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Both Na^+ and Cl^- gradients energize NaCl/L-glutamate cotransport in lobster hepatopancreatic brush border membrane vesicles

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Previous work with L-[^{3}H]glutamate transport by lobster (*Homarus americanus*) hepatopancreatic brush border membrane vesicles (BBMV) indicated that the transport of this amino acid was stimulated by the presence of both Na^+ and Cl^- ions in the external medium, however, the specific catalytic or energetic role of each monovalent ion in amino acid transfer was not established (Ahearn and Clay (1987) J. Exp. Biol. 130, 175–191). The present study employs a variety of experimental treatments with this membrane preparation to clarify the nature of the ion dependency in the cotransport process. A zero-trans time course experiment using inwardly-directed transmembrane Na^+ or Cl^- gradients led to similar transient accumulations of the amino acid above equilibrium values in the presence of equilibrated concentrations of the respective counterions. The uptake overshoots observed in the presence of single ion gradients were significantly increased when gradients of both Na^+ and Cl^- were used simultaneously. When vesicles were pre-equilibrated with L-[^{3}H]glutamate and either of the monovalent ions, an inwardly-directed gradient of each counterion led to the transient accumulation of additional labelled amino acid above its equilibrium concentration, indicating that either ion gradient was capable of energizing the net flow of L-glutamate. A cotransport stoichiometry of 1 Na^+/1 Cl^-/1 L-glutamate was established using the Static Head analysis where a balance of ion and amino acid driving forces were attaincd with a 7:1 Na^+ or Cl^- gradient (o > i) against a 7:1 L-glutamate gradient (i > o).

Introduction

Epithelial transport of the acidic amino acids in mammals and birds occurs by a Na-dependent carrier process which is energized by the transmembrane Na gradient and has a somewhat restricted substrate range generally only including the dicarboxylic acids [2–8]. In some intestinal and renal cell types the Na/L-glutamate cotransport event is also coupled with, and energized by, the simultaneous efflux of potassium [4,6,9–12]. In other vertebrate cells K may only serve as an activator of the transport system and not participate in energizing transmembrane amino acid transfer [13–15]. Hydrogen ion has also been implicated as being a third inorganic driving substrate for this carrier system replacing external Na [4,14]. The role of the membrane potential as an additional factor influencing electrogenic L-glutamate transport in vertebrate intestinal and renal epithelial brush border membranes has also been reported [16,17]. As a result of three cations, Na, K, and H, and the transmembrane electrical potential, being associated with acidic amino acid transport across plasma membranes, a wide variety of both electrogenic [8,10,15–17] and electoneutral [5–7,12,18] models have been proposed for this mechanism. Two recent reviews have been published which summarize the independent roles of cation gradients and the transmembrane electrical potential as driving forces for acidic amino acid transport [19,20].

In addition to the roles described above for cations in acidic amino acid transport across vertebrate plasma membranes, the anion, Cl^-, has also been shown in rat and fish epithelia to display an activating effect on the operation of this transporter [13,15]. In no instance has

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a transmembrane Cl gradient been found to directly, and independently, energize the accumulation of an acidic amino acid against a concentration gradient.

Recently, in a communication describing the characteristics of Na/L-glutamate cotransport in epithelial brush border membrane vesicles of the crustacean hepatopancreas (absorptive diverticulum of the gastrointestinal tract), a specific stimulating effect of Cl on the symport event was disclosed, but the nature of the anion effect was unclear [1]. The present investigation is a continuation of this earlier study examining the effect of Cl on acidic amino acid transport in the hepatopancreas. Results, which have previously appeared in abstract form [21], suggest that both transmembrane Na and Cl gradients can, independently, drive the concentrative uptake of L-glutamate across the epithelial brush border membrane of this structure.

Materials and Methods

Live intemolt Atlantic lobsters (*Homarus americanus*; 0.5 kg each) were purchased from commercial dealers in Hawaii and maintained unfed at 10 °C for up to 1 week in filtered seawater. Hepatopancreatic brush border membrane vesicles (BBMV) were prepared from fresh organs of individual lobsters using a magnesnium precipitation technique developed previously [22]. Purity of BBMV prepared by this method was assessed by comparing the activities of membrane-bound enzymes in the final membrane pellet with those of the original tissue homogenate. These comparisons showed that alkaline phosphatase, Na/K-ATPase, and cytochrome-*c* oxidase were enriched by factors of 15.26 $\pm$ 4.65, 1.04 $\pm$ 0.42, and 0.19 $\pm$ 0.04, respectively, suggesting that brush border membrane preparations made in this manner were highly enriched in apical membranes and contained minimal contamination from basolateral and organelle sources.

Transport studies using BBMV were conducted at 15 °C using the Millipore filtration technique developed by Hopfer et al. [23]. Both long-term and short-term incubations of membrane vesicles were conducted in this study using radioisotopic and liquid scintillation methods previously described [22,24,25]. In long-term incubations a volume of membrane vesicles (e.g. 20 μl) was added to a volume of radiolabelled medium (e.g. 160 μl) containing L-[3,4-^{3}H]glutamate (ICN Radiochemicals). After incubation for 15 s, 1, 2, 5, 10, 20, 120, and 180 min, a known volume of this reaction mixture (20 μl) was withdrawn and plunged into 1.5 ml of ice-cold stop solution (composition generally the same as the respective outside medium except lacking the isotope) to stop the uptake process. The resulting suspensions were rapidly filtered through Millipore filters (0.65 μm) to retain the vesicles and washed with another 5–10 ml of stop solution. Filters were then added to a scintillation cocktail and counted for radioactivity in a Beckman LS-8100 scintillation counter.

In short-term incubations (10 s or less), 5 μl of membrane suspension was mixed for predetermined time intervals with 45 μl of external medium containing the isotope using a rapid-uptake apparatus (Inovativ Labor AG, Adliswil, Switzerland). Following isotope incubation, an ice-cold stop solution was injected into the membrane-isotope mixture. Vesicles in stop solution were then treated as described above for long-term incubation.

The composition of the intravesicular medium was established by resuspending the penultimate membrane pellet in the appropriate internal solution with a Potter-Elvehjem homogenizer and allowing this mixture to stand at room temperture (23 °C) for 90 min prior to the final 30-min high-speed centrifugation. When radiolabelled L-glutamate was equilibrated across the vesicle membranes, the isotope was added to the final resuspended pellet and allowed to stand for an additional 30 min before beginning an experiment. Vesicles therefore had normally been incubated in internal media for at least 150 min, and for at least 30 min in radiolabelled L-glutamate, before a transport experiment was initiated.

L-[^{3}H]Glutamate uptake values in this paper are expressed as nmol/mg protein per filter (using specific activity of isotope in the medium) or as percent change in isotope content of preloaded vesicles over a period of time. The Bio-Rad protein assay was used to estimate vesicle protein content. Each experiment was repeated two or three times using membranes prepared from different animals. Within a given experiment 3–5 replicates were used, and data are presented as means with their associated standard errors.

CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) and other reagent grade chemicals were obtained from Sigma Chem. Co.

Results

Effects of Na and Cl gradients on long-term L-glutamate uptake

The influences of separate inwardly-directed gradients of sodium or chloride on the accumulative time course of 0.05 mM L-[^{3}H]glutamate uptake by lobster BBMV were examined by loading the vesicles at pH 4.0 (20 mM Mes/H_2SO_4) with 50 μM CCCP and either 200 mM NaCl, 200 mM TMA-Cl (tetramethylammonium chloride), 200 mM Na-gluconate, or 400 mM mannitol and incubating them in a medium at the same pH containing 200 mM NaCl and the labelled amino acid. Under these conditions vesicles experienced either transmembrane Na, Cl, NaCl, or no ion gradients. When the effects of single ion gradients were examined, the respective counterion was equili-

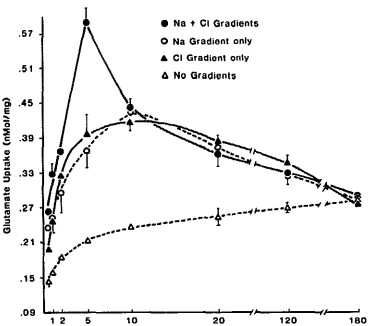

Fig. 1. Effect of inwardly-directed transmembrane gradients of Na, Cl, or NaCl, in the presence of equilibrated concentrations of respective counterions, on the time course of L-[^{3}H]glutamate uptake. Outside medium was composed of 200 mM NaCl, 20 mM Mes/H_2SO_4, and 0.05 mM L-[^{3}H]glutamate at pH 4.0. The four different internal media were prepared at the same pH with 50 μM CCCP (proton ionophore) and comtained one of the following combinations: (a) 400 mM mannitol (Na + Cl gradients); (b) 200 mM TMA-Cl (Na gradient); (c) 200 mM Na-gluconate (Cl gradient); and (d) 200 mM NaCl (no gradients). Results shown are means ± S.E. from a single experiment using 3–5 replicates per point. An identical replicate experiment provided qualitatively and quantitatively similar results.

brated across the vesicle membrane. Fig. 1 shows that all test conditions, except the one lacking ion gradients, led to transient uptake overshoots of labelled L-glutamate, where the intravesicular amino acid concentration transiently exceeded that observed at equilibration (180 min of incubation). Accumulative overshoots were approximately equal in vesicles prepared with either a transmembrane Na or Cl gradient, but both were exceeded by BBMV displaying combined Na and Cl gradients. All conditions led to equilibration by 180 min of incubation suggesting that the different treatments did not produce variations in intravesicular volume. These results suggest that either monovalent ion was capable of driving amino acid transport against a concentration gradient to about an equal extent so long as the respective counter ion was present on both vesicle surfaces. The fact that concentrative L-glutamate uptake was greater with gradients of both Na and Cl suggests that a synergistic effect on amino acid transport occurred between the two separate ionic driving forces.

Equilibrium shift demonstration of Na and Cl gradient driving forces

If single monovalent ion gradients are able to serve as sole driving forces for L-glutamate transport in lobster BBMV, and inwardly-directed gradient of either Na or Cl should be able to displace, or shift, an equilibrated intravesicular quantity of L-[^{3}H]glutamate from one concentration to another. Under these conditions, the concentration of all solutes on both membrane surfaces must be equal prior to the imposition of the single driving force, i.e., addition of excess monovalent ion to the exterior of the vesicle. Two separate equilibrium shift experiments were conducted to ascertain whether transmembrane Na and Cl gradients, individually, could drive concentrative uptake of L-[^{3}H]glutamate away from a previously equilibrated intravesicular condition.

In the first experiment vesicles were equilibrated with two different media containing 0.05 mM L-

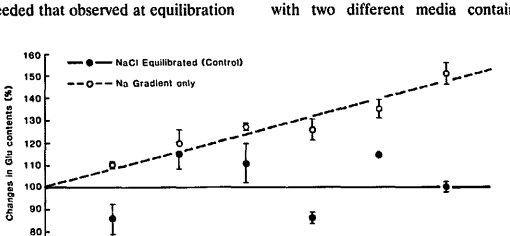

Fig. 2. Equilibrium shift experiment testing the effect of inwardly-directed Na gradient alone as a potential driving force for net influx of L-[^{3}H]glutamate. Vesicles were equilibrated with two different media containing 0.05 mM L-[^{3}H]glutamate, 20 mM Mes/H_2SO_4 at pH 4.0, 50 μM CCCP, and one of the following combinations: (a) 200 mM NaCl, or (b) 200 mM TMA-Cl. These equilibrated vesicles were then exposed to media containing 0.05 mM L-[^{3}H]glutamate and 200 mM NaCl at the same pH. Values of the two treatments differed significantly ($P < 0.05$) after 30 s. Results shown are means ± S.E. from a single experiment using 3–5 replicates per point. Two identical replicate experiments provided qualitatively and quantitatively similar results.

[^{3}H]glutamate, 20 mM Mes/H_2SO_4 at pH 4.0, 50 μM CCCP, and either 200 mM NaCl or 200 mM TMA-Cl. These equilibrated vesicles were then exposed to media containing 0.05 mM L-[^{3}H]glutamate and 200 mM NaCl at the same pH for time periods of 10 to 60 s. Fig. 2 shows that in vesicles equilibrated with TMA-Cl and incubated in NaCl an approximately 50% increase in intravesicular L-[^{3}H]glutamate occurred over a 60 s period in the presence of the inwardly-directed Na gradient. Control vesicles equilibrated and incubated in NaCl (no gradients) did not exhibit a shift in equilibrated L-[^{3}H]glutamate content over the course of the experiment. This experiment suggests that a transmembrane Na gradient can act as a single driving force for the net movement of L-glutamate across the vesicular membrane.

In the second experiment vesicles were equilibrated with 0.05 mM L-[^{3}H]glutamate, 50 μM CCCP, 20 mM Mes/H_2SO_4, and either 200 mM NaCl or 200 mM Na-gluconate at pH 4.0. After equilibration these vesicles were incubated for periods of 10 to 60 s in a medium at the same pH containing 0.05 mm L-[^{3}H]glutamate and 200 mM NaCl. The results in Fig. 3 indicate that approximately a 50% increase in intravesicular radiolabelled L-glutamate occurred over a 60 s period in vesicles equilibrated in Na-gluconate and incubated in NaCl, a condition producing a transmembrane Cl gradient as a potential single ion driving force. As in Fig. 2, intravesicular L-[^{3}H]glutamate content of the the control vesicles did not significantly change over the incubation interval in the absence of a driving force. This experiment suggests that, in the presence of equilibrated Na ion, a transmembrane Cl gradient alone is able to drive the concentrative net uptake of labelled L-glutamate.

Static Head demonstration of Na / Cl / L-glutamate transport stoichiometry

Figs. 1–3 indicated that both transmembrane Na and Cl gradients were able to independently energize the concentrative transport of L-[^{3}H]glutamate in BBMV of lobster hepatopancreas in the presence of the respective counterion. Because Fig. 1 suggested that combined gradients of both Na and Cl were synergistic in their amino acid transport stimulatory capacity, and because it has been previously reported that the simultaneous presence of both ions are required for concentrative L-glutamate uptake [1], the cotransport of both Na and Cl with the amino acid was suspected.

To ascertain the stoichiometric relationship between the simultaneous fluxes of Na, Cl, and L-glutamate in these vesicles, the Static Head method of transport analysis of Turner and Moran [26] was applied to hepatopancreatic NaCl-dependent L-glutamate transport. In this procedure the Na/L-glutamate and Cl/L-glutamate cotransport stoichiometries were independently determined with vesicles equilibrated with the respective counterions.

When Na/L-glutamate cotransport stoichiometry was determined vesicles were equilibrated with 200 mM Cl and had an L-glutamate gradient (0.5 mM inside, 0.07 mM outside) established across the vesicle wall which served as a fixed driving force for Na/L-

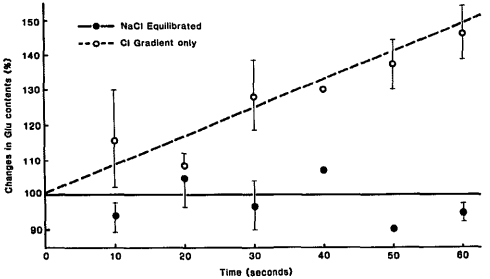

Fig. 3. Equilibrium shift experiment testing the effect of inwardly-directed Cl gradient alone as a potential driving force for net influx of L-[^{3}H]glutamate. Vesicles were equilibrated with two different media containing 0.05 mM L-[^{3}H]glutamate, 20 mM Mes/H_2SO_4 at pH 4.0, 50 μM CCCP, and one of the following combinations: (a) 200 mM NaCl, or (b) 200 mM Na-gluconate. These equilibrated vesicles were then exposed to media containing 0.05 mM L-[^{3}H]glutamate and 200 mM NaCl at the same pH. Values of the two treatments differed significantly ($P < 0.05$) after 20 s. Results shown are means ± S.E. from a single experiment using 3–5 replicates per point. Two identical replicate experiments provided qualitatively and quantitatively similar results.

glutamate cotransport. This fixed outwardly-directed driving force was opposed by a series of inwardly-directed Na gradients (20 mM inside; 92.0, 105.2 140.0, 154.4, and 173.6 mM outside). When the driving forces from L-glutamate and Na gradients were balanced, no net Na-dependent L-[^{3}H]glutamate flux across the membrane would be observed. The thermodynamic equation that describes the condition of no net coupled Na flux or Na-dependent L-[^{3}H]glutamate flux by way of a Na/L-glutamate cotransporter, under short-circuited conditions (50 μM CCCP), is shown below:

$$\ln(Glu_i/Glu_o) = n(\ln([Na]_o/[Na]_i))$$

where n is the number of Na ions simultaneously transported for each L-glutamate (e.g. transport stoichiometry).

Two groups of BBMV were preloaded for 30 min with 0.5 mM L-[^{3}H]glutamate and 50 μM CCCP at pH 4.0 (Mes/H_2SO_4). One group contained 20 mM NaCl and 180 mM choline chloride, while the other had 200 mM choline chloride (Na free). Each of these preloaded groups of BBMV was then incubated for 5 and 10 s in external media at the same pH containing the above Na concentrations made by mixing NaCl and choline chloride solutions to yield the desired Na gradient and 0.07 mM L-[^{3}H]glutamate (same specific activity as internal medium). One external medium consisted of 200 mM choline chloride (Na free). Preloaded vesicle samples were taken and analyzed for L-[^{3}H]glutamate content at the end of the preloading period for comparison with vesicle isotopic content following exposure to each external medium.

Fig. 4 shows the relationship between the intravesicular L-[^{3}H]glutamate content and incubation time with various transmembrane Na gradients where values of 'n' on the figure are the stoichiometries which would be calculated from the equation above if the respective external Na concentration were to result in static head conditions (i.e., no net coupled movement of Na or L-[^{3}H]glutamate). The figure indicates that when n = 1.0 (20 mM Na inside; 140 mM Na outside) the intravesicular isotopic content at 5 and 10 s were approximately equal to vesicles which were preloaded and incubated in Na-free medium, suggesting that under these conditions the only net flow of amino acid occurred by Na-independent processes. At all other values of 'n' a net movement of amino acid into or out of the vesicles occurred which exceeded that under Na-free conditions. These results provide strong support for a Na/L-glutamate cotransport stoichiometry of 1.0.

In a similar manner the cotransport stoichiometry of Cl/L-glutamate was determined with vesicles which were equilibrated with 200 mM Na and possessed a fixed outwardly-directed transmembrane L-[^{3}H]glutamate gradient of the same magnitude as described

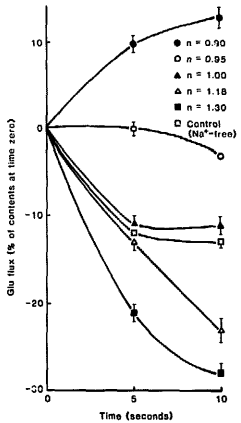

Fig. 4. Static head determination of the Na/L-glutamate cotransport stoichiometry using equilibrated Cl (200 mM), fixed transmembrane L-[^{3}H]glutamate gradient (0.5 mM inside, 0.07 mM outside), and variable transmembrane Na gradients (20 mM inside; 92,0, 105.2, 140.0, 154.4, and 173.6 mM outside). Values of 'n' on the figure are the stoichiometries which would be calculated from the equation in text if the respective external Na concentrations were to result in static head conditions (e.g., no net movement of amino acid or Na by coupled reactions). The 'control' condition represents vesicles lacking Na, equilibrated with Cl (200 mM), and exposed to a fixed transmembrane L-[^{3}H]glutamate gradient (same as above), and therefore represents an index of net Na-independent L-[^{3}H]glutamate flux. Results shown are means ± S.E. from a single experiment using 3–5 replicates per point. An identical replicate experiment provided qualitatively and quantitatively similar results.

above. This outward amino acid gradient was opposed by a variety of inwardly-directed chloride gradients (20 mM inside; 92.0, 105.2, 140.0, 154.4, and 173.6 mM outside). Elimination of Cl-dependent L-glutamate flux would occur when a balance was achieved between the fixed outward amino acid driving force and the inward anion driving force.

Two groups of BBMV were prepared for this test as described above for Na gradient conditions except in this case vesicles with variable transmembrane Cl gradients and Cl-free conditions were employed. Fig. 5 illustrates the relationship between net L-[^{3}H]glutamate flux and incubation time under conditions of variable transmembrane Cl gradients. When n = 1.0 (20 mM Cl inside, 140 mM outside), net amino acid flux could be described as the result of transfer by Cl-independent processes only, while all other n-values led to net inward or outward flows of labelled L-glutamate which were significantly different than those

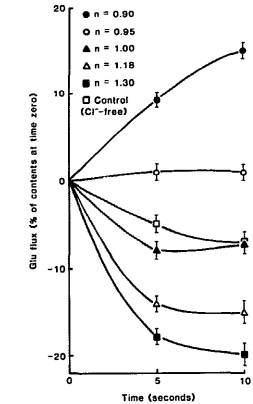

Fig. 5. Static head determination of the Cl/L-glutamate cotransport stoichiometry using equilibrated Na (200 mM), a fixed transmembrane L-[^{3}H]glutamate gradient as in Fig. 4, and variable transmembrane Cl gradients of the same magnitude as the Na gradients in Fig. 4. Values of 'n' are as described in Fig. 4. The 'control' condition in this instance represents vesicles lacking Cl, equilibrated with Na (200 mM Na), and exposed to the standard fixed transmembrane L-[^{3}H]glutamate gradient and is therfore an index of net Cl-independent amino acid flux. Results shown are means ± S.E. from a single experiment using 3–5 replicates per point. Two identical replicate experiments provided qualitatively and quantitatively similar results.

occurring under Cl-free conditions. This result indicates that the Cl/L-glutamate cotransport stoichiometry was approximately 1.0. Taken together the data displayed in Figs. 4 and 5 are evidence for a simultaneous cotransport stoichiometry of the two monovalent ions and amino acid of 1 Na/1 Cl/1 L-glutamate.

Discussion

Results of this study and one published previously [1] suggest that the amino acid, L-glutamate, is transferred across the brush border membrane of the lobster hepatopancreatic epithelium by a cotransport carrier protein which simultaneously binds both Na and Cl with the zwitterionic form of the amino acid in an electroneutral fashion at the physiologically acidic pH (pH 4–5) found in crustacean gastric contents [24,27]. Evidence from previous work indicates that an outwardly-directed K gradient does not enhance NaCl-dependent L-glutamate transport in these vesicles, and in fact this cation acts as a strong inhibitor for 1 Na/1 Cl/1 L-glutamate cotransport [1]. The mechanism of potassium inhibition was not disclosed in this previous study, but one possible way that K might reduce Na/Cl/glutamate cotransport would be for external inhibition of Na binding by potassium ion carried over to the reaction medium during mixing of preloaded vesicles with isotopically-labelled external saline. Alternatively, internal K might act as an allosteric inhibitor of amino acid entry from the intravesicular surface without exerting an appreciable influence on ion binding to the external vesicular surface.

Na/Cl/glutamate cotransport in crustacean hepatopancreas has been shown to be pH dependent, increasing significantly as the bilateral pH was lowered from pH 7.0 to pH 4.0 [1]. Effects of a transmembrane pH gradient on L-glutamate transport by these vesicles was not explored. Other studies with hepatopancreatic BBMV have also demonstrated a strong pH dependency for the transport of glucose [22], alanine [28], lysine [29], and leucine [30]. In the case of L-alanine transport, a pH gradient across the vesicle membrane ($pH_i > pH_o$) was no more effective in accelerating the transport of amino acid than was an equally lowered bilateral pH, suggesting the absence of a proton gradient as a driving force for vesicular accumulation of this amino acid. Because the transport of each of these three amino acids at low pH, in the absence of Na, was accelerated by a transmembrane electrical potential (inside negative), the suggestion was made that the role of protons in accelerating amino acid transfer across hepatopancreatic vesicle membranes was due to the protonation of the respective organic solute and its conversion into a cationic species. At physiologically acidic pH in crustacean gut, protonation of L-glutamate would produce an electrically neutral zwitterion that, by itself, would be unresponsive to membrane potential. At present, it is not known if protons have a further role, such as allosteric regulation, in L-glutamate transport in these invertebrates. Recently, several papers using vertebrate cells have presented alternative transport models of possible activating and energizing roles of Na, K, and H gradients for L-glutamate transport [17,19,20].

Results from the present study and those of our previous paper [1] strongly suggest a cotransport stoichiometry for L-glutamate transfer by hepatopancreatic BBMV of 1 Na/1 Cl/1 L-glutamate. Support for this transport stoichiometry is provided from the results of the static head analyses shown in Figs. 4 and 5 of the present investigation as well as from previous experiments indicating that uptake of the amino acid into vesicles from an external medium containing both Na and Cl was unresponsive to an imposed transmembrane electrical potential. In addition, our previous paper presented results of a Hill analysis of Na/Cl/L-glutamate cotransport and indicated that only single monovalent ions were associated with the transport of this amino acid. This is the first reported instance of

electroneutral L-glutamate transport being brought about by the use of Na and Cl as cotransported substrates. In vertebrates, electroneutral Na/L-glutamate cotransport is attained as a result of the anionic form of the amino acid being transported at approximately pH 7.0 in conjuction with the symport of Na and the antiport of K [4–7,12].

The use of Cl as a cotransported substrate in lobster hepatopancreatic BBMV is unique in two regards. First, as mentioned above, electroneutral Na/L-glutamate cotransport is attained in this invertebrate epithelium as a result of protonation of the amino acid at acidic pH and symport of the zwitterion with both a single cation (Na) and anion (Cl). No apparent antiport of NaCl/L-glutamate cotransport with intravesicular (intracellular) cations occurs. While Cl may have a possible role in activation of vertebrate Na/L-glutamate cotransport [13,15], there is no reported instance of this anion energizing transmembrane L-glutamate transfer in these animals.

The second unique aspect of the use of Cl as a cotransported substrate in lobster BBMV is the fact that this is the first instance of a transmembrane anion gradient energizing the concentrative transport of an amino acid in invertebrate cells in much the same way as a transmembrane Na gradient does for a wide assortment of organic solutes. Although the transport of other amino acids, such as taurine, in vertebrates have a reported strong Cl dependency [31,32], the only other published instance for an unequivocal energizing role of a transmembrane Cl gradient in a sodium-dependent cotransport mechanism is that for β-alanine transport in dog renal brush border membrane [33]. In this study, as reported in the present investigation in Figs. 2 and 3, equilibrium shfit experiments were used to show that concentrative uptake of β-alanine could occur in response to a single transmembrane Cl gradient driving force if vesicles were equilibrated with the cotransported substrate, Na. It is possible that as more investigators examine the potential energizing role of a transmembrane Cl gradient in Na-dependent organic solute transport, this phenomenon will be found more widespread than currently thought.

In order for a transapical Na or Cl gradient to provide energy for concentrative uptake of a cotransported organic solute, the respective ions must occur at a higher electrochemical potential on the outside of the cell than in the cell interior so that the downhill flow of the ion can impart energy to the uphill movement of the cotransported organic solute. The ATP-dependent basolateral Na/K-ATPase provides a necessary primary energy input to the cotransport event on the apical membrane by exporting intracellular Na and generating the transapical Na gradient. In a similar manner intracellular Cl must be maintained at a lower chemical or electrochemical potential than in the external solution if downhill Cl diffusion into the cell is to provide energy for uphill cotransported amino acid transfer. At present there are no reported values of transapical membrane potential or for intracellular Cl activity in lobster hepatopancreatic cells to use in the calculation of the transmembrane chemical or electrochemical gradients for Cl. However, data are available for these measurements in gut cells of another marine invertebrate, the mollusc *Aplysia californica*. Gut epithelial cells from this animal were electrically negative by -68.2 ± 2.2 mV compared to the mucosal solution, while intracellular Cl activity, measured by liquid ion-exchange microelectrodes, was 10.1 ± 0.5 mM compared to seawater Cl activity at 340 mM [34] Using these values, the authors calculated that intracellular Cl activity was maintained in these cells at approximately half of the value expected from a passive Nernst distribution, therefore providing a finite measure of stored energy that could be used by a cotransport event coupled to transmembrane Cl movement into the cell. It was suggested in this and later studies by the same authors that low intracellular Cl activity was maintained in *Aplysia* cells by a primary ATP-dependent Cl pump transferring the anion from cytoplasm to blood across the basolateral membrane against an electrochemical potential for Cl [35]. Such a Cl pump, or some other means of affecting the net uphill efflux of Cl from cytosol to blood, present in lobster hepatopancreatic cells, could provide a transapical chemical gradient for Cl to be used by electroneutral 1 Na/1 Cl/1 L-glutamate cotransport for concentrative amino acid transfer.

Acknowledgements

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