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Review

Renal Transport of Drugs: An Overview of Methodology with Application to Cimetidine

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The development of new methods to study transport processes in renal epithelia has greatly enhanced our knowledge of the mechanisms involved in the transport of a number of endogenous compounds. More recently, these methods have been applied to study mechanisms of specific drug transport. This article is intended to provide an overview of the various methods used to study renal elimination of compounds. References to more detailed reviews of the individual methods are provided. Studies of the renal transport of cimetidine, a histamine H_2-receptor antagonist, are presented to illustrate the application of these methods to the study of specific drugs. Methods such as clearance techniques and the Sperber chicken preparation used to study renal elimination of compounds in whole animals are briefly described. Techniques to identify the site of renal transport including stop flow, isolated perfused tubules, and micropuncture methods are discussed and references to more technical reviews are cited. The more recently developed methods of isolated membrane vesicles for studying transport across the individual polar membranes of the proximal tubule are discussed along with the relevant studies of the use of these membranes in elucidating the mechanisms involved in the renal transport of cimetidine. Finally, the use of cultured renal epithelial cell lines in studying renal transport is described. Knowledge of drug transport mechanisms in the kidney is important both in drug targeting to the kidney and in understanding the pharmacokinetics of renally eliminated drugs. As exemplified by the studies with cimetidine, only by combining the data from experiments using diverse methodology can the mechanisms involved in the renal excretion of compounds be delineated. With the use of existing methods and the development of new technologies, many of the questions related to drug transport mechanisms can be addressed.

KEY WORDS: organic cation transport; cimetidine; membrane transport; techniques; proximal tubule transport.

INTRODUCTION

Recent advances in the development of new methods to study transport processes in renal epithelia have greatly enhanced our knowledge of the mechanisms of transport of a number of endogenous compounds as well as drugs. In particular, the development of procedures to isolate and purify brush border or luminal membranes from basolateral or antiluminal membranes has led to a new understanding of the molecular mechanisms involved in the transport of substances across the individual epithelial cell membranes. With micropuncture techniques, transport sites along the nephron have been specifically identified. The use of *in vitro* microperfusion methods has allowed the investigator to measure fluxes under carefully controlled experimental conditions. The driving forces and characteristics of a number of transport systems in specific segments of the tubule have been identified. Monoclonal cell lines have provided a powerful tool for studying the regulation of transport systems and for examining the characteristics of transport processes in homogeneous cell types.

Although these methods have been used primarily to study the mechanisms of transport of endogenous substances, it is clear that these same techniques can be used in examining the transport of specific drugs. Knowledge of specific transport mechanisms is important in targeting drugs to or away from the kidney as well as in understanding the pharmacokinetics of renally eliminated drugs and their metabolites. This review is intended to provide an overview of the various methods used to study the renal elimination of compounds including the more recent methods developed to study transport mechanisms at the brush border and basolateral membrane. References to appropriate articles that provide detailed reviews of the individual methods are cited. Studies of the renal transport of cimetidine, a clinically important histamine H_2-receptor antagonist, are presented to illustrate the application of these methods to the study of specific drug transport. Because of its clinical importance and the fact that cimetidine is actively secreted by the kidney, most of the techniques discussed in this article have been used to study the renal elimination of cimetidine. Thus, cimetidine is well suited as an exemplary compound.

RENAL CLEARANCE METHODS

Renal clearance is defined as the ratio of renal excretion

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rate to plasma concentration. Renal clearance computed in this manner actually represents renal excretory clearance and does not include clearance by biotransformation in the kidney. Clearance methods are straightforward and can be carried out in humans and in whole animals as well as in isolated perfused kidneys. The techniques allow the investigator to ascertain the net excretory functions of the kidney as a whole. By comparing the renal clearance of a compound to the total-body clearance, the contribution of renal excretion to the elimination of a compound can be determined. When a marker for glomerular filtration rate (GFR) such as inulin or creatinine is included in the study, the net clearance by secretion or reabsorption can be calculated. Although reabsorption and secretion cannot be quantitatively differentiated, information about whether these processes are involved in the renal clearance of a compound can be obtained by comparing the renal clearance of the compound to its clearance by filtration (GFR $\times f_u$, where f_u is the fraction of drug unbound in plasma). For example, active secretion is involved if the renal clearance is greater and reabsorption is involved if the renal clearance is less than the clearance by filtration. A dependency of renal clearance on urine pH or urine flow suggests that reabsorption occurs via nonionic diffusion. The ability of selected compounds to inhibit the renal clearance of a particular compound may be indicative of the involvement of a specific transport system. Several reviews including a recent review by Maack on clearance methods and isolated kidney perfusion techniques have been published (1,2).

Renal clearance methods have been applied to study the excretion of cimetidine in both humans and animals (3–9). Evidence for net renal secretion has been obtained from studies in healthy subjects in which the renal clearance was shown to be three- to fourfold greater than the glomerular filtration rate (3). Based upon a number of studies demonstrating that cimetidine reduces the renal clearance of several basic drugs including ranitidine (4), triamterene (5), and procainamide (6,7), the organic cation transport system is thought to be involved in the renal secretion of cimetidine in humans. This system appears to be responsible for the active secretion of a variety of organic bases (see Ref. 10 for review). Cimetidine also reduces the renal clearance of the neutral, endogenous compound, creatinine, which is commonly used as a marker of glomerular filtration rate (8).

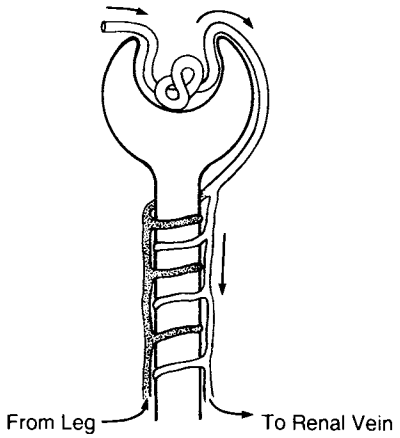

Fig. 1. Circulation of the nephron of the avian kidney. Shaded vessels, from leg, bypass the glomerulus and supply blood only to the renal tubule. Unshaded vessels supply blood to the entire nephron (see Ref. 10).

Weiner and Roth (9) determined the renal clearance of cimetidine in anesthetized rats under steady-state conditions. The ratio of cimetidine renal clearance to glomerular filtration rate (determined by inulin clearance) decreased from 2.6 to 1.3 when cimetidine concentrations in plasma increased from 2 to 200 μg/ml, indicating that the renal secretion of cimetidine was saturable. Renal clearance was found to be dependent upon urine pH, suggesting that cimetidine may be reabsorbed by nonionic diffusion in the kidney. No evidence for a dependency of renal clearance on urine flow was obtained. Consistent with the involvement of the organic cation transport system, cimetidine inhibited the renal clearance of the organic cation, tetraethylammonium, but not the renal clearance of the organic anion, para-aminohippuric acid.

SPERBER CHICKEN PREPARATION

A variation of the renal clearance method was developed by Sperber, who took advantage of the renal–portal circulatory system of avians, which partially supplies blood to peritubular capillaries of the kidney without passing through the glomeruli (Fig. 1) (11). A compound injected into a leg vein of a chicken will be secreted by the ipsilateral kidney before entering the systemic circulation. By comparing the amount of the compound excreted in the urine by the ipsilateral kidney to the amount excreted by the contralateral kidney, an apparent tubular extraction fraction can be obtained as the difference in the amount excreted by the two kidneys divided by the infusion rate of the drug. Because a substantial fraction of the compound may be excreted by the kidney before entering the systemic circulation, toxicologic or pharmacologic effects which might interfere with the measurement of renal transport are minimal. This is particularly advantageous when studying the transport of organic cations, many of which are pharmacologically active. The major disadvantage of this technique is that it can be used only in avian species. Findings from avian kidneys may not be applicable to mammalian kidneys.

Using the Sperber chicken preparation, Rennick and co-workers studied the renal secretion of cimetidine (12). Renal transport of radiolabeled cimetidine was found to be saturable and was shown to occur at 88% of the transport rate of para-aminohippuric acid, a compound which at low concentrations is completely secreted in one pass through the kidney. Consistent with studies in mammalian kidney, organic cations, such as ranitidine and procainamide, produced concentration-dependent inhibition of cimetidine transport, suggesting the involvement of the organic cation transport system. Surprisingly, the organic cation, quinine, was ineffective as an inhibitor of cimetidine transport. The lack of effect of quinine on cimetidine secretion is unexplained. Organic anions were not tested as potential inhibitors of cimetidine renal transport in this study.

RENAL CORTICAL SLICES

Renal cortical slices have been used extensively in early studies of drug transport in the kidney (13). The cortex of

the kidney can be cut into thin slices with a microtome. The slices can be incubated in a physiologic oxygenated medium with radiolabeled substrates. The ratio of the substrate concentration per gram of tissue to the concentration of substrate in the medium (T:M) can be computed. A ratio greater than one suggests that the compound accumulates in the kidney. Potential inhibitors of uptake can be studied. The effects of metabolic intermediates and inhibitors of cellular metabolism on the uptake of the substrate can be determined. However, cellular binding cannot be easily distinguished from transport. To do so, one must use specific metabolic inhibitors to stop active transport processes. Such compounds may also interfere with binding processes. The information obtained from studies in renal cortical slices is presumed to reflect the function of the basolateral membrane since the lumen collapses in these preparations (13). The accumulation of certain compounds in the slices may not relate to the intact kidney. Thus, this technique, although often employed, has specific limitations.

Cacini *et al.* (14) conducted experiments in which the uptake of radiolabeled cimetidine was studied in cortical slices prepared from canine kidney. Cimetidine accumulated in the slices by a saturable process. At 10^{-6} *M*, cimetidine achieved a T:M ratio of 3 at equilibrium. No evidence of biotransformation was observed. To distinguish transport from membrane binding, the uptake studies were performed in the presence of nitrogen and sodium cyanide, inhibitors of cellular metabolism. These inhibitors reduced the uptake of cimetidine to 60 and 30% of the control, respectively, indicating that the majority of uptake of cimetidine was dependent on metabolic energy and presumably represented active transport rather than binding. The organic cations, quinine and cyanine 863, at concentrations of 10^{-3} and 10^{-4} *M*, respectively, produced 60 to 70% inhibition of the equilibrium uptake of cimetidine. Interestingly, probenecid (10^{-4} *M*), an organic anion, produced a 20% inhibition of cimetidine uptake. Thus, the investigators concluded that cimetidine uptake is saturable and energy dependent, that the uptake is mediated by the organic cation transport system but is sensitive to probenecid, and that the basolateral membrane may be responsible for cimetidine transport.

STOP FLOW

The method of stop flow is used to locate the transport site of a compound (15). The ureter of an animal (usually a dog) is catheterized and ligated. An intravenous infusion of an organic compound in mannitol solution is started while the ureteral catheter is clamped shut so that the GFR in that kidney is very close to zero. The clamp is then released and the rapid outflowing urine is collected in serial samples. The first samples represent tubular fluid trapped in the most distal portions of the tubule, whereas the later samples represent tubular fluid trapped in more proximal portions of the tubule. Secreted compounds are present at increased concentrations in certain samples. The time of collection of these samples infers the location of the secretion site. For example, increased concentrations of a compound in samples which also show increased concentrations of para-aminohippuric acid, a reference compound secreted in the proximal tubule, may suggest that the secretion site is in the proximal areas. Because water is reabsorbed in both proximal and distal parts of the nephron, inulin is usually coadministered to trace the water movement. This method is excellent for locating the transport site of compounds; however, artifacts from the clamped ureters must be considered. The method is also less accurate for compounds transported in the proximal tubules since the samples must travel over a relatively long distance to reach the catheters. Thus, lateral diffusion and passive reabsorption may obscure the results. This technique has not been specifically applied to study the location to cimetidine transport; however, it has been applied to identify the location of organic cation transport (16), which appears to be in the proximal tubule.

MICROPUNCTURE TECHNIQUES

Several excellent reviews have been published on micropuncture techniques (17–20). Briefly, a single cortical surface nephron in the exposed kidney is punctured with a micropipette under a microscope. Fluids containing compounds of interest are perfused and collected by separate micropipettes located at various distances along the tubule. Considerable information can be obtained about the exact sites of transport of a compound and the direction in which the compound is being transported. Transport rates can be quantitatively assessed. However, the technique is tedious and requires skill. Only certain portions of the nephron are available for micropuncture. The nephrons used are surface nephrons of proximal cortical tubules, which may not be representative of all the nephrons. Therefore care must be taken in extrapolation of the results to the entire kidney. To date, the technique has not been applied to the study of cimetidine renal transport.

ISOLATED PERFUSED AND NONPERFUSED TUBULES

A segment of renal tubule can be isolated and simply incubated with radiolabeled substrates (nonperfused) or perfused with a buffer of suitable composition (Fig. 2) (see reviews in Refs. 21–23). In the nonperfused tubule, the lumen is collapsed and the data obtained, similar to data from the renal cortical slice preparation, presumably reflect the functions of the basolateral membrane. In the perfused tubule, both the brush border membrane (luminal membrane) and the basolateral membrane (antiluminal membrane) are functional. Both secretion and reabsorption of compounds can be determined quantitatively. Perfused tubules have some advantages over micropuncture methods, which are limited to the nephrons and portions of nephrons which are at the surface of the kidney. By perfusing various segments of the tubule, the location of specific transport processes can be ascertained. However, the transport properties of the brush border and basolateral membranes can not be studied separately. As with micropuncture, considerable technical skill is required to obtain good data.

McKinney and co-workers studied the renal transport of cimetidine in isolated perfused superficial proximal tubules from rabbit kidney (24,25). The individual tubules were perfused while in a bath containing radiolabeled cimetidine. Fluid from the perfused tubule was collected and the concentration of labeled cimetidine in the tubular fluid determined. Cimetidine concentrations were found to be 15 to 25 times higher in the tubular fluid than in the bath, indicating that the compound was actively secreted (24). Temperature- and concentration-dependent cimetidine transport

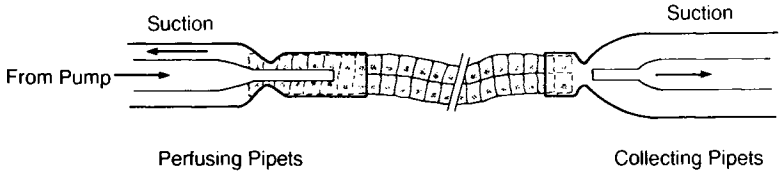

Fig. 2. Technique used in microperfusion of isolated tubules (17). Perfusion pipettes (left) are concentric. The outer portion is used to supply suction to mount the tubule. The inner portion, connected to a perfusion pump, functions in perfusing the tubule. On the right, a single pipette supplies suction to mount the tubule. A collecting pipette is inserted periodically to collect fluid (see reviews in Refs. 18 and 19).

was also demonstrated. The rate of cimetidine transport from lumen to bath (reabsorptive direction) was 10 to 20% of the bath-to-lumen transport rate and was only slightly dependent upon the temperature, suggesting that the reabsorption was passive in nature (24).

The organic cations, quinine, quinidine, tolazoline, procainamide, *N*-acetylprocainamide, and cimetidine sulfoxide, at concentrations ranging from 10^{-5} to 10^{-3} *M*, produced concentration-dependent inhibition of cimetidine transport from bath to lumen (24,25). Interestingly, the organic anions, probenecid and para-aminohippuric acid, produced concentration-dependent inhibition of the bath-to-lumen transport of cimetidine. The endogenous compound, creatinine, also inhibited the transport of cimetidine. These detailed studies represented the first reports of cimetidine transport in isolated tissue and suggested that cimetidine is transported by the organic cation transport system. The effect of the anions, probenecid and para-aminohippuric acid, on cimetidine secretion raised a number of questions concerning the nature of cimetidine transport and the structural specificity of the organic cation transport system. Because the data were obtained in intact tissue, it was possible that probenecid and para-aminohippuric acid were altering renal cellular function and indirectly affecting cimetidine transport. Subsequent studies in isolated membrane vesicles from this laboratory (26) as well as from the laboratory of McKinney *et al.* (27) have clarified these data. These studies are presented below.

ISOLATED BRUSH BORDER AND BASOLATERAL MEMBRANE VESICLES

For a compound to be secreted in the kidney, the compound has to be transported from the blood across the basolateral membrane into the tubule cell and subsequently transported from the cell across the brush border membrane into the tubular fluid. To accomplish this type of vectoral transport the two membranes are necessarily polar (Fig. 3). Methods to separate the brush border from the basolateral membrane have been developed, and several reviews published on the use of renal plasma membrane vesicles to study transport (28–31). The methods take advantage of the distinct physical differences between the two membranes. For example, the membranes differ in their buoyancy and surface charge density, properties which can be exploited in centrifugation or electrophoresis procedures to isolate and purify the membranes.

The most commonly used technique for preparing purified brush border membrane vesicles, developed by Booth and Kenny (32), takes advantage of the high surface density of negative charge of the brush border membrane. Following homogenization of the renal cortex, divalent cations such as Mg^{2+} are added to the homogenate. Brush border membranes, which have a high negative surface charge density, can be separated from basolateral membranes and intracellular organelles which aggregate in the presence of divalent cations. Brush border membranes isolated using this technique form right-side-out spherical vesicles which are osmotically reactive. The vesicles are fairly pure as indicated by reported enhancements of specific enzyme markers such as maltase, alkaline phosphatase, trehalase, and γ-glutamyltranspeptidase. In general, an enhancement in the specific activity of one of these enzymes in the final membrane preparation in comparison to its activity in the initial homogenate suggests the presence of brush border membranes. Contamination by basolateral membranes can be monitored by assessing the specific activity of Na^+/K^+ ATPase, a specific enzyme marker for the basolateral membrane.

Basolateral membranes do not have the rigid cytoskeletal structure of the brush border membrane. Accordingly, more gentle procedures are required in isolating these membranes. Furthermore, because there is no single property, such as a high negative surface charge density, which can be taken advantage of to isolate these membranes, more tedious procedures are required. The most commonly used procedures involve centrifugation procedures that incorporate sucrose or Percoll gradients (31). The final membrane fraction isolated in this manner is usually enriched in basolateral membranes but not brush border membranes as as-

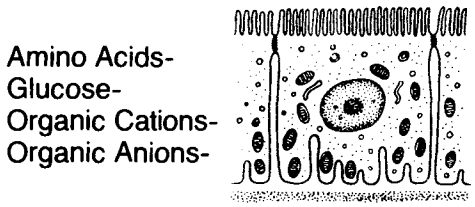

Fig. 3. Two-dimensional drawing of a renal proximal tubule cell depicting the polar nature of the plasma membranes. The brush border or luminal membrane is on top and lines the tubule lumen. The basolateral or antiluminal membrane, shown as the lower membrane, is in close proximity to the capillary endothelia. Examples of organic compounds that are transported in the proximal tubule are listed.

sessed by the specific enzyme markers cited above. However, the membrane vesicles are not uniformly right-side-out and there may be contamination by cytoplasmic membranes such as endoplasmic reticulum.

Unlike studies in intact tissue, studies in isolated membrane vesicles allow the mechanisms of transport at each membrane to be characterized separately. Membrane vesicles are advantageous to study drug transport since biotransformation is minimized. Cofactors are not included and preparations are virtually devoid of drug metabolizing enzymes. Driving forces for transport such as specific ion gradients can be identified in vesicles since the composition of the internal and external media is controlled by the investigator. Kinetic properties of transport can be studied under precise experimental conditions. For example, metabolites, which may interfere with the transport of a drug, can be eliminated or added to the media. The disadvantages of studies in isolated membrane vesicles include contamination by other components of cells that cannot be totally eliminated. Also, the membranes are generally derived from a variety of cell types in the kidney and, as such, may be heterogeneous in terms of their transport characteristics; however, this disadvantage may be overcome if vesicles are prepared from monoclonal cell lines. The general difficulty in the extrapolation of *in vitro* data to the *in vivo* situation has to be considered.

We (26) and others (27,33) have specifically examined the transport of cimetidine in isolated membrane vesicles. Studies in our laboratory (26) and that of McKinney and coworkers (27) have demonstrated that cimetidine accumulates in brush border membrane vesicles prepared from rabbit renal cortex and that its accumulation is sensitive to changes in extravesicular osmolarity, suggesting that the compound is transported into an osmotically reactive intravesicular space. Michaelis–Menten studies have revealed that the drug is transported into brush border membrane vesicles by both a saturable and a nonsaturable process (26). The K_m for transport is about 5 μM, which is considerably lower than comparable values for other organic cations in brush border membrane vesicles prepared from rabbit renal cortex (34–39). As illustrated in Fig. 4, cimetidine uptake in the presence of an outwardly directed proton gradient (or inwardly directed hydroxyl gradient) is enhanced to values exceeding its equilibrium accumulation (overshoot phenomenon) in brush border membrane vesicles prepared from rat (33) or rabbit (26,27) renal cortex. The overshoot phenomenon suggests that cimetidine temporarily accumulates in the vesicles against a concentration gradient in the presence of a pH gradient. When the pH gradient dissipates, concentrative transport of cimetidine cannot be maintained and the uptake returns to equilibrium values. Presumably at equilibrium the concentrations of cimetidine in the intravesicular and extravesicular media are identical. Demonstration of an overshoot phenomenon suggests that the pH gradient which exists from lumen to cell in the proximal tubule may be the driving force for cimetidine transport. In basolateral membrane vesicles prepared from rat renal cortex, a pH gradient could not drive the uptake of cimetidine (33). Previously, a pH gradient was reported to be the driving force for the uptake of the organic cations, N^1-methylnicotinamide and tetraethylammonium, in brush border membrane vesicles, but not basolateral membrane vesicles, prepared from the cortex of rat, rabbit, and dog kidney (36–38). The pH gradient-stimulated uptake of cimetidine in brush border membrane vesicles can be inhibited by other organic cations including cimetidine sulfoxide, procainamide, ranitidine, and N^1-methylnicotinamide (26,27) as well as the neutral molecule, creatinine (40). Interestingly, the organic anion, probenecid, can inhibit cimetidine transport at the brush border membrane (26,27). These data, which are consistent with data obtained in the isolated perfused tubules from rabbit kidney (24,25) and in cortical slices from dog kidney (14), suggest that the effect of probenecid on cimetidine transport involves, at least in part, a transporter in the brush border membrane. Recently, we addressed the question of whether the observed interaction between probenecid and cimetidine is specific for these two compounds or whether organic anions generally inhibit the transport of organic cations (41). Our data demonstrate that probenecid as well as furosemide inhibits the transport of the organic cation, N^1-methylnicotinamide, suggesting that the observed interaction between probenecid and cimetidine may be representative of a general inhibition of organic cation transport by organic anions.

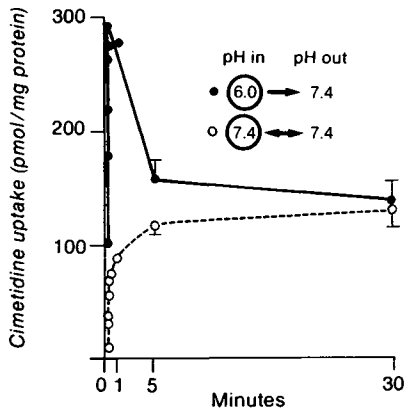

Fig. 4. The uptake of cimetidine in brush border membrane vesicles from rabbit renal cortex. Filled circles represent the uptake in the presence of an outwardly directed proton gradient (pH_{in}:pH_{out} = 6.0:7.4). Open circles represent the uptake at pH 7.4 in the absence of a proton gradient. The overshoot phenomenon suggests concentrative uptake of cimetidine in the presence of a pH gradient. Data are from Gisclon *et al.* (26).

RENAL CELL CULTURE

Renal cell culture has been used extensively to understand the factors involved in the growth and function of kidney cells (42–48). Table I lists some examples of commonly used continuous renal cell lines. In addition, methods for obtaining primary cell cultures have also been described (47,48). For studies of renal transport processes, cultured epithelia may provide specific advantages. Cells may be grown on permeable membranes to allow the convenient study of transport. Cells derived from cultured cell lines, such as LLC-PK and MDCK cells, are homogeneous, which is an important advantage since the kidney is an extremely heterogeneous organ. Transport characteristics delineated

Table I. Commonly Used Renal Epithelial Cell Lines

Cell line	Origin	Reference No.
A6	*Xenopus laevis* kidney	39
LLC-PK1	Pig kidney	40
MDCK	Dog kidney	41
OK	Opossum kidney	42

using methods such as kidney slice or membrane vesicles prepared from renal cortex necessarily reflect characteristics from a number of cell types. Cultured epithelia provide an excellent means for studying the regulation of transport by specific agents which may be included in the culture media. Recently, methods to culture well-defined segments of the human nephron have been developed which should allow important advances to be made in the understanding of transport processes in the human kidney (47). Care must be taken in selecting a specific culture to study drug transport. Certain cell lines retain selected transport properties but may be otherwise inappropriate for transport studies. For example, LLC-PK cells are reflective of proximal tubule cells and contain specific transporters including the organic cation transporter (42). In contrast, MDCK cells do not exhibit proximal tubule transport properties. Primary culture offers the advantage of being less dedifferentiated but may be less homogeneous. To date, there have been no studies characterizing cimetidine transport in cultured renal epithelia.

CONCLUSIONS

As exemplified by the studies of cimetidine, mechanisms involved in renal excretion can be delineated only by comparing and combining the data from experiments using diverse methodology. Clearance studies revealed that cimetidine is actively secreted. Stop-flow and isolated proximal tubule studies established the site of secretion for organic cations. The use of isolated brush border and basolateral membranes delineated the different characteristics of the transport processes in the two membranes. In the brush border membrane, cimetidine was shown to exchange with protons (or to be cotransported with hydroxyl ions), whereas in the basolateral membrane cimetidine transport did not appear to be driven by a pH gradient. Some questions remain unanswered. For example, it is not known whether the transport of cimetidine in the basolateral membrane involves an active or a passive process. The mechanisms of the interaction between organic anions and the organic cation transport system have to be delineated. Systems responsible for the transport of cimetidine and other drugs have not been successfully isolated and characterized. With the use of new technologies such as monoclonal cell lines and biotechnology, many of these questions may be resolved in the near-future.

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