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BASOLATERAL TRANSPORT OF TAURINE IN EPITHELIAL CELLS OF ISOLATED, PERFUSED *MYTILUS CALIFORNIANUS* GILLS

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Summary

We found that the basolateral surface of the gill epithelium of the marine mussel *Mytilus californianus* possesses a carrier-mediated process capable of concentrating taurine within epithelial cells. We used retrograde perfusion of gill sections to demonstrate the kinetics, specificity and ion-dependence of taurine transport. [^{3}H]taurine was concentrated relative to a space marker ([^{14}C]mannitol); this accumulation was blocked by the inclusion of 10 mmol l^{-1} unlabeled taurine in the perfusate. The drop in [^{3}H]taurine uptake at increasing concentrations of unlabeled taurine was fitted to Michaelis–Menten kinetics and indicated a basolateral process with a taurine concentration at which transport is half-maximal (K_t) of 35.3 μmol l^{-1} and a maximal flux (J_{max}) of 0.35 μmol g^{-1} wet mass h^{-1}. Taurine accumulation on the apical surface had a higher affinity (K_t=9.5 μmol l^{-1}) and a higher maximum rate of transport (J_{max}=1.23 μmol g^{-1} h^{-1}). Basolateral transport was inhibited by inclusion in the perfusate of 1 mmol l^{-1} of another β-amino acid (β-alanine), but not by inclusion of α-alanine, glutamic acid or betaine. The dependence of basolateral taurine transport on Na$^+$ (when replaced with *N*-methyl-D-glucamine) was sigmoidal with an apparent Hill coefficient of 2.3, indicating that more than one Na$^+$ is necessary for the transport of each taurine molecule. Complete substitution of Cl$^-$ in bathing media reduced taurine accumulation by 90 % and 70 % on the apical and basolateral surfaces, respectively. Taurine accumulation on both surfaces was reduced by only 20 % when Cl$^-$ was reduced from 496 to 73 mmol l^{-1}, suggesting that taurine uptake is not significantly influenced by the changes in Cl$^-$ concentration accompanying the salinity fluctuations normally encountered by mussels. We estimate that the various Na$^+$ and Cl$^-$ gradients naturally encountered by epithelial cells are capable of providing ample energy to maintain a high intracellular concentration of taurine. We suggest that the ability of epithelial cells to accumulate taurine across the basolateral surface from the hemolymph plays a significant role in the intracellular regulation of this important osmolyte and may effect osmolality-dependent changes in the intracellular concentration of taurine.

Key words: taurine, amino acid transport, gill, *Mytilus californianus*, osmolyte.

Introduction

Organic solutes serve critical intracellular roles in the epithelia of marine invertebrates such as bivalve molluscs and, in many cases, are much more concentrated inside the cell than in either the external environment or the hemolymph. The ability of epithelial cells to regulate internal concentrations of organic solutes and to sustain large gradients of these compounds between the intra- and extracellular compartments must be attributed to either transmembrane movements or metabolic processes. In the gill cells of bivalves, which represent a primary interface between the hemolymph and the external environment, multiple pathways for the transport of organic substances have been demonstrated on the apical (seawater-facing) surface (Manahan *et al.* 1983; Wright and Pajor, 1989). While such pathways are clearly capable of concentrating amino acids within the cell against a large chemical gradient, their role under natural conditions is less certain given the extremely low concentration of many of the substrates in the natural waters to which the apical surface is exposed (Braven *et al.* 1984). Alternatively, the algal diet of bivalves could serve as a major source for many organic substances (Bishop *et al.* 1983), reaching the tissues *via* the circulatory system. Amino acids are, in fact, usually at higher concentrations in the hemolymph than in the external environment (Zurburg and De Zwaan, 1981; Braven *et al.* 1984), raising the possibility that transmembrane transport across the basolateral surface of the gill epithelium is an important effector in the overall regulation of amino acid levels in the cytosol. Despite the likelihood of important transmembrane processes between the hemolymph and the intracellular compartment, there are no studies specifically examining solute transport on the basolateral surface of the gill epithelium of bivalves.

The possibility of basolateral transport is particularly relevant to the regulation of the levels of taurine, an organic solute present in gill cells at much higher concentrations than any other solute except betaine (Bricteux-Gregoire *et al.* 1964). Gill cells of *Mytilus* maintain cytoplasmic taurine concentrations at approximately 200 mmol l^{-1} in full-strength sea water (D. S. Neufeld and S. H. Wright, unpublished data), despite its apparent absence from the environment (Braven *et al.* 1984). The taurine concentration in tissues of many bivalves varies with changes in external osmolality such as those regularly experienced by estuarine organisms (Bricteux-Gregoire *et al.* 1964; Livingstone *et al.* 1979). Changes in the intracellular concentration of taurine serve to counteract changes in cell volume that would occur if intracellular osmolality were not adjusted during a change in extracellular osmolality. While endogenous taurine synthesis may occur (Bishop *et al.* 1983), the energetic cost of relying solely on synthesis would probably be prohibitive considering the large taurine gradient that is maintained and the possibility of repeated losses and gains of taurine by the gill cells caused by the regular salinity changes to which such estuarine animals are exposed (Hawkins and Hilbish, 1992). The large concentration of taurine inside gill cells, its presence in the hemolymph and its evident absence from natural waters, the regulatory changes in tissue taurine associated with salinity changes and the unlikelihood of significant endogenous synthesis of taurine all support the hypothesis that the basolateral membrane of gill cells can effect a carrier-mediated flux of taurine from the hemolymph. We describe here the use of isolated, perfused *Mytilus* gills to characterize the basolateral transport of taurine, a process that may be integral to the regulation of intracellular taurine levels.

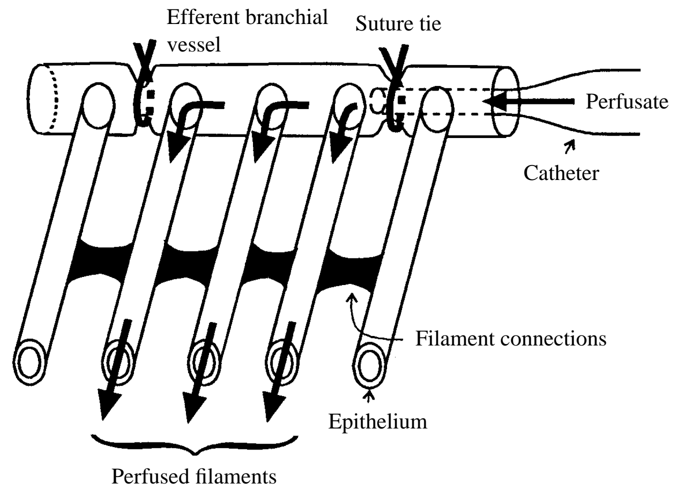

Fig. 1. Schematic diagram of perfused section of gill demonstrating the flow of perfusate from the catheter into the efferent branchial vessel and through the filaments.

Materials and methods

Animals

Specimens of *Mytilus californianus* (Conrad) 5–10 cm in length were purchased from the Bodega Bay Marine Laboratory, Bodega Bay, California, or from Oregon State University, Corvallis, Oregon, and shipped overnight to the University of Arizona. Mussels were maintained in recirculating aquaria of artificial sea water (Instant Ocean) kept at 13 °C. They were not fed and were used within 8 weeks. Gill demibranchs were dissected from mussels and kept in ice-cold artificial sea water until use, up to 6 h following the dissection. Artificial sea water (ASW) used in all experiments on isolated gills was made from the individual salts (in mmol l^{-1}): NaCl, 423; $MgCl_2$, 23; $MgSO_4$, 26; $CaCl_2$, 9; KCl, 9; $NaHCO_3$, 2 (Cavanaugh, 1956). For studies on the Na^+-dependence of uptake, Na^+ was replaced by *N*-methyl-D-glucamine. Cl^--dependence was determined by substituting Cl^- with either gluconate or isethionate. We adjusted the pH of all solutions to between 7.6 and 7.8 with 1 mol l^{-1} NaOH, KOH, HCl or H_2SO_4.

Perfusion technique and vascular space measurement

All experiments determining vascular space or basolateral uptake were performed at room temperature in ASW containing 5-hydroxytryptamine (5-HT). 5-HT stimulates the lateral cilia, causing the ASW to mix well with water at the gill surface and thus washing away labeled substrate in the fluid adhering to the external surface of the gill. We catheterized the efferent branchial vessel (EBV) of demibranch sections with polyethylene tubing drawn to a fine point (approximately 200 μm) and secured in place using suture thread (size 7-0). The EBV was tied shut with suture thread several centimeters distal to the catheter, forcing any perfusate introduced into the gill section to flow through the filaments and to leave at the cut ends of the filaments (Fig. 1).

We estimated vascular space by perfusing approximately 100 mg sections of gill with 0.5 ml (representing more than 10 times the volume of the vascular space in the perfused section) of ASW containing labeled [^{14}C]mannitol or [^{14}C]polyethylene glycol (PEG, relative molecular mass 4000). After perfusion, sections were rinsed for 5 min in ice-cold ASW containing 10 μmol l^{-1} 5-HT. After washing, the perfused section of the tissue was cut into pieces, blotted on filter paper and weighed to the nearest 0.1 mg wet mass. Radioactivity in the tissue was extracted for at least 1 h in 80 % ethanol before liquid scintillation counting, sufficient time for complete extraction of the radiolabeled compounds (D. S. Neufeld and S. H. Wright, unpublished observations). Dual-label counting was performed with correction for variable quench for each isotope and correction for 'spillover' of counts between the ^{3}H and ^{14}C counting windows.

Basolateral uptake experiments

We used the perfusion technique described above to measure taurine uptake selectively across the basolateral surface of epithelial cells in the gill. At the start of each experiment, 0.5 ml of ASW was perfused through the gill section and the tissue was preincubated in ASW for 30 min at room temperature. We then perfused the gill with approximately

0.5 ml of the test solution containing labeled substrate over the course of 2 min, followed by a 0.5 ml flush of ASW. After perfusion, the gill section was rinsed for 5 min in ice-cold ASW containing 5-HT to wash any [^{3}H]taurine from the external surface.

All test solutions contained 0.05 μmol l^{-1} [^{3}H]taurine (55 kBq ml^{-1}) for measurement of uptake and 17 μmol l^{-1} [^{14}C]mannitol (19 kBq ml^{-1}) to indicate the volume of perfusate not flushed from the vasculature. Kinetic variables were determined by varying the concentration of unlabeled taurine in the test solution. Substrate specificity was determined by measuring uptake from a test solution containing 0.5 μmol l^{-1} unlabeled taurine and 1 mmol l^{-1} of various nitrogenous compounds. The dependency of taurine uptake on Na^+ was determined by substitution with *N*-methyl-D-glucamine. We determined the effect on the rate of taurine uptake of various combinations of gluconate and isethionate as Cl^- substitutes. Isethionate salts of Ca^{2+}, Mg^{2+} and K^+ were not available; total replacement of Cl^- was therefore accomplished by using the gluconate salts of these cations in combination with the Na^+ salt of either gluconate or isethionate. The degree of calcium chelation by Cl^- substitutes was estimated by measuring the concentration of free Ca^{2+} in all solutions using a Ca^{2+}-selective electrode (Microelectrodes, Inc.) standardized to ASW samples containing known concentrations of Ca^{2+}.

Perfusate leaves the gill filaments through the open ends distal to their connections with the EBV, entering the bath solution and thus exposing the apical surface of the gill epithelium to any substrates in the perfusate (Fig. 1). In order to minimize apical uptake, sections were held in an external bath of 0.5 l of ASW containing 10 μmol l^{-1} 5-HT, providing a 1000-fold dilution of the solution leaving the vascular space of filaments. Apical uptake was further reduced by the inclusion of unlabeled taurine (1 mmol l^{-1}) in the bath, which acts as a competitive inhibitor of [^{3}H]taurine uptake. The unlabeled taurine in the bath was sufficient to inhibit apical uptake of [^{3}H]taurine by more than 99 %. We monitored the rate of apical uptake of [^{3}H]taurine under these conditions by measuring [^{3}H]taurine accumulation in unperfused sections of gill that were pinned underneath the perfused section and thus exposed to the same conditions as the perfused gill.

Apical uptake

In trials measuring the apical uptake of taurine, sections were presoaked for 5 min in 200 ml of ASW containing 10 μmol l^{-1} 5-HT in order to activate the lateral cilia. We then transferred the sections to beakers with 40 ml of ASW containing 10 μmol l^{-1} 5-HT, 0.35 nmol l^{-1} (0.38 kBq ml^{-1}) [^{3}H]taurine and various concentrations of unlabeled taurine. After 2 min in the test solution, sections were transferred to a 200 ml ASW bath with 5-HT to wash external taurine from the section. As with trials involving basolateral uptake, we blotted pieces of gill on filter paper, weighed them and extracted them in 80 % ethanol before liquid scintillation counting.

Analytical and statistical treatment

Kinetic variables were determined using a non-linear regression algorithm (SigmaPlot) and are reported as means ± S.E.M. of the individual variables calculated for each animal. Variables describing Na^+-dependence were similarly calculated using the Hill analysis (Segel, 1975). Differences were tested for statistical significance with Student's *t*-test.

Results

Perfusion technique and vascular space

Perfusion of the gill with ASW containing dye (Toluidine Blue) produced an even distribution of color in those filaments arising from the EBV between the suture ties. We observed dye leaving from the cut ends of the filaments, indicating that the solution traversed the entire length of individual filaments. No dye was visible in filaments outside the area bounded by the suture ties, suggesting that all the solution passed through the perfused filaments and that it did not flow through fleshy connections between adjacent filaments (Fig. 1). In experiments involving perfusion of the gill with solution containing radiolabeled substrate, the label was distributed evenly among the pieces of the gill that received perfusate, also suggesting that the perfusion technique provided an even distribution of solution throughout the gill section.

At an external taurine concentration of 50 μmol l^{-1}, apical uptake of taurine in gills kept ice-cold for 6 h following dissection (1.29±0.36 μmol g^{-1} h^{-1}) was equivalent to the rate of uptake immediately after dissection (1.38±0.46 μmol g^{-1} h^{-1}; P>0.05, paired *t*-test, N=4), suggesting that results were unaffected by the period between dissection from the animal and use in perfusion experiments. The volume of perfusate remaining in the tissue sections after flushing, indicated by the quantity of [^{14}C]mannitol remaining in the tissues, averaged 18 % of the estimated vascular space, indicating that the majority of perfusate was successfully flushed from the sections. The uptake in the unperfused gill sections pinned beneath the perfused sections averaged 1.6 % of the uptake into the perfused sections, verifying that accumulation occurred *via* the basolateral surface rather than *via* the apical surface as a consequence of perfusate entering the external bath.

Vascular space estimated by perfusion of gill sections with [^{14}C]mannitol (0.22±0.01 ml g^{-1} wet mass, N=6) was equivalent (P>0.05) to vascular space estimated using [^{14}C]PEG (0.24±0.02 ml g^{-1} wet mass, N=5).

Kinetics of basolateral uptake

We performed uptake experiments on gill sections with perfusate containing both [^{3}H]taurine and [^{14}C]mannitol. Since [^{14}C]mannitol does not accumulate in gill tissue over the period of the trial (data not shown), a higher ^{3}H/^{14}C ratio in the gill tissue relative to the ratio in the perfusate should indicate accumulation of taurine in the epithelial cells *via* the basolateral membrane. Indeed, when gill sections were perfused with solution lacking unlabeled taurine and followed

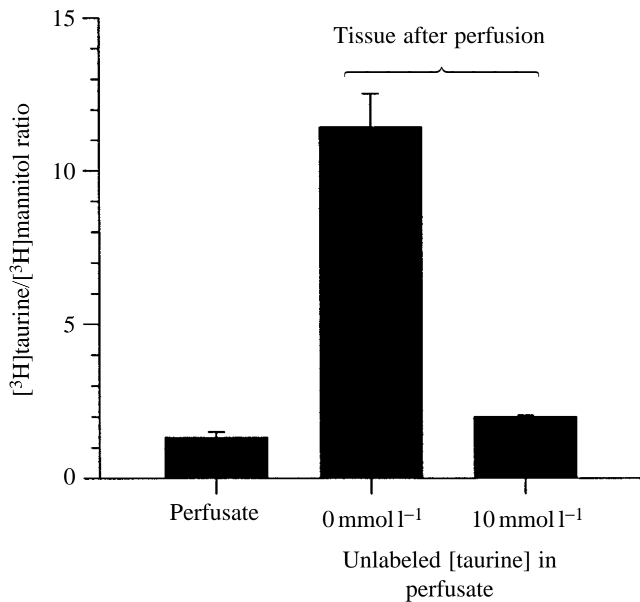

Fig. 2. Ratios of [^{3}H]taurine to [^{14}C]mannitol in perfusate and gill sections, showing the ability of gill sections to accumulate [^{3}H]taurine and the inhibition of this accumulation by the inclusion of 10 mmol l^{-1} unlabeled taurine in the perfusate. Values are means + 1 S.E.M. (N=3).

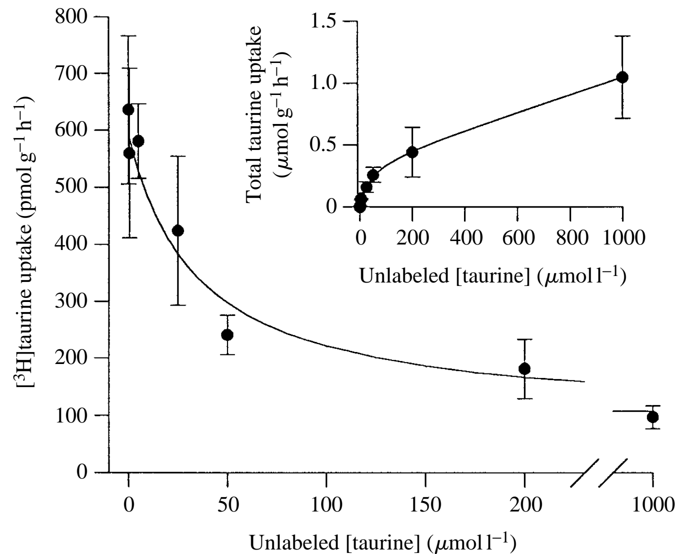

Fig. 3. Effect of increasing concentrations of unlabeled taurine on the basolateral uptake of [^{3}H]taurine in perfused gill sections. The plot of [^{3}H]taurine uptake was not corrected for the quantity of perfusate remaining after flushing, as indicated by the vascular space marker ([^{14}C]mannitol), and represents total radioactivity accumulated in gill sections. Total uptake of taurine (inset) was plotted after correction for the amount of [^{3}H]taurine remaining in the vascular space as estimated by the vascular space marker. Values are means ± 1 S.E.M. (N=4–6).

by an ASW flush, the ^{3}H/^{14}C ratio in the tissue was 8.5 times higher than that in the perfusate, indicating that taurine accumulated in the gill section (Fig. 2). When 10 mmol l^{-1} unlabeled taurine was included in the perfusate, the ^{3}H/^{14}C ratio in tissues was only 1.6 times higher than the ratio in the perfusate, indicating that accumulation was substantially blocked in the presence of sufficient unlabeled taurine (Fig. 2).

The response of [^{3}H]taurine uptake to unlabeled taurine in the perfusate is adequately described by Michaelis–Menten kinetics and was fitted to an equation describing one transport site plus diffusion:

$$J_{Tr} = [(J_{max} \times Tr)/(K_t + S + Tr)] + c\,,$$

where J_{Tr} is the rate of tracer uptake at a total taurine concentration of S, J_{max} is the maximum uptake rate, Tr is the tracer concentration ([^{3}H]taurine), K_t is the taurine concentration at which transport is half-maximal and c is a constant reflecting accumulation of tracer not blocked by unlabeled taurine. This equation allowed determination of kinetic variables without needing to correct the uptake for the mannitol space, thus incorporating the smallest number of assumptions in the treatment of the data. Kinetics were calculated for eight separate experiments in which the highest total taurine concentration was 1 mmol l^{-1} and indicate a transport process with a K_t of 35.3±9.6 μmol l^{-1}, a J_{max} of 0.35±0.09 μmol g^{-1} h^{-1} and a constant of 90.6±17.4 pmol g^{-1} h^{-1} (Fig. 3). Since data were not corrected for mannitol space, the first-order constant represents, in part, that amount of substrate that was not completely flushed from the vasculature following perfusion.

The inset of Fig. 3 shows the total accumulation of taurine, corrected for mannitol space, as a function of external taurine concentration. These data suggest that, in addition to a high-affinity transport site, accumulation of taurine involves a second process that could be either a low-affinity transport or binding site, or a diffusional process. The nature of the taurine accumulation not accounted for by a high-affinity transporter was investigated by comparing taurine uptake at 10 mmol l^{-1} and 50 mmol l^{-1} in a separate group of mussels. [^{3}H]taurine accumulation at 50 mmol l^{-1} (35.0±4.7 pmol g^{-1} h^{-1}) was significantly less (P<0.05, paired t-test, N=4) than [^{3}H]taurine accumulation at 10 mmol l^{-1} (65.1±15.5 pmol g^{-1} h^{-1}), indicating the presence of either a low-affinity transport or a binding site. Kinetic variables calculated for the high-affinity taurine transporter were equivalent when data were analyzed using the equations either for one transport site plus diffusion or for two transport sites plus diffusion. Accurate calculation of variables for a low-affinity site was precluded because of low [^{3}H]taurine uptake at high concentrations of unlabeled taurine; data are therefore presented as calculated using the equation for one transport site plus diffusion.

Kinetics of apical uptake

In order to facilitate a direct comparison of basolateral with apical uptake of taurine, we repeated the experiments reported elsewhere (Wright and Secomb, 1984) measuring the kinetics of taurine uptake across the apical surface of cells.

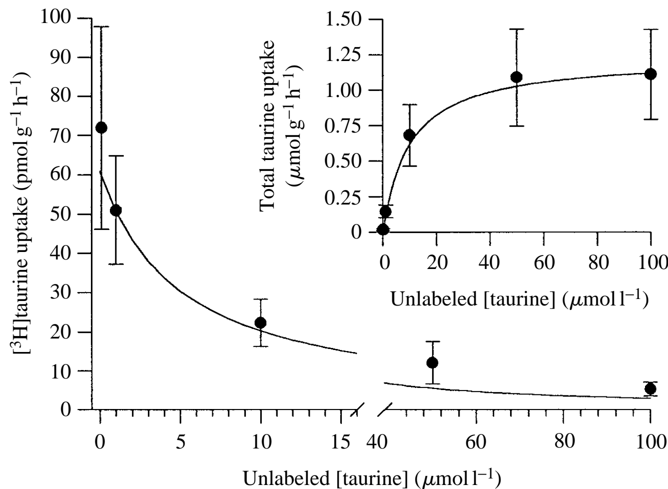

Fig. 4. Kinetics of the apical uptake of taurine expressed as the effect of unlabeled taurine concentration on [^{3}H]taurine uptake and the dependence of total uptake of taurine on the total taurine concentration in the external solution. Values are means ± 1 S.E.M. (N=6).

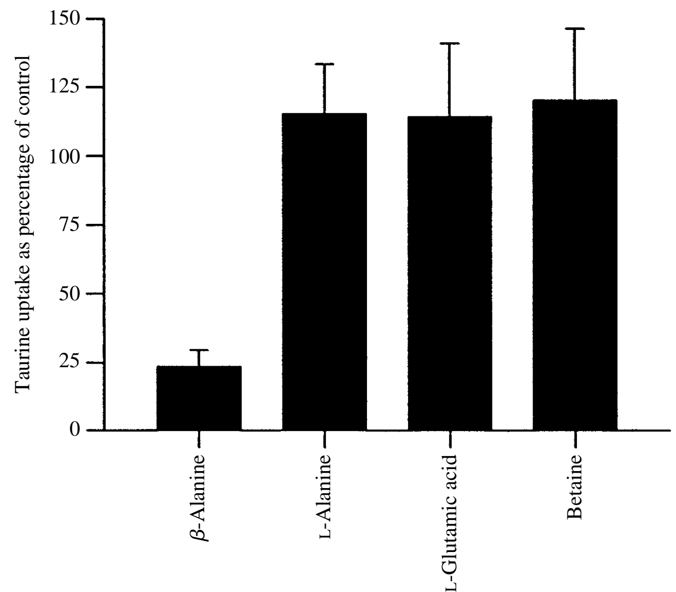

Fig. 5. Specificity of basolateral transport as indicated by the inhibition of basolateral accumulation of taurine in the presence of 1 mmol l^{-1} of various nitrogenous compounds. Uptake was measured at a total taurine concentration of 0.5 μmol l^{-1}. Data are standardized to the rate of uptake in the absence of inhibitor. Values are means + 1 S.E.M. (N=4).

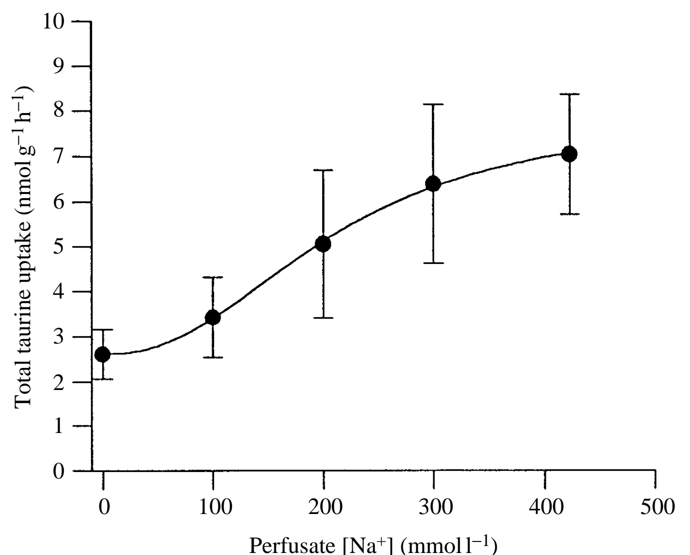

Fig. 6. Sigmoidal dependence of basolateral uptake of taurine on [Na^+] when replaced by N-methyl-D-glucamine, indicating an apparent Hill coefficient of 2.3, a K_{50} of 211 mmol l^{-1} and a $J^{0.5}_{max}$ of 5.3 nmol g^{-1} h^{-1}. Uptake was measured at a total taurine concentration of 0.5 μmol l^{-1}. Values are means ± 1 S.E.M. (N=4).

Accumulation of [^{3}H]taurine *via* the apical surface of the gill responded to progressively higher concentrations of unlabeled taurine in a manner consistent with Michaelis–Menten kinetics and indicated a transport process with a K_t of 9.5±2.5 μmol l^{-1} and a J_{max} of 1.23±0.36 μmol g^{-1} h^{-1} (Fig. 4). Taurine uptake within an animal fitted well to Michaelis–Menten kinetics; the large variability in mean kinetic variables was due to a large interanimal variation in absolute uptake.

Specificity of basolateral uptake

At a total taurine concentration of 0.5 μmol l^{-1}, the uptake of [^{3}H]taurine was blocked by 76.5±6.9 % in the presence of 1 mmol l^{-1} of β-alanine, another β-amino acid (Fig. 5). Taurine uptake was unaffected by 1 mmol l^{-1} concentrations of the nitrogenous compounds L-alanine, L-glutamic acid and betaine (Fig. 5).

Na^+-dependence of basolateral uptake

The basolateral uptake of taurine was clearly sensitive to the concentration of Na^+ in the perfusate; at a total taurine concentration of 0.5 μmol l^{-1}, the uptake ($J^{0.5}$) was a sigmoidal function of external [Na^+] with an apparent Hill coefficient of 2.3 and a K_{50} of 211 mmol l^{-1} (Fig. 6). Complete removal of Na^+ from the perfusate did not eliminate basolateral uptake of taurine. Uptake remained at 41 % of control values when Na^+ in the perfusate was completely replaced by N-methyl-D-glucamine.

Cl^--dependence of apical and basolateral uptake

Taurine uptake on both the apical and basolateral surfaces was only slightly decreased by replacement of NaCl with sodium isethionate (Figs 7, 8). Further reduction of the Cl^- concentration by gluconate replacement caused a significant decrease (P<0.05) in the rate of taurine uptake both apically and basolaterally. As an estimate of the magnitude of any indirect effects on uptake, such as those caused by calcium

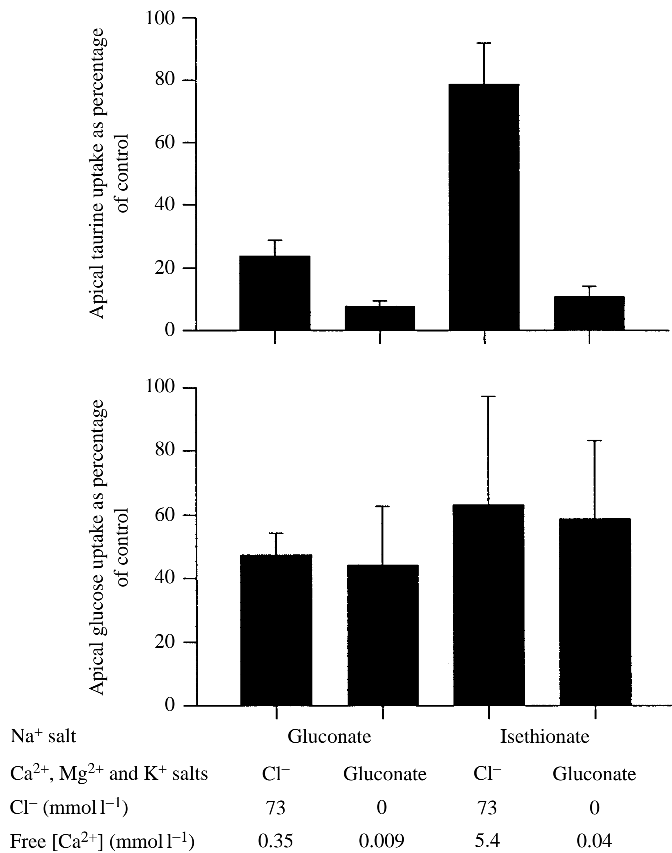

Fig. 7. Effect of gluconate and isethionate as Cl^- substitutes on the apical uptake of taurine and glucose. Trials represent either partial Cl^- replacement using gluconate or isethionate as the Na^+ salt, or complete replacement of Cl^- by substituting the remaining Cl^- salts with gluconate. Data are standardized to uptake in normal artificial sea water (ASW) (Cl^- concentration of 496 mmol l^{-1}) at a total taurine or glucose concentration of 1 μmol l^{-1}. Values are means + 1 S.E.M. (N=3).

chelation by the Cl^- substitutes, we measured the effect of Cl^- substitution on apical uptake of glucose, the transport of which is insensitive to Cl^- availability but is related to membrane potential (Pajor *et al.* 1989). Apical uptake of glucose was significantly decreased and highly variable in all solutions where Cl^- substitutes were present, but did not appear to correlate with the concentration of free Ca^{2+} in the solution (Fig. 7).

There was a 30-fold reduction in the concentration of free Ca^{2+} when NaCl alone was replaced with the Na^+ salt of gluconate, while use of the Na^+ salt of isethionate as a Cl^- replacement reduced free [Ca^{2+}] by only half (Fig. 7). Complete substitution of Cl^- with gluconate reduced free [Ca^{2+}] by 1000-fold. Replacement of all Cl^- by a combination of isethionate and gluconate salts also resulted in a large reduction of free [Ca^{2+}] (200-fold). The lower degree of Ca^{2+} chelation by isethionate salts made isethionate the anion of choice for Cl^- substitution, although in experiments where Cl^- was completely replaced, gluconate salts were also used

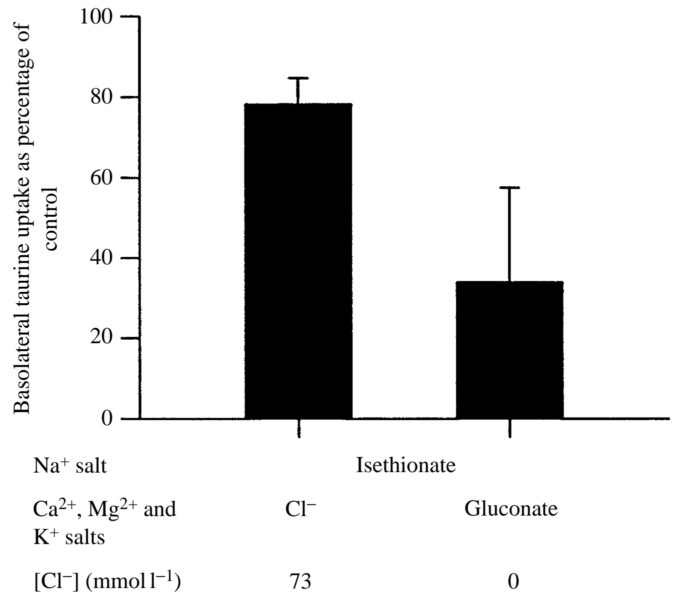

Fig. 8. Dependence of basolateral uptake of taurine on the partial replacement of Cl^- (using isethionate as the Na^+ salt) and on the complete replacement of Cl^- (substituting gluconate for the remaining Cl^- salts). Data are standardized to uptake in normal ASW (Cl^- concentration of 496 mmol l^{-1}) at a total taurine concentration of 0.5 μmol l^{-1}. Values are means + 1 S.E.M. (N=5).

since isethionate salts were not available for Ca^{2+}, Mg^{2+} and K^+.

Discussion

Previous studies identified the presence of transporters for various organic substances, including taurine, on the apical surface of mollusc gills (reviewed by Wright, 1988). These transporters may play an important role either in the acquisition of compounds for nutritive purposes (Wright and Manahan, 1989) or in the re-accumulation of compounds lost by diffusion from the apical surface of the gill epithelium (Wright and Secomb, 1984). While net acquisition of taurine *via* the apical surface is unlikely, because of the effective absence of taurine in natural waters, it is present at concentrations of approximately 0.5 mmol l^{-1} in the hemolymph of bivalves (Zurburg and De Zwaan, 1981). A comprehensive picture of solute balance within the gill cell must, therefore, include the potential role of basolateral exchange with the pool of organic solutes present in the hemolymph.

We present here the first direct evidence for the existence of a carrier-mediated transport process on the basolateral surface of the gill epithelium in *Mytilus*, that for the transport of taurine. The identification of basolateral transport is especially pertinent in view of the physiological role of taurine as an osmolyte in gill cells. A long-term reduction in external osmolarity is correlated with a reduction in the intracellular concentration of taurine (Bricteux-Gregoire *et al.* 1964), serving to minimize cell volume changes while reducing the

deleterious effects on cell metabolism brought about by substantial changes in the concentrations of inorganic osmolytes (Yancey *et al.* 1982). Animals in estuarine habitats can be exposed to long-term fluctuations in salinity that may cause changes in the intracellular concentration of taurine. The basolateral transporter is therefore in a position to maintain the 200 mmol l^{-1} intracellular concentration of taurine and to act in a regulatory capacity during salinity challenges that cause changes in the taurine concentrations of gill cells. A basolateral site for taurine transport is also consistent with suggestions that the gill serves as a central site of amino acid deamination for the organism during a salinity drop (e.g. Henry and Mangum, 1980). Amino acids released by other tissues into the hemolymph during a volume regulatory decrease induced by decreased salinity could reach a deamination site in the gill cell *via* the basolateral transporter.

Taurine transport on the basolateral surface of the *Mytilus* gill has characteristics similar to taurine transport on the apical surface. Taurine transporters on both surfaces are clearly capable of accumulating taurine from very low ambient concentrations. While the K_t of 35.3 μmol l^{-1} for basolateral transport was statistically greater than the K_t on the apical surface reported here (9.5 μmol l^{-1}) or by Wright and Secomb (1984; 13.1 μmol l^{-1}), it may be premature to conclude that this reflects a real difference in the true value of the kinetic variable of the transporter. The overall error reported here is based on the best fits for individual experiments, the additional error from the calculation of kinetic variables for each individual experiment is therefore not reflected in the calculated K_t. In addition, the differing methodologies used to measure taurine accumulation across the apical and basolateral surfaces may have differentially affected the estimations of K_t.

The J_{max} of the apical transporter in both this study (1.23 μmol g^{-1} h^{-1}) and the study of Wright and Secomb (1984; 4.4 μmol g^{-1} h^{-1}) was considerably greater than that of the basolateral transporter (0.35 μmol g^{-1} h^{-1}). The basolateral transporter, however, probably contributes relatively more than the apical transporter to the total flux across the cell membrane, since the concentration of taurine in sea water is quite low. Indeed, we are unaware of any reports of measurable concentrations of taurine in samples of nearshore water, with the exception of one report noting submicromolar concentrations of taurine in tide pools (Almeida *et al.* 1989). Thus, despite the higher transport potential of the apical pathway, influx of exogenous taurine is likely to be insignificant. Instead, apical taurine transport may serve to recover endogenous taurine lost from the gill by passive processes (Wright and Secomb, 1986). Unlike the apical transporter, the basolateral transporter probably operates near its maximum capacity (0.35 μmol g^{-1} h^{-1}) since the concentration of taurine in the hemolymph of bivalves is approximately 0.5 mmol l^{-1} (Zurburg and De Zwaan, 1981), 15 times greater than the K_t (35 μmol l^{-1}) of the basolateral transporter reported here. Assuming a steady-state taurine content in gills of 100 μmol g^{-1} wet mass (D. S. Neufeld and S. H. Wright, unpublished data), basolateral uptake is equivalent to a turnover of approximately 8 % of the intracellular taurine pool every day. A transporter operating at this rate over a period of days could therefore effect a slow change in intracellular taurine concentration, consistent with the slow changes in intracellular taurine concentration observed in response to long-term changes in salinity (Livingstone *et al.* 1979).

The comparatively high affinities of the taurine transporters on both the apical and basolateral surfaces reported here are consistent with the affinities of taurine transporters in most of the other systems where taurine transport has been studied (e.g. Sanchez-Olea *et al.* 1991; Jones *et al.* 1993). Other pathways for taurine movement across the cell membrane are present in other tissues either as a separate, low-affinity transporter (e.g. Jones *et al.* 1993) or as a diffusional component (Sanchez-Olea *et al.* 1991). The data for apical uptake presented here fit well with the presence of a single high-affinity transporter, suggesting that a single transporter is present and that the rates of diffusion are low, in agreement with previous studies (Wright *et al.* 1989). Our data are not conclusive as to the presence of diffusion on the basolateral surface. While there is a continued increase in taurine uptake at high taurine concentrations that is not due to the high-affinity transporter and may include diffusion, this is at least partially due to the presence of a low-affinity transport or binding process for taurine. Since metabolic control of intracellular taurine levels is minimal (Bishop *et al.* 1983), some diffusional efflux of taurine probably occurs on the basolateral surface to offset the high rate of basolateral uptake (approximately 8 % of the intracellular taurine pool per day) and to prevent a continual increase in the intracellular concentration of taurine. Net loss of taurine *via* the apical surface is predictably low (0.12 μmol g^{-1} h^{-1}; Wright and Secomb, 1986), given that net efflux of taurine into sea water represents a permanent loss of taurine. A basolateral location for diffusional efflux, in contrast, would serve to maintain a constant intracellular concentration of taurine while preventing excessive losses to the external environment from where it cannot be recovered.

While the affinity and capacity of taurine transport may differ slightly between the apical and basolateral surfaces, the specificities of both processes appear to be effectively identical to that of other taurine transporters. As with the basolateral uptake of taurine found in this study, accumulation *via* the apical surface is strongly inhibited by β-amino acids, such as β-alanine, while taurine uptake is unaffected by the presence of both zwitterionic (L-alanine) and α-anionic (L-glutamic acid) amino acids (Wright and Secomb, 1984). Betaine, the other nitrogenous compound present in large quantities inside gill cells, is also ineffective in blocking taurine uptake. There are separate pathways for these nitrogenous compounds on the apical surface (Wright and Pajor, 1989; Wright *et al.* 1992), and it is likely that an analogous situation exists on the basolateral surface. The transporters responsible for taurine uptake in other systems studied are also specific to β-amino acids (e.g. King *et al.* 1982; Chesney *et al.* 1985).

The energetic dependence on Na^+ for the uphill transport of

organic substances is a hallmark not only for taurine uptake in other systems (e.g. King *et al.* 1982; Chesney *et al.* 1985) but also for the transport of other amino acids (Stephens, 1988). From the apparent Hill coefficient, it can be calculated that 2 or 3 Na^+ are needed for the transport of a taurine molecule. The apparent Hill coefficient for taurine transport on the apical surface of *Mytilus* gills is 3.2 (Silva and Wright, 1992); the Na^+-dependence of transporters on both the apical and basolateral surfaces in *Mytilus* gills is therefore in general agreement with findings for other taurine transporters, where the apparent Hill coefficient is typically 2 or 3 (e.g. Jones *et al.* 1993; Wolff and Kinne, 1988). The coupling ratio of 2.3 in *Mytilus* indicates only that more than one Na^+ is needed for activation of transport, and is no assurance that the Na^+ is translocated across the membrane.

Taurine uptake on both the apical and basolateral surfaces of the gill epithelium was sensitive to Cl^- concentration; however, the degree of Cl^--sensitivity was less than that of other taurine transport systems (e.g. Wolff and Kinne, 1988; Chesney *et al.* 1985). Taurine uptake was only slightly reduced by lowering the Cl^- concentration from 496 to 73 mmol l^{-1} using isethionate as a Cl^- substitute. Since the reduction in Cl^- concentration normally encountered by mussels in estuarine habitats is less than this, Cl^--dependent changes in taurine uptake probably do not play a significant role in taurine balance in the natural environment. The reduction in taurine uptake in the absence of Cl^- may indicate a direct dependence of the transporter on Cl^- or, alternatively, could be an indirect consequence of changes in membrane potential induced by the substitution of Cl^-, perhaps by calcium chelation. However, the lack of any systematic effect of Cl^- replacement on glucose transport, which is independent of the Cl^- gradient but related to the transmembrane electrical potential (Pajor *et al.* 1989), suggests a direct dependence of taurine uptake on Cl^-, as indicated for other systems where there is a 1:1 dependence of taurine transport on Cl^- (Chesney *et al.* 1985; Wolff and Kinne, 1988).

Taurine accumulation *via* a system of secondary active transport is consistent with the mechanism of transport for other amino acids (Stephens, 1988). On the basis of the thermodynamic calculation of a transport process that is coupled to ion gradients (Aronson, 1981), it is possible to calculate the maximum gradient that can be sustained by secondary active transport. If intracellular taurine concentration is approximately 200 mmol l^{-1} (D. S. Neufeld and S. H. Wright, unpublished data) and hemolymph taurine concentration is approximately 0.5 mmol l^{-1} in 100 % sea water (Zurburg and De Zwaan, 1981), a 400:1 gradient is maintained across the basolateral membrane. Gradients in excess of 10^7:1 can be sustained across the apical membrane (Wright *et al.* 1989) by coupling 3 Na^+ to the transport of each taurine molecule. Given the other similarities between the transport of taurine on the apical and basolateral membranes, we consider it likely that 3 Na^+ are also coupled to each taurine molecule on the basolateral surface. Sufficient energy is, in fact, provided by Na^+:taurine coupling ratios of either 2 or 3 to sustain a taurine gradient that is orders of magnitude greater than that found across the basolateral surface, regardless of whether taurine transport is Cl^--dependent (Table 1). During a reduction in salinity of the type normally encountered by estuarine animals, Na^+ and Cl^- concentrations also change in the hemolymph (Stickle and Denoux, 1976), although at a slower rate than that of the ambient sea water (Livingstone *et al.* 1979). The driving force for taurine uptake would therefore be smaller as ion availability is reduced during a salinity decrease, but we calculate that sufficient driving force would still be present to maintain the observed taurine concentrations (Table 1). The ambiguity as to whether taurine transport in the mussel gill is coupled to Cl^- has little bearing on the energetics of taurine uptake, since the Cl^- gradient is close to its equilibrium potential. It is evident that sufficient driving force is provided solely by the Na^+ gradients existing across the range of salinities normally encountered by mussels.

While it is expected that gill cells have access to the relatively high concentrations of various organic solutes present in the hemolymph, this is the first characterization of such a transport process on the basolateral surface. The high affinity and ion-dependence of the taurine transporter, in addition to its access to a relatively large source of taurine, are

Table 1. *The taurine gradient that can be sustained across the basolateral surface at various Na^+ and Cl^- concentrations and coupling ratios*

	100 % seawater		60 % seawater	
	Intracellular	Hemolymph	Intracellular	Hemolymph
$[Na^+]$ (mmol l^{-1})	17	425	17	255
$[Cl^-]$ (mmol l^{-1})	50	496	50	298

	Cl^- coupling ratio	Taurine gradient sustained: Na^+ coupling ratio 2	Na^+ coupling ratio 3	Na^+ coupling ratio 2	Na^+ coupling ratio 3
Taurine gradient sustained	0	8.1×10^4	2.3×10^7	2.9×10^4	5.0×10^6
	1	7.1×10^4	2.0×10^7	1.5×10^4	2.6×10^6

The taurine gradient sustained was calculated based on the equation for secondary active transport where Na^+ and Cl^- are both cotransported (Aronson, 1981): $[\text{Taurine}]_i/[\text{Taurine}]_o = ([Na^+]_o/[Na^+]_i)^{(n\text{Na}/n\text{Taurine})} \times ([Cl^-]_o/[Cl^-]_i)^{(n\text{Cl}/n\text{Taurine})} \times \exp[F/\boldsymbol{RT}(\psi_o-\psi_i)] \times [(nNa^+/n\text{Taurine})Z_{Na} + (nCl^-/n\text{Taurine})Z_{Cl}]$. $[\text{Taurine}]_i/[\text{Taurine}]_o$ is the ratio of taurine maintained inside the cell relative to the hemolymph. Na^+ and Cl^- concentrations are similarly represented and are based on concentrations reported by Potts (1958) and Gerencser (1983). The coupling ratios of taurine to Na^+ and Cl^- are represented by (nNa^+/nTaurine) and (nCl^-/nTaurine). Z_{Na} and Z_{Cl} are the charges on Na^+ and Cl^-, $\psi_o-\psi_i$ is the potential difference across the basolateral membrane (60 mV inside negative; Murakami and Takahashi, 1975) and F, $\boldsymbol{R}$ and T have their usual values.

consistent with its role in maintaining high concentrations of taurine inside the cell. Given the primary role of taurine as an osmolyte, the basolateral transporter probably plays a key role in the regulatory response of cell volume to the long-term salinity changes naturally encountered by mussels.

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