J. exp. Biol. **162**, 265–279 (1992)
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INTEGUMENTAL TAURINE TRANSPORT IN *MYTILUS* GILL: SHORT-TERM ADAPTATION TO REDUCED SALINITY

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Accepted 30 August 1991

Summary

Taurine, a principal osmolyte in molluscan integument, is actively transported from sea water by *Mytilus* gill by means of a Na^+-dependent process. In this study we examined the response of this transport to reductions in external salinity, i.e. the response to reductions in osmotic concentration as well as Na^+ concentration. Acute exposure of isolated gill tissue to 60 % artificial sea water (ASW) resulted in a greater than 85 % inhibition of taurine uptake, substantially more than the 45 % inhibition predicted on the basis of the acute reduction in external [Na^+]. Within 60 min, however, taurine transport recovered to the level predicted by the Na^+ concentration in dilute sea water. Isolated gills acutely exposed to 60 % ASW made isosmotic to normal (100 %) ASW with mannitol had rates of taurine uptake comparable to gills acclimated for 60 min. Taurine uptake by gill tissue exposed to 60 % ASW for 60 min and then returned to 100 % ASW for 90 min was not significantly different from that of control gills held in 100 % ASW. Glucose uptake by the gill during acute exposure to reduced salinity responded in a pattern similar to that of taurine. Gill tissue increased by 20 % in wet mass within 2 min of exposure to 60 % ASW, but returned to control mass within 30–60 min, presumably reflecting cell volume regulation. Long-term (12 days) exposure to reduced salinities was not accompanied by increases in taurine transport over that of gills observed following the 60 min 'short-term' acclimation. These results suggest that *Mytilus* gill undergoes a rapid (albeit incomplete) recovery from the extreme inhibition of transport associated with abrupt changes in salinity, and the extent of recovery is defined by the availability of Na^+ in the external medium. The extreme sensitivity of taurine uptake observed after acute exposure of gills to reduced salinity is related to the osmotic concentration of the medium, and is possibly linked to a change in cell volume.

Introduction

Intertidal animals may be exposed to abrupt, but transient, changes in external salinity (Stickle and Ahokas, 1974). For osmoconforming animals, including marine mussels of the genus *Mytilus*, this can result in large changes in the osmotic concentration of hemolymph (Shumway, 1977), necessitating compensatory vol-

Key words: salinity, transport, integument, *Mytilus edulis*, *Mytilus californianus*, gill, epithelia.

ume regulatory responses in the cells of these animals. One of the primary strategies of osmoconforming animals for coping with changes in ambient osmotic concentration is the manipulation of intracellular levels of organic osmolytes, including free amino acids, thereby sparing intracellular inorganic ion concentrations (Gilles, 1987; Yancey *et al.* 1982). Changes in cell amino acid content can involve changes in the permeability of the plasma membrane to amino acids, as noted in studies of the response of isolated ventricular tissue to acute exposure to reduced salinity (Pierce and Greenberg, 1972; Strange and Crowe, 1979).

The first tissues exposed to changes in ambient salinity are those of the general integument. Indeed, for a filter feeder, such as *Mytilus*, the epithelial surfaces of the gill are immediately exposed to the full shock of exposure to dilute media, down to ambient salinities of 15‰ or less (<50% full strength sea water; see Shumway, 1977). Although a few studies have examined the effect of acclimation to reduced salinity on rates of integumental amino acid transport in bivalves (Anderson, 1975; Rice and Stephens, 1988), nothing appears to be known about the immediate response of these processes to acute or transient changes in external salinity. Such changes should have a profound effect on transport, owing to the requisite cotransport of amino acids with Na^+ in integumental tissues (Wright, 1987; Wright and Pajor, 1989). However, exposure of the gill to dilute sea water involves not only a reduction in Na^+ concentration, but also a reduction in osmotic pressure of the external solution. To our knowledge, nothing is known about the combined influence of these two variables on integumental transport. Therefore, the current study was undertaken to examine the effect of abrupt changes in external salinity on integumental transport of taurine in *Mytilus* gill.

We found that, immediately following exposure of gills to reduced salinity, uptake of taurine, the major constituent of the intracellular free amino acid pool, was reduced to a greater extent than could be explained by a requirement of the transporter for Na^+. However, within 60 min, transport recovered to the predicted, Na^+-limited level. Transport rates returned to control levels within 90 min of being reintroduced to normal salinity (32‰). Acclimating animals to reduced salinities for several days did not result in rates of transport any greater than those noted in gills exposed for 1 h to the reduced salinity. Gills exposed to hypo-osmotic media initially gained weight, but tissue mass returned to the control value within 60 min, suggesting that recovery of transport may have been associated with recovery of normal cell volume.

Materials and methods

Animals

Specimens of *Mytilus californianus* (Conrad) and *Mytilus edulis* (L.) were purchased from Bodega Marine Labs, Bodega Bay, CA, and were held in a commercial artificial sea water (Tropic Marin, salinity 32 ‰) in recirculating aquaria at 13°C. The animals were not fed and were normally used within 8 weeks of collection.

Transport experiments

Gills of *M. californianus* were dissected and individual demibranchs secured at one end with nylon fishing line as previously described (Wright and Secomb, 1986). Prior to a transport measurement, tissue was maintained for 60 min at room temperature (23 °C) in artificial sea water (ASW; Cavanaugh, 1956), which, at normal strength (i.e. 100 % ASW; salinity of 32 ‰), included 425 mmol l^{-1} Na^+ and had an osmolality of approximately 980 mosmol kg^{-1} (range 950–1010 mosmol kg^{-1}, determined using a Wescor vapor pressure osmometer, model 5500XR). Immediately prior to the experiments, individual demibranchs were pre-equilibrated for 1 min in a slowly stirred solution of ASW of the proper composition containing 10 μmol l^{-1} 5-hydroxytryptamine (5-HT), to activate lateral cilia (Wright, 1979). Tissue was then suspended for 2 min in 200 ml of a slowly stirred solution of ASW of the appropriate composition containing 10 μmol l^{-1} 5-HT, 1 μCi of [^{14}C]taurine and sufficient unlabeled substrate to produce a final taurine concentration of 0.5 μmol l^{-1}.

The structure of *M. edulis* gills precluded their being suspended by a thread, so a different procedure was used for these experiments. Gills were removed and maintained as with *M. californianus,* and then cut into several small pieces, approximately 25 mg (wet mass) each. The pieces of tissue were placed into flasks containing the appropriate composition of ASW and 10 μmol l^{-1} 5-HT and pre-equilibrated for 1 min on a shaker at 90 revs min^{-1}. Tissue sections were then transferred with a spatula to flasks containing 100 ml of ASW, 10 μmol l^{-1} 5-HT, 0.5 μCi of [^{14}C]taurine, and sufficient unlabeled taurine to produce a final taurine concentration of 0.5 μmol l^{-1}.

Following the test incubation, demibranchs were rinsed for 5 min in 200 ml of ice-cold ASW containing 10 μmol l^{-1} 5-HT and then blotted on tissue paper. Small (7 mm diameter) disks were then cut from intact *M. californianus* demibranchs. Tissue disks and sections were weighed to the nearest 0.1 mg, and extracted for several hours in 80 % ethanol before being assayed for radioactivity. Uptake rates are expressed as pmol taurine mg^{-1} wet mass 2 min^{-1}.

Long-term acclimation to reduced external salinity

Four *M. californianus* were acclimated in 10 % steps to 50 % (16 ‰; 490 mosmol kg^{-1}) ASW. Animals spent 2 days in each 10 % step reduction, followed by 4 days at 50 % ASW prior to transport experiments.

Data analysis

Kinetic constants were calculated from uptake data according to the Hill equation (Segel, 1976) using a non-linear regression algorithm (Enzfitter, Biosoft). Statistical analysis of data was made using an analysis of variance (ANOVA) and comparisons made with Sheffe's *F*-test (Statview II by Abacus). The significance of observed changes in wet tissue mass was gauged using a repeated-measures

ANOVA, and comparisons were made with Fisher's PLSD test. Differences were considered to be significant at the $P<0.05$ level.

Chemicals

[^{14}C]taurine (92.1 mCi mmol^{-1}) and D-[^{14}C]glucose (305 mCi mmol^{-1}) were purchased from New England Nuclear. All other chemicals were obtained from Sigma chemical corporation.

Results

Taurine uptake in gills of *M. californianus* was dependent on external [Na^+] when Na^+ was iso-osmotically replaced with Li^+ (Fig. 1), consistent with previous reports (Wright, 1987; Wright and Pajor, 1989). The activation of taurine uptake was a sigmoidal function of external [Na^+] and was adequately described by the Hill equation (Segel, 1975):

$$J = (J_{max}[Na^+]^n)/([K_{Na}]^n + [Na^+]^n),$$

in which J is the rate of uptake of 0.5 μmol l^{-1} taurine at an external concentration of Na^+ of [Na^+], J_{max} is the maximal rate of taurine uptake at a saturating Na^+ concentration, K_{Na} is the concentration of Na^+ producing half-maximal taurine

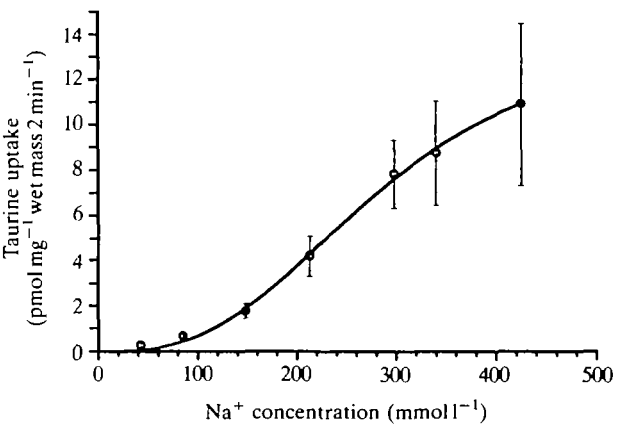

Fig. 1. Effect of Na^+ concentration on taurine uptake in intact *Mytilus californianus* gills. Gills were incubated for 2 min in normal strength artificial sea water (ASW, 980 mosmol kg^{-1}) containing 1 μCi of [^{14}C]taurine, sufficient unlabeled taurine to produce a final taurine concentration of 0.5 μmol l^{-1}, and increasing concentrations of Na^+ (Na^+ isosmotically replaced with Li^+). Uptakes are means±S.E. from three experiments, each using tissue from a different animal. The line was calculated using the Hill equation, and variables were estimated from the means±S.E. from three separate experiments using a non-linear regression algorithm. K_{Na} was 370±120 mmol l^{-1}, J_{max} was 19.3±7.4 pmol mg^{-1} 2 min^{-1}, and the apparent Hill coefficient, n, was 3.2±0.58.

uptake, and n is the apparent Hill coefficient. For the three experiments summarized in Fig. 1, J_{max} was $19.3\pm7.4\,pmol\,mg^{-1}\,2\,min^{-1}$, K_{Na} was $370\pm120\,mmol\,l^{-1}$ and n was 3.2 ± 0.58, in general agreement with previously reported (Wright, 1987) values for these variables.

A decrease in external salinity reduces the external Na^+ concentration and, as suggested by the results in Fig. 1, should reduce the rate of taurine uptake by the gill. For example, the Na^+ concentration of 60% normal strength ASW ($580\,mosmol\,kg^{-1}$; 19.2‰) is $255\,mmol\,l^{-1}$. If rates of transport are defined only by the Na^+ concentration of the external medium, then the rate of uptake of taurine in 60% ASW would be approximately 55% of that noted in full-strength ASW ($425\,mmol\,l^{-1}$ Na^+). We tested this simple hypothesis by measuring taurine transport in gills of *M. californianus* and *M. edulis* acutely exposed to 60% ASW (Fig. 2). Transport was reduced by more than 85%, much greater than the inhibition predicted to occur on the basis of the reduction in external $[Na^+]$. However, when gill tissue was allowed to 'acclimate' to the dilute medium for

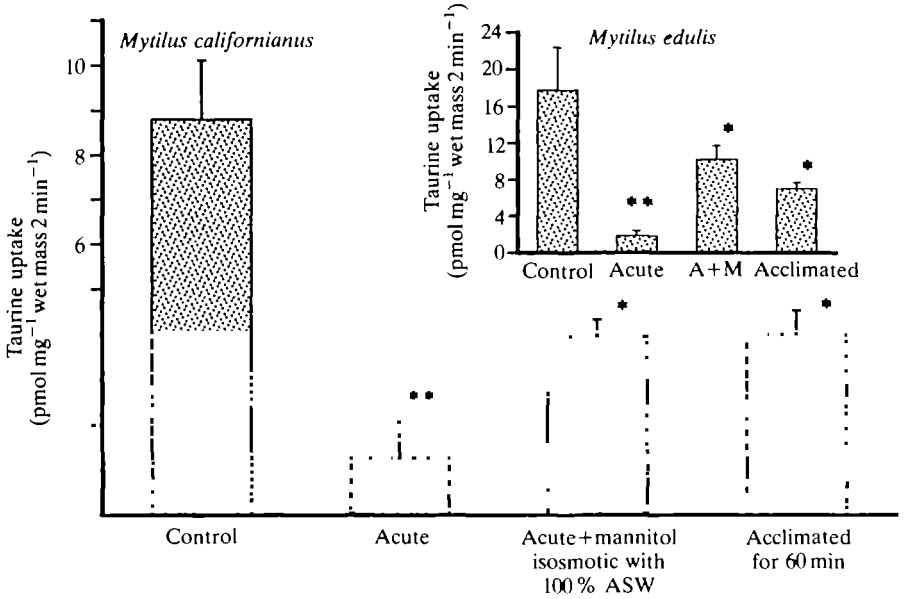

Fig. 2. Effect of decreased external salinity on taurine uptake in intact *Mytilus* gill. Gills from *M. californianus* and *M. edulis* were incubated for 2 min in 100% ASW ($980\,mosmol\,kg^{-1}$), 60% ASW ($590\,mosmol\,kg^{-1}$) or 60% ASW+mannitol ($980\,mosmol\,kg^{-1}$) (A+M), containing $10\,\mu mol\,l^{-1}$ 5-HT, $1\,\mu Ci$ of [^{14}C]taurine and sufficient unlabeled taurine to produce a final taurine concentration of $0.5\,\mu mol\,l^{-1}$. Acclimation experiments exposed gill tissue to 60% ASW for a minimum of 60 min prior to incubation in 60% ASW with [^{14}C]taurine. Uptakes are means+S.E. from three experiments using tissue from different animals. Single asterisks indicate uptake values significantly different from control uptakes; double asterisks indicate significant differences from all other groups. Differences were considered significant at $P<0.05$.

60 min, rates of taurine transport increased to 45 % (*M. californianus*) or 57 % (*M. edulis*) of control, i.e. to levels very similar to that predicted if Na^+ were the only modulating factor involved in defining rates of uptake. In a paired series of measurements, we examined the effect on transport of reducing salinity (i.e. salt), while maintaining the osmotic concentration equal to control levels by adding mannitol (Fig. 2). The rate of taurine transport in gills acutely exposed to 60 % salinity at 100 % osmolality was the same as the rate observed in tissues given 60 min to recover from the combined effects of reduced salinity and osmotic dilution.

We examined more directly the issue of whether the reduced availability of Na^+ was the limiting factor in the ability of gills to recover transport capability following acute exposure to dilute media. Reduction of $[Na^+]$ in the experimental medium was accomplished by either (i) partial replacement of Na^+ in sea water with Li^+; or (ii) simple dilution of ASW with distilled water to achieve the desired Na^+ concentration. Uptake of taurine was measured (i) during an acute exposure to the Li^+–ASW; or (ii) after a 60 min acclimation period in the dilute sea water. As shown in Fig. 3, both methods of reducing $[Na^+]$ significantly reduced uptake of taurine when ambient Na^+ levels were reduced from 425 to 255 or 213 mmol l^{-1} (60 % and 50 % ASW, respectively). However, as implied by the results of the experiment presented in Fig. 2, there was no difference between the two reduced

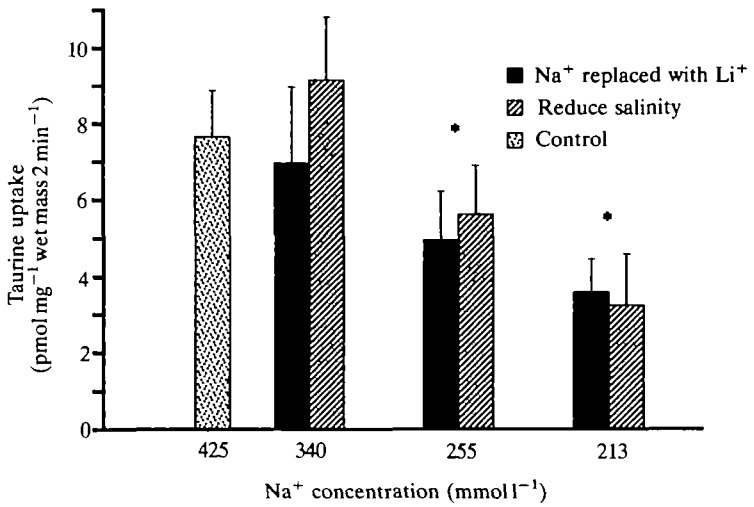

Fig. 3. Effect of exposure to reduced external $[Na^+]$ on taurine uptake in intact *Mytilus californianus* gills by (i) replacement with Li^+ and (ii) short-term acclimation to decreased external salinity. Transport measurements with Li^+ replacement are as in Fig. 1. Acclimation studies exposed gill tissue to decreasing steps (20 min per 10 % step) of salinity prior to incubation in decreasing concentrations of ASW containing 10 μmol l^{-1} 5-HT, 1 μCi of [^{14}C]taurine and sufficient unlabeled taurine to produce a final taurine concentration of 0.5 μmol l^{-1}. Uptakes are means+S.E. from seven experiments using tissue from different animals. Asterisks indicate uptakes significantly different from other groups at the $P<0.05$ level.

$[Na^+]$ conditions; tissues allowed to acclimate for 60 min to dilute sea water had rates of uptake that were the same as those that were exposed only to a reduction in external $[Na^+]$.

Rice and Stephens (1988) reported that the rate of integumental amino acid uptake by the bivalve *Mercenaria mercenaria* following long-term acclimation (5–7 days) to 50 % ASW does not differ from the rate observed in animals held in normal strength sea water. They did not, however, examine the short-term effects of exposure to reduced salinity. Therefore, we tested whether the rates of integumental transport in *Mytilus* gill following long-term acclimation differed significantly from those observed following short-term acclimation. Taurine uptake in gills from *M. californianus* acclimated over 2 weeks to 50 % ASW (including 4 full days in 50 % ASW) was not significantly different from the rate observed in tissues following a 60 min acute exposure to 50 % ASW (3.09 ± 1.12 *vs* 3.25 ± 1.34 pmol mg^{-1} $2\,min^{-1}$, respectively; $N=4$). Furthermore, the Na^+ activation curve for taurine uptake in tissues from animals acclimated over 2 weeks was virtually identical to that observed in tissues from animals held in 100 % ASW (refer to Fig. 1), with a J_{max} of 14.1 ± 3.9 pmol mg^{-1} $2\,min^{-1}$, K_{Na} of 319 ± 56 mmol l^{-1} and a Hill coefficient, n, of 3.1 ± 0.22.

In a previous study we reported that the response of *Mytilus* gill to an acute exposure to 60 % ASW includes a loss of endogenous taurine to the ambient medium (Wright *et al.* 1987). Loss of endogenous substrate can reduce the ambient specific activity of a radioactively labeled substrate and thereby reduce the accumulation of label into the tissue. Indeed, such a mechanism was found to be the basis for apparent inhibition of serine transport by sea urchin larvae produced by preloading larvae with unlabeled serine (Davis and Stephens, 1984). Under the conditions used in the present set of experiments, loss of taurine could have resulted in an increase in the ambient taurine concentration from a starting level of 0.5 μmol l^{-1} to approximately 2 μmol l^{-1} after a 2 min incubation in 200 ml of medium, or a mean concentration of approximately 1 μmol l^{-1}. The half-saturation constant (K_t) for taurine transport by *M. californianus* gill is 4 μmol l^{-1} (Wright, 1987). This combination of factors could have combined to reduce the apparent accumulation of taurine by approximately 10 %, a small effect compared to the 85 % inhibition caused by acute exposure to dilute ASW noted here.

Despite the argument above, it was important to assess whether other integumental transport pathways were similarly inhibited by acute exposure to hypo-osmotic media. Glucose uptake by *Mytilus* integument is by a Na^+-dependent cotransport process (Pajor *et al.* 1989) similar to, but separate from, those for taurine and other amino acids. Furthermore, the free concentration of glucose in the gill is very low, ($\ll 1$ mmol l^{-1}; Pajor *et al.* 1989), so leakage of glucose from the gill into hypo-osmotic media should not influence the specific activity of an exogenous labeled compound during measurement of transport. Based on the Na^+ activation characteristics of glucose transport in the gill (Pajor *et al.* 1989), the Na^+ concentration of 60 % ASW should reduce uptake by only 15 %. Yet, consistent with our observations with taurine, rates of glucose uptake

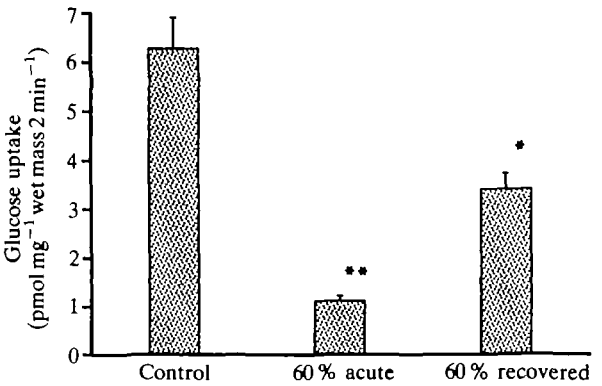

Fig. 4. Effect of decreased external salinity on glucose uptake in intact *Mytilus californianus* gills. Gills were incubated in 100 % ASW (980 mosmol kg^{-1}) or 60 % ASW (590 mosmol kg^{-1}) containing 10 μmol l^{-1} 5-HT, 1 μCi of [^{14}C]taurine and sufficient unlabeled taurine to produce a final taurine concentration of 0.5 μmol l^{-1}. Acclimation experiments exposed gill tissue to 60 % ASW for a minimum of 60 min prior to incubation in 60 % ASW with [^{14}C]taurine. Uptakes are means+S.E. from three experiments using tissue from different animals. Single asterisk represents uptake significantly lower ($P<0.05$) from all groups; double asterisks represent uptake significantly lower than control uptake.

by the gill were decreased by more than 85 % following acute exposure to 60 % ASW compared to control gills held in 100 % ASW (Fig. 4). Following a short-term (60 min) acclimation to 60 % ASW, glucose transport did recover significantly, to a level approximately 54 % of the control value (Fig. 4). We draw two conclusions from this and the observations described above. First, Na^+-dependent integumental transporters respond to an acute exposure to dilute media with a decrease in the rate of substrate influx that is larger than that predicted to arise simply through the associated reduction in external [Na^+]. Second, this large initial inhibition is transient; after a relatively brief (60 min) acclimation period in dilute sea water, transport of both amino acid and glucose recovers significantly and, in the case of taurine, is limited apparently only by the availability of Na^+ to the transport process.

As discussed earlier, intertidal animals are routinely exposed to relatively abrupt fluctuations in salinity, with a decrease in salinity followed in several hours by an increase in salinity. Therefore, we felt that it would be of interest to determine the rate of taurine transport in gills that were allowed to acclimate briefly to 60 % ASW, and then were returned to 100 % ASW. Fig. 5 shows that the rate of gill taurine uptake returned to the control rate within 90 min of the reintroduction of normal strength sea water. This demonstrates that integumental transport can adapt relatively rapidly to a range of salinities common to the intertidal environment, with the extent of adaptation at any given salinity (i.e. >16 ‰) ultimately defined by the external [Na^+].

A sudden reduction in external osmolality increases the water activity at the

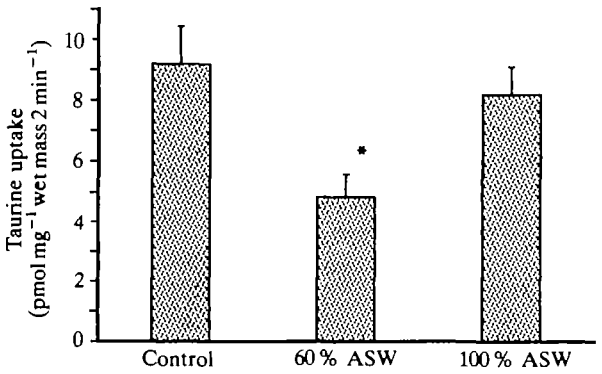

Fig. 5. Recovery of taurine uptake in *Mytilus californianus* gills following exposure to 60 % ASW. Gills were incubated in 60 % ASW for 60 min, and then returned to 100 % ASW for an additional 90 min. Individual demibranchs were incubated for 2 min in 100 % ASW or 60 % ASW containing 10 μmol l^{-1} 5-HT, 1 μCi of [^{14}C]taurine and sufficient unlabeled taurine to produce a final taurine concentration of 0.5 μmol l^{-1}. Uptakes are means+S.E. from three experiments using tissue from different animals. The asterisk represents a rate of uptake significantly different from the control rate at the $P<0.05$ level.

external surface of the gill. As an osmoconformer, *Mytilus* is incapable of sustaining an osmotic gradient between cells of the gill and the surrounding medium, so exposure to reduced external salinity should result in an influx of water into the gill, and would be reflected by an increase in tissue wet mass. In Fig. 6, we show the time course of the change in gill wet mass, expressed as the percentage of the wet mass at time zero, in intact *M. californianus* gills following exposure to 60 % ASW. Within 2 min of the exposure of the gill to the dilute medium there was a rapid increase (20±8 %) in wet tissue mass. In the isolated gills, the ends of branchial hemolymph vessels are open to the external medium, so it is unlikely that this accumulation of water by the gill represented a substantial loading of the hemolymph space of the gill. Instead, we presume that it indicated an increase in volume of the epithelial cells of the gill. Within 60 min gill wet mass returned to the control value (Fig. 6). This response suggests that the gill is capable of rapid cell volume regulation. It is also interesting to note that integumental transport recovers from the transient, steep inhibition that follows acute exposure to dilute sea water within the same time period (Fig. 2).

Discussion

The focus of the current study was to examine how two variables of salinity – [Na^+] and osmotic concentration – affect integumental transport in *Mytilus* gill. Based on our previous observations of the Na^+-dependence of taurine transport in the gill (Wright, 1987), our initial hypothesis was that exposure of the gill to a decrease in external salinity would reduce transport because of the associated

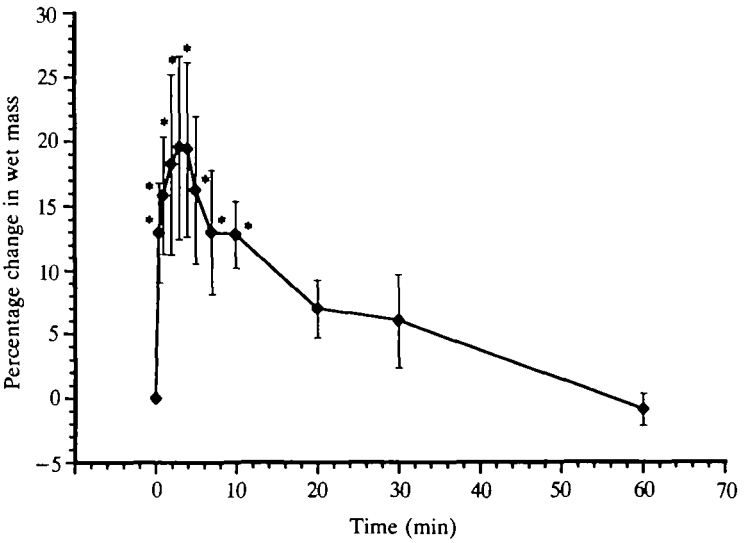

Fig. 6. Change in wet mass of *Mytilus californianus* gills following exposure to 60% artificial sea water. Gills were excised and exposed to 60% (590 mosmol kg^{-1}) ASW. Masses were recorded at selected sampling intervals from 30 s to 60 min following exposure. Points±S.E. are calculated from the mean percentage change in wet mass from time zero, using 12 demibranchs from four different animals. Asterisks represent significant differences from control mass at the $P<0.05$ level.

reduction in $[Na^+]$. The actual pattern of short-term response to an acute exposure to dilute sea water was different. The immediate effect was a dramatic, almost complete, inhibition of uptake (Fig. 2). However, within 60 min, taurine transport recovered to a level consistent with that predicted by the 'Na^+-limit' hypothesis (Figs 2 and 3). Fig. 7 is a compilation of all the data measuring taurine transport as a function of either $[Na^+]$ or salinity (following either short- or long-term acclimation). The rate of transport in acclimated tissues always appeared to be defined by the kinetics of Na^+ as an essential activator of the transport process (see Wright and Pajor, 1989).

Changes in osmotic and ionic concentration have been shown to influence the permeability to amino acid of bivalve membranes. Pierce and Greenberg (1972, 1973) reported that ventricles isolated from *Modiolus demissus* lose amino acid to the extracellular space in response to acute exposure to hypo-osmotic media. This response arises from changes in the external Ca^{2+} concentration associated with dilution of the external medium. For the ventricle, the 'external medium' is normally hemolymph, and changes in hemolymph osmotic and ionic composition lag behind those occurring in ambient sea water (Shumway, 1977). Reduction in external divalent cation concentration has, however, also been shown to influence amino acid flux in bivalve gill, a tissue whose cells are directly exposed to the ambient medium. For example, Swinehart *et al.* (1980) found that reduction in seawater Mg^{2+} concentration inhibits uptake and accelerates efflux of amino acid

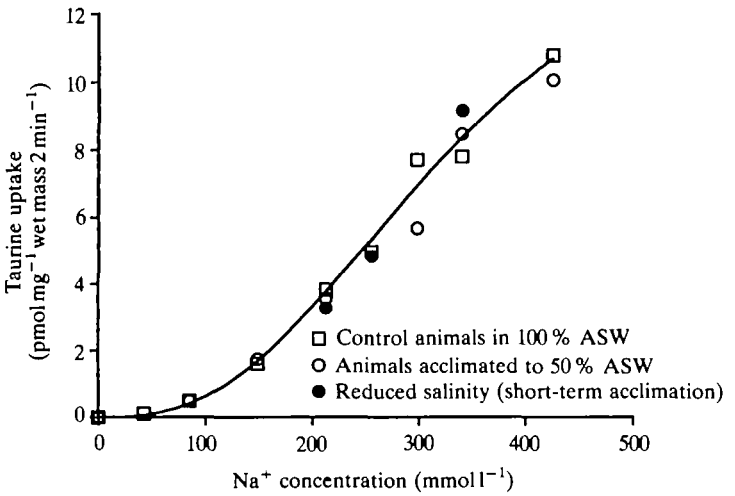

Fig. 7. Summary of data on taurine uptake by intact *Mytilus californianus* gills as a function of external [Na^+]. Uptakes are means from control animals in 100 % ASW, animals short-term acclimated to reduced salinity and animals acclimated to 50 % ASW, taken from Figs 1 and 3 and values presented in the text.

in gills isolated from *M. californianus*, but the reduction in external [Mg^{2+}] required to produce these effects is greater than that arising from exposure to 50 % or 60 % ASW, the conditions employed in the present study. The basis for the steep, transient inhibition of transport that immediately followed acute exposure to reduced salinity is less clear. The decrease in external Ca^{2+} concentration known to influence the permeability of *Modiolus* ventricle does not appear to be involved in the acute inhibition of integumental transport: uptake of taurine into *Mytilus* gill is still reduced by 85 % when 60 % ASW is augmented with Ca^{2+} to the concentration found in 100 % ASW (i.e. 9.7 mmol l^{-1}; A. L. Silva and S. H. Wright, unpublished observations). Changes in intracellular [Ca^{2+}] have, however, been linked to transport-mediated cell volume regulation in several systems (e.g. Cala, 1983; McCarty and O'Neil, 1990). Thus, Ca^{2+} (and Mg^{2+}) may still play an important regulatory role in the cycle of events that follows acute exposure of *Mytilus* gill to dilute sea water.

It is interesting to speculate on the possible adaptive role of the recovery from the initial, large inhibition of amino acid transport in the hypo-osmotically shocked gill. Acute exposure of bivalve tissues to reduced salinity is associated with a loss of amino acid, but the amount of endogenous substrate lost over the course of 30 min is small compared to the size of the total pool (4 %; Wright *et al.* 1987) and, therefore, is unlikely to play a significant role in a short-term volume regulatory decrease (VRD). Indeed, the loss of an organic substrate, such as taurine, as a means of regulating cell volume during cyclic exposures of an intertidal animal to reduced salinities is, arguably, a metabolically expensive strategy. In fact, short-term cell volume regulation is typically associated with

changes in inorganic ion content (Gilles, 1987). Integumental transport has been shown to play a role in recovering endogenous solutes, such as taurine, lost to the sea water through passive processes. Gomme (1981) estimates that some 40% of the D-glucose lost from the integument of the marine polychaete *Nereis diversicolor* could be recycled back into the surface epithelium. We estimated that up to 50% of the taurine lost from *Mytilus* gill is re-accumulated under normal circumstances; (Wright and Secomb, 1986), and Gomme (1982) suggested that up to 90% could be recovered by the gill. Therefore, the rapid recovery of integumental transport following exposure of the gill to dilute sea water could play a role in reducing losses of endogenous organic osmolytes during a period of VRD that (presumably) involves the efflux of other osmotically active solutes.

The capacity of the gill to effect a VRD was apparent from the change in wet tissue mass that followed exposure to a dilute medium (Fig. 6). Immediately following introduction of the gill into 60% ASW, tissue mass began to increase, reaching a maximum of approximately 120% of the control mass within 2 min. We assume that this reflected, primarily, an increase in cell water arising from the difference in osmotic concentration across the apical, brush-border membrane of the gill epithelium. After 2 min, tissue mass began to decline, representing a VRD (Gilles, 1987; Pierce, 1971). Indeed, within 60 min, tissue mass (cell volume) was back to control levels. It is tempting to suggest that the parallel between recovery of integumental transport and apparent recovery of cell volume is indicative of a causal link between the two processes. Changes in cell volume have been shown to cause changes in the flux of both inorganic (see Larson and Spring, 1987) and organic solutes (Nakanishi and Burg, 1989) associated with volume regulatory responses. Although it is premature to conclude that the observed correlation between recovery of transport and recovery of cell volume represents such a link in *Mytilus* gill, it warrants further examination.

The ability of the gill to respond rapidly to large, acute osmotic shocks is clearly advantageous for an animal that can experience such changes several times a day. The estuarine habitat of *M. edulis* can undergo tidally driven changes in salinity that can shift the ambient osmotic concentration from 1000 to approximately 0 mosmol kg^{-1} and back to 1000 mosmol kg^{-1} twice in 24 h (Davenport, 1985). Although *M. californianus* is typically found in a rocky intertidal habitat not normally associated with such broad or routine changes in ambient salinity, it is capable of living in salinities as low as 20‰ for several months (Young, 1941) and thus represents a valid model for studying adaptive responses to salinity stress. There is some controversy in the literature (see Davenport, 1985) concerning the extent to which *Mytilus* is exposed to fluctuation in ambient salinity; by closing its valves, the animal can effectively isolate itself from changes in the external salinity. However, it is clear that *M. edulis* continues to ventilate the mantle cavity actively, thereby irrigating the gill and other integumental surfaces, until the external osmotic concentration drops to approximately 45% ASW (14.4‰; Shumway, 1977), well below the lowest salinity employed in the acute studies used in the present experiments. Integumental transport is extremely sensitive to acute

changes in external salinity. The ability of transport to recover from the inhibition of transport following acute osmotic shocks suggests that the gill has volume regulatory responses that come rapidly into play and permit the gill to regain function quickly after exposure to an altered salinity.

Rates of taurine transport in gills from animals acclimated over several days to reduced salinity were significantly lower than those measured in animals acclimated to 100 % ASW. We found this somewhat surprising in the light of Rice and Stephens' (1988) observation that alanine uptake in intact *Mercenaria mercenaria* was the same in animals acclimated for 5–7 days in media of either 34 or 17 ‰. They did not, however, measure the acute response of transport to decreased salinity, or the effect of external Na^+ concentration on transport. We cannot dismiss the possibility that an increase in integumental transport may follow a longer time course of acclimation than that used in the present study. Stephens (1964) reported that the introduction of *Nereis limnicola* into sea water (19 ‰) from fresh water (0.2 ‰) results in a 10-fold increase in glycine transport over a 9 week period, and less than 60 % of this upregulation occurs within 2 weeks of the exposure to elevated salinity.

In summary, the integumental transport of taurine is sensitive to external salinity. The response to a decrease in salinity involves both a 'salt' (Na^+) and an osmotic component. The initial, steep inhibition of transport upon exposure of gill tissue to a decrease in external salinity was related to the decreased osmotic concentration of the medium. Following a modest period (60 min) of acclimation to the dilute medium, integumental transport rapidly recovered to the rate predicted by the external Na^+ concentration. Following this short-term acclimation to reduced salinity, gill tissue returned to 100 % ASW for 90 min had rates of uptake similar to control gills. Increasing the period of acclimation to reduced salinity to several days did not result in an increase in taurine transport over rates measured following the 60 min acclimation. A rapid and, apparently, complete VRD was also observed following exposure of isolated gills to reduced external salinity. This temporal correlation between the recovery of rates of transport and cell volume suggests a possible causal link between integumental transport and cell volume status.

This work was supported by NSF grant DCB8819367 and by NIH training grant HL-07249.

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