CO- AND COUNTER-TRANSPORT MECHANISMS IN BRUSH BORDER MEMBRANES AND BASAL-LATERAL MEMBRANES OF INTESTINE AND KIDNEY

BY H. MURER, G. AHEARN, J. BIBER, G. CASSANO, P. GMAJ AND B. STIEGER

Department of Physiology, University of Zurich, Rämistrasse 69, CH-8028 Zurich, Switzerland

SUMMARY

One way to obtain a deeper understanding of the complex function of the small intestinal and renal proximal tubular epithelium is to dissect it into single components and then, having defined the components under well-controlled conditions, try to describe the behaviour of the whole system on the basis of the properties of the single components.

Brush border and basal-lateral membranes can be isolated by different methods, including free flow electrophoresis, differential and gradient centrifugation. Transport can be analysed in vesiculated membrane fractions by tracer techniques and spectrophotometric techniques.

Different sodium-solute co-transport mechanisms were identified in the brush border membrane. Until now, studies with vesicles failed to document a sodium-chloride co-transport mechanism satisfactorily. On the other hand, a sodium/proton and a chloride/hydroxyl exchange mechanism were documented. These two exchange mechanisms could represent partial reactions of the postulated electroneutral sodium-chloride co-transport. In addition to different sodium-independent transport systems, the basal-lateral membrane contains an ATP-driven transport system for calcium as well as a sodium/calcium exchange mechanism.

Studies with membrane vesicles isolated from animals which have been exposed to different dietary conditions or in which the parathyroid hormone or 1·25(OH)₂Vit D₃ level has been altered show altered transport of calcium and inorganic phosphate. Thereby, it might be possible to identify the biochemical mechanisms involved in transport regulation.

GENERAL REMARKS

Vectorial transcellular transport consists of at least three different steps: (a) transport across the luminal membrane, (b) transport across the cytosol and (c) transport across the basal-lateral (serosal) membrane. For vectorial transport processes at least quantitative differences in the transport properties have to exist between the luminal and basal-lateral membranes of the epithelial cell. From an energetic point of view, the transport across each of the two different plasma membranes of an epithelial cell can be active or passive. In primary-active transport an energy providing reaction

Key words: Kidney, intestine, membrane transport.
(e.g. ATP-hydrolysis) is directly linked to the transport process (e.g. via transmembrane ATPases). In secondary-active transport a primary-active transport pathway leads to a transmembrane electrochemical potential difference for a cosubstrate (Na or H). A flux-coupling mechanism (co-transport, symport, antiport, exchange) leads to the movement of a second substrate against its chemical or electrochemical potential difference.

In secondary-active transepithelial transport a spatial separation between the primary active sodium pump (Na-K-ATPase) and the Na coupled transport system for different solutes (flux coupling mechanisms) is frequently observed (for reviews see: Frizzell, Field & Schultz, 1979; Murer & Kinne, 1980; Sacktor, 1977; Schultz, 1981). As in most epithelia (with the exception of the choroid plexus), in the proximal tubule and small intestine the Na-K-ATPase is located in the basal-lateral membrane. Na/H-exchange, Na-glucose co-transport and Na-phosphate co-transport, examples of flux-coupling mechanisms, are located in the luminal membrane, whereas Na/Ca-exchange is located in the basal-lateral membrane. The co-transport systems in the luminal membrane lead to a substrate accumulation in the cell. Transcellular transport is completed by a passive efflux through the basal-lateral membrane. In Na-dependent secondary-active secretion, the polar distribution of the transport system would be reversed. If the cellular concentration is below equilibrium distribution in absorptive systems, the active transport mechanism(s) must be located in the basal-lateral membrane. Transepithelial transport of calcium is an example of this situation (see below).

The luminal membrane of the small intestinal and renal proximal tubular epithelial cell (brush border membrane) is a plasma membrane with highly specialized transport functions. At least with respect to transport functions, the basal-lateral membranes of these epithelial cells resemble plasma membranes of non-polarized cells (for review see: Murer & Kinne, 1980). Not only various Na-independent facilitated diffusion systems but also various Na-dependent transport systems are found in the basal-lateral membranes (e.g. Mircheff, Van Os & Wright, 1980). These transport systems are involved in the cellular uptake of different solutes from the interstitial fluid and can also represent the contraluminal exit mechanism in transcellular transport. The intracellular substrate concentrations would decide whether substrates would leave or be taken up by the cell.

Membrane mechanisms involved in transcellular transport can be analysed by various in vivo and in vitro techniques using intact epithelial preparations, cellular suspensions or membrane preparations, respectively. In this article we will restrict the discussion solely to studies with membrane vesicles.

**Na-solute co-transport mechanisms**

Na-solute co-transport mechanisms represent part of the secondary active (re)-absorptive transport mechanisms (see above). In studies with brush border membrane vesicles, co-transport mechanisms have been identified for sugars, different groups of amino acids, different di- and tricarboxylic acids, dipeptides, water-soluble vitamins and different inorganic anions (for review see: Murer & Kinne, 1980; Murer & Burckhardt, 1983). Affinity labelling-, membrane solubilization- and membrane...
Membrane mechanisms in transport of inorganic ions

Experiments have been performed in order to characterize the transport proteins more closely (Crane, Malathi & Preiser, 1976; Fairclough, Malathi, Preiser & Crane, 1979; Gibbs et al. 1982; Im, Ling & Faust, 1982; Kinne & Faust, 1977; Koepsell et al. 1980; Lin, da Cruz, Riedel & Kinne, 1981). A successful isolation of one of the transport molecules has not yet been reported.

A typical example of the study of Na-solute co-transport mechanisms by means of isolated membrane vesicles is presented in Fig. 1 (Stieger, Stange, Biber & Murer, 1983). The transport of L-cysteine by rat renal brush border membrane vesicles is stimulated by an inwardly directed Na-gradient as compared to an inwardly directed K-gradient. For a kinetic evaluation, linear initial velocity conditions are important (compare: Hopfer & Liedtke, 1981; Kaunitz, Gunther & Wright, 1982; Semenza, 1982; Turner & Silvermann, 1978). Solute dependent Na-flux, as well as the kinetic evaluation of Na-activation of solute transport (Hill analysis), provides information for the determination of the coupling coefficient between Na- and solute-fluxes (e.g. Kaunitz et al. 1982).

Electrophysiological studies on intact epithelia and tracer studies with isolated membrane vesicles using ion gradients (K and/or H) and ionophores (valinomycin and FCCP) documented a potential dependence of various Na-solute co-transport systems (for reviews see: Frömter, 1979; Murer & Kinne, 1980). In studies with vesicles, alterations in the transmembrane electrical potential difference can be recorded by using fluorescent cyanine dyes (Beck & Sacktor, 1978; Stieger et al. 1983; Wright, Krasne, Kippen & Wright, 1981). An example of this technique is presented in Fig. 2. Addition of d-glucose to Na-pre-equilibrated rat renal brush border membrane vesicles provokes a transient increase in the fluorescence of 3,3'-diethylthiadicarbocyanine. This refers to a transient increase of an inside positive transmembrane

---

Fig. 1. Time course and sodium-dependence of cysteine-uptake by rat proximal tubular brush border membranes. Membranes were loaded with 300 mmol l⁻¹ mannitol, 20 mmol l⁻¹ Hepes adjusted with Tris to pH 7.4. The incubation media contained in addition (final concentrations): 100 mmol l⁻¹ NaCl (●) or KCl (■), respectively; 0.1 mmol l⁻¹ L-³⁵S-cysteine and 1.0 mmol dithiothreitol. This Figure is taken from Stieger, Stange, Biber & Murer (1983).
Sodium equilibrated:

\[ \text{D-Glucose addition: } 10.8 \text{ mmol l}^{-1} \]

Potassium addition in the presence of valinomycin: \(7.5 \text{ mmol l}^{-1}\)

Potassium equilibrated:

\[ \text{D-Glucose addition: } 10.8 \text{ mmol l}^{-1} \]

Fig. 2. Effect of Na-d-glucose co-transport on the fluorescence of 3'-3-diethylthiadicarbocyanine iodide. Rat renal brush border membranes were prepared in either 74 mmol l\(^{-1}\) Na\(_2\)SO\(_4\), 1 mmol l\(^{-1}\) K\(_2\)SO\(_4\), 20 mmol l\(^{-1}\) Hepes-Tris pH 7.4 (sodium equilibrated) or in 75 mmol l\(^{-1}\) K\(_2\)SO\(_4\), 20 mmol l\(^{-1}\) Hepes adjusted with Tris to pH 7.4 (potassium equilibrated). The solutes to be added during the experiment were suspended in the same buffer. For all experiments, 250 \(\mu\)g of membrane protein were used per cuvette (2 ml). Dye concentration was 3 \(\mu\)mol l\(^{-1}\). This Figure is adapted from Stieger, Stange, Biber & Murer (1983).

electrical potential difference as also indicated by the observed increase in fluorescence after injection of K in the presence of valinomycin.

**Na / H-exchange**

In small intestinal and renal proximal tubular epithelia, uphill secretion of H is partially coupled to the downhill movement of Na (e.g. Chan & Giebisch, 1981; Malnic & Giebisch, 1979; Ullrich, Rumrich & Baumann, 1975). In studies with brush border membrane vesicles, the imposition of a Na-gradient (\(\text{Na}_o > \text{Na}_i\)) led to an acidification of the extravesicular medium. Likewise a \(\Delta p\text{H}\) provided the driving force for the transient uptake of Na above the equilibrium value. Na/H exchange is electroneutral, accepts lithium and ammonium and is inhibited by amiloride (Kinsella & Aronson, 1981a,b; Liedtke & Hopfer, 1977; Murer, Hopfer & Kinne, 1976). These findings are in agreement with observations on the intact tubule (Chan & Giebisch, 1981; Ullrich *et al.* 1975). In studies with isolated proximal tubular brush border membrane vesicle preparations it has been shown that the rate of Na/H-exchange is under the influence of glucocorticoid hormones and allosterically regulated by an increase in the intracellular H-concentration (Aronson, Nee & Suhm, 1982; Freiberg, Kinsella & Sacktor, 1982).
Two different experimental approaches can be used to study Na/H-exchange by the acridine-orange fluorescence quench technique (Cohn, Hruska, Klahr & Hammerman, 1982; Reenstra, Warnock, Yee & Forte, 1981; Warnock, Reenstra & Yee, 1982). (1) The internal acidification occurring during Na-efflux can be monitored (Fig. 3). (2) A ΔpH can be preset and its breakdown can be studied under the influence of different Na concentrations (Fig. 4). In Figs 3 and 4 experiments with brush border membranes isolated from rat jejunum are presented. Na-efflux is accompanied by intravesicular acidification as indicated by the transient fluorescent quenching (Fig. 3). This quenching is partially due to indirect coupling between conductive Na-efflux (leak) and conductive H-entry (leak). The signal observed under conditions of 'short-circuited membrane potential' (K at equal concentrations on both membrane sides in the presence of valinomycin) is related to Na/H exchange only. In studies with brush border membrane vesicles, it is most important to analyse Na/H-exchange under conditions of a short-circuit membrane potential as significant non-specific conductive pathways have been observed (e.g. Reenstra et al. 1981). The use of amiloride as a potential inhibitor of Na/H exchange is complicated in these studies because of two facts: (1) amiloride at concentrations higher than 0.1 mmol l⁻¹ shows strong quenching of the dye fluorescence (Sabolic & Burckhardt, 1983); (2) amiloride used at higher concentrations shunts the ΔpH and thus 'competes' for the

Inside
Na⁺-gluconate (100)  |  Outside
TMA⁺-gluconate (100)
K⁺-gluconate (50)    |  K⁺-gluconate (50)

H-T, pH 7.5
(10)

+ Ethanol

+ Valinomycin

Fig. 3. Measurement of Na/H-exchange in rat small intestinal brush border membrane vesicles by the acridine orange fluorescence quench technique. The methods used for this experiment are similar to that described by Reenstra, Warnock, Yee & Forte (1981). Important experimental conditions are indicated in the Figure (G. Cassano & H. Murer, unpublished observations). Concentrations are given in mmol l⁻¹. H-T = Hepes adjusted with Tris, TMA = tetramethylammonium.
Experimental conditions: 1000 μg protein (20 μl)/cuvette (2 ml) IN MES (50)-Tris pH 6.4 OUT Hepes (50)-Tris pH 8.0 Valinomycin 0.005

H. Murer and others

Directly coupled transport of Na and Cl

For transcellular Cl-flux in the small intestine, the luminal Cl-entry mechanism is Na-dependent. These findings suggested the existence of a Na/Cl-co-transport mechanism (for reviews see: Duffey, Thompson, Frizzell & Schultz, 1979; Frizzell et al. 1979; Schultz, 1979, 1981). An alternative mechanism is the coupled action of a Na/H and a Cl/HCO₃-exchange (Turnberg, Fordtran, Carter & Rector, 1970). In rat proximal tubular epithelial cells, the intracellular chloride activity was also found...
Membrane mechanisms in transport of inorganic ions

be above equilibrium distribution (Cassola, Gebler & Frömter, 1981). The localization and nature of the active transport step, however, is at present not known for mammalian proximal tubule.

Liedtke & Hopfer (1982a) denied the existence of a Na/Cl-co-transporter in kinetic experiments with brush border membrane vesicles isolated from rat small intestine. Powell & Fan (1982) reported coupled transport of Na and Cl in intestinal brush border membranes isolated in the absence of calcium, although indirect coupling of Na/H- and Cl/OH-exchange could not be excluded. High intravesicular calcium concentrations inhibited 'coupled' entry of Na and Cl (Powell & Fan, 1982). As high calcium concentrations are normally used in the preparation of brush border membrane vesicles, this finding raises the possibility that a coupled mechanism could be inhibited during vesicle preparation. The existence of a Na-K-2Cl-co-transport [a co-transport mechanism observed in many different cells including epithelia (e.g. Greger & Schlatter, 1982; Murer & Greger, 1982; Oberleitner, Giebisch, Lang & Wenhui, 1982)] has never been rigorously tested in studies with vesicles. However, studies on intact epithelia did not provide evidence for the existence of a Na-K-2Cl co-transport in the luminal membrane of mammalian small intestine or proximal tubule.

TRANSPORT MECHANISMS FOR Cl

A Cl/OH-exchange (a possible component of electroneutral 'coupled' transport of sodium and chloride) was documented in tracer experiments for rat and rabbit small intestinal and proximal tubular brush border membrane vesicles (Liedtke & Hopfer, 1982b; Murer, Kinne-Saffran, Beauwens & Kinne, 1980a; Warnock & Yee, 1981; Warnock & Eveloff, 1982). In experiments using a pH-sensitive fluorescent dye no clear evidence could be obtained for a Cl/OH-exchange mechanism in studies with rat proximal tubular and rat jejunal brush border membrane vesicles (G. Cassano & H. Murer, unpublished observations). Surprisingly, measured under the corresponding conditions as Na/H-exchange (see Fig. 3) no internal acidification was obtained by applying an inwardly directed chloride gradient (Fig. 5). The simultaneous application of an outwardly directed chloride and sodium gradient was unable to reduce the internal acidification as compared to the simultaneous application of outwardly directed gradients for sodium and gluconate (G. Cassano & H. Murer, unpublished observations). If Na/H-exchange and Cl/OH-exchange mechanisms operated in the same membrane and with comparable rates, the signal in the outwardly directed Cl-gradient condition should be considerably lower than the signal in the presence of Na gluconate. Thus, it seems that the Cl/OH-exchange mechanism has a much smaller rate than the Na-H-exchange mechanism. It is likely, that HCO₃⁻ is the preferentially accepted substrate of the anion exchanger (Knickelbein, Aronson & Dobbins, 1982).

Studies using proximal tubular and small intestinal membrane preparations provided evidence for a conductive pathway for chloride-entry (G. Cassano & H. Murer, unpublished observations; Reenstra et al. 1981). The physiological significance of this observation is not clear.
Fig. 5. Lack of Cl/OH-exchange in rat small intestinal brush border membrane vesicles (measured by acridine orange fluorescence quench technique). The experimental conditions are given in the Figure (G. Cassano & H. Murer, unpublished observations).

TRANSPORT MECHANISMS FOR Ca

Studies using intact tissue preparations revealed that transepithelial transport of Ca in the small intestine and renal proximal tubule are partially dependent on the presence of Na (for reviews see: Martin & DeLuca, 1969; Murer & Hildmann, 1981; Ng, Peraino & Suki, 1982; Ullrich, Rumrich & Klöss, 1976). Due to the presence of a high affinity Ca-ATPase in basal-lateral membranes isolated from renal proximal tubular and small intestinal epithelium (Ghijsen & Van Os, 1979; Ghijsen, De Jong & Van Os, 1982a; Gmaj, Murer & Carafoli, 1982; Van Os & Ghijsen, 1981), Ca can also be transported in a primary-active Na-independent manner. In view of the intra-/extracellular concentration difference for Ca, transport from the cytosol into the serosal interstitium represents an energy-requiring 'uphill' movement whereas influx into the cell from the lumen is a downhill movement.

In general, plasma membranes of non-excitable mammalian cells contain two types of Ca-transport mechanisms: (1) Ca/Mg-ATPase with high affinity sites for Ca and (2) Na/Ca-exchange systems. Evidence has been found for the existence of both mechanisms in basal-lateral membrane vesicles isolated from small intestinal and renal proximal tubular epithelia (Ghijsen & Van Os, 1982; Ghijsen et al. 1982a,b; Gmaj, Murer & Kinne, 1979; Hildmann, Schmidt & Murer, 1982; Murer & Hildmann, 1981). The addition of ATP leads to an acceleration of initial Ca-uptake as compared to the situation without ATP. Inhibition of ATP driven Ca-uptake by orthovanadate can be observed (Fig. 6B). It has been shown that the high affinity Ca-ATPase located in small intestinal and renal proximal tubular epithelia (De Jon...
Membrane mechanisms in transport of inorganic ions

Membranes: 160 mmol l⁻¹ KCl
20 mmol l⁻¹ Heps adjusted with Tris to pH 7.4

Incubation: 160 mmol l⁻¹ KCl(NaCl), 20 mmol l⁻¹ Heps-Tris, 1 mmol l⁻¹ MgCl₂, 2.5 mmol l⁻¹ ouabain,
5 μg ml⁻¹ oligomycin, 5 mmol l⁻¹ MgATP, 56 μmol l⁻¹ Mg-ATP

A Effect of sodium

B Effect of inhibitors

Fig. 6. Effect of 'inhibitors' on ATP-driven Ca-uptake by renal basal-lateral membrane vesicles. The
experimental conditions are given in the Figure (P. Gmaj, E. Carafoli & H. Murer, unpublished
observations).

Ghijsen & Van Os, 1981; Ghijsen, 1982; Gmaj et al. 1982) is inhibited by anti-calmodulin drugs and stimulated by the addition of calmodulin. Similarly, ATP-dependent Ca-uptake was inhibited by anti-calmodulin drugs (kidney, Fig. 6B) and stimulated by addition of calmodulin (Ghijsen, 1982; P. Gmaj, E. Carafoli & H. Murer, unpublished observations; Nellans & Popovitch, 1981).

Evidence for a Na/Ca-exchange mechanism is also available from studies with basal-lateral membrane vesicles isolated from renal proximal tubule and small intestine (e.g. Ghijsen, 1982; Gmaj et al. 1979; Hildmann et al. 1982). In both membrane preparations, ATP-driven Ca-uptake was maximal in a KCl-medium, i.e. in the complete absence of Na. Without inhibiting the Ca-ATPase activity, increased Na-concentration in the medium prevented ATP-driven intravesicular Ca-accumulation (see Fig. 6A). These findings suggested a Na-dependent pathway for Ca in the same vesicular structure that contained the ATP-driven Ca-transport mechanism. Further evidence for a Na/Ca-exchange mechanism was obtained from the experiments on the efflux of Ca from preloaded vesicles in the presence of different transmembrane ion gradients (Gmaj et al. 1979; Hildmann et al. 1982).

In agreement with observations mainly obtained on the intact intestinal epithelium, studies with isolated brush border membrane vesicles indicate the existence of a
passive, carrier-mediated entry mechanism for Ca at the luminal cell pole (e.g. Matsu-
sumoto, Fontaine & Rasmussen, 1980; Miller & Bronner, 1981; Murer & Hildmann,
1981). Studies with chicken brush border membrane vesicles demonstrated that the
passive entry-mechanism is regulated by 1-25(OH)2Vit D3 level in the intact animal
(Matsumoto et al. 1980; Rasmussen, Fontaine, Max & Goodman, 1979). This ex-
pression of the regulatory effect of 1-25(OH)2Vit D3 on the passive entry-mechanism
in the luminal membrane has also been confirmed in studies with rat and rabbit small
intestinal brush border membrane vesicles (Miller & Bronner, 1981; Murer & Hild-
mann, 1981).

More recently, it has been shown that the Ca-ATPase and the ATP-dependent Ca-
uptake is higher in basal-lateral membranes isolated from 1-25(OH)2Vit D3 replete
rats than in basal-lateral membranes from Vitamin D deficient rats (Ghijsen & Van
Os, 1982). It remains to be determined whether this effect is the consequence of
indirect effects (e.g. via the Ca-calmodulin complex or the Ca-binding protein) or
whether it is a 1-25(OH)2Vit D3-induced direct effect on the ‘Ca pump’. Na/Ca-
exchange was not influenced by the 1-25(OH)2Vit D3 level in the intact animal (Ghij-
sen, 1982; Ghijsen & Van Os, 1982).

TRANSPORT MECHANISMS FOR PO4

PO4-transport against an electrochemical potential difference in the renal proximal
tubule and the small intestine depends on the presence of Na, suggesting Na-PO4 co-
transport in the brush border membrane (for reviews see: Bikle, Morrissey, Zolock
& Rasmussen, 1981; Dennis, Stead & Myers, 1979; Murer et al. 1980b; Murer & Hild-
mann, 1981; Ullrich & Murer, 1983). Na-PO4 co-transport mechanisms have
been identified in renal and intestinal brush border membrane preparations isolated
from different animals (Berner, Kinne & Murer, 1976; Cheng & Sacktor, 1981;
Danisi, Murer & Straub, 1982; Fuchs & Peterlik, 1980; Hoffmann, Thees & Kinne,

In both intact epithelia and isolated membrane vesicles, a decrease in luminal pH
increased PO4-transport in intestine and decreased it in the kidney (Baumann, Rum-
rich, Papavassiliou & Klöss, 1975; Berner et al. 1976; Burckhardt, Stern & Murer,
1981; Danisi et al. 1982; Dennis, Woodhall & Robinson, 1976; Hoffmann et al.
1976; Sacktor & Cheng, 1981; Ullrich et al. 1978). This has been taken as evidence
for the preferential transport of monovalent or divalent PO4, respectively (Baumann
Sacktor & Cheng, 1981). Three different lines of experimental evidence are available
against a preferential transport of monovalent or divalent PO4 in rat renal membrane
preparations: (1) high sodium concentrations diminished the pH-sensitivity of the
transport system (Burckhardt et al. 1981); (2) increasing pH from 6.3 to 6.9 increased
the apparent $K_m$ about twofold and $V_{max}$ more than threefold. Assuming that the
transport system accepts only divalent PO4 and that pH has no direct effect on the
transport system itself, an increase in pH should decrease $K_m$ values in an analysis
based on total PO4 concentrations. (3) In electrophysiological experiments and in
studies with rat renal brush border membranes, Na-PO4 co-transport carries a
positive charge only at acidic pH values (Barrett & Aronson, 1982; Burckhardt et al.
Membrane mechanisms in transport of inorganic ions

This is explained by the operation of a transport mechanism which always accepts two sodium ions but can accept monovalent and divalent PO₄. Experiments with rabbit small intestinal brush border membrane vesicles suggested that the pH-dependence of Na-PO₄ co-transport in small intestine is also provoked by a pH-sensitivity of the transporter and does not reflect preferential transport of monovalent PO₄ (Danisi et al. 1982).

Small intestinal and renal proximal tubular PO₄-transport is influenced by various control mechanisms. In the intestine regulatory phenomena are mediated by 1-25(OH)₂Vit D₃. The effect of 1-25(OH)₂Vit D₃ is expressed at the level of the brush border membrane and can be analysed in isolated brush border membrane vesicles (for reviews see: Bikle et al. 1981; Murer & Hildmann, 1981; Rasmussen, Matsumoto, Fontaine & Goodman, 1982; Fig. 7).

Proximal tubular PO₄-reabsorption is inhibited by parathyroid hormone. This effect is intracellularly mediated by cAMP (Aurbach & Heath, 1974; Ullrich et al. 1977). The regulatory effects are expressed at the level of the brush border membrane (Evers, Murer & Kinne, 1978; Hammerman, Karl & Hruska, 1980). It was suggested that cAMP-dependent membrane phosphorylation is responsible for a specific reduction of Na-dependent PO₄-transport (Hammerman, Hansen & Morrissey, 1982).

**Fig. 7.** Influence of 1-25(OH)₂Vit D₃ on the sodium gradient-dependent uptake of PO₄ by duodenal brush border vesicles. To induce 1-25 D₃-deficiency, two rabbits were injected s.c. with 40 mg kg⁻¹ EHDP in the morning of three consecutive days. 8-10 h before killing them, one EHDP-treated rabbit and a sham-treated rabbit received an i.v. injection of 600 ng kg⁻¹ 1-25 D₃. Membrane suspension prepared in 300 mmol l⁻¹ mannitol, 20 mmol l⁻¹ Hepes/Tris, pH 7.4 was added to incubation media containing in addition 100 mmol l⁻¹ sodium chloride and different concentrations of KH₂PO₄. EHDP = disodium ethane-1-hydroxy-1-1-diphosphonate; this drug is known to lower plasma levels of 1-25-dihydroxy-vitamin D₃. This Figure is taken from Hildmann, Schmidt & Murer (1982). In vivo treatments: ■, control; ○, EHDP; □, 1-25(OH)₂Vit D₃; ○, EHDP + 1-25(OH)₂Vit D₃.
However, a causal link between cAMP-dependent membrane phosphorylation inhibited Na-dependent PO₄-transport is missing (Biber, Murer & Malmstrom, 1983). The tubular transport mechanism for PO₄ responds in a parathyroid hormone-independent manner to alterations in the dietary PO₄-intake (Bonjour, Troehler, Preston & Fleisch, 1978; Steele & DeLuca, 1976; Ullrich et al. 1977). This effect is expressed at the level of the isolated brush border membranes (Caverzasio, Murer, Fleisch & Bonjour, 1982; Kempson et al. 1980; Stoll et al. 1979; Stoll, Murer, Fleisch & Bonjour, 1980).

An interesting theory attempts to relate the regulation of PO₄-transport in the proximal tubule to alterations in the cellular NAD/NADH ratio (Dousa & Kempson, 1982). In this respect, the inhibition of Na-dependent PO₄-transport in vesicles by NAD/NADH is interesting (Kempson et al. 1981). As NAD/NADH is hydrolysed very rapidly by brush border membrane preparations (Angielski, Zielkrewicz & Dzierzko, 1982), the inhibition of PO₄-transport by NAD/NADH seems to be indirect. It was shown that inhibition of alkaline phosphatase activity releases the inhibition of PO₄-transport by NAD/NADH (Tenenhouse & Chu, 1982). Hammerman et al. (1982) suggested a ribosylation reaction to be involved in inhibition of PO₄-transport by NAD. However, a causal link is missing between membrane protein-ribosylation and inhibition of PO₄-transport by NAD. Inhibition of transport is rather related to the hydrolytic breakdown of the NAD and to an indirect inhibition of PO₄-transport via released products (P. Gmaj, St. Angielski, J. Biber & H. Murer, unpublished observations).

The Na-PO₄-co-transport mechanism operates independently of Ca (Bernerei et al. 1976; B. Hildmann, C. Storelli & H. Murer, unpublished observations; Matsumoto et al. 1980). PO₄- and Ca-transport respond to stimulation by 1,25(OH)₂Vit D₃ with different time courses, and the polyene antibiotic filipin mimics only the 1,25(OH)₂Vit D₃ stimulation of transmembrane Ca-flux, but not of transmembrane PO₄-flux (Matsumoto et al. 1980; Rasmussen et al. 1979; Rasmussen et al. 1982).

**Transport mechanism for SO₄**

SO₄ is reabsorbed in the ileum against its concentration gradient in a Na-dependent way (Anast, Kennedy, Volk & Adamson, 1965; Smith, Orella & Field, 1981). Proximal tubular SO₄-reabsorption is also Na-dependent (Brazy & Dennis, 1981; Ullrich & Murer, 1983; Ullrich et al. 1980).

Studies with brush border membrane vesicles isolated from rat ileum and rat and rabbit proximal tubule revealed a Na-SO₄-co-transport (Fig. 8 for rabbit ileum). Na-SO₄-co-transport is electroneutral (Lücke, Stange & Murer, 1979, 1981; Schneider, Durham & Sacktor, 1980) in agreement with electrophysiological data obtained with rabbit ileal epithelium (Smith et al. 1981) and proximal tubules in vivo (Samarzija et al. 1981). In isolated rabbit proximal tubules, the stilbene derivative SITS inhibited Na-dependent SO₄-transport across the luminal membrane (Brazy & Dennis, 1981). Langridge-Smith, Sellin & Field (1982) postulated a model for Na-sulphate co-transport in rabbit ileum with varying stoichiometry between Na- and SO₄-flux. At low SO₄-concentrations the stoichiometry factor is one; electroneutrality would then be obtained by the additional involvement of H-flux. At high SO₄-concentrations, two Na-
Membrane mechanisms in transport of inorganic ions

are involved in transmembrane SO\textsubscript{4}-movement. Studies with rabbit ileal brush border vesicles should be useful to test this model (G. Ahearn & H. Murer, unpublished observations).

Langridge-Smith & Field (1981) provided evidence for a stilbene-sensitive anion exchange-mechanism for SO\textsubscript{4} in the basolateral membranes of isolated rabbit ileal epithelia. Similar findings were obtained in isolated rabbit proximal tubules (Brazy & Dennis, 1981). Stilbene derivatives also inhibited SO\textsubscript{4}-flux in basolateral membrane vesicles isolated from rat small intestine and dog kidney cortex (Grinstein, Turner, Silverman & Rothstein, 1980), but were ineffective in rat proximal tubules in vivo (Ullrich et al. 1980). Most recently a stilbene derivative sensitive anion exchange mechanism was detected in isolated basal-lateral membrane vesicles from rat proximal tubule, (J. Löw & G. Burckhardt, unpublished observations.)

CONCLUDING REMARKS

In this brief review we have summarized experiments with membrane vesicles isolated from renal proximal tubules and small intestine. Such experiments represent useful tools for the analysis of membrane transport mechanisms involved in trans-epithelial transport. We have only given the examples related to the transport of inorganic ions.

For Ca it has been observed that the basal-lateral membrane contains two 'uphill' transport mechanisms, whereas influx into the cell is a passive process. For the transport of Na the passive entry into the cell across the luminal membrane is coupled
to the uphill movement of a second substrate (co-transport). This was discussed for an antiport mechanism, namely Na/H-exchange, as well as for different Na-coupled movements of anions as summarized in Fig. 9. Inspection of the Figure may indicate where future efforts can complete our knowledge of the mechanisms involved in transepithelial inorganic anion transport.

In more recent time, studies with vesicles have also been used to determine regulatory mechanisms involved in the regulation of transepithelial transport. As discussed above, regulation of transcellular PO$_4$- and Ca-flux most probably proceeds via altered membrane transport (luminal influx only?). Certainly, studies with vesicles can help to define the cellular mechanisms leading to the final event of altered membrane transport.

REFERENCES


Membrane mechanisms in transport of inorganic ions


Membrane mechanisms in transport of inorganic ions


