MEMBRANES AND MEMBRANE PROCESSES

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PREFACE

During the past two decades Membrane Science and Technology has made tremendous progress and has changed from a simple laboratory tool to large scale processes with numerous applications in Medicine and Industry.

In this volume are collected papers presented at the First Europe-Japan Congress on Membrane and Membrane processes, held in Stresa in June 1984. Other contributions to the Conference will be published in a special issue of the Journal of Membrane Science.

This Conference was organized by the European Society of Membrane Science and Technology and the Membrane Society of Japan, to bring together European Scientists and Engineers face to face with their colleagues from Japan; in both countries membrane processes will play a strategic role in many industrial areas in the 1990s, as predicted by the Japanese project for Next Generation Industries and by the EEC Project on Basic Technological Research (BRITE).

The large number of participants, of about four hundred from twenty-six countries including USA, Australia, China and Brazil, the quality of the Plenary Lectures and Scientific Communications made the Conference a significant international success.

We are deeply grateful to the following organizations that contributed financial support for the Conference:

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We acknowledge also in particular the Italian Oriented Program on Fine Chemistry (CNR) and its Director Prof. L. Caglioti for the continuous support to this event.

All the papers published in this volume have been reviewed by members of the Scientific Committee or international experts in the field. We are particularly grateful for their cooperation to: G. Alberti, P. Aptel, T. Fane, C. Fell, A. Gliozzi, T. Hoshi, G. Jonsson, N. Kamo, S. Katoh, O. Kedem, H. Kimizuka, S. Kimura, Y. Kobatake, T. Kondo, P. Meares, T. Nakagawa, S. Nakao, J. Neel, A. Nidola, Y. Nozawa, H. Ohya, D. Paterson, M. Pegoraro, R. Rautenbach, G. Sarti, G. Semenza, M. Seno, H. Strathmann.

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CONTENTS

TRANSPORT MECHANISMS OF ORGANIC IONS IN RAT RENAL BRUSH BORDER AND BASOLATERAL MEMBRANE VESICLES

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Renal handling of organic ions is a complex phenomenon involving glomerular filtration, tubular secretion and tubular reabsorption[1,2]. The primary function of the renal organic ion secretory system is the elimination of foreign compounds, and organic anions and cations are actively secreted by the proximal renal tubules. The sequence of movement of organic ions is transport across basolateral membranes, accumulation in the cells, followed by efflux from the cell across brush border membranes into tubular fluid. However, it has been difficult to characterize the specific membrane events underlying the transepithelial transport of organic ions, because of its complex structure, being composed of two distinct membranes, luminal brush border and contraluminal basolateral membranes. The two membranes differ in the enzyme composition and in the transport mechanisms for solutes.

In recent years a methodology has been developed to use vesicles of the isolated brush border and basolateral membranes for the analysis of tubular transport. In particular, many studies have been presented on the mechanisms for D-glucose and neutral amino acid transport in brush border membranes[3]. However, there have been only a few reports concerning the plasma membrane transport of organic anions and cations[4]. Thus we were prompted to assess the transport of various organic ions by brush border and basolateral membrane vesicles: p-aminohippurate (anion)[5], tetraethylammonium (cation)[6], and cephalexin (amphoteric compound)[7,8].

ISOLATION OF BRUSH BORDER AND BASOLATERAL MEMBRANES

Brush border membrane vesicles were isolated from the renal cortex of rats according to the calcium precipitation of Evers et al.[9]. On the other hand, the isolation of basolateral membranes has been difficult, because the densities of brush border and basolateral membranes are so close. We have recently developed a simple method for the isolation of basolateral membranes using Percoll density gradient centrifugation[10]. As shown in Figure 1, $(Na^+ + K^+)$-ATPase, the marker enzyme for basolateral membranes, was enriched 22-fold in the basolateral membrane preparation compared with that found in the homogenate. Alkaline phosphatase and aminopeptidase, the marker enzymes for brush border membranes, were enriched 10-fold in brush border membrane preparation. In both prepar-

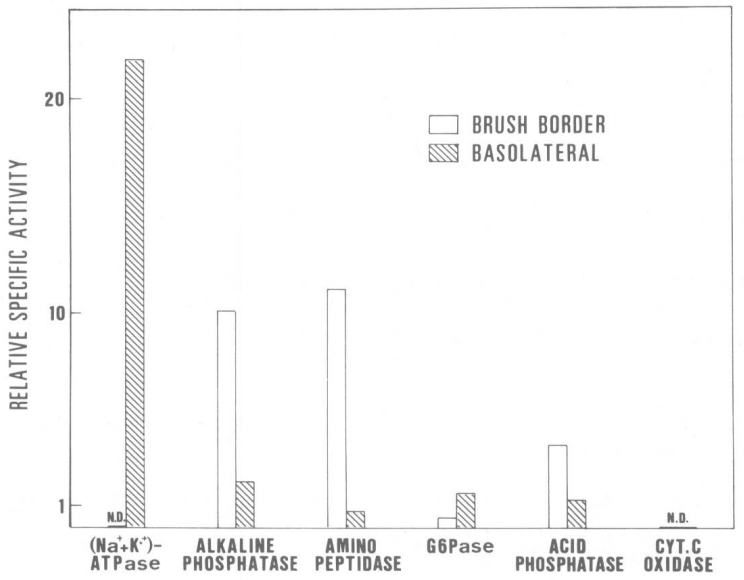

Fig. 1. Relative specific activities of marker enzymes in brush border and basolateral membranes.

ations, the contamination by mutual membranes, mitochondria, lysosomes, endoplasmic reticulum and cytosol was small.

The uptake of substrates was measured by a rapid filtration technique. In the regular assay, the reaction was initiated rapidly by adding 20 µl of substrate mixture to 20 µl of membrane vesicle suspension at 25°C. At the stated time points, the incubation was stopped by diluting a reaction sample with 1 ml of ice-cold stop solution. The tube contents were immediately poured onto Millipore filters (0.45 µm). The radioactivity of p-amino[^{3}H]hippurate or [^{3}H]tetraethylammonium trapped on the filters was determined by liquid scintillation counting.

p-AMINOHIPPURATE TRANSPORT

The effect of probenecid and 4,4'-diisothiocyano-2,2'-disulfonic stilbene (DIDS) on p-aminohippurate uptake by brush border and basolateral membrane vesicles was studied. DIDS, a specific inhibitor for anion transport into red blood cells, inhibited more strongly the uptake of p-aminohippurate than probenecid. The degree of the inhibition in brush border and basolateral membrane vesicles was similar. In the concentration dependence of p-aminohippurate uptake, the relationship between concentration and rate of uptake was nonlinear in basolateral membranes, providing evidence for saturability. However there was no evidence for saturation of the uptake by brush border membrane vesicles.

In order to confirm the differences in p-aminohippurate transport by brush border and basolateral membrane vesicles, we have studied the effect of countertransport on p-aminohippurate uptake. As is evident from Figure 2, vesicles preloaded with a high concentration of unlabeled p-aminohippurate showed enhancement of p-amino[^{3}H]hippurate accumulation by countertransport only in basolateral membranes, while no change of the uptake was observed in brush border membranes[5].

Furthermore, the temperature dependence of p-aminohippurate uptake by brush border and basolateral membrane vesicles was studied. The Arrhenius

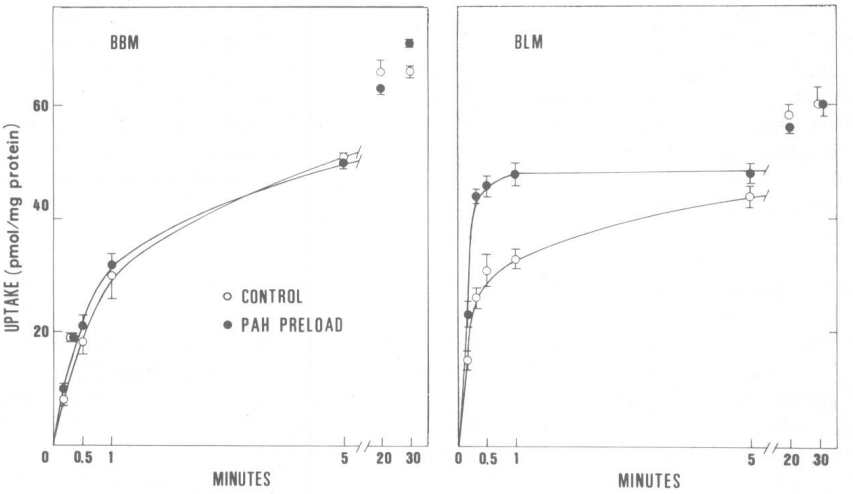

Fig. 2. Countertransport effect on p-aminohippurate uptake by brush border (BBM) and basolateral membrane (BLM) vesicles. [From Reference 5].

plot for the uptake by brush border membrane vesicles was linear over the temperature range used (5-37°C). In contrast, the Arrhenius plot for the uptake by basolateral membrane vesicles was biphasic. As Smedt and Kinne[11] also reported a biphasic Arrhenius plot with respect to Na^+-dependent D-glucose transport by brush border membrane vesicles, the present data suggest the contribution of a carrier-mediated transport system for p-aminohippurate in basolateral membranes.

The role of membrane potential as a driving force for p-aminohippurate uptake by brush border and basolateral membrane vesicles was studied by applying different anion gradients with sodium directed into the vesicles. The more permeant lipophilic anion, SCN^-, thought to facilitate a more rapid development of interior negative membrane potential, was compared with less permeant anions, such as Cl^- and SO_4^{2-}. Anion permeability to biological membrane generally follows in the order of $SCN^- > Cl^- > SO_4^{2-}$. p-Aminohippurate uptake by brush border membrane vesicles was higher when chloride was replaced by sulfate, and lower when chloride was replaced by thiocyanate. On the other hand, this effect for the uptake induced by anion gradients was small in extent in basolateral membrane vesicles. These results suggest that a decrease of the inside negative membrane potential increases p-aminohippurate uptake, and this effect is more evident in brush border membranes compared with basolateral membranes.

Based on the above results, the uptake of p-aminohippurate by basolateral membrane vesicles satisfies some of the criteria for carrier-mediated process; namely, the process is saturable, temperature dependent, inhibited by anion transport inhibitors, and undergoes a countertransport effect. In contrast, brush border membrane vesicles failed to display the capacity to accelerate the exchange of p-aminohippurate, saturability of the uptake, and biphasic Arrhenius plot, although probenecid and DIDS reduced p-aminohippurate transport. Okamoto et al.[12] discussed that if an ion is conducted via a carrier, the transport is affected by temperature, which controls the fluidity of lipids in the membrane, and that if it is conducted via a channel, the effect of fluidity is rather small. Therefore, it may be reasonable to assume that p-aminohippurate is transported across brush border membranes by a gated channel, which responds to anionic charge, rather than by a simple diffusion. Furthermore, p-amino-

hippurate uptake by brush border membrane vesicles was influenced more sensitively by the alteration of the membrane potential compared with that by basolateral membrane vesicles, and it was significantly stimulated by the membrane potential induced with various anion gradients, which renders the intravesicular space more positive. This finding is compatible with the secretion of p-aminohippurate at the luminal side in vivo, because the intracellular compartment has more negative electrical potential than the luminal fluid compartment (Figure 5).

TETRAETHYLAMMONIUM TRANSPORT

It is well-known that the organic cation transport system is clearly separable from the anion transport system. In the concentration dependence of [^{3}H]tetraethylammonium uptake by brush border and basolateral membrane vesicles, the relationship between concentration and uptake rate was nonlinear in both membranes, providing evidence for saturability. After the correction for the nonsaturable component, the values of Km and Vmax were 0.8 mM and 7.4 nmol/mg protein per min in brush border membranes, and 2.5 mM and 5.6 nmol/mg protein per min in basolateral membranes, respectively. Vesicles preloaded with high concentration of unlabeled tetraethylammonium showed enhancement of [^{3}H]tetraethylammonium accumulation by countertransport in brush border and basolateral membranes. These data suggest the contribution of a carrier-mediated transport system for tetraethylammonium in both membranes. Furthermore, the temperature dependence of tetraethylammonium uptake by brush border and basolateral membrane vesicles was studied. The Arrhenius plot for the uptake was biphasic in both membranes.

Based on the above results, the uptake of tetraethylammonium by brush border and basolateral membrane vesicles satisfied some of the criteria for carrier-mediated process; namely, the process is saturable, temperature dependent, inhibited by other organic cations, and undergoes a countertransport effect.

In order to get further information about the driving force for the transport, we have studied the effect of various ionic conditions on the uptake of tetraethylammonium. As is evident from Figure 3, there was no

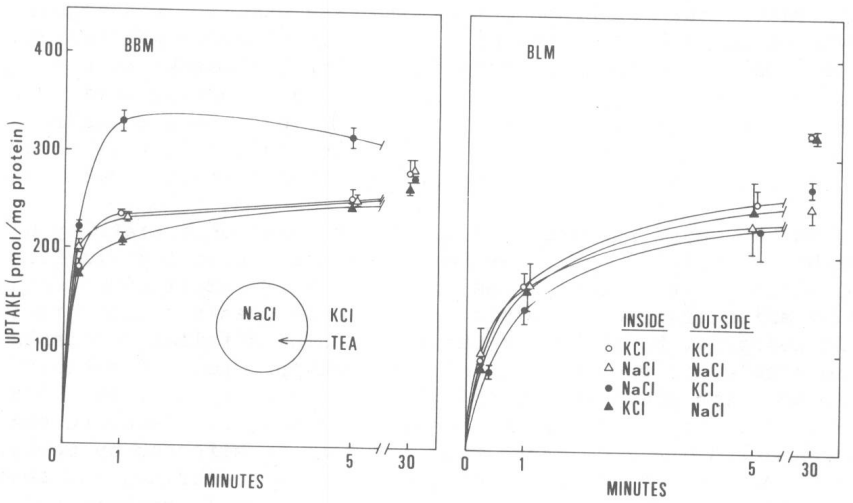

Fig. 3. Effect of various ionic conditions on tetraethylammonium uptake by brush border (BBM) and basolateral membrane (BLM) vesicles. [From Reference 6).

effect of ionic conditions on tetraethylammonium uptake by basolateral membranes[6]. However, there was the stimulation of tetraethylammonium uptake by brush border membrane vesicles in the presence of Na^+ gradient: sodium chloride is inside of the vesicles and potassium chloride is outside. Thus, this ionic condition can be a driving force for tetraethylammonium transport.

As intravesicular acidification of brush border membrane vesicles was confirmed in the presence of $[Na^+]i$ and $[K^+]o$ using Acridine orange, the effect of H^+ gradient on tetraethylammonium uptake by brush border and basolateral membrane vesicles was studied (Figure 4)[6]. In brush border membranes, the presence of an H^+ gradient (inside pH 6.0, outside pH 7.5) induced a transient uphill transport of tetraethylammonium (overshoot phenomenon). When the gradient was reversed (lower pH in outside), uphill transport was not observed. The final levels of the uptake were all identical, indicating that the pH gradient did not effect the vesicle size. In contrast, an H^+ gradient was ineffective in basolateral membrane transport of tetraethylammonium. The concentrative uptake of tetraethylammonium driven by H^+ gradient was completely inhibited by mercuric chloride.

Furthermore, it is important to clarify the role of membrane potential as a driving force for tetraethylammonium uptake by brush border and basolateral membrane vesicles. Valinomycin in the presence of K^+ gradient (inside>outside) was employed to produce an inside-negative membrane potential. Tetraethylammonium uptake by brush border membrane vesicles was unaffected by valinomycin, suggesting electroneutral antiport of H^+ and tetraethylammonium. In contrast, a valinomycin-induced inside-negative membrane potential stimulated significantly the initial rate of tetraethylammonium uptake by basolateral membrane vesicles.

Based on the above results, tetraethylammonium is transported from blood to cell across basolateral membrane via a carrier-mediated system and this process is stimulated by the intracellular negative potential. Tetraethylammonium transport from cell to urine across brush border membranes is driven by an H^+ gradient via an electroneutral H^+-tetraethylammonium antiport system. This H^+ gradient can be created by an Na^+-H^+ antiport system and/or an ATP-driven H^+ pump in brush border membranes (Figure 5).

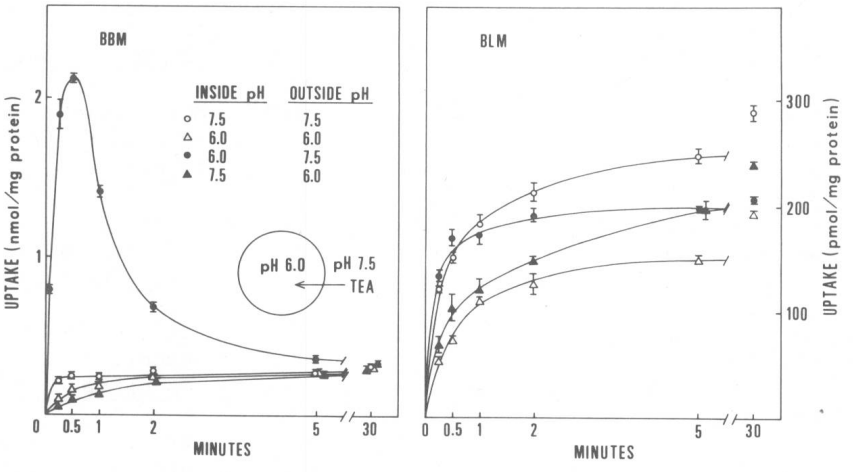

Fig. 4. Effect of H^+ gradient on tetraethylammonium uptake by brush border (BBM) and basolateral membrane (BLM) vesicles. [From Reference 6].

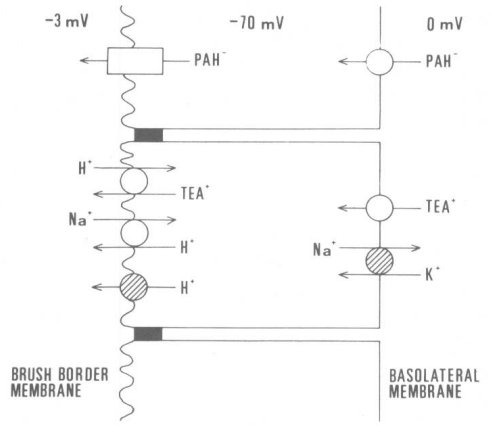

Fig. 5. Model for transepithelial transport of p-aminohippurate (PAH) and tetraethylammonium (TEA) in proximal tubular cells.

From the studies described above, it is evident that renal epithelial cells possess a striking polarity with respect to the transport properties of organic ions across brush border and basolateral membranes (Figure 5). These results can represent useful information to further the study of tubular transport mechanisms of organic anions and cations.

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