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 H2-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 H2-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...
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), trimaterene (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).

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

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**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).