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ION TRANSPORT AND ACID–BASE BALANCE IN FRESHWATER BIVALVES

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Summary

Blood acid–base and ionic balance in freshwater bivalves is affected by the relative activities of epithelial Na^+ and Cl^- transporters. In the unionid *Carunculina texasensis*, the Na^+/H^+ exchanger is the predominant epithelial transporter that affects acid–base state, while Cl^-/HCO_3^- exchange is of lesser importance. In the corbiculid *Corbicula fluminea*, Cl^- and Na^+ transport are both significant components affecting acid–base state. Serotonin (5-hydroxytryptamine, 5-HT) stimulates Na^+ and Cl^- transport in both species. In *C. texasensis*, the effect of exogenous serotonin is four times greater on Na^+/H^+ exchange than on Cl^-/HCO_3^- transport, resulting in an increase in acid secretion and a rise in blood pH. In a Na^+-free environment, serotonin had no effect on blood acid–base state in *C. texasensis*. In *C. fluminea*, the acid–base consequences of serotonin stimulation of Na^+/H^+ exchange were offset by similar increases in Cl^-/HCO_3^- exchange and by alterations in blood P_{CO_2} in medium containing Na^+. In Na^+-free medium, stimulation of the Cl^- transporter with 5-HT resulted in a decrease in blood pH. The differences between these two species are related to the reliance of *C. fluminea* on Cl^- as the major anion in the blood, requiring high levels of epithelial Cl^- transport. In *C. texasensis*, the anionic component of the blood consists of both Cl^- and HCO_3^- and these ions are interchangeable over a wide concentration range. Extracellular acid–base balance in freshwater bivalves is governed, in part, by epithelial ion transporters.

Key words: acid–base balance, serotonin, Cl^- transport, HCO_3^- transport, ion transport, bivalve, *Carunculina texasensis*, *Corbicula fluminea*.

Introduction

Although freshwater bivalves are a polyphyletic group, they display striking convergence in some of their physiological adaptations to a dilute medium. In particular, the osmolality of their body fluids is remarkably low, ranging from 35 to 50 mosmol kg^{-1} in *Dreissena polymorpha* (Dreissenidae) to 50–65 mosmol kg^{-1} in *Corbicula fluminea* (Corbiculidae), and with values for members of the Unionidae averaging 40–50 mosmol kg^{-1} (Potts, 1954; Dietz, 1979; Byrne *et al.* 1989; Horohov *et al.* 1992; Dietz *et al.* 1996). In general, Na^+ is the predominant cation, with HCO_3^- and Cl^- being the major anions. There are significant differences between freshwater bivalve groups: *Dreissena* and *Corbicula* have Cl^- as their predominant anion, whereas the unionids typically employ Cl^- and HCO_3^- equally as major anions in their blood (Dietz, 1979; Horohov *et al.* 1992).

In order to maintain even these low levels of solute, freshwater bivalves must actively transport the major ions from the dilute medium. The gills are the site of both Na^+ (Dietz and Findley, 1980) and Cl^- uptake (Dietz and Hagar, 1990). For the unionids and corbiculids, a Na^+/H^+ exchanger and a Cl^-/HCO_3^- exchanger are the main transporters present, as shown by transport kinetic studies and the consequences of application of specific inhibitors (Dietz, 1978; Dietz and Branton, 1979).

Na^+ transport is consistently stimulated in unionids and corbiculids by exogenous application of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) in the bathing medium (Dietz *et al.* 1982; Zheng, 1996). Serotonin also stimulates Cl^- transport in corbiculids (Zheng, 1996) and in *D. polymorpha* (Horohov *et al.* 1992).

The blood (more accurately the extracellular fluid) of freshwater and marine bivalve groups is very low in protein (Byrne and McMahon, 1991; Booth *et al.* 1984). Thus, the non-bicarbonate buffering capacity of the blood, which is usually directly related to the protein content, is also extremely low. Therefore, changes in the controlling components of acid–base balance, such as P_{CO_2}, and changes in the strong ion difference (SID) (Stewart, 1978) result in relatively large changes in blood pH (Byrne *et al.* 1991, 1995; Byrne and McMahon, 1991, 1994).

The interaction between ion transport and acid–base balance has been well studied in aquatic vertebrates, and particular attention has been given to mechanisms involving the gills of freshwater fish (Maetz *et al.* 1976; Perry *et al.* 1982; for a

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review, see McDonald *et al.* 1989). A combination of Na^+/H^+; Na^+/NH_4^+ and Cl^-/HCO_3^- exchange on the apical (bath side) surface of gill epithelia is largely responsible for gill-mediated ion exchange. This combination of cation and anion transport exchanges a strong ion for an acid or base equivalent. Thus, measurement of net movements of Na^+ and Cl^- yields information on excretion or uptake of acid equivalents (McDonald *et al.* 1989).

In the present study, we examined the following questions. Can measurements of Na^+ and Cl^- transport aid in the understanding of acid–base state in freshwater bivalves? Can neuromodulators that alter ion transport variables exert predictable effects on acid–base state? Are there differences in response or mechanism between phyletically distinct groups of freshwater bivalve, and does this information aid in the understanding of their physiology?

Materials and methods

Rationale

Experiments were designed to examine Na^+ and Cl^- fluxes and blood acid–base variables of clams exposed to a pondwater medium and to compare these responses with those of animals incubated in medium deficient in either Na^+ or Cl^-. Animals were also stimulated with the neuromodulator serotonin so that the acid–base effects of changes in epithelial ion transport would be more apparent.

Animals

Specimens of the corbiculid *Corbicula fluminea* (Müller) were collected, under permit, either from Anococo Creek, 10 km northwest of Leesville, Louisiana, or from the Tangipahoa River at Percy Quin State Park, Mississippi. We collected the unionid *Carunculina texasensis* (Lea) from local ponds in the Baton Rouge area. Animals were returned to the laboratory and maintained unfed in artificial pondwater (APW, in mmol l^{-1}: NaCl, 0.5; $NaHCO_3$, 0.2; KCl, 0.05; $CaCl_2$, 0.4; Dietz and Branton, 1975) at ambient temperature (22–24 °C) for at least a week prior to use.

Treatments

We measured blood acid–base variables and ion composition in bivalves following incubation in a series of ion-restricted media, with or without the addition of serotonin. In addition, we measured the components of ion flux (net flux, influx and efflux) in these bathing media and in the presence or absence of 5-HT.

Blood ion and acid–base status experiments

Animals (N=5–17 per treatment category) were incubated in individual containers (volume 50 ml) for 3 h at 23 °C in one of the following media: artificial pondwater ± 0.1 mmol l^{-1} serotonin; 1 mmol l^{-1} choline chloride (Na^+-free medium) ± 0.1 mmol l^{-1} serotonin; 0.5 mmol l^{-1} Na_2SO_4 (Cl^--free medium) ± 0.1 mmol l^{-1} serotonin. At the end of the incubation period, each animal was removed and a blood sample was taken by pericardiac puncture (Fyhn and Costlow, 1975). The pH of the sample was measured immediately using a micro pH electrode (Microelectrodes Inc., MI-730) fitted to a thermostatted blood-gas cell (Cameron Instruments) maintained at 23 °C by a recirculating refrigerated water bath. The electrode was connected to a blood-gas analyzer (Cameron Instruments). Blood total CO_2 (C_{CO_2}) was measured immediately by gas chromatography using the methods described in Horohov *et al.* (1992). Briefly, 50 µl of blood was introduced into a 10 ml gas-tight syringe containing 2.0 ml of N_2 (CO_2-free)-equilibrated 0.10 mol l^{-1} HCl and a 3.0 ml gas space filled with N_2. The syringe contents were sealed and the contents mixed on a shaker for 5 min to allow equilibration of the gas space with CO_2 liberated from the sample. The gas space from the syringe was injected through a drier (magnesium perchlorate) into the input port of a Hach gas chromatograph (80 mesh Porapak column). The resulting CO_2 peak was compared with peaks for bicarbonate standards, and blood C_{CO_2} was expressed as mmol l^{-1}. Blood P_{CO_2} (mmHg; 1 mmHg=133.3 Pa) and apparent HCO_3^- concentration ($[HCO_3^-]_{app}$, mmol l^{-1}; *sensu* Heisler, 1986) were calculated by rearrangement of the Henderson–Hasselbalch equation:

$$pH = pK_{app} + \log[(C_{CO_2} - \alpha_{CO_2}P_{CO_2})/\alpha_{CO_2}P_{CO_2}], \quad (1)$$

where pK_{app} (apparent pK) at 23 °C for freshwater bivalve blood is 6.324 (Byrne *et al.* 1991) and α_{CO_2} is the solubility of CO_2 in a salt solution similar to clam blood at the experimental temperature (0.046 mmol l^{-1} $mmHg^{-1}$, see Cameron, 1986). Apparent bicarbonate concentration $[HCO_3^-]_{app}$ was calculated as:

$$[HCO_3^-]_{app} = C_{CO_2} - \alpha_{CO_2}P_{CO_2}. \quad (2)$$

The remaining blood sample was centrifuged for 3 min at 9000 ***g*** to remove particulate matter and stored at 4 °C for later ion analyses. Blood $[Na^+]$ was determined by flame photometry on diluted samples and blood $[Cl^-]$ was measured by electrometric titration.

The non-bicarbonate buffer value (β_{NB}) for freshwater bivalve blood at 23 °C was taken from Byrne *et al.* (1991) as 0.99 µmol pH $unit^{-1}$ g^{-1} (slykes). Base excess (mmol l^{-1}) was calculated as:

$$\text{base excess} = [HCO_3^-]_{app,pw} - [HCO_3^-]_{app,exp} - \beta_{NB}(pH_{pw} - pH_{exp}), \quad (3)$$

where the subscripts ‘pw’ and ‘exp’ refer to values for pondwater control animals and experimental animals, respectively (Booth *et al.* 1984).

Ion flux experiments

Specimens of *Corbicula fluminea* and *Carunculina texasensis* (N=5–10 per treatment category) were incubated for 3 h in 50 ml of one of the following eight media: artificial pondwater containing 6×10^3–14×10^3 cts min^{-1} ml^{-1} ^{22}Na or ^{36}Cl ± 0.1 mmol l^{-1} serotonin; 1 mmol l^{-1} choline chloride (Na^+-free medium) + ^{36}Cl ± 0.1 mmol l^{-1} serotonin; 0.5 mmol l^{-1} Na_2SO_4 (Cl^--free medium) + ^{22}Na ± 0.1 mmol l^{-1} serotonin. Bath samples (5.0 ml) were taken at time zero and

after the 3 h incubation. Animals were removed, and the soft tissues were dried (90 °C; 24 h) and weighed. The radioactivity of bath samples was measured on 0.5 ml subsamples using liquid scintillation methods (Weigman *et al.* 1975). Bathing medium [Na^+] was measured by flame photometry and [Cl^-] by electrometric titration. Net ion flux was determined from the change in [Na^+] or [Cl^-] in the bathing medium and was expressed as $\mu mol\ g^{-1}\ dry\ mass\ h^{-1}$. Ion influx ($J_i$) was determined by the loss of isotope from the bathing medium using standard methods (Graves and Dietz, 1982), and ion efflux (J_o) was calculated as the algebraic difference between influx and net flux (J_{net}).

Statistical analyses

All data are expressed as means ± S.E.M.; significance was accepted at $P<0.05$. Overall differences between means were determined by single-factor analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) to determine differences between specific means. Data from individual species were analyzed separately. Analyses were performed following Statview (Abacus Systems) procedures.

Results

The acid–base responses of both species to serotonin treatment and treatment with ion-restrictive media are summarized in a pH–HCO_3^- (Davenport) diagram (Fig. 1). The blood of pondwater-acclimated *Corbicula fluminea* is characterized as having a relatively low pH of approximately 7.9 and a low HCO_3^- concentration, whereas the blood of pondwater-acclimated *Carunculina texasensis*, in common with that of most other unionid species, has a higher pH and a higher bicarbonate content (Fig. 1).

Corbicula fluminea *blood acid–base variables and ion composition*

Application of exogenous serotonin to pondwater-acclimated *Corbicula fluminea* had no effect on blood acid–base variables or ion composition (Table 1; Fig. 1). However, animals incubated in Cl^--free medium developed a significant base excess demonstrated by an increase in blood [HCO_3^-] with no significant change in blood pH or P_{CO_2} (Table 1; Fig. 1). Application of serotonin to these animals resulted in a further increase in base excess, this time with a concomitant increase in P_{CO_2}, but with no change in blood pH (Table 1; Fig. 1). Serotonin stimulation of clams bathed in Cl^--free medium did not affect blood [Na^+], but blood Cl^- levels did decline significantly compared with values for animals bathed in Na^+-free medium (Table 1). When specimens of *C. fluminea* were incubated in a Na^+-free medium, a base deficit occurred relative to pondwater-acclimated controls, as HCO_3^- content fell, but with non-significant changes in P_{CO_2} or in blood pH (Table 1; Fig. 1).

Treatment of these animals with serotonin resulted in a significant decrease in blood pH with no further change in [HCO_3^-], but with a 25 % increase in mean blood P_{CO_2} that was not statistically significant (Table 1; Fig. 1). Blood Cl^- levels increased to 30 mmol l^{-1}, whereas blood Na^+ levels were lower than those of pondwater-bathed animals.

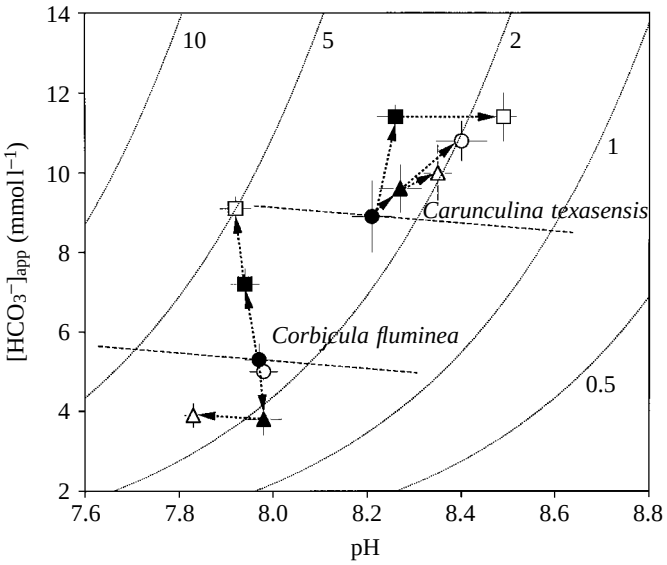

Fig. 1. A pH–bicarbonate (Davenport) diagram illustrating the effects of incubation of the corbiculid *Corbicula fluminea* and the unionid *Carunculina texasensis* in pondwater (circles), Cl^--free medium (0.5 mmol l^{-1} sodium sulfate) (squares) and Na^+-free medium (1 mmol l^{-1} choline chloride) (triangles) at 23 °C. Filled symbols are for animals incubated for 3 h in the respective medium, open symbols represent values for animals incubated in the respective medium with the addition of 0.1 mmol l^{-1} serotonin. The *x* and *y* error bars are standard errors of the mean (N=5–17). The dashed straight line represents the non-bicarbonate buffer line for freshwater bivalve blood at 23 °C (from Byrne *et al.* 1991) and was fitted through the values for pondwater-acclimated individuals for both species. The isopleths are for P_{CO_2} values in equilibrium with clam blood; values are in mmHg (1 mmHg=133.3 Pa).

Carunculina texasensis *blood acid–base variables and ion composition*

The effects of serotonin and the compounding effects of the ion-restricted medium are more apparent on blood acid–base variables and ion composition in the unionid *Carunculina texasensis* (Table 2; Fig. 1). Blood pH and HCO_3^- levels for pondwater-acclimated *C. texasensis* are elevated significantly over those of *C. fluminea* (Fig. 1). Blood pH was significantly elevated in pondwater-acclimated *C. texasensis* when stimulated with serotonin (Table 2; Fig. 1). There was a significant serotonin-induced base excess, with blood HCO_3^- content increasing and a significant reduction in blood P_{CO_2}, resulting in a combined respiratory and non-respiratory alkalosis (Table 2; Fig. 1). Although blood [Na^+] was unaltered, blood Cl^- levels were significantly reduced following serotonin treatment, and there was an equivalent increase in [HCO_3^-] (Table 2). Incubation in Cl^--free medium resulted in an elevation in base excess compared with that of

Table 1. *Effects of 3 h incubation in artificial pondwater in ion-restricted media (Cl^--free, 0.5 mmol l^{-1} sodium sulfate; Na^+-free, 1 mmol l^{-1} choline chloride) with and without 0.1 mmol l^{-1} serotonin on blood acid–base variables and ion composition in* Corbicula fluminea

	Pondwater control	Pondwater +5-HT	Cl^--free control	Cl^--free +5-HT	Na^+-free control	Na^+-free +5-HT
pH	7.974±0.030[b] (17)	7.975±0.038[b] (17)	7.944±0.030[b] (5)	7.924±0.033[b] (7)	7.979±0.038[b] (6)	7.833±0.018[a] (8)
C_{CO_2} (mmol l^{-1})	5.4±0.4[b] (17)	5.2±0.3[b] (15)	7.4±0.4[c] (5)	9.3±0.3[d] (7)	3.9±0.4[a] (6)	4.0±0.3[a] (8)
P_{CO_2} (mmHg)	2.7±0.3[a,b] (17)	2.8±0.4[a,b] (15)	3.8±0.3[b,c] (5)	5.0±0.3[c] (7)	1.9±0.4[a] (6)	2.6±0.1[a,b] (8)
$[HCO_3^-]_{app}$ (mmol l^{-1})	5.3±0.4[b] (17)	5.0±0.2[b] (15)	7.2±0.4[c] (5)	9.1±0.3[d] (7)	3.8±0.4[a] (6)	3.9±0.3[a] (8)
$[Na^+]$ (mmol l^{-1})	31.8±0.5[b] (17)	30.9±0.5[b] (15)	28.1±1.4[a] (5)	28.9±0.5[a] (7)	28.6±0.8[a] (6)	27.1±0.9[a] (8)
$[Cl^-]$ (mmol l^{-1})	27.8±0.9[a,b] (17)	26.5±0.7[a] (15)	28.5±1.3[a,b] (5)	25.7±0.6[a] (7)	30.0±0.7[b] (6)	30.0±0.8[b] (8)

Data are expressed as means ± S.E.M. (*N*).
Means within a row with different letters are significantly different from one another ($P<0.05$; Fisher PLSD after ANOVA).
5-HT, serotonin.
1 mmHg=133.3 Pa.

pondwater-incubated controls similar to that noted with *C. fluminea*. There was no change in blood pH, $[Na^+]$ or $[Cl^-]$, but P_{CO_2} increased (Table 2; Fig. 1). When stimulated with serotonin, there was a significant alkalosis in these animals due entirely to a reduction in blood P_{CO_2} with no change in $[HCO_3^-]$ (Table 2; Fig. 1). Blood $[Na^+]$ remained at pondwater-acclimated levels but blood $[Cl^-]$ declined significantly (Table 2). In the absence of external Na^+, blood pH was not significantly altered compared with that of pondwater-incubated controls; none of the other variables measured was altered (Table 2). Application of serotonin to animals incubated in a Na^+-free medium resulted in no significant change in blood pH compared with Na^+-free controls, but blood pH was significantly elevated over that of pondwater-acclimated animals (Table 2; Fig. 1).

Na^+ fluxes

Serotonin stimulated Na^+ influx in both *C. fluminea* and *C. texasensis* (Table 3). In pondwater, both species were essentially in steady state with regard to Na^+, having net fluxes

Table 2. *Effects of 3 h incubation in artificial pondwater in ion-restricted media (Cl^--free, 0.5 mmol l^{-1} sodium sulfate; Na^+-free, 1 mmol l^{-1} choline chloride) with and without 0.1 mmol l^{-1} serotonin on blood acid–base variables and ion composition in* Carunculina texasensis

	Pondwater control	Pondwater +5-HT	Cl^--free control	Cl^--free +5-HT	Na^+-free control	Na^+-free +5-HT
pH	8.205±0.042[a] (11)	8.401±0.054[c] (10)	8.267±0.039[a,b] (6)	8.493±0.027[c] (6)	8.271±0.045[a,b] (6)	8.347±0.030[b,c] (6)
C_{CO_2} (mmol l^{-1})	9.0±0.4[a] (15)	10.3±0.4[b,c] (14)	11.5±0.3[c] (6)	11.5±0.6[c] (6)	9.7±0.6[a,b] (6)	10.1±0.7[a,b,c] (6)
P_{CO_2} (mmHg)	2.2±0.2[b,c] (6)	1.5±0.2[a] (6)	2.9±0.3[d] (6)	1.7±0.1[a,b] (6)	2.4±0.3[c,d] (6)	2.0±0.2[a,b,c] (6)
$[HCO_3^-]_{app}$ (mmol l^{-1})	8.9±0.9[a] (17)	10.8±0.5[b] (6)	11.4±0.3[b] (6)	11.4±0.6[b] (6)	9.6±0.6[a] (6)	10.0±0.7[a,b] (6)
$[Na^+]$ (mmol l^{-1})	18.0±0.5[a] (15)	17.2±0.4[a] (14)	17.3±1.0[a] (6)	16.7±0.5[a] (6)	17.8±0.6[a] (6)	17.0±0.5[a] (6)
$[Cl^-]$ (mmol l^{-1})	13.0±0.2[c] (17)	11.2±0.3[b] (14)	12.2±0.8[c] (6)	10.1±0.3[a] (6)	13.1±0.6[c] (6)	12.2±0.4[c] (6)

Data are expressed as means ± S.E.M. (*N*).
Means within a row with different letters are significantly different from one another ($P<0.05$; Fisher PLSD after ANOVA).
5-HT, serotonin.
1 mmHg=133.3 Pa.

Table 3. *Effects of external serotonin stimulation (0.1 mmol l^{-1}) on the components of the whole-animal Na^+ flux in specimens of* Corbicula fluminea *and* Carunculina texasensis *incubated in artificial pondwater and in Cl^--free medium (0.5 mmol l^{-1} sodium sulfate)*

	Na^+ flux (μmol g^{-1} h^{-1})		
	J_{net}	J_i	J_o
Corbicula fluminea			
Pondwater	–0.08±0.55[a]	4.05±1.05[a]	4.12±0.86[a]
Pondwater + 5-HT	4.14±0.58[b]	9.05±1.17[b]	4.91±0.73[a]
Cl^--free	–1.32±0.62[a]	3.15±0.43[a]	4.47±0.50[a]
Cl^--free + 5-HT	5.72±1.16[b]	13.53±1.94[c]	7.81±1.86[b]
Carunculina texasensis			
Pondwater	–0.19±0.16[a]	1.19±0.22[a]	1.38±0.15[a]
Pondwater + 5-HT	2.77±0.21[b]	5.02±0.51[b]	2.25±0.46[a]
Cl^--free	–0.65±0.16[a]	0.70±0.14[a]	1.35±0.26[a]
Cl^--free + 5-HT	3.04±0.39[b]	4.67±0.62[b]	1.64±0.30[a]

Values are means ± S.E.M.; N=9 for each *C. fluminea* treatment and N=10 for each *C. texasensis* treatment.

For each species, means within a column with different letters are significantly different from one another (P<0.05; Fisher's PLSD after ANOVA).

5-HT, serotonin.

J_{net}, net flux; J_i, influx; J_o, efflux.

Table 4. *Effects of external serotonin stimulation (0.1 mmol l^{-1}) on the components of the whole-animal Cl^- flux in specimens of* Corbicula fluminea *and* Carunculina texasensis *incubated in artificial pondwater and in Na^+-free medium (1 mmol l^{-1} choline chloride)*

	Cl^- flux (μmol g^{-1} h^{-1})		
	J_{net}	J_i	J_o
Corbicula fluminea			
Pondwater	–1.31±0.43[a]	2.15±0.26[a]	3.46±0.58[a]
Pondwater + 5-HT	2.27±0.93[b]	5.66±0.77[b]	3.38±0.67[a]
Na^+-free	–1.80±0.39[a]	2.97±0.40[a]	4.77±0.52[a]
Na^+-free + 5-HT	1.21±0.67[b]	5.88±0.91[b]	4.66±0.74[a]
Carunculina texasensis			
Pondwater	–0.36±0.16[a]	1.28±0.37[a]	1.65±0.42[b,c]
Pondwater + 5-HT	0.62±0.33[b]	2.35±0.24[b]	1.73±0.39[c]
Na^+-free	0.17±0.13[a]	0.92±0.10[a]	0.75±0.42[a]
Na^+-free + 5-HT	1.12±0.15[b]	1.93±0.17[b]	0.81±0.12[a,b]

Values are means ± S.E.M.; N=9 for each *C. fluminea* treatment and N=10 for each *C. texasensis* treatment.

For each species, means within a column with different letters are significantly different from one another (P<0.5; Fisher's PLSD after ANOVA).

5-HT, serotonin.

J_{net}, net flux; J_i, influx; J_o, efflux.

of approximately zero. There were no differences between fluxes (J_{net}, J_i and J_o) in control animals in pondwater and in Cl^--free medium. Serotonin stimulated Na^+ influx by more than twofold for *C. fluminea* and by approximately fivefold for *C. texasensis* when bathed in pondwater (Table 3). Efflux was not affected by either bathing medium or serotonin stimulation in *C. texasensis*. Pondwater-bathed *C. fluminea* displayed no alteration in efflux in response to serotonin stimulation. However, when *C. fluminea* were stimulated with serotonin in a Cl^--free medium, Na^+ efflux and Na^+ influx increased. Examination of fluxes of individual animals demonstrated that those with high influxes also had the highest effluxes (data not shown), suggesting high turnover rates in these individuals.

Cl^- fluxes

Specimens of *C. fluminea* utilized as controls in this study were experiencing net Cl^- losses both in pondwater and in Na^+-free bathing medium, whereas *C. texasensis* were in approximate steady state (Table 4). In both species, serotonin stimulation resulted in an increase in Cl^- influx in both bathing media. In *C. fluminea*, there was an approximate doubling of the influx, resulting in positive net Cl^- fluxes, while efflux was not significantly altered. There was no effect of Na^+ absence on any of the fluxes in *C. fluminea* (Table 4). In *C. texasensis*, serotonin stimulated Cl^- influx to a similar extent in both pondwater and Na^+-free medium (Table 4). Unlike *C. fluminea*, incubation of *C. texasensis* in Na^+-free medium resulted in a reduction in Cl^- efflux to approximately half the rate observed in pondwater-incubated controls. The effluxes were not further affected by serotonin application.

Interactions between ion transport and acid–base/ionic status

Summary diagrams for both *C. fluminea* (Fig. 2) and *C. texasensis* (Fig. 3) highlight changes in components of acid flux and blood acid–base balance. The net acid flux can be expressed as the algebraic difference between the net flux of Cl^- and the net flux of Na^+ (McDonald *et al.* 1989). Pondwater-acclimated *C. fluminea* were in net acid loss because Cl^- net flux was lower than Na^+ net flux (Fig. 2). Serotonin stimulation did not alter blood pH or the net loss of acid equivalents, despite net increases in Na^+ and Cl^- flux (Fig. 2). In pondwater, Na^+ influx is approximately twice Cl^- influx in *C. fluminea*, and serotonin stimulation doubles the influx of both Na^+ and Cl^-, thereby maintaining the relative stoichiometry (Tables 3, 4). The elevated ion fluxes could result in an increase in acid secretion with consequent changes in blood variables. However, the relative difference between the net Na^+ and Cl^- fluxes was maintained upon serotonin stimulation, resulting in no net change in acid secretion (Fig. 2).

The responses of these species to ion-deficient media accentuate the significance of the differences both in ion-transporting properties and in the ionic composition of the blood. In the absence of external Cl^-, there were offsetting increases in base excess and P_{CO_2} in *C. fluminea* (Fig. 2). Stimulation by serotonin resulted in a large increase in Na^+ net flux and caused an equally large increase in net acid equivalent loss. Blood pH was not altered, despite further increases in base excess, because of a compensatory increase in P_{CO_2}. In Na^+-free controls, there was no net movement of acid

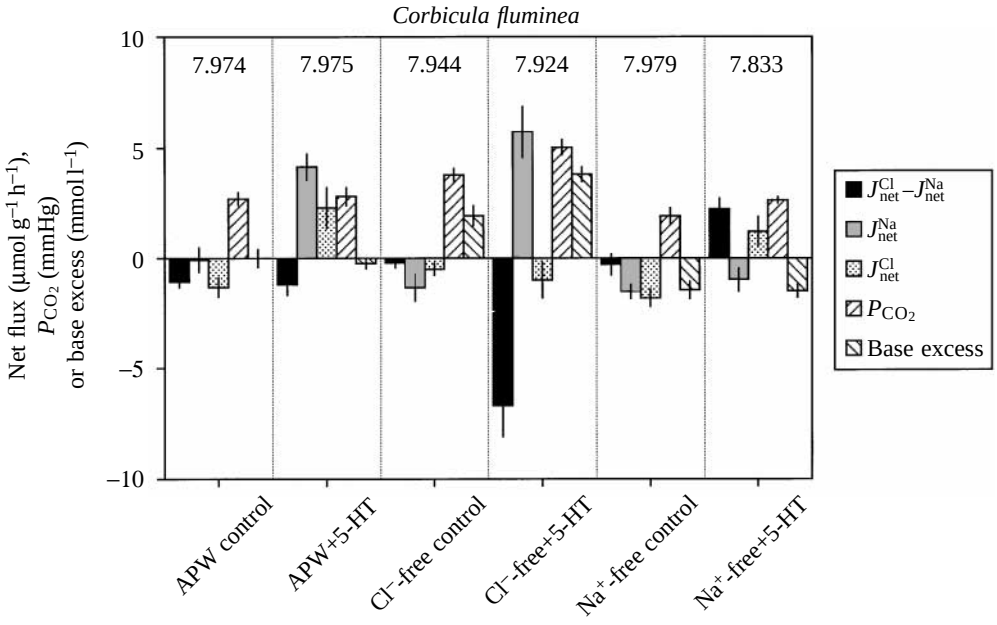

Fig. 2. Effects of incubation in pondwater, Cl^--free medium (0.5 mmol l^{-1} sodium sulfate) and Na^+-free medium (1 mmol l^{-1} choline chloride) with or without 0.1 mmol l^{-1} serotonin (5-HT) on variables affecting acid–base status in *Corbicula fluminea*. The value over the bar in each treatment block is blood pH. Net Cl^- flux (J^{Cl}_{net}) minus net Na^+ flux (J^{Na}_{net}) represents net H^+ flux (J^{H}_{net}) (after McDonald *et al.* 1989). Error bars are standard errors of the mean (*N*=5–17). APW, artificial pondwater.

equivalents because Na^+ and Cl^- net fluxes were similar (Fig. 2). Base excess was negative (=base deficit), but P_{CO_2} was lower than in pondwater-acclimated animals, resulting in no significant change in blood pH. Serotonin stimulated Cl^- transport and, as net Na^+ flux was unaltered in the absence of exogenous Na^+, net acid flux was positive (the animals retained protons). The base deficit was not changed, but P_{CO_2} increased, causing a significant decline in blood pH (Fig. 2).

Carunculina texasensis was in a steady state in pondwater with acid flux at around zero (Fig. 3). There was a significant net loss of acid equivalents when these animals were stimulated with serotonin, with a concurrent increase in base excess and a decline in blood P_{CO_2} combining to cause a rise in blood pH (Fig. 3). In a Cl^--free medium, there was a slightly positive net acid flux (proton retention), but a large base excess was offset by an increase in blood P_{CO_2}. Serotonin stimulation significantly increased net Na^+ flux in this medium, resulting in a loss of acid equivalents, base excess was not altered significantly and blood P_{CO_2} declined, the end result being an increase in blood pH. Stimulation of Cl^- flux in *C. texasensis* in a Na^+-free medium did not result in a significant change in acid uptake (proton retention); base excess was not affected, nor was blood P_{CO_2}, resulting in no significant change in blood pH (Fig. 3). Thus, in *C. texasensis*, serotonin affects Na^+ influx to a significantly greater extent than it affects Cl^- influx. Consequently, the differences in net Na^+ and Cl^- flux have a significantly greater effect on acid secretion than that observed in *C. fluminea*.

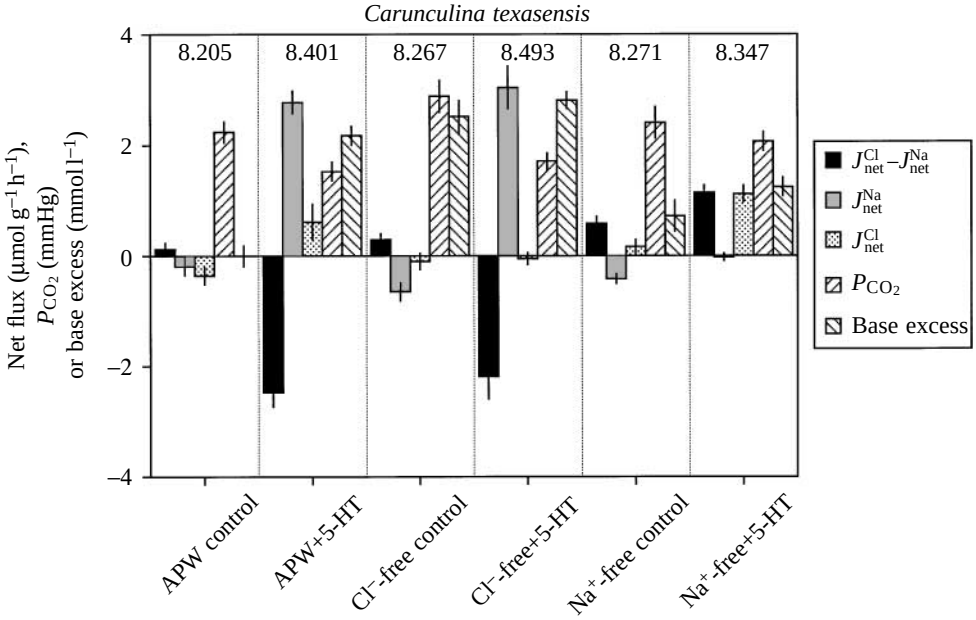

Fig. 3. Effects of incubation in pondwater, Cl^--free medium (0.5 mmol l^{-1} sodium sulfate) and Na^+-free medium (1 mmol l^{-1} choline chloride) with or without 0.1 mmol l^{-1} serotonin (5-HT) on variables affecting acid–base status in *Carunculina texasensis*. The value over the bar in each treatment block is blood pH. Net Cl^- flux (J^{Cl}_{net}) minus net Na^+ flux (J^{Na}_{net}) represents net H^+ flux (J^{H}_{net}) (after McDonald *et al.* 1989). Error bars are standard errors of the mean (*N*=5–17). APW, artificial pondwater.

Discussion

The interrelationships between Na^+/Cl^- transport and acid–base balance in these two species of freshwater bivalve are dependent on the relative activities of the transporters and the overall blood ion composition of each species. *Corbicula fluminea*, in common with other freshwater bivalve species with relatively short histories in fresh water (Keen and Casey, 1969), has a blood composition relying on Cl^- as the major anion (Dietz, 1979). Consequently, rates of Cl^- transport in *C. fluminea* are high and are apparently under neural control as shown by serotonin stimulation of Cl^- transport in this species (this study; Zheng, 1996). In contrast, *Carunculina texasensis* conforms to the unionid model of having approximately equal amounts of Cl^- and HCO_3^- in the blood (Dietz, 1979). In the present study, we report the first instance of effective stimulation of Cl^- influx by exogenously applied serotonin in a unionid species, but the level of stimulation is significantly lower than that of Na^+ stimulation and lower than that observed in *C. fluminea.*

The disturbances in blood acid–base state in these species both in ion-deficient media and in response to serotonin stimulation are consistent with the actions of largely independent Na^+/H^+ and Cl^-/HCO_3^- exchangers in the gill epithelia. Blood pH in *C. texasensis* was alterable to a greater extent than that of *C. fluminea.* The change in pH was primarily due to the greater activity of the Na^+/H^+ exchanger relative to the Cl^-/HCO_3^- exchanger in *C. texasensis* so that, under conditions of serotonin stimulation, acid secretion increased. Also, a higher blood pH is subject to greater perturbations by addition/deletion of acid equivalents than a lower blood pH, given similar buffering environments. This is a consequence of measuring pH on a logarithmic scale. Although the HCO_3^- buffering capacity increases with increasing HCO_3^- content, changes in P_{CO_2} have a greater influence at higher pH. In both species, incubation in a Cl^--free medium resulted in retention of HCO_3^- in the blood as a result of a reduced ability to exchange HCO_3^- with the environment and continued Na^+/H^+ exchange, with H^+ excretion being equivalent to HCO_3^- retention.

For the unionid, maintenance of ionic homeostasis requires greater reliance on active Na^+ uptake than on uptake of Cl^-. The anionic component of blood consists of a combination of Cl^- and HCO_3^-, with substitution of one for the other over a substantial concentration range (Scheide and Dietz, 1982). As HCO_3^- can be mobilized or removed relatively easily by metabolic and respiratory means, the requirement for a tightly regulated Cl^- transport system, controlled by hormonal and/or neural means, is much less important. In contrast, *C. fluminea* must regulate Cl^- uptake in order to maintain blood Cl^- at a high and relatively constant level. Although blood HCO_3^- levels increase and decrease in response to treatment, *C. fluminea* has a much less well-developed capacity to substitute Cl^- and HCO_3^- in the blood.

It is uncertain whether bivalve molluscs actively regulate blood pH. Acid–base responses to changes in environmental gases result in predictable and large changes in blood pH in the unionids *Elliptio complanata* (Byrne *et al.* 1995) and *Anodonta grandis simpsoniana* (Byrne and McMahon, 1990). Although some compensatory mechanisms to alleviate perturbations in blood pH have been described for these species, the acid–base responses are generally regulated only within wide limits. Booth *et al.* (1984) concluded that the marine bivalve *Mytilus edulis* had a limited ability to regulate blood pH and that maintenance of blood pH within narrow limits may be unnecessary. This view was supported by a related study (Walsh *et al.* 1984) in which it was demonstrated that intracellular pH was not strictly dependent on extracellular pH in this species. The gas-carrying characteristics of respiratory pigments are functionally dependent on blood pH; thus, the absence of such pigments from the blood of most marine and all freshwater bivalve species would remove a major reason for maintenance of close extracellular pH regulation (Booth *et al.* 1984).

However, shell-forming animals, such as bivalve molluscs, must maintain an overall acid–base balance because the deposition of shell ($CaCO_3$) is accompanied by the release of acid equivalents (Wilbur, 1983). Therefore, if a bivalve is growing a shell, it must excrete protons in order to remove the accumulated proton load due to carbonate deposition. The time course of this pH regulation may encompass longer periods than are generally utilized in laboratory experiments. Indeed, shell deposition normally occurs in a distinctly discontinuous manner as shown by the presence of growth lines. Zheng (1996) demonstrated that *C. fluminea* maintains a constant ratio of Na^+ and Cl^- net flux, the result of which is a constant rate of acid secretion over a wide range of Na^+ and Cl^- net fluxes, suggesting that, over time, acid excretion is maintained as a function of the regulation of specific ions.

Thus, control of blood acid–base balance in the long term may have more to do with regulating shell deposition/reabsorption and of maintaining Ca^{2+} and HCO_3^- levels than with maintaining short-term extracellular homeostasis. Shell formation is influenced by environmental factors such as hypoxia (Wilbur, 1983) and pH (Machado *et al.* 1988). Calcium stores in unionid bivalve species are defended during environmental hypoxia by sequestration of blood Ca^{2+} released from the shell into calcium phosphate concretions located extracellularly in the gills (Silverman *et al.* 1983). These studies suggest that perturbations in blood acid–base balance and the associated changes in calcium dynamics affect the process of shell formation occurring in the separate extrapallial compartment. Coimbra *et al.* (1993) postulate that the dynamics of shell deposition are ultimately under the control of $[Ca^{2+}]$ in the blood and of the transepithelial electrical potential difference across the outer mantle epithelium. These factors exert a controlling effect on the direction of Ca^{2+} movement into or out of the extrapallial compartment (Coimbra *et al.* 1993). This is well illustrated when bivalves are exposed to air, which results in a respiratory acidosis because the pathway for metabolic CO_2 exchange with the environment is severely compromised. When *C. fluminea* is exposed to air, Ca^{2+} and HCO_3^- are mobilized from the shell and appear in the blood (Byrne *et al.* 1991). The respiratory

acidosis causes alterations in the Ca^{2+} and HCO_3^- dynamics between the blood/extrapallial compartments, resulting in shell reabsorption. The effect on blood acid–base balance is to partially alleviate the acidosis by providing a large base excess of HCO_3^- (Byrne *et al.* 1991). In addition, blood P_{CO_2} could rise to high levels without resulting in severe acidosis; this provides a sufficient gradient for gas loss to the environment, thereby achieving a steady-state acid–base balance (Byrne *et al.* 1991; Byrne and McMahon, 1994). The blood acid–base responses to emersion were similar in the unionid *Anodonta grandis simpsoniana* (Byrne and McMahon, 1991), suggesting that broadly similar processes were occurring in both species and that the importance of the regulation of blood acid–base balance is to provide a suitable environment to control the deposition and reabsorption of shell calcium.

The two species of freshwater bivalve utilized in this study represent lineages with sharply different histories in fresh water. Unionids have inhabited fresh water since the Cambrian, whereas corbiculids invaded fresh water in the relatively recent geological past (Triassic) (Keen and Casey, 1969). Other bivalve groups with a recent history in fresh water are some members of the Dreissenidae, represented in North America by *Dreissena polymorpha*. *D. polymorpha* conforms to the model that recent invaders of fresh water retain Cl^- as the major anion and have a relatively lower level of HCO_3^- in the blood (Horohov *et al.* 1992). This species also possesses Na^+ and Cl^- transporters capable of high rates of transport; both these transporters are stimulated by serotonin (Horohov *et al.* 1992).

Pondwater-acclimated bivalves having a greater reliance on Cl^- as the major anion have a comparatively lower blood pH. Blood with a low level of HCO_3^- may be the more archetypal condition for bivalves coming into fresh water, a position supported by the low levels of HCO_3^- found in the blood of marine bivalve species and in *D. polymorpha* (Booth *et al.* 1984; Horohov *et al.* 1992). The significance to those unionids that shift to a blood with higher $[HCO_3^-]$ is open to speculation. It is possible that, because all ions tend to be at low concentration in fresh water, reducing the dependence on one of these ions (Cl^-) and replacing it with an ion that can be endogenously produced from metabolism (HCO_3^-) would be an adaptive strategy. The relationship between blood acid–base balance and the dynamics of shell formation seems to be one of shifting equilibria so that a change in blood pH brought on by retaining HCO_3^- would need only to require a compensatory shift in the ionic components of the extrapallial fluid compartment in order to achieve a new equilibrium. The maintenance of freshwater bivalve blood pH and acid–base balance may be important to this interrelationship.

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