter does not take into account the small (unmeasured) electrical gradient across the gill (McWilliams & Potts, 1978). In these calculations, for each 3-h flux period, the initial (0·5 h) and final (2·5 h) plasma measurements were related to the means of the 0 h and 1 h, and 2 h and 3 h water measurements, respectively.

Net branchial flux rates of total ammonia (J_{net}^{Amm}) were calculated as:

$$J_{net}^{Amm} = \frac{(T_{Amm,i} - T_{Amm,f}) \times V}{t \times W}, \quad (6)$$

where i and f refer to initial and final concentrations in water in $\mu mol\,ml^{-1}$, V is the volume of the system in ml (corrected for sampling deficits), t is the elapsed time in h, and W is the fish weight in kg. Several experiments were run with known T_{Amm} but no fish present to check for ammonia loss from the water to the atmosphere in alkaline exposures (pH = 9·54); this proved to be negligible.

Net branchial sodium flux rates (J_{net}^{Na}) were calculated by an equation analogous to (6). Unidirectional influx rate (J_{in}^{Na}) was calculated as outlined by Maetz (1956):

$$J_{in}^{Na} = \frac{(R_i - R_f) \times V}{SA \times t \times W}, \quad (7)$$

where R_i and R_f are initial and final radioactivities of water in c.p.m. ml^{-1}, SA is the mean specific activity of water (c.p.m. $\mu equiv^{-1}$) over the flux period in question, and the other symbols are as in equation (6). In practice, reliable changes in R could only be detected over 3 h, so a single value for average J_{in}^{Na} was determined for each

3-h period. Internal SA never exceeded 10% of external SA, so correction for radioisotopic backflux in equation (7) was unnecessary (cf. Maetz, 1956). Unidirectional efflux rates (J_{out}^{Na}) were calculated by the conservation equation:

$$J_{out}^{Na} = J_{net}^{Na} - J_{in}^{Na}. \quad (8)$$

For all fluxes, losses by the animal have a negative sign, gains a positive sign.

Data have been expressed as mean ± 1 S.E.M. (N) where N equals the number of animals sampled. Changes in selected parameters within flux periods are illustrated in the figures, while averaged values over each 3-h treatment period are summarized in the Tables. Student's two tailed t-test ($P < 0{\cdot}05$) has been used for comparisons within groups, with each animal serving as its own control.

RESULTS

Control series and resting values

J_{net}^{Amm}, measured on an hourly basis, declined during each 3-h flux period, and then recovered after the flush (Fig. 1D), a pattern also seen in the control periods and the experimental periods of all the other experimental series (Figs 2D–6D). A similar pattern was seen by Kormanik & Cameron (1981*b*) in their work on blue crabs. In the present study, the fish were preadapted to the experimental chambers for at least 48 h, so it is unlikely to be a stress effect. Rather, this decline in J_{net}^{Amm} appeared to be correlated with the rise in external T_{Amm} in the closed system over time. Plasma T_{Amm}, while variable, did not increase significantly over the same period (Fig. 1A). Consequently both ΔP_{NH_3} and ΔNH_4^+ progressively fell (Fig. 1B,C); again the same patterns were seen in the control periods of all other protocols (Figs 2–6). There was no significant variation in plasma acid-base status (i.e. pH_a, HCO_3^-, Pa_{CO_2}; data not shown) within the 3-h periods.

Averaged data for each 3-h period are summarized in Table 1. All measured parameters were unchanged in the experimental period relative to control I. However, in control II, both plasma and water P_{NH_3}, NH_4^+, and thus T_{Amm}, were significantly lower relative to control I. This lowering of average ammonia levels in the system was due both to a small reduction in J_{net}^{Amm} in control II, at least relative to the experimental period, and to slightly lower starting ammonia levels (cf. Fig. 1A). This probably resulted from the longer flush performed prior to control II, which was performed to duplicate the experimental protocols where thorough flushes were employed to ensure washout of the experimental water.

Fig. 1. Changes over time in selected parameters related to branchial ammonia excretion in rainbow trout subjected to a control protocol identical to that employed in the various experimental series. During the 3-h experimental period, the flux box was simply flushed with fresh acclimation water at control pH. Means ± 1 S.E.M. (A) Total ammonia levels (T_{Amm}) in plasma (●) and water (○), $N = 5$. (B) The calculated partial pressure gradient for NH_3 (ΔP_{NH_3}; inside minus outside) across the gills, $N = 5$. (C) The calculated concentration gradient for NH_4^+ (ΔNH_4^+; inside minus outside) across the gills, $N = 5$. (D) The net rate of ammonia excretion at the gills (J_{net}^{Amm}) during each hour, $N = 8$. (E) The mean unidirectional (J_{in}^{Na}, J_{out}^{Na}) and net (J_{net}^{Na}; cross-hatched) flux rates of sodium across the gills during each 3-h period, $N = 8$.

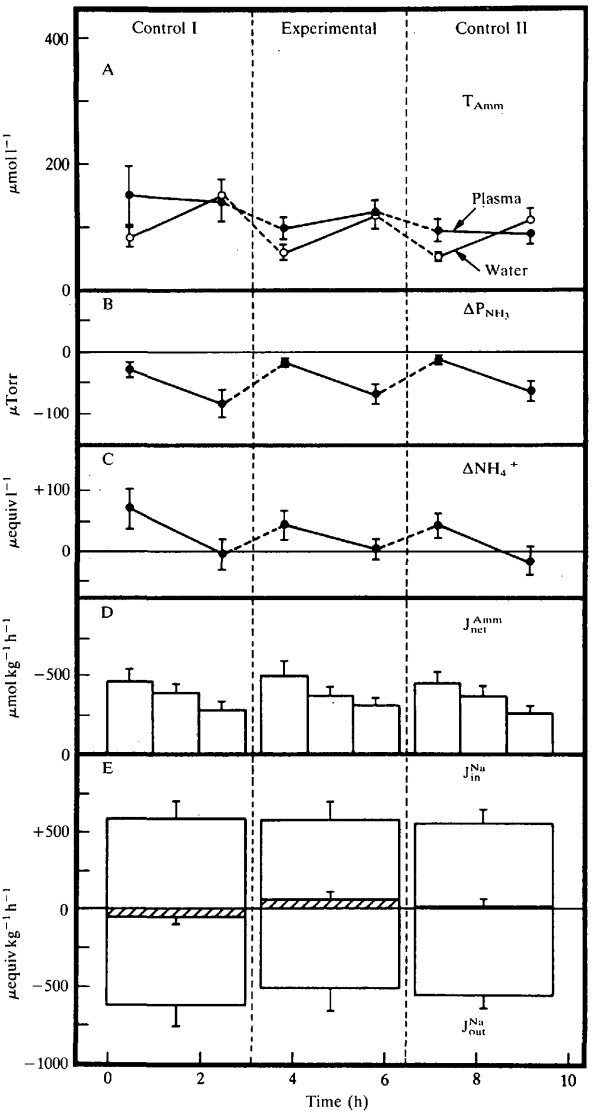
Control I
Experimental
Control II
A
T_{Amm}
Plasma
Water
μmol l⁻¹
400
200
0
B
ΔP_{NH_3}
μTorr
0
−100
C
ΔNH_4^+
μequiv l⁻¹
+100
0
D
J_{net}^{Amm}
μmol kg⁻¹ h⁻¹
−500
0
E
J_{in}^{Na}
J_{out}^{Na}
μequiv kg⁻¹ h⁻¹
+500
0
−500
−1000
0
2
4
6
8
10
Time (h)

Fig. 1

ble 1. *Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to the control protocol*

	N	Control I	Experimental	Control II
ater pH	8	8·10 ± 0·04	8·11 ± 0·04	8·09 ± 0·04
asma pH_a	7	7·79 ± 0·02	7·80 ± 0·02	7·81 ± 0·02
asma Pa_{CO_2} (Torr)	7	2·94 ± 0·27	2·75 ± 0·20	2·54 ± 0·24
asma HCO_3^- (mequiv l^{-1})	7	6·41 ± 0·42	6·26 ± 0·26	5·76 ± 0·44
asma P_{NH_3} (μTorr)	5	42 ± 11	33 ± 5	27 ± 5*
ater P_{NH_3} (μTorr)	5	99 ± 17	77 ± 10	65 ± 7*
P_{NH_3} (μTorr)	5	−57 ± 12	−44 ± 8	−38 ± 10
asma NH_4^+ (μequiv l^{-1})	5	145 ± 38	108 ± 18	89 ± 20*
ater NH_4^+ (μequiv l^{-1})	5	113 ± 17	86 ± 12	78 ± 8*
NH_4^+ (μequiv l^{-1})	5	+32 ± 34	+22 ± 17	+11 ± 25
$^{mm}_{st}$ (μmol kg^{-1} h^{-1})	8	−387 ± 57	−394 ± 61	−358 ± 51†
^{a} (μequiv kg^{-1} h^{-1})	8	+579 ± 121	+573 ± 137	+550 ± 88
$^{a}_{ut}$ (μequiv kg^{-1} h^{-1})	8	−625 ± 124	−513 ± 145	−549 ± 117
$^{a}_{st}$ (μequiv kg^{-1} h^{-1})	8	−46 ± 33	+60 ± 54	+1 ± 57

± 1 S.E.M. * $P < 0·05$ relative to control I. † $P < 0·05$ relative to experimental.

ble 2. *Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to severe acid exposure during the experimental period*

	N	Control I	Severe acid	Control II
'ater pH	10	7·96 ± 0·06	4·06 ± 0·03*	7·85 ± 0·05†
lasma pH_a	7	7·78 ± 0·02	7·72 ± 0·03*	7·73 ± 0·02
lasma Pa_{CO_2} (Torr)	7	2·64 ± 0·18	2·99 ± 0·21	3·10 ± 0·20
lasma HCO_3^- (mequiv l^{-1})	7	5·70 ± 0·26	5·34 ± 0·24	5·81 ± 0·53
lasma P_{NH_3} (μTorr)	7	73 ± 21	70 ± 14	52 ± 11†
'ater P_{NH_3} (μTorr)	7	91 ± 17	0 ± 0*	48 ± 8*†
P_{NH_3} (μTorr)	7	−18 ± 15	+70 ± 14	+4 ± 11†
lasma NH_4^+ (μequiv l^{-1})	7	220 ± 51	281 ± 62	194 ± 40†
'ater NH_4^+ (μequiv l^{-1})	7	123 ± 22	84 ± 19*	95 ± 22
NH_4^+ (μequiv l^{-1})	7	+97 ± 41	+197 ± 44*	+99 ± 32†
$^{mm}_{st}$ (μmol kg^{-1} h^{-1})	10	−379 ± 53	−273 ± 38*	−354 ± 53†
^{a} (μequiv kg^{-1} h^{-1})	7	+433 ± 68	−55 ± 13*	+265 ± 44*†
$^{a}_{ut}$ (μequiv kg^{-1} h^{-1})	7	−363 ± 73	−396 ± 93	−310 ± 107
$^{a}_{st}$ (μequiv kg^{-1} h^{-1})	7	+70 ± 53	−451 ± 90*	−45 ± 82†

± 1 S.E.M. * $P < 0·05$ relative to control I. † $P < 0·05$ relative to severe acid.

Resting levels of plasma T_{Amm}, P_{NH_3}, NH_4^+, acid-base status, ΔNH_4^+ across the gills and J_{net}^{Amm} were very similar to those found by Cameron & Heisler (1983), who employed comparable methodology, though ΔP_{NH_3} across the gills was very different. This difference was probably due to the very different control water pH levels employed in the two studies (see Discussion). The present fish started each control period with ΔP_{NH_3} close to zero, which became significantly negative by 3 h as water P_{NH_3} increased (Figs 1B–6B). Thus our fish excreted ammonia in the apparent absence of or even against a P_{NH_3} gradient, but in the direction of the NH_4^+ gradient (Tables 1–6).

J_{in}^{Na} and J_{out}^{Na} were rather variable both within and between different experimental groups (400–700 μequiv $kg^{-1} h^{-1}$ in control I; Tables 1–6), but a J_{net}^{Na} close to zero balance was usual (Figs 1E–6E). These unidirectional fluxes were approximately twice as large as those measured by Wood *et al.* (1984) in trout in water of the same ionic composition and temperature. J_{net}^{Amm} in the present study was also twice as high, perhaps because Wood *et al.* (1984) allowed external T_{Amm} to rise to a level 3–5 times greater. Elevated external T_{Amm} may well interfere with both Na^+/NH_4^+ exchange and diffusive ammonia efflux.

Severe acid exposure

The aim here was to apply a condition [water pH = 4·06 ± 0·03 (10)] which would enhance ΔP_{NH_3} while simultaneously blocking J_{in}^{Na} (cf. McDonald, 1983*b*). If branchial Na^+/NH_4^+ exchange is unimportant and NH_3 diffusion predominates, the predicted result would be an increase in J_{net}^{Amm}. Since at this pH essentially all ammonia is protonated, the water becomes an immense sink for NH_3.

J_{net}^{Amm}, rather than increasing, declined significantly by 28% relative to control I and then recovered in control II (Fig. 2B, Table 2). This occurred despite a reversal of ΔP_{NH_3} to a positive value as water P_{NH_3} became zero (Fig. 2B, Table 2). ΔNH_4^+ also increased (Fig. 2C) because the reduction in J_{net}^{Amm} both raised plasma T_{Amm} (and thus NH_4^+) and lowered water T_{Amm} (Fig. 2A, Table 2). This rise in internal ammonia shows that the reduction in J_{net}^{Amm} could not be attributed to a reduction in endogenous ammonia production. As anticipated, J_{in}^{Na} was abolished so that J_{net}^{Na} became highly negative and equal to J_{out}^{Na} (Fig. 2E, Table 2), an effect which was only partially reversed in control II. A slight but significant blood acidosis occurred during severe acid exposure attributable to both respiratory (Pa_{CO_2} elevation) and metabolic (HCO_3^- reduction) components, neither of which was significant by itself (Table 2). The magnitude of this acidosis after 3 h at pH = 4·06 was approximately equal to that which would be predicted from an earlier study under comparable conditions (McDonald & Wood, 1981).

These results indicate a significant contribution of Na^+/NH_4^+ exchange to resting J_{net}^{Amm}. However 72% of control J_{net}^{Amm} continued in the complete absence of J_{in}^{Na}, suggesting that diffusive processes, probably enhanced by the elevated gradients, were important in sustaining J_{net}^{Amm} under conditions where Na^+/NH_4^+ exchange was blocked.

Moderate acid exposure

The goal of this treatment was again to elevate ΔP_{NH_3} by reducing water P_{NH_3} close to zero, but without inducing a blockage of J_{in}^{Na}. A water pH of $6{\cdot}64 \pm 0{\cdot}04$ (17) was calculated to represent the minimum acidity level to ensure the former objective.

J_{net}^{Amm} increased only slightly (+7 %) and non-significantly relative to control I during moderate acid exposure (Fig. 3D, Table 3). While this J_{net}^{Amm} was significantly higher (+23 %) than in control II, the same trend was also seen in the control series (Table 1). As planned, ΔP_{NH_3} was reversed to a positive value (Fig. 3B, Table 3), though not as high as in the severe acid series (Table 2) because internal ammonia levels fell significantly (Table 3), an effect which was not corrected during control II. It is not clear whether this was the result of the marginally elevated J_{net}^{Amm}, or reflected a true decrease in the endogenous ammonia production, though the severe acid results would argue against this. In any event, one important consequence was a significant reduction in ΔNH_4^+ during the experimental period (Fig. 3C, Table 3). The goal of preserving sodium exchanges at control levels was largely achieved, with only a non-significant 23 % decrease in J_{in}^{Na} and continued positive J_{net}^{Na} occurring at pH = 6·64 (Fig. 3E, Table 3). There were no further changes in these parameters in control II. As with severe acid exposure, a slight blood acidosis of compound respiratory and metabolic origin again occurred (Table 3).

Under these conditions, a significant elevation of ΔP_{NH_3} had only a very small stimulating effect on J_{net}^{Amm}, and ΔNH_4^+ again changed in the opposite direction from J_{net}^{Amm}. J_{net}^{Amm} was therefore only slightly altered by a treatment whereas J_{in}^{Na} was largely unaffected.

Severe alkaline exposure

Exposure to alkaline water was selected as a treatment to separate the relative importance of NH_3 and NH_4^+ diffusion across the gills, for elevated water pH should increase external P_{NH_3} and decrease external NH_4^+. An alkaline pH [$9{\cdot}54 \pm 0{\cdot}04$ (9)] close to the pK′ was initially tested to ensure pronounced changes in ΔP_{NH_3} and ΔNH_4^+. As alkaline effects on sodium transport in trout have not been studied previously, it was not known what changes, if any, might occur in J_{in}^{Na}.

J_{net}^{Amm} declined dramatically by 80 % during severe alkaline exposure and then recovered to a level significantly above control I (+22 %) during control II (Fig. 4D, Table 4). Plasma T_{Amm} increased 2·5-fold during the experimental period and then dropped during recovery (Fig. 4A), showing that the reduction in J_{net}^{Amm} was not due to a blockage of endogenous ammonia production. At the same time, ΔNH_4^+ increased over 4-fold to a highly positive value, while ΔP_{NH_3} fell from its initial level close to zero to a very negative value (Fig. 4B,C, Table 4). These alterations in gradients, which were the intention of the experiment, were

Fig. 2. The influence of severe acid exposure (pH = 4·06) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout. $N = 7$ in (A) – (C), 10 in (D) and 7 in (E). Other details as in legend of Fig. 1.

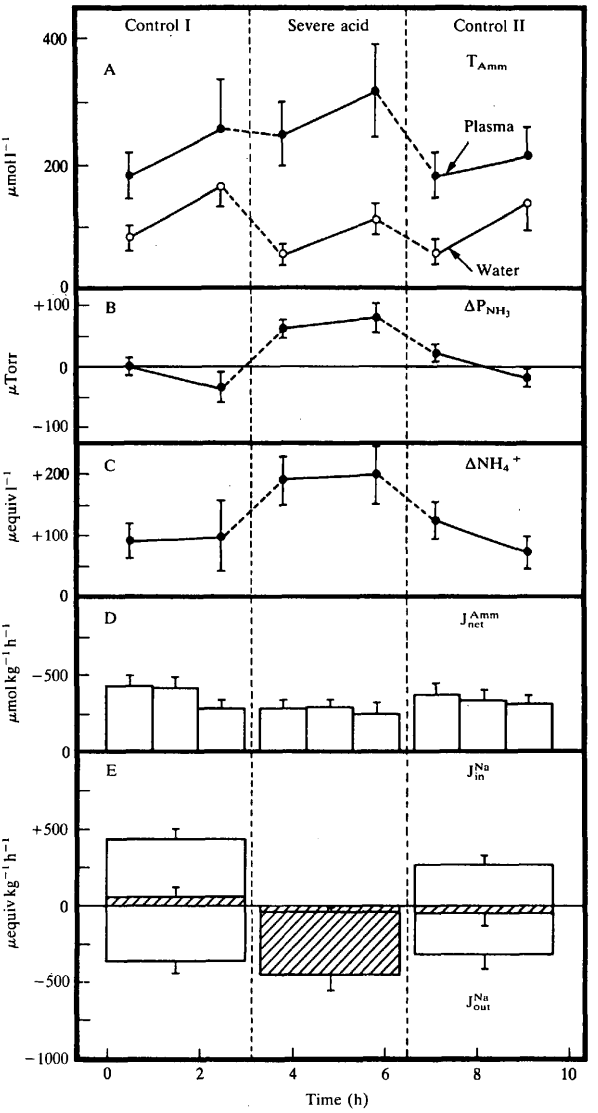
Control I
Severe acid
Control II
A
T_{Amm}
Plasma
Water
400
200
0
μmol l^{-1}
B
ΔP_{NH_3}
+100
0
−100
μTorr
C
ΔNH_4^+
+200
+100
0
μequiv l^{-1}
D
J_{net}^{Amm}
−500
0
μmol kg^{-1} h^{-1}
E
J_{in}^{Na}
J_{out}^{Na}
+500
0
−500
−1000
μequiv kg^{-1} h^{-1}
0
2
4
6
8
10
Time (h)

Fig. 2

reversed during control II. High water pH also markedly affected J_{in}^{Na}, which fell by 55 % during the experimental period, and returned to normal in control II (Fig. 4E, Table 4). J_{net}^{Na} mirrored these changes. A pronounced blood alkalosis (+0·3 pH units) of wholly respiratory origin occurred during severe alkaline exposure and partially persisted during recovery (Table 4). Pa_{CO_2} was approximately halved while $[HCO_3^-]$ remained unchanged because water at pH = 9·54 essentially acts as a vacuum for CO_2.

Of all the experimental treatments, this caused the largest drop in J_{net}^{Amm}. In this situation, where ΔNH_4^+ increased over 4-fold and where ΔP_{NH_3} was rendered highly negative and therefore the net outward diffusion of NH_3 clearly prevented, the decreases ($\sim -270\,\mu equiv\,kg^{-1}\,h^{-1}$) in J_{net}^{Amm} and J_{in}^{Na} were equal in size (Table 4). These data were therefore consistent with the results of the two acid series.

Moderate alkaline exposure

A much more moderate alkaline pH was tested in the hope that a very negative ΔP_{NH_3} could still be attained without affecting J_{in}^{Na}. A pH of 8·69 ± 0·04 (17) was selected as the minimum level of alkalinity to ensure the former objective.

As intended, moderate alkaline exposure changed ΔP_{NH_3} to a very negative value (Fig. 5B, Table 5) almost equal to and less variable than that during severe alkaline exposure (Fig. 4B, Table 4). However in this case, the drop in J_{net}^{Amm}, while significant, was only 23 % (Fig. 5D, Table 5). Even at this much more moderate alkaline pH, J_{in}^{Na} declined significantly by 28 % (Fig. 5E, Table 5). The absolute size of this decrease was adequate to explain the observed fall in J_{net}^{Amm}. As with severe alkaline exposure, plasma T_{Amm} and ΔNH_4^+ both rose during the experimental period as ammonia accumulated internally (Fig. 5A,C), and a respiratory alkalosis again developed (Table 5). Most changes were reversed during control II.

Thus in water of pH = 8·69, 77 % of control I J_{net}^{Amm} persisted in the face of a very negative ΔP_{NH_3}. This small reduction in J_{net}^{Amm} was accompanied by a comparable fall in J_{in}^{Na}.

Amiloride exposure

The results of the previous four experiments suggested that a large decrease in J_{net}^{Amm} would be the predicted result of a treatment which blocked J_{in}^{Na} without altering ΔP_{NH_3} or ΔNH_4^+. The drug amiloride, a specific sodium transport inhibitor (Benos, 1982), was selected for this purpose.

As intended, amiloride at $10^{-4}\,mol\,l^{-1}$ in the external water (pH = 8·12 ± 0·05) had no effect on ΔP_{NH_3} or ΔNH_4^+ (Fig. 6B,C, Table 6), but essentially obliterated J_{in}^{Na}, with only 6 % of the control I influx persisting (Fig. 6E, Table 6). J_{net}^{Na} therefore became very negative. These effects were largely reversed during control II. However, contrary to prediction, the fall in J_{net}^{Amm}, while significant, was only

Fig. 3. The influence of moderate acid exposure (pH = 6·64) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout. $N = 15$ in (A)–(C), 17 in (D) and 9 in (E). Other details as in legend of Fig. 1.

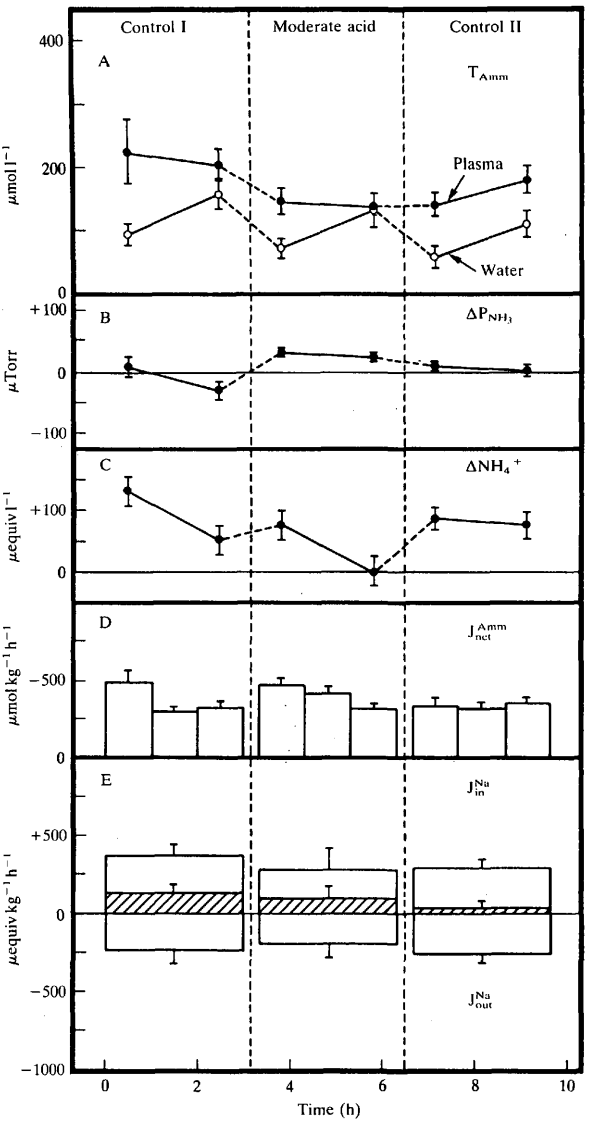
Control I
Moderate acid
Control II
A
T_{Amm}
Plasma
Water
μmol l^{-1}
400
200
0
B
ΔP_{NH_3}
μTorr
+100
0
−100
C
ΔNH_4^+
μequiv l^{-1}
+100
0
D
J_{net}^{Amm}
μmol kg^{-1} h^{-1}
−500
0
E
J_{in}^{Na}
J_{out}^{Na}
μequiv kg^{-1} h^{-1}
+500
0
−500
−1000
0
2
4
6
8
10
Time (h)

Fig. 3

ble 3. *Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to moderate acid exposure during the experimental period*

	N	Control I	Moderate acid	Control II
ater pH	12	7·85 ± 0·08	6·64 ± 0·04*	7·61 ± 0·06†
asma pH_a	15	7·80 ± 0·01	7·73 ± 0·02*	7·75 ± 0·02
asma Pa_{CO_2} (Torr)	15	2·65 ± 0·12	2·97 ± 0·24	2·64 ± 0·19
asma HCO_3^- (mequiv l^{-1})	15	6·12 ± 0·32	5.42 ± 0·32	5·28 ± 0·38
asma P_{NH_3} (μTorr)	15	56 ± 6	32 ± 5*	35 ± 3*
ater P_{NH_3} (μTorr)	15	62 ± 10	3 ± 0*	32 ± 6*†
P_{NH_3} (μTorr)	15	−6 ± 14	+29 ± 5*	+3 ± 9†
asma NH_4^+ (μequiv l^{-1})	15	198 ± 33	129 ± 23*	137 ± 23*
ater NH_4^+ (μequiv l^{-1})	15	121 ± 18	102 ± 14*	78 ± 12*†
NH_4^+ (μequiv l^{-1})	15	+77 ± 32	+27 ± 31*	+59 ± 24
$^{amm}_{et}$ (μmol $kg^{-1} h^{-1}$)	17	−375 ± 38	−403 ± 30	−327 ± 28†
$^{a}_{a}$ (μequiv $kg^{-1} h^{-1}$)	9	+370 ± 66	+285 ± 143	+297 ± 43
$^{a}_{ut}$ (μequiv $kg^{-1} h^{-1}$)	9	−238 ± 86	−188 ± 84	−261 ± 69
$^{a}_{et}$ (μequiv $kg^{-1} h^{-1}$)	9	+132 ± 55	+97 ± 88	+36 ± 40

ean ± 1 S.E.M. *$P < 0.05$ relative to control I. †$P < 0.05$ relative to moderate acid.

ble 4. *Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to severe alkaline exposure during the experimental period*

	N	Control I	Severe alkaline	Control II
ater pH	9	7·97 ± 0·06	9·54 ± 0·04*	7·99 ± 0·08†
lasma pH_a	5	7·78 ± 0·02	8·08 ± 0·07*	7·89 ± 0·02*†
lasma Pa_{CO_2} (Torr)	5	2·95 ± 0·50	1·61 ± 0·12*	2·12 ± 0·47†
lasma HCO_3^- (mequiv l^{-1})	5	6·60 ± 0·83	6·91 ± 0·86	6·76 ± 0·69
lasma P_{NH_3} (μTorr)	5	59 ± 10	285 ± 65*	83 ± 13*†
ater P_{NH_3} (μTorr)	5	81 ± 20	424 ± 89*	95 ± 22†
P_{NH_3} (μTorr)	5	−22 ± 17	−139 ± 83	−12 ± 26
lasma NH_4^+ (μequiv l^{-1})	5	186 ± 22	408 ± 51*	215 ± 21†
ater NH_4^+ (μequiv l^{-1})	5	103 ± 18	16 ± 3*	97 ± 20†
NH_4^+ (μequiv l^{-1})	5	83 ± 22	392 ± 49*	+118 ± 34†
$^{amm}_{et}$ (μmol $kg^{-1} h^{-1}$)	9	−345 ± 91	−68 ± 13*	−420 ± 45*†
$^{a}_{a}$ (μequiv $kg^{-1} h^{-1}$)	7	+496 ± 56	+226 ± 60*	+465 ± 46†
$^{a}_{ut}$ (μequiv $kg^{-1} h^{-1}$)	7	−530 ± 66	−745 ± 193	−381 ± 82
$^{a}_{et}$ (μequiv $kg^{-1} h^{-1}$)	7	−34 ± 33	−519 ± 194*	+84 ± 66

ean ± 1 S.E.M. *$P < 0.05$ relative to control I. †$P < 0.05$ relative to severe alkaline.

23% relative to control I (Fig. 6D, Table 6). This inhibition disappeared during control II. Blood acid-base status was not significantly affected by amiloride (Table 6). The effects of amiloride on J_{in}^{Na} and J_{net}^{Amm} were therefore very similar to those of severe acid exposure (cf. Fig. 2, Table 2). This occurred despite the fact that elevated ΔP_{NH_3} (and ΔNH_4^+) gradients, interpreted as sustaining J_{net}^{Amm} when Na^+/NH_4^+ exchange was blocked at pH = 4·06, were not seen with amiloride.

DISCUSSION

Ammonia gradients

There are numerous sources of uncertainty in the estimation of ammonia gradients across the gills. As Cameron & Heisler (1983) point out, relatively small errors in physical constants and pH may have large effects on calculated ΔP_{NH_3}. Employing Cameron & Heisler's physical constants, we have obtained internal P_{NH_3} and NH_4^+ levels similar to their values, which at least suggests consistency between the two studies, and that our procedure of predicting mean plasma T_{Amm} levels from arterial T_{Amm} was not a major source of error. The pH values are a more serious concern. While blood plasma pH is a routine and precise measurement (±0·01 units), the value obtained represents an equilibrium condition in the electrode which may or may not be the same as that in gill blood plasma depending on the extent of carbonic anhydrase catalysis. One may even question whether blood plasma pH is the correct internal value to use. The relevant gradient could in fact be from the cytoplasm of branchial epithelial cells to the water, rather than from blood to water, especially if ammonia is transported rather than diffuses across the baso-lateral boundary (cf. Claiborne *et al.* 1982). The intracellular pH of the gill epithelium is unknown, but pH_i in other trout tissues is 0·4–0·5 pH units below pH_a (Hōbe, Wood & Wheatly, 1984*b*; C. L. Milligan & C. M. Wood, unpublished data). Furthermore, while branchial ammonia production appears to be of little importance (see Introduction), it must be noted that the enzymes involved in ammoniagenesis have been located in the gills (Goldstein & Forster, 1961; Makarawicz & Zydowo, 1962). It remains conceivable that under appropriate conditions, ammonia production within the epithelial cells could be turned on.

The measurement of water pH (±0·02 units) is somewhat less precise than that of plasma because of the lower ionic strength. However, a much more serious concern is that like other workers (e.g. Maetz, 1972, 1973; Cameron & Heisler, 1983), we have employed equilibrium pH measurements in the external bath where water P_{CO_2} is kept relatively constant. Lloyd & Herbert (1960) theorized that P_{CO_2} was higher and pH lower in lamellar water, assuming that the CO_2 hydration reaction was rapid. However in the absence of external carbonic anhydrase, the CO_2/HCO_3^- system is undoubtedly not at equilibrium in lamellar water. Holeton & Randall (1967*b*) reported that mixed expired water pH, at equilibrium, was ~0·2 pH units below inspired in rainbow trout examined in very soft water. Our water is more alkaline and better buffered, so a smaller difference is anticipated. However actual lamellar water pH has yet to be measured and is difficult to predict accurately, for it will depend on the extent of this disequilibrium, the size of the unstirred layer and the magnitude of the CO_2 flux relative to the ventilatory flow, as

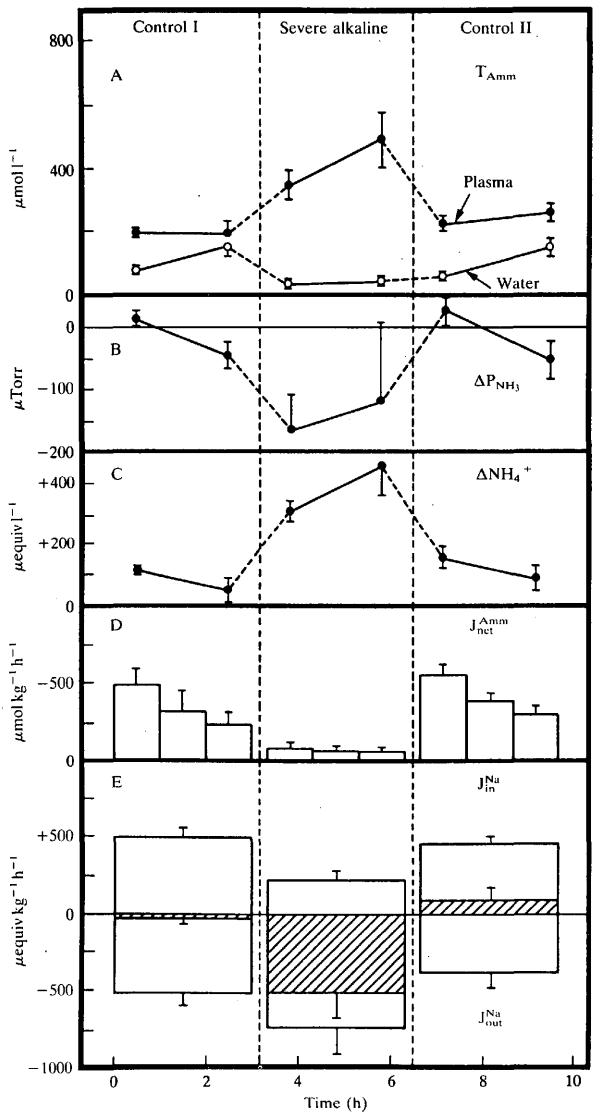
Control I
Severe alkaline
Control II
A
T_{Amm}
Plasma
Water
B
ΔP_{NH_3}
C
ΔNH_4^+
D
J_{net}^{Amm}
E
J_{in}^{Na}
J_{out}^{Na}
μmol l⁻¹
μTorr
μequiv l⁻¹
μmol kg⁻¹ h⁻¹
μequiv kg⁻¹ h⁻¹
800
400
0
−100
−200
+400
+200
−500
+500
−1000
0
2
4
6
8
10
Time (h)

Fig. 4

well as the fluxes of other acidifying (H^+) or alkalinizing species (e.g. NH_3, HCO_3^-, OH^-). In view of these difficulties, we have much more confidence in relative changes in ammonia gradients caused by experimental treatments than in their absolute values.

Experimental responses

The immediate and complete blockage of J_{in}^{Na} during severe acid exposure has been observed and analysed repeatedly (see Wood & McDonald, 1982; McDonald, 1983*a* for reviews). However, the simultaneous reduction in J_{net}^{Amm}, in the face of greatly elevated ΔP_{NH_3} during the initial exposure period has received little attention, though it has been noted in three other recent studies (Ultsch, Ott & Heisler, 1981; McDonald, Walker & Wilkes, 1983; Hōbe *et al.* 1984*a*,*b*). Instead, most authors have concentrated on the fact that J_{net}^{Amm} increases well above the control level during longer term acid exposure despite minimal recovery of J_{in}^{Na} (e.g. Ultsch *et al.* 1981; McDonald & Wood, 1981; Booth, Jansz & Holeton, 1982; McDonald, 1983*a*,*b*; McDonald *et al.* 1983). Plasma T_{Amm} also rises during longer term exposure (McDonald, 1983*b*; Hōbe *et al.* 1984*a*) so elevated ammoniagenesis is probably involved. Moderate acid exposure, which also elevated ΔP_{NH_3}, did not significantly alter J_{in}^{Na} and only minimally elevated J_{net}^{Amm}. These observations are all consistent with the interpretation that a large Na^+/NH_4^+ exchange under the control conditions of the present laboratory experiments is blocked by severe acid exposure; the continuation of ammonia efflux is then dependent upon increased NH_3 diffusion due to the elevated ΔP_{NH_3}.

Trout branchial function appears very sensitive to alkaline conditions, for raising the water pH to only 8·69 depressed both J_{net}^{Amm} and J_{in}^{Na} by ~25 %, and 9·54 reduced these parameters by 80 % and 55 % respectively, the latter representing an equimolar reduction. We are aware of no previous reports of alkaline effects on ammonia excretion, and only one on sodium exchange. In contrast to the present data, Maetz & De Renzis (1978) found a 10-fold stimulation of J_{in}^{Na} in *Tilapia mossambica* raised from water pH = 7·8 to 9·7, though their tests were conducted in 10 % sea water. The inhibitory effect of low pH on J_{in}^{Na} has been variously explained by H^+ competition for the carrier, H^+ titration of negative charges on channels leading to the carrier, or a direct effect on the carrier itself (cf. McDonald, 1983*a*). Only the latter would seem a reasonable mechanism for high pH inhibition of J_{in}^{Na}, though the effect was reversible in the present study. The persistence of over 75 % of control J_{net}^{Amm} in the face of a very negative ΔP_{NH_3} (at pH = 8·69) and the large equimolar reduction in both J_{in}^{Na} and J_{net}^{Amm} (at pH = 9·54) were again consistent with a dominant role for Na^+/NH_4^+ exchange under the present control conditions.

The observed 94 % reduction in J_{in}^{Na} caused by amiloride agrees with previous studies showing a 70–92 % reduction in various trout preparations (Kirschner, Greenwald & Kerstetter, 1973; Kerstetter & Keeler, 1976; Payan, 1978; Perry,

Fig. 4. The influence of severe alkaline exposure (pH = 9·54) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout. $N = 5$ in (A)–(C), 9 in (D) and 7 in (E). Other details as in legend of Fig. 1.

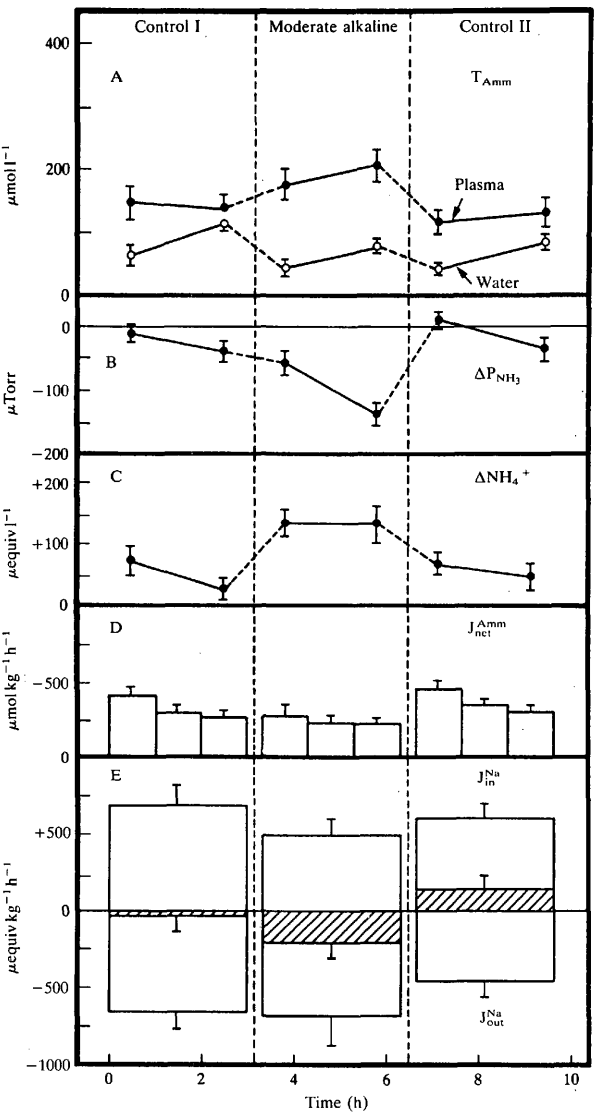
Control I
Moderate alkaline
Control II
A
T_{Amm}
Plasma
Water
μmol l^{-1}
400
200
0
B
ΔP_{NH_3}
μTorr
0
−100
−200
C
ΔNH_4^+
μequiv l^{-1}
+200
+100
0
D
J_{net}^{Amm}
μmol kg^{-1} h^{-1}
−500
0
E
J_{in}^{Na}
J_{out}^{Na}
μequiv kg^{-1} h^{-1}
+500
0
−500
−1000
0
2
4
6
8
10
Time (h)

Fig. 5.

Haswell, Randall & Farell, 1981; Perry & Randall, 1981). Amiloride also reduced J_{net}^{Amm} by 23%, a figure consistent with 30% reductions reported in perfused and/or irrigated trout gill preparations (Kirschner *et al.* 1973; Payan, 1978). A much larger inhibition of J_{net}^{Amm} would be expected if Na^+/NH_4^+ were the dominant process, for no elevation of diffusion gradients occurred to sustain excretion as during severe acid exposure. Recently Perry & Randall (1981) have shown that 10^{-4} mol l^{-1} amiloride also inhibits J_{in}^{Cl} by 55% in intact trout, an effect they attributed to a predicted acidosis within the branchial epithelial cells. As this could alter the effective driving gradients for NH_3 and NH_4^+ movements at both the baso-lateral and apical surfaces (see above), the interpretation of this experiment is unclear.

The mechanism(s) of ammonia excretion

The results of all five experimental series were consistent with the conclusion that Na^+/NH_4^+ exchange plays a significant role in J_{net}^{Amm} in trout under the present control conditions, and all but the amiloride experiment suggest it may be the dominant mechanism. NH_3 diffusion also occurs, and this becomes more important in treatments where ΔP_{NH_3} is elevated and/or J_{in}^{Na} is inhibited. While NH_4^+ diffusion cannot be ruled out, it is probably of minor quantitative importance; for J_{net}^{Amm} always changed in the opposite direction from ΔNH_4^+. It must be noted that we did not take the transepithelial potential (TEP) into account. However, this was probably unimportant to our conclusion, for McWilliams & Potts (1978) showed TEP in the brown trout, *Salmo trutta*, to be stable down to pH = 6·0, and then to become progressively more positive at lower pH values. Our calculated elevation in ΔNH_4^+ at pH = 4·06 was therefore probably an underestimate. The effect of alkaline pH on TEP has not been studied in trout, but is reported to elevate the TEP in goldfish *Carassius auratus* (Eddy, 1975). Our calculated elevation in ΔNH_4^+ at pH = 8·69 and 9·54 would again be an underestimate if this were also true in trout. In any event, when one compares the relative gradients available to drive the passive effluxes of Na^+ and NH_4^+ across the gills, only a very small NH_4^+ diffusion is theoretically likely. This argument has been developed in detail by Kormanik & Cameron (1981*a*).

We have attempted to relate the influx of Na^+ (J_{in}^{Na}) to NH_4^+ excretion ($J_{net}^{NH_4}$) in all series by assuming that at pH = 4·06, net ammonia excretion (J_{net}^{Amm}) was entirely by NH_3 diffusion (i.e. $J_{net}^{NH_4} = 0$) since J_{in}^{Na} was obliterated and NH_4^+ diffusion appears insignificant. The diffusivity of the gills to NH_3 ($D_{NH_3} = J_{net}^{Amm}/\Delta P_{NH_3}$) under these conditions was $4\,\mu mol\,kg^{-1}\,h^{-1}\,\mu Torr^{-1}$, which is about 65% of the value estimated by Cameron & Heisler (1983) for the same species. This D_{NH_3} value was then applied to the other series and the NH_3 contribution ($J_{net}^{NH_3}$) to J_{net}^{Amm} determined from the prevailing ΔP_{NH_3}. $J_{net}^{NH_4}$ was then calculated as the difference between J_{net}^{Amm} and $J_{net}^{NH_3}$, signs considered. The analysis therefore relies on the absolute ΔP_{NH_3} values (see above), assumes that

Fig. 5. The influence of moderate alkaline exposure (pH = 8·69) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout. $N = 13$ in (A)–(D) and 7 in (E). Other details are in legend of Fig. 1.

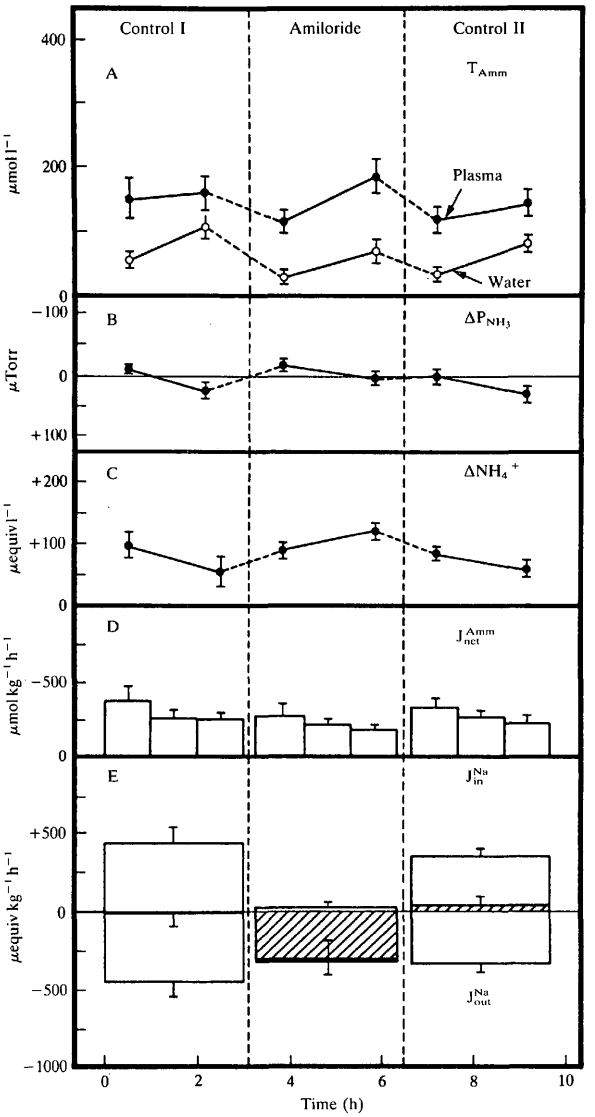
Control I
Amiloride
Control II
A
T_{Amm}
Plasma
Water
μmol l^{-1}
400
200
0
B
ΔP_{NH_3}
μTorr
−100
0
+100
C
ΔNH_4^+
μequiv l^{-1}
+200
+100
0
D
J_{net}^{Amm}
μmol kg^{-1} h^{-1}
−500
0
E
J_{in}^{Na}
J_{out}^{Na}
μequiv kg^{-1} h^{-1}
+500
0
−500
−1000
0
2
4
6
8
10
Time (h)

Fig. 6

NH_3 excretion is diffusion limited (i.e. any ventilatory or cardiovascular effects of the various treatments were unimportant), and assumes that D_{NH_3} is the same under all treatments and in both directions of NH_3 flux: all of which are possible sources of error.

Fig. 7 illustrates the relationship between $J_{net}^{NH_4}$ and J_{in}^{Na} based on this analysis. The two parameters appear to be more or less linearly related and increase with pH from 4·1 to 8·7. If the analysis is valid, Na^+/NH_4^+ exchange clearly dominates over diffusive processes in the control pH range. At pH = 9·5, where $J_{net}^{NH_4}$ appears greatly to exceed J_{in}^{Na}, any overestimation in the very negative (and highly variable) ΔP_{NH_3} measurement will greatly exaggerate the calculated $J_{net}^{NH_4}$. Under these conditions, there may well have been a significant branchial H^+ efflux or HCO_3^- uptake, lowering the pH of lamellar water and decreasing the ΔP_{NH_3} below the measured value.

These conclusions about the dominance of Na^+/NH_4^+ exchange relative to NH_3 diffusion under our control conditions agree completely with the work of Maetz (1972, 1973) on goldfish where control pH (~7·9) was similar to the present. Maetz later questioned the absolute values of the ammonia gradients calculated in his experiments, but not the basic conclusions (Maetz *et al.* 1976). In contrast, Cameron & Heisler's (1983) investigation of rainbow trout, while not ruling out Na^+/NH_4^+ exchange, concluded that NH_3 diffusion was sufficient to explain all resting J_{net}^{Amm}, assuming that NH_3 and CO_2 have similar permeabilities in gill tissue. This difference is probably related to the lower control pH = 7·0 used in their study. Thus comparable internal and external T_{Amm} in the two studies gave rise to a reasonable ΔP_{NH_3} (+55 μTorr) to drive resting J_{net}^{Amm} by passive NH_3 diffusion at pH = 7·0 (Cameron & Heisler, 1983), but negligible or negative ΔP_{NH_3} at pH = 8·0 (present study) due to the approximately 10-fold higher water P_{NH_3}. In any event, absolute values of ΔP_{NH_3} should be viewed with caution and relative changes may well be more informative (see above). Indeed, Cameron & Heisler (1983) postulated a high rate of Na^+/NH_4^+ exchange to explain the response to a treatment (pH = 8·0, elevated external T_{Amm}) which changed ΔP_{NH_3} to a very negative value. Hence there appears to be no conflict in observation between our study and that of Cameron & Heisler (1983), but subtle differences in interpretation.

We favour a flexible model in which J_{net}^{Amm} is achieved by a variable combination of Na^+/NH_4^+ exchange and NH_3 diffusion. The exact proportions occurring through each pathway will depend upon the relative ΔP_{NH_3}, the extent of J_{in}^{Na}, and quite possibly the acid-base status of the animal. Ammonia excretion *via* Na^+/NH_4^+ exchange represents acidic equivalent excretion, while NH_3 efflux is neutral in net acid-base terms, though inequalities between NH_3 production and excretion rates will affect internal acid-base status. Branchial Na^+/H^+ exchange may also occur (e.g. Kerstetter *et al.* 1970; Kirschner *et al.* 1973), and the relative ΔH^+ and ΔNH_4^+ gradients will determine which mechanism is an energetically

Fig. 6. The influence of exposure to 10^{-4} mol l^{-1} amiloride in water at control pH during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout. N = 8 in (A)–(C), 9 in (D) and 5 in (E). Other details as in legend of Fig. 1.

ıble 5. *Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to moderate alkaline exposure during the experimental period*

	N	Control I	Moderate alkaline	Control II
/ater pH	17	7·98 ± 0·06	8·69 ± 0·04*	8·11 ± 0·06†
lasma pH_a	13	7·79 ± 0·02	7·87 ± 0·02*	7·83 ± 0·02
lasma Pa_{CO_2} (Torr)	13	3·13 ± 0·22	2·61 ± 0·14*	2·67 ± 0·16
lasma HCO_3^- (mequiv l^{-1})	13	6·91 ± 0·44	7·09 ± 0·29	6·44 ± 0·47†
lasma P_{NH_3} (μTorr)	13	39 ± 6	60 ± 9*	35 ± 6†
/ater P_{NH_3} (μTorr)	13	58 ± 9	160 ± 16*	52 ± 7†
P_{NH_3} (μTorr)	13	−19 ± 12	−100 ± 18*	−17 ± 11†
lasma NH_4^+ (μequiv l^{-1})	13	144 ± 21	187 ± 26*	120 ± 22†
/ater NH_4^+ (μequiv l^{-1})	13	90 ± 8	53 ± 5*	60 ± 6*
NH_4^+ (μequiv l^{-1})	13	+54 ± 17	+134 ± 24*	+60 ± 20†
J_{net}^{Amm} (μmol $kg^{-1} h^{-1}$)	13	−342 ± 34	−262 ± 28*	−380 ± 37†
J_{in}^{Na} (μequiv $kg^{-1} h^{-1}$)	7	+688 ± 147	+492 ± 125*	+600 ± 105
J_{out}^{Na} (μequiv $kg^{-1} h^{-1}$)	7	−658 ± 121	−698 ± 171	−458 ± 91
J_{net}^{Na} (μequiv $kg^{-1} h^{-1}$)	7	+30 ± 133	−206 ± 105	+142 ± 78†

$\bar{X}$ ± 1 S.E.M. *$P<0.05$ relative to control I. †$P<0.05$ relative to moderate alkaline.

ıble 6. *Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to 10^{-4} mol l^{-1} amiloride in water at control pH during the experimental period*

	N	Control I	Amiloride	Control II
Vater pH	9	7·97 ± 0·10	8·12 ± 0·05	8·14 ± 0·05
Plasma pH_a	8	7·84 ± 0·06	7·81 ± 0·04	7·79 ± 0·03
Plasma Pa_{CO_2} (Torr)	8	2·88 ± 0·29	2·45 ± 0·19	2·56 ± 0·34
Plasma HCO_3^- (mequiv l^{-1})	8	7·05 ± 0·54	6·02 ± 0·43	5·54 ± 0·48
Plasma P_{NH_3} (μTorr)	8	51 ± 18	42 ± 9	33 ± 6
Vater P_{NH_3} (μTorr)	8	61 ± 24	39 ± 16	49 ± 16
P_{NH_3} (μTorr)	8	−10 ± 12	+3 ± 16	−16 ± 12†
Plasma NH_4^+ (μequiv l^{-1})	8	128 ± 27	127 ± 15	106 ± 15
Vater NH_4^+ (μequiv l^{-1})	8	75 ± 19	44 ± 13*	53 ± 11*†
NH_4^+ (μequiv l^{-1})	8	+53 ± 29	+83 ± 20	+53 ± 22
J_{net}^{Amm} (μmol $kg^{-1} h^{-1}$)	9	−306 ± 61	−237 ± 45*	−290 ± 40†
J_{in}^{Na} (μequiv $kg^{-1} h^{-1}$)	5	+444 ± 101	+28 ± 49*	+363 ± 40†
J_{out}^{Na} (μequiv $kg^{-1} h^{-1}$)	5	−447 ± 105	−319 ± 65	−323 ± 54
J_{net}^{Na} (μequiv $kg^{-1} h^{-1}$)	5	−3 ± 91	−292 ± 125	+40 ± 62†

$\bar{X}$ ± 1 S.E.M. *$P<0.05$ relative to control I. †$P<0.05$ relative to amiloride.

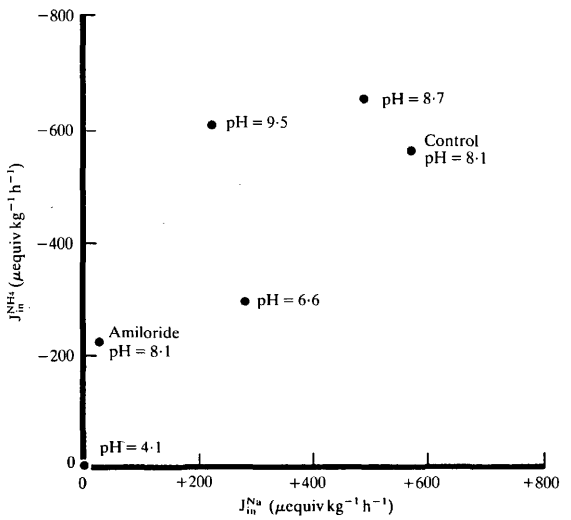

Fig. 7. The relationship between estimated $J_{net}^{NH_4}$ and measured J_{in}^{Na} in rainbow trout in each experimental series. See text for details of the $J_{net}^{NH_4}$ calculation.

more favourable means of acidic equivalent excretion in any given situation. Na^+/H^+ exchange plus NH_3 diffusion is equivalent to Na^+/NH_4^+ exchange in terms of both mass and acid-base balance. Na^+/Na^+ exchange diffusion also undoubtedly occurs in the gills (cf. Maetz *et al.* 1976; Wood *et al.* 1984). The factors controlling the relative rates of these various processes is a challenging field for future investigation.

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