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SODIUM ION UPTAKE BY PERFUSED GILLS OF THE BLUE CRAB *CALLINECTES SAPIDUS*: EFFECTS OF OUABAIN AND AMILORIDE

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Summary

Sodium ion uptake in the isolated perfused posterior gill of the blue crab, *Callinectes sapidus* Rathbun, acclimated to 5‰ salinity and 20°C was measured using ^{22}Na as a tracer. Unidirectional influxes across individual gills occurred against large sodium concentration differences (internal perfusate=273 mmol l^{-1}; external perfusate=1–80 mmol l^{-1}) and depended strongly on the concentration of sodium ions present in the external perfusate. The maximum rate of influx was 3.24 μmol g^{-1} min^{-1} and the K_m for this process was 23.0 mmol l^{-1}. Ouabain added to the internal perfusate reduced the influx to an average of 51.3% of the control values and had its maximal effect at 1 mmol l^{-1}. Ouabain (1 mmol l^{-1}) added to the external perfusate had no significant effect on sodium influx. Amiloride (0.1 mmol l^{-1}) added to the external perfusate reduced the influx to an average of 38.9% of the control values. During all treatments, perfusion pressures showed no treatment-dependent effects, indicating that the observed changes in fluxes were due to specific effects of the treatment on the epithelium and not due to some indirect effect on perfusion pattern within the gill. Treatment of the gill with the metabolic poisons cyanide and iodoacetate resulted in a rapid decline of sodium influx to 20–30% of the control values. This was correlated with a large reduction in gill ATP content to 13% of the control. The overall results of this study are consistent with the general picture of epithelial sodium uptake across gills, supporting the existence of a ouabain-sensitive Na^+ pump on the basolateral membrane and an amiloride-sensitive Na^+/H^+ exchanger on the apical membrane.

Introduction

Although the crustacean gill has been implicated for many years in ion transport

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as well as gas exchange, cellular mechanisms remain unclear. In whole-animal studies, Na^+ uptake across the branchial epithelium is inhibited by external amiloride (Kirschner *et al.* 1973; Cameron, 1979; Pressley *et al.* 1981), suggesting that apical Na^+/H^+ exchange is one component of transepithelial Na^+ movement. In addition, the specific activity of Na^+,K^+-ATPase increases upon acclimation of crabs to dilute salinity (Towle *et al.* 1976; Neufeld *et al.* 1980; Pequeux *et al.* 1984), in which Na^+ uptake processes are enhanced. Na^+,K^+-ATPase in crab gill epithelial cells is restricted to basolateral membranes (Towle and Kays, 1986), where it may exchange Na^+ for NH_4^+ as well as Na^+ for K^+ (Towle and Hølleland, 1987). The coordinated operation of apical Na^+/H^+ exchange and basolateral ATP-dependent Na^+/K^+ (or Na^+/NH_4^+) exchange would provide a plausible mechanism of Na^+ uptake from medium to blood.

Perfusion of isolated gills offers the possibility of designing experiments to clarify cellular mechanisms of transport by modifying compositions of internal and external perfusates. Many crustacean gills are particularly suited to perfusion because of the simple circulatory pathway between afferent and efferent blood vessels. Hemolymph passes directly from the afferent vessel through the lamellae to the efferent vessel. Each lamella is composed of an envelope of cells, one cell-layer in thickness, surrounded by a thin cuticle (Johnson, 1980). Two cell types predominate, a thin respiratory cell with basolateral membranes parallel to the cuticle, and a thicker transporting cell with basolateral membranes perpendicular to the cuticle (Copeland and Fitzjarrell, 1968). The latter cell type, rich in Na^+,K^+-ATPase activity (Neufeld *et al.* 1980), predominates in the posterior gills of many osmoregulating crabs such as the blue crab *Callinectes sapidus* (Aldridge and Cameron, 1982).

Previous studies on perfused crustacean gills have provided potentiometric evidence for active Na^+ transport (Mantel, 1967; King and Schoffeniels, 1969; Smith and Linton, 1971; Gilles and Pequeux, 1985; Siebers *et al.* 1985, 1986) and other studies have demonstrated uptake of ^{22}Na directly (Koch, 1954; Mantel, 1967; Pequeux and Gilles, 1981). For example, large differences in Na^+ transporting capacity have been demonstrated between the anterior and posterior gills of the Chinese crab *Eriocheir sinensis* (Pequeux and Gilles, 1981). The more recent studies have supported the existence of an apical Na^+/H^+ antiporter and a basolateral Na^+,K^+-ATPase in crab gill epithelium (Lucu and Siebers, 1986; Siebers *et al.* 1986, 1987; Pequeux and Gilles, 1988). In most of these studies, gills were perfused externally and internally with identical salt solutions, because large net sodium effluxes were observed during the more physiological condition, involving asymmetric perfusion with concentrated internal medium and diluted external medium (Lucu and Siebers, 1986). Whether the conventional model of Na^+ uptake applies to the asymmetric condition has been difficult to assess.

In addition, some of the previous studies have employed unphysiological perfusion patterns or flow rates as well as internal perfusion media that did not closely match the composition of the crab hemolymph. We decided to investigate Na^+ transport in perfused gills of the blue crab *Callinectes sapidus*, which is known

for its ion-regulating ability (Engel *et al.* 1974), under conditions closely approximating those found *in vivo*. Respiratory properties of such preparations have been described in detail in a previous paper (Burnett, 1984).

Materials and methods

Male blue crabs (*Callinectes sapidus*) in intermolt were obtained from local suppliers in Carteret County, North Carolina, or from Gulf Specimens Co., Inc. Crabs were maintained in natural sea water diluted to 5‰ with deionized water. The dilute sea water was aerated and recirculated *via* a non-metallic pump through a gravel filter. Crabs were acclimated to laboratory conditions at 20°C (or 25°C in several instances) for at least 5 days before use.

The composition of artificial sea water at 5‰ (=external perfusate) was modified from Prosser (1973) (Table 1). External perfusate containing 1 mmol l^{-1} NaCl was obtained by substituting choline chloride. The composition of the internal perfusate was based on published values for hemolymph of *C. sapidus* acclimated to reduced salinity (Table 1). Concentrations of inorganic salts, amino acids, urea, glucose and lactate, as well as buffering capacity, closely matched those reported for hemolymph of the blue crab.

In early experiments, asymmetric perfusion with the described media lacking glutathione in the internal perfusate resulted in a substantial net sodium efflux, similar to a previous report (Lucu and Siebers, 1986). Inclusion of 0.1 mmol l^{-1} glutathione in the internal perfusate effectively blocked most or all of the sodium efflux and permitted measurements of unidirectional fluxes under asymmetric conditions similar to those found *in vivo*.

A crab was killed by bisection and gill 7 (out of a total of 8, counting from the

Table 1. *Compositions of the internal and the external perfusates*

Internal perfusate				External perfusate	
NaCl	273	Glycine	0.43	NaCl	1
$MgSO_4$	13	Histidine	0.04	Choline chloride	66.9
$CaCl_2$	12	Isoleucine	0.01	$CaCl_2$	1.5
$KHCO_3$	5	Leucine	0.04	$MgCl_2$	3.7
NH_4Cl	0.14	Lysine	0.04	$MgSO_4$	4
Hepes	5	Methionine	0.02	$KHCO_3$	1.4
Urea	0.5	Ornithine	0.05	$Sr(NO_3)_2$	0.02
Glucose	0.65	Phenylalanine	0.03	KBr	0.1
Lactate	0.15	Proline	0.16	H_3BO_3	0.1
Glutathione	0.1	Serine	0.08		
Alanine	0.28	Taurine	0.37		
Arginine	0.12	Threonine	0.09		
Aspartate	0.03	Tyrosine	0.01		
Cysteine	0.01	Valine	0.05		
Glutamine	0.05				

All concentrations are mmol l^{-1}.

anterior) was removed at its base under cold sea water (5 ‰). The gill was flushed immediately with a small amount of cold internal perfusate (see below) and placed in a vial containing ice-cold sea water (5 ‰). The gill was then cannulated and mounted in a perfusion chamber, as described by Burnett (1984). The external perfusate was recirculated (25 ml min^{-1}) through the chamber (total volume of external perfusate=50 ml) *via* a peristaltic pump, while the internal perfusate was pumped (peristaltic pump) through the gill (2 ml min^{-1}) and then collected. The pressure pulses generated by the pumps were significantly attenuated by a hydraulic buffer. The external perfusate passed across the surfaces of the gill within the perfusion chamber, and then down a column filled with glass beads. This column was gassed with humidified CO_2-free air to remove CO_2 and ammonia gas from the perfusate. In this way, the external perfusate was recirculated and maintained at or above pH 7.8. Temperature was maintained at 20±0.2 °C during an experiment, or at 25 °C in two instances.

The influx of sodium ions across the gill was measured by following the movement of ^{22}Na (Dupont/NEN) added to the external perfusate, from the external to the internal perfusate. The specific activity of ^{22}Na in the external perfusate was between 10 and 20 μCi $mmol^{-1}$ Na^+. Care was taken to ensure that the external perfusate was air-equilibrated both to remove CO_2 and to reoxygenate the saline prior to recirculation. External perfusate pH, temperature and sodium ion concentration (measured on samples with an atomic absorption spectrophotometer or continuously with a specific ion electrode and a Radiometer ION83 meter) were monitored. The hydrodynamic pressure necessary to drive the internal perfusate through the gill and the pressure of the external perfusate within the gill perfusion chamber were measured using manometers.

To determine the unidirectional influx (medium to hemolymph) of Na^+, the following protocol was generally followed. First, 0.1 ml of external perfusate was sampled. Next, the internal perfusate leaving the gill was collected for exactly 1 min directly into a tared scintillation vial, providing an accurate measure of the minute volume of the internal perfusate. Three samples of the internal perfusate were taken in rapid succession. Finally, 0.1 ml of the external perfusate was again sampled. Ready-Mix (Beckman; 12 ml) or Aquasol (New England Nuclear; 15 ml) was added to each vial, and the radioactivity measured, using a Beckman LS 3801 liquid scintillation counter, and compensated for quench.

Unidirectional fluxes, expressed as μmol g wet gill $mass^{-1}$ min^{-1}, were calculated using the formula:

$$\frac{\text{disints min}^{-1}}{\text{ml IP}} \times \frac{\text{ml IP}}{\text{min}} \times \frac{\mu\text{mol Na}^+}{\text{ml EP}} \times \frac{1}{\text{disints min}^{-1}\,\text{ml}^{-1}\,\text{EP}} \times \frac{1}{\text{g gill}} = \mu\text{mol Na}^+\,\text{g}^{-1}\,\text{min}^{-1},$$

where IP is internal perfusate, EP is external perfusate, and the radioactivity of the perfusate is measured in disints min^{-1}. Using this system it was also possible to determine the net flux of Na^+ by measuring changes of sodium ion concentrations

in the external perfusate. However, in the presence of glutathione in the internal perfusate, net fluxes either did not occur or were negligible.

Transport parameters including the maximum rate of transport (J_{max}) and the point of half saturation of the transport mechanism(s) (K_m) were measured on a number of gills. A small amount of concentrated NaCl was added to the external perfusate to increase the sodium ion concentration (range=1–80 mmol l^{-1}). After each addition of NaCl and a period of 10–15 min, the sodium influx was measured.

The effects of two specific transport inhibitors on sodium flux were determined. Ouabain (Sigma) was added directly to the internal or external perfusates in separate experiments. Amiloride (a gift from Merck, Sharp & Dohme) was added only to the external perfusate. Either the drugs were dissolved directly in the perfusates or concentrated solutions were made up in 50 % ethanol and subsequently added to the perfusates.

We were also interested in determining the effects of metabolic poisons on sodium ion influx. After determining initial sodium fluxes in untreated gills, metabolic poisons (monofluoroacetate or iodoacetate in combination with sodium cyanide) were added to the internal perfusate and sodium uptake was measured. The concentrations of all poisons was 10 mmol l^{-1}.

In addition, the content of ATP in several sets of gills was determined. In these experiments gills 5, 6, 7 and 8 were used since they contain significant amounts of ion transporting tissue (Neufeld *et al.* 1980). Gills were removed from a crab and flushed immediately with internal perfusate as above to remove the hemolymph. Gills in a control group, which received no further treatment, were placed in plastic vials containing 5 ‰ sea water. Gills in the experimental group were flushed with internal perfusate to which both sodium cyanide and sodium iodoacetate (10 mmol l^{-1}) had been added and placed individually in vials containing 5 ‰ sea water. Both groups were allowed to stand for 30–45 min after which the gills were blotted and pressed to remove liquid within and around the gill and immediately freeze-clamped. Individual gills were weighed and homogenized in 2 ml of ice-cold 12 % trichloroacetic acid and allowed to stand for at least 5 min. The homogenate was centrifuged to produce a clear supernatant and ATP was assayed according to Sigma Technical Bulletin no. 366-UV. One additional group of gills was removed from the animal, freeze-clamped immediately, and assayed for ATP as described above.

Means are given ±1 S.E.M. (N).

Results

The sodium-transporting systems of isolated blue crab gill remained viable for at least 3–4 h (Fig. 1). Under control conditions, Na^+ influx rates were stable, as were physical parameters, including external perfusate pressure, internal perfusate pressure, external perfusate temperature and pH, and internal perfusate flow rate (Fig. 1).

A linear relationship existed between the inverse of Na^+ concentration and the

rate of sodium influx (Fig. 2). The maximum rate of sodium uptake was $3.24\pm0.53\,\mu mol\,g^{-1}\,min^{-1}$ ($N=9$). This value is based on the uptake rate per gram of gill perfused, accounting for any portions of the gill which were not perfused. The K_m for the transport process was $23.0\pm5.7\,mmol\,l^{-1}$ ($N=9$). This value compares favorably with the K_m for Na^+ ($34\,mmol\,l^{-1}$) in measurements of Na^+/H^+ exchange in membrane vesicles from the gills of *Carcinus maenas* (Shetlar *et al.* 1987).

Ouabain added to the internal perfusate significantly reduced the influx of sodium ions (Table 2). The maximum effect of this inhibition occurred at $10^{-3}\,mol\,l^{-1}$ ouabain. The addition of ouabain to the internal perfusate produced no changes in any of the measured physical parameters (Fig. 3, which we illustrate

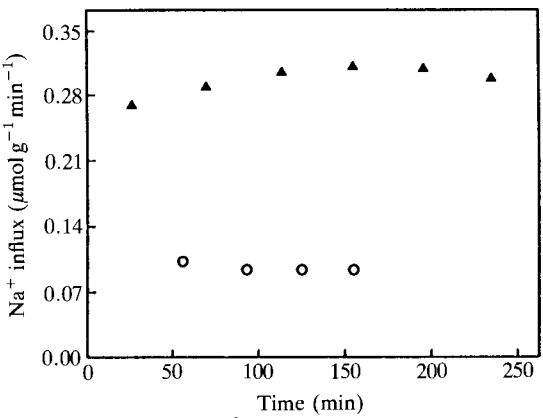

Fig. 1. The relative uptake of sodium ions in two perfused gill preparations as a function of time after the initiation of perfusion. In one preparation (triangles) the sodium ion concentration of the external perfusate was constant at $47\,mmol\,l^{-1}$, while in the other (circles) it was $1\,mmol\,l^{-1}$.

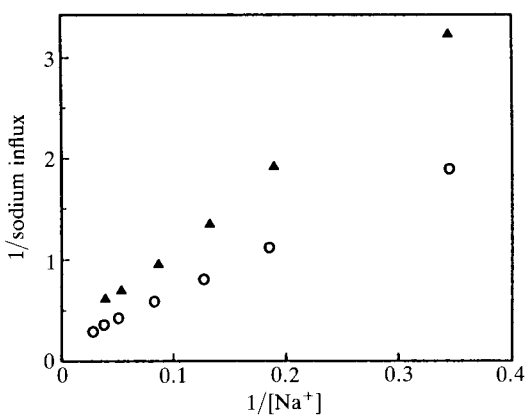

Fig. 2. A linear relationship exists between the inverse of sodium ion concentration ($mmol\,l^{-1}$) in the external perfusate and the inverse of sodium influx ($\mu mol\,g^{-1}\,min^{-1}$), as shown in these two gill preparations represented by different symbols.

Table 2. *Effects of ouabain and amiloride on sodium ion uptake from the external medium*

	Ouabain ($mmol\,l^{-1}$)				Amiloride ($mmol\,l^{-1}$)	
	IP			EP	EP	
	0.1	1	5	1	0.01	0.1
Mean	67.8	53.2	51.3	100.2	65.5	38.9
N	5	6	6	4	2	3
S.E.M.	3.4	3.9	5.3	1.9	0.3	1.9

Ouabain was added to the internal perfusate (IP) and the external perfusate (EP), while amiloride was added to the external perfusate.
Values are percent of control.

with data from a single experiment). In particular, pressure and flow rate of internal perfusate were unaffected by ouabain. Ouabain ($10^{-3}\,mol\,l^{-1}$) added to the external perfusate had no significant effect on sodium ion influx (Table 2).

Addition of amiloride (10^{-5} or $10^{-4}\,mol\,l^{-1}$) to the external perfusate produced a substantial decline in ^{22}Na influx into the internal perfusate, in a concentration-dependent manner (Table 2). No other measured physical parameter was affected by amiloride.

Metabolic poisons (cyanide and iodoacetate) added to the internal perfusate produced a significant and rapid decrease in the sodium influx. In all cases the major decline of sodium influx occurred within the first 20–30 min of exposure to

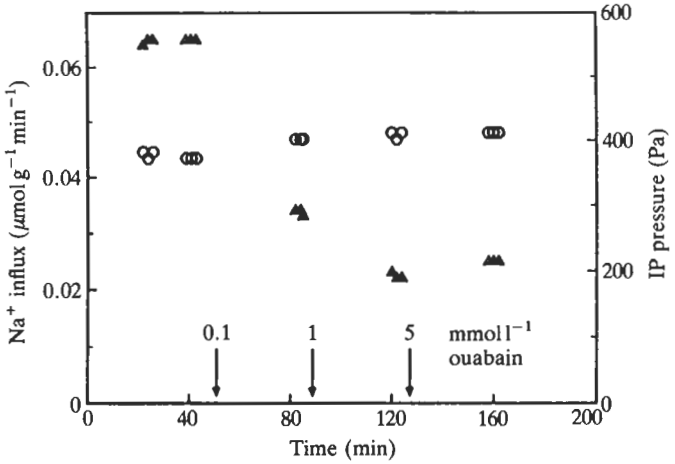

Fig. 3. The effects of different concentrations of ouabain, added to the internal perfusate, on the influx of sodium ions across the gill (triangles) and the pressure driving internal perfusate (IP) through the gill (circles).

the poison (Fig. 4). Subsequent exposure resulted in only small decreases in sodium flux.

We then tested the hypothesis that the movement of CO_2 through the gill from the hemolymph side to the external medium was in part responsible for the sodium flux remaining after the gill had been perfused with the metabolic poisons. In two experiments iodoacetate and cyanide were added to the internal perfusate and the sodium fluxes were measured for 1 h, after which a carbonic anhydrase inhibitor, 10^{-3} mol l^{-1} acetazolamide in one experiment and 10^{-3} mol l^{-1} ethoxzolamide in another, was added to the internal perfusate in the presence of the metabolic poisons. The internal perfusate in the experiment where ethoxzolamide was added to the internal perfusate was also free of any form of carbon dioxide. In both experiments a small decrease in sodium flux was noted (Fig. 5): however, this decrease in the flux was associated with a small increase (98–147 Pa) in the pressure driving the perfusate through the gill. In other words, the small decline in sodium ion flux was attributable to an increase in branchial resistance, possibly shunting internal perfusate around sections of gill epithelium. These results underscore the importance of making measurements simultaneously of the hydrodynamic pressures of the perfusates as well as the ionic fluxes.

Gills perfused with cyanide and iodoacetate show a significant reduction in ATP content (Table 3), but surprisingly large amounts of ATP (13 % of control) are still present after 30–45 min.

Discussion

The gills in our preparation showed either no net sodium efflux or a small efflux (see Materials and methods). Thus, the unidirectional sodium efflux either

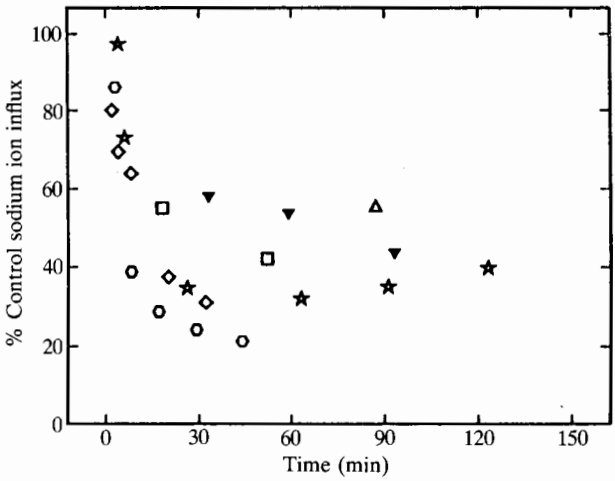

Fig. 4. The effect of the metabolic poisons cyanide together with iodoacetate (both 10 mmol l^{-1}) on the influx of sodium ions across isolated perfused gills. The different symbols indicate data for gills of separate preparations.

equalled or exceeded sodium influx. This result is hardly surprising since the sodium ion concentration difference between the internal and external perfusates was large (193–272 mmol l^{-1}). The concentration difference was larger than that which the crab experiences *in vivo*, a situation brought about by the need to lower the external perfusate sodium ion concentration in order to maximize the specific activity of ^{22}Na.

Ouabain-sensitive Na^+,K^+-ATPase has been implicated in transepithelial Na^+ movement in both teleost and crustacean gills, but the evidence has been somewhat circumstantial (Towle, 1981). Studies on perfused teleost gill have been hampered by the large increase in perfusion pressure induced by ouabain (e.g. Farmer and Evans, 1981). Similarly, demonstration of a ouabain effect on ammonia excretion by teleost gill is made problematic by the simultaneous circulatory effects of the drug (Claiborne *et al.* 1982). One early investigation on the isolated gill of the Chinese crab *Eriocheir sinensis* failed to demonstrate any effect of internal ouabain on Na^+ uptake or ammonia excretion, but physical parameters were not well described (Pequeux and Gilles, 1981). In this study we have shown that perfusion of gills with ouabain has no effect on perfusion pressure or flow rate in isolated gills of *Callinectes sapidus* (Fig. 3), and thus we have been able to separate circulatory effects from ouabain-specific effects on sodium ion transport.

Ouabain in the internal perfusate clearly inhibited a large fraction of Na^+ uptake by the perfused gill (Table 2), and its concentration dependence strongly suggests the presence of a saturable receptor, namely Na^+,K^+-ATPase, on the basolateral membrane of the transporting cell. Our findings are quantitatively similar to those of Siebers *et al.* (1986), who found that ouabain, applied

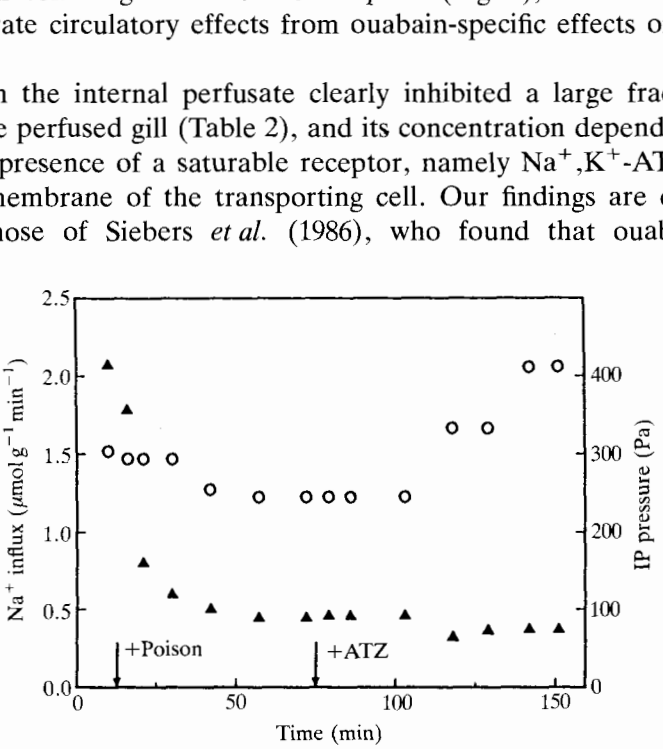

Fig. 5. The effect of the carbonic anhydrase inhibitor acetazolamide (ATZ) (1 mmol l^{-1}) on sodium influx (triangles) and internal perfusate (IP) pressure (circles) in a gill treated with sodium cyanide and sodium iodoacetate (both 10 mmol l^{-1}). The sodium concentration of the external perfusate was 25 mmol l^{-1}.

Table 3. *ATP content of gills*

Gill no.	ATP (μmol g^{-1})	Conditions
5	1.104	Fresh
6	0.868	
6	1.059	
6	1.118	
8	0.371	
8	0.791	
8	0.994	
Mean	0.901	
5	1.930	Perfused IP saline; 30 min
6	1.758	
7	1.679	
5	1.073	Infused IP saline; 45 min
6	1.133	
Mean	1.515	
5	0.172	Infused IP saline+cyanide/iodoacetate; 45 min
6	0.223	
6	0.183	
7	0.217	
7	0.165	
Mean	0.192	

Gills designated 'Fresh' were removed and freeze-clamped immediately after the crab had been killed. Other gills were either perfused continuously with internal perfusate (IP) or infused initially and allowed to incubate in 5‰ sea water.

internally, caused a large reduction in the sodium influx across symmetrically perfused gills of the shore crab *Carcinus maenas*. An inhibitory effect of ouabain was clearly observed in the present study with asymmetrically perfused gills, supporting a central role of Na^+,K^+-ATPase under conditions similar to those *in vivo*. Unlike Pequeux and Gilles (1981), however, we were unable to demonstrate an effect of ouabain on Na^+ uptake when it was added to the external perfusate. Our results with externally applied ouabain are similar to those of Siebers *et al.* (1985) and to later results of Pequeux and Gilles (1988), who found that ouabain, applied externally, had no effect on the potential difference across the gill.

The lack of complete inhibition of Na^+ influx by ouabain added to the internal perfusate has several possible explanations. Crustacean Na^+,K^+-ATPase is notably insensitive to inhibition by ouabain compared to other arthropod and vertebrate Na^+,K^+-ATPases (Towle, 1984). This low sensitivity, coupled with the potential problem of access of ouabain to transport sites in the invaginated basolateral membrane, suggests that some fraction of the Na^+,K^+-ATPase molecules were inhibited. An alternative explanation is that other Na^+-transporting systems reside in the basolateral membrane, independent of the Na^+,K^+-

ATPase. A $Na^+/K^+/Cl^-$ cotransport system inhibited by furosemide has been described in teleost gill (Farmer and Evans, 1981). In preliminary experiments, however, we were unable to show any effect of internal or external furosemide (10^{-3} mol l^{-1}) on Na^+ influx across the perfused gill (data not shown). When internal ouabain was provided after treatment with external amiloride, little additional inhibitory effect was noted.

The inhibitory effect of amiloride on the apical membrane of the gill suggests the presence of an amiloride-sensitive sodium transport pathway. Membrane vesicle preparations from posterior gills of *C. maenas* have been shown to contain an amiloride-sensitive Na^+/H^+ antiporter which appears to exhibit a unique stoichiometry of $2Na^+:1H^+$, unlike the electroneutral vertebrate Na^+/H^+ antiporter (Shetlar *et al.* 1987). External amiloride has been shown previously to reduce Na^+ uptake and to increase the transepithelial potential in perfused gills of *C. maenas* (Lucu and Siebers, 1986; Siebers *et al.* 1987), supporting the existence of an apical Na^+/H^+ antiporter. Our results suggest that this pathway is a major route for sodium entry into the gill (61 %) and is accordingly a rate-limiting step for transepithelial Na^+ transport.

The lack of the complete elimination of the sodium influx in the presence of metabolic poisons is somewhat surprising. We first did a set of experiments to determine the role in the uptake of sodium (and chloride) ions, if any, of CO_2 movement across the apical membrane of the gill (as HCO_3^- and H^+) into the ambient medium. We reasoned that in the absence of active mechanisms a portion of the sodium influx, as well as chloride influx, could be driven solely by Na^+/H^+ and Cl^-/HCO_3^- exchanges supported by carbonic anhydrase. Our results, however, did not corroborate this hypothesis. The only result of inhibition of carbonic anhydrase in both cases and the elimination of CO_2 from the internal perfusate in one case was a small decline in sodium influx which was associated with an increase in the branchial resistance (Fig. 5). This increase in branchial resistance in the presence of carbonic anhydrase inhibitors was not found in other studies of perfused gills where carbonic anhydrase was inhibited (Burnett, 1984; Burnett and McMahon, 1985) and may have resulted from the combination of the inhibitors plus the metabolic poisons. In any case, inhibition of carbonic anhydrase following metabolic arrest did not appear to reduce sodium influx significantly, indicating that Na^+/H^+ exchange is dependent on an intact ATP-producing system driving basolateral Na^+,K^+-ATPase.

The persistence of a significant sodium ion influx in the presence of cyanide and iodoacetate is not surprising, given that ATP is still present within the gill (Table 3). However, the resulting ATP levels must be interpreted with caution since the driving force for the hydrolysis of ATP, the ratio of ATP to ADP, may be low.

Our data obtained with asymmetrically perfused *Callinectes* gills under closely monitored physiological conditions support previous conclusions of the central importance of apical Na^+/H^+ exchange and basolateral ATP-dependent Na^+/K^+ (or Na^+/NH_4^+) exchange in the uptake of Na^+ from the medium. Incomplete

inhibition of Na^+ uptake by amiloride and ouabain suggests, however, that other pathways exist.

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