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# Effect of procainamide on renal tubular transport of cimetidine in the isolated perfused rat kidney

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**The effect of procainamide on renal tubular transport of cimetidine was studied in isolated perfused rat kidney based on the multiple indicator dilution (MID) technique. T-1824-labeled albumin (a vascular reference), [$^{14}$C]creatine (an extracellular reference), and [$^{3}$H]cimetidine were rapidly injected into the renal artery of isolated perfused rat kidney in the presence or absence of procainamide (100 $\mu$M) in the perfusate, and normalized outflow–time patterns were secured from rapidly sampled renal perfusate. A distributed two-compartmental model was fitted to the dilution data by non-linear least-squares regression, and the influx, efflux and sequestration rate constants were estimated. Net transport and influx processes of cimetidine were competitively inhibited by procainamide (PA), while the efflux and sequestration processes were increased. The increase in the values of the efflux and sequestration rate constants by addition of procainamide may be explained by the increase in the tissue binding of cimetidine. However, these three processes were not significantly affected by *p*-aminohippurate (PAH). These results suggest that both cimetidine and procainamide are secreted into the lumen by an organic base transport mechanism in the perfused kidney, in which the spatial organization and cell polarity of the kidney are maintained.**

## Introduction

Cimetidine, a histamine $H_2$-receptor antagonist, has been widely used for the treatment of peptic ulcer. Renal excretion plays a major role in the elimination of this drug. Depending on dose, sex and species (rat, dog or man), some 40–70% of an intravenous dose of cimetidine is excreted in the urine as unchanged drug within 24 h after administration [1]. Weiner and Roth [2] reported that the renal clearance of cimetidine was 2.6-times as large as the glomerular filtration rate at low plasma cimetidine concentration from their in vivo study in dogs. The results of various investigations, e.g., the perfusion of rabbit proximal tubules in vitro by McKinney et al. [3,4] indicated that cimetidine, an organic base, is actively and saturably secreted into the proximal tubules by the organic base transport system.

We previously reported that the MID technique is a sensitive new method with which to study the rate-limited process of renal tubular transport in rats [5–7]. Using this new technique, we examined the renal tubular transport of cimetidine in rats, and found that both the influx and sequestration processes (based on the unbound cimetidine concentration) were accounted for by the two mechanisms, i.e., Michaelis-Menten-type saturable transport and linear type passive diffusion, whereas the efflux process (from intracellular to the extracellular space) was independent of the intracellular cimetidine concentration. We observed also that, at the low dose of cimetidine, the influx clearance was larger than those of the efflux and sequestration, whereas at the high dose (above 100 $\mu$M) no significant difference was observed among these three clearances due to the large decrease in the influx clearance [8].

The purpose of the present study was to examine the inhibitory effect of PA, an organic cationic drug, on the renal tubular transport of cimetidine using isolated perfused rat kidney based on the MID method. The effect of PAH, an organic anionic drug, was also examined.

## Methods

### *Materials*

[*N-methyl*-$^{3}$H]Cimetidine (2.1 Ci/mmol) and [*carbonyl*-$^{14}$C]creatinine hydrochloride (80 $\mu$Ci/mg) were

Abbreviations: MID, multiple indicator dilution; PAH, *p*-aminohippurate; PA, procainamide; BSA, bovine serum albumin.

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purchased from Amersham International U.K. [G-$^3$H]Inulin (217 mCi/g) was obtained from New England Nuclear (Boston, MA). Bovine serum albumin (fraction V) and cimetidine (Tagamet®) were purchased from the Sigma Chemical, St. Louis, MO and Fujisawa Pharmaceutical Industry, Osaka, Japan, respectively. All other chemicals were obtained from the commercial sources and were of analytical grade.

*Kidney isolation and perfusion.* Male Wistar rats weighing 380–430 g were anesthetized with diethyl ether and operated on the right kidney by the method described by Nishitsutsuji-Uwo et al. [9] and the renal artery, the renal vein and the ureter were cannulated. An 18-gauge needle connected with a silicon tube (i.d. 2 mm, e.d. 4 mm) was used for the arterial cannula, a polyethylene tube (i.d. 2 mm, e.d. 3 mm) for the venous cannula, and a PE 10 polyethylene tubing (i.d. 0.58 mm, e.d. 0.61 mm) for the ureter cannule. Immediately after the operation, perfusion was started using a constant infusion pump (Mitsumi Science, Tokyo, Japan), then the right kidney was isolated and connected to the perfusion apparatus. The kidney was maintained at 37°C and the perfusion pressure was kept at $100 \pm 10$ mmHg during the experimental period. Krebs-Henseleit buffer (NaCl 118 mM/KCl 4.7 mM/$CaCl_2$ 2.5 mM/$KH_2PO_4$ 1.19 mM/$MgSO_2 \cdot 7H_2O$ 1.19 mM/$NaHCO_3$ 25 mM) containing 6% bovine serum albumin, 10 mM glucose and 20 mg/dl creatinine oxygenated with 95% $O_2$/5% $CO_2$ used as the perfusate.

In all rats, mannitol was added to the perfusate at the concentration of 0.1% to ensure adequate urine sample volumes to permit all the necessary chemical analysis for each renal function [10].

*Renal function of isolated perfused kidney.* The glucose reabsorption ratio and the urinary pH were measured by the glucose oxidase method and with a glass electrode, respectively. GFR was determined according to the method described by Whiteside and Silverman [11]. All experiments were performed within 40 min after initiation of perfusion. During perfusion, isolated kidneys maintained a normal uniform color without molting. Perfusion rates were 6–10 ml/min per g kidney with a perfusion pressure of $100 \pm 10$ mmHg. Urine flow was 60–100 $\mu l \cdot min^{-1}$. During all experiments (40 min), GFR and the reabsorption ratio of glucose were 180–200 $\mu l \cdot min^{-1}$ and 90–96%, respectively.

*MID method.* The pulse-injection MID technique originally developed by Goresky et al. [12–16] was used. The injection solution (0.2 ml) consists of T1824-labeled albumin (plasma reference, 0.7 mg of T1824 and 12 mg of BSA were added), 0.17 $\mu$Ci of [$^{14}$C]creatinine or [$^{14}$C]sucrose (extracellular reference), and 1.7 $\mu$Ci of [$^3$H]cimetidine. For the measurement of GFR, [$^3$H]inulin with T1824-labeled albumin was injected. In order to raise the cimetidine level from 0.37 $\mu$M to 10 mM, unlabeled cimetidine was added to the perfusate. After 10 min perfusion to obtain the steady state, 0.2 ml of the injection solution was administered with a pulse-injection through the silicone-tube cannula to the right renal artery. The usual collections consisted of 20 perfusate samples collected at a rate of 1 s per sample. At 10 min intervals, in order to obtain steady-state, three or four injections were repeated. At the end of each experiment, the right kidney was excised, weighed and examined for any gross abnormality. The mean transit time for each material was calculated using the outflow fraction during 20 s by using the following equation:

$$\bar{t} = \sum_{t=0}^{20} C(t)t / \sum_{t=0}^{20} C(t) \qquad (3)$$

where $t$ and $C(t)$ represent the time and the concentration of a material at time, $t$, respectively. The mean transit time was corrected for the catheter delay by subtracting the catheter mean transit time (0.82 s) calculated from the ratio of the catheter volume to the perfusion rate. The delay became a part of the large vessel transit time ($t_0$).

*Effect of PA and PAH on cimetidine secretion.* 100 $\mu$M PA or PAH was added to the perfusate to examine the effect of PA and PAH on cimetidine secretion. The changes in the values of $K_1 (= k_1 \cdot \theta/(1+\gamma))$, $K_2$ and $K_3$ (see Data analysis) were determined. Concentrations of cimetidine, PA and PAH used in each run are listed in Table I.

*Protein binding of cimetidine.* The unbound fraction of cimetidine in the perfusate was determined by equilibrium dialysis at 37°C for 4 h using semimicrocelles [17] and a Spectrapor membrane tubing (type 2, Spectrum Medical Industries, Los Angeles, CA) against Krebs-Henseleit buffer (pH 7.4), containing 1 $\mu$M–1 mM cimetidine.

*Tissue binding of cimetidine.* Rat renal cortex was homogenized with 3-fold excess volume of 50 mM Tris-HCl buffer (pH 7.0) in a Teflon-glass homogenizer. 1 ml of homogenate containing $^3$H-labeled cimetidine (1.5 $\mu$Ci), unlabeled (1–1000 $\mu$M) and PA or PAH (1 mM) was applied to the membrane cone (Amicon Centriflo Ultrafiltration Membrane Filter Cone, type CF-25, Lexington, MA) after incubation at 37°C for 30 min. The radioactivity of [$^3$H]cimetidine was determined and the unbound fraction of tissue was calculated according to the equation described by Lin et al. [18]. The adsorption ratio of cimetidine (14%) was corrected.

*Assays.* Radioactivity of [$^3$H]inulin (or cimetidine) and [$^{14}$C]creatinine in the sample solution was determined in a Tri-Carb liquid scintillation spectrometer (Packard Instruments; model 3255, Downers Grove, IL). 50 $\mu$l of perfusate sample was added to a scintillation vial containing 10 ml of counting solution (2 l toluene/1 l Triton X-100/ 8 g PPO/0.2 g POPOP. The appropriate crossover correction was given to separate

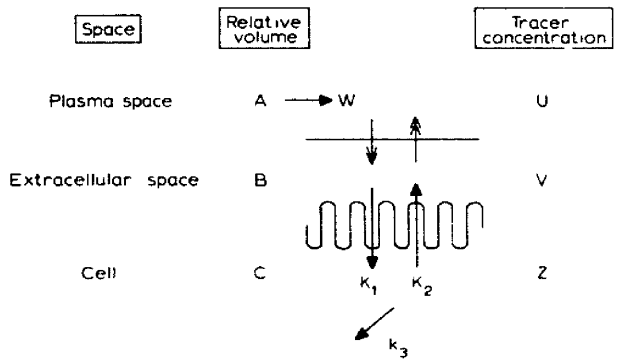


Fig. 1. Kinetic models utilized to derive fitted parameters from experimental indicator dilution curves for cimetidine. Arrows show the directions of flow and transport for substances. Double arrows show the flow-limited distribution of cimetidine.

the radioactivities of $^3H$ and $^{14}C$. For T1824-labeled albumin, 50 μl of perfusate was diluted with 3 ml distilled water and was immediately measured at 610 nm in a Hitachi 557 double-wavelength double-beam spectrophotometer (Hitachi, Tokyo, Japan). Creatinine and glucose were measured using Creatinine-Test Wako and Glucose-B-Test Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively. Absorbance at 505 nm for creatinine and at 520 nm for glucose were measured in a Hitachi 557 double-wavelength double-beam spectrophotometer, respectively.

*Data analysis*

*Analysis of indicator dilution curves.* Data were analyzed according to the flow-limited diffusion model of Goresky et al. [12–16] and Sawada et al. [6] (Fig. 1). The assumptions for the development of the model were described previously [7]. The concentration of cimetidine in the effluent at time, $t$, is expressed by

$$C_{cim}(t) = \exp(-K_1 \cdot t) \cdot C_{cre}(t) + \exp\{-(K_2 + K_3) \cdot t\} \times \int_0^t \exp\{-(K_1 - K_2 - K_3) \cdot \tau\} \times C_{cre}(\tau) \times \sqrt{\frac{\tau \cdot K_1 \cdot K_2}{t - \tau}} \times I_1 \cdot [2 \cdot K_1 \cdot K_2 \cdot \tau \cdot (t - \tau)] d\tau \quad (5)$$

and

$$K_1 = k_1 \theta / (1 + \gamma) \quad (6)$$

where $\gamma$ is the ratio of the extracellular space to the plasma space, $\theta$ is the ratio of the cellular space to the plasma space, $\tau$ is the capillary transit time (dummy variable), $C_{cre}(t)$ is the concentration of the extracellular reference appearing at the outflow, $C_{cim}(t)$ is the concentration of cimetidine appearing at the outflow, $k_1$ is the influx rate constant from the extracellular space to the cellular space, $K_2$ the efflux rate constant from the cellular space to the extracellular space and $K_3$ the sequestration rate constant from the cellular space to the luminal side.

Experimental data were fitted to Eqn. 5 to obtain the values of $K_1$, $K_2$ and $K_3$ by a nonlinear least-squares method using a digital computer as described previously in Refs. 6 and 19). The coefficient of variation of the fit was quantitated as

$$\text{C.V.} = \sqrt{\frac{\sum_{i=1}^{n} (\log \exp_i - \log \text{calc}_i)^2 / (n-3)}{(1/n) \sum_{i=1}^{n} \log \exp_i}} \quad (7)$$

where $n$ is the total number of data points, $\exp_i$ is the experimentally observed value at point $i$, and $\text{calc}_i$ is the corresponding calculated value. At the early phase after the bolus injection, when cimetidine returning from the cells contributes no quantitatively important component to the outflow curves, Eqn. 5 may be transformed into:

$$C_{cim}(t) = \exp(K_1 \cdot t) \cdot C_{cre}(t) \quad (8)$$

so that

$$\ln[C_{cre}(t)/C_{cim}(t)] = K_1 \cdot t \quad (9)$$

A semilogarithmic plot of the ratio of [$^{14}$C]creatinine to [$^3$H]cimetidine versus time generated a straight line with positive slope over the initial second following the bolus injection. As defined by Goresky et al. [12,17], the magnitude of this slope is equivalent to $K_1$ ($= k_1 \cdot \theta/(1 + \gamma)$) (see Eqn. 9). In this paper, the relationships between $\ln(C_{cre}/C_{cim})$ and time ($t$) are shown in Fig. 3. The apparent initial slope of a plot of the natural logarithm of the ratio of the [$^{14}$C]creatinine to [$^3$H]cimetidine outflow fraction was used as the initial estimate of $K_1$ for determining the rate constants using a nonlinear least-squares program [6,19].

The distribution volumes of albumin and creatinine were calculated by means of the following equations:

$$Vd_{alb} = F \cdot (\bar{t}_{alb} - t_0) \quad (10)$$

$$Vd_{cre} = F \cdot (\bar{t}_{cre} - t_0) \quad (11)$$

where $F$, $\bar{t}_{alb}$, $\bar{t}_{cre}$ and $t_0$ are the total flow through the system, the mean transit time of albumin, the mean transit time of creatinine and the large vessel transit time, respectively.

*Statistical analysis.* All means are presented with their standard errors (the mean ± S.E.). Statistical significance was analyzed according to Student's $t$-test, using two tails.

## Results

Fig. 2 shows a typical dilution curve of [$^3$H]cimetidine, [$^{14}$C]creatinine and T-1824-albumin. Fig. 3 shows

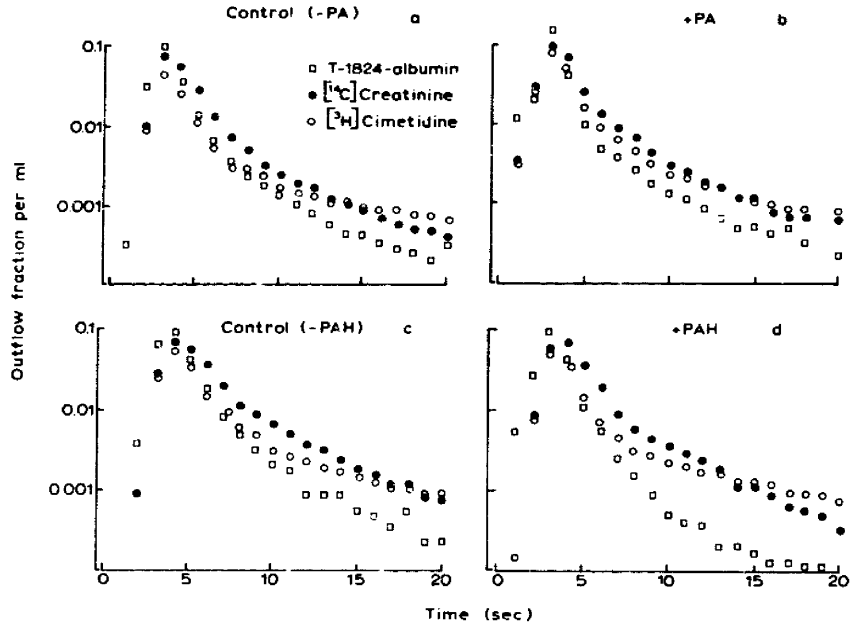


Fig. 2. Renal vein outflow curves for [$^3$H]cimetidine (○), [$^{14}$C]creatinine (●) and T-1824-albumin (□). Abscissae: time in seconds; ordinates: outflow fraction. (a) Control kidney; (b) PA-treated kidney; (c) control kidney; (d) PAH-treated kidney.

a typical natural logarithm of the ratio $C(t)_{cre}/C(t)_{cim}$ vs. time in the presence or absence of PA or PAH. The effects of PA and PAH on the mean transit time and the tubular transport rate constants for cimetidine are listed in Table I. The mean transit times of cimetidine and creatinine were longer than that of albumin, similarly to the case without any inhibitors [18]. In the control studies, the distribution volumes were $0.31 \pm 0.02$ ml/g kidney and $0.46 \pm 0.03$ ml/g kidney ($n = 15$) for albumin ($V_{d.alb}$) and creatinine ($V_{cre}$) at the cimetidine concentration of 1 μM. At the same concentration of cimetidine, these volumes were $0.26 \pm 0.02$ ml/g kidney for albumin and $0.35 \pm 0.10$ ml/g kidney for creatinine in the presence of PA, and $0.35 \pm 0.10$ ml/g kidney for albumin, $0.54 \pm 0.11$ ml/g kidney for creatinine in the presence of PAH ($n = 3$). Therefore, PAH or PA did not significantly change either the plasma volume or the extracellular volume.

The secretory parameters for cimetidine determined by computer fitting [6,19] are shown in Table I and Fig. 4. In the presence of 100 μM PA, the influx rate constant decreased as compared with that for the control (Table II). Assuming one Michaelis-Menten equa-

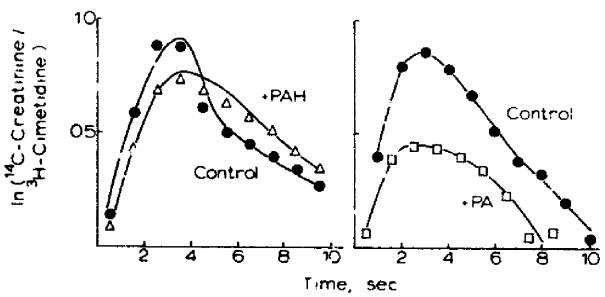


Fig. 3. Representative natural logarithm curves of the ratio (creatinine outflow per ml/cimetidine outflow fraction per ml) vs. time. Lines were calculated by the MID-SALS method [6,19] using a digital computer. Panel a: effect of PAH (100 μM) on [$^3$H]cimetidine uptake; panel b: effect of PA (100 μM) on [$^3$H]cimetidine uptake. Key: ●, control; △, with PAH; and □, with PA.

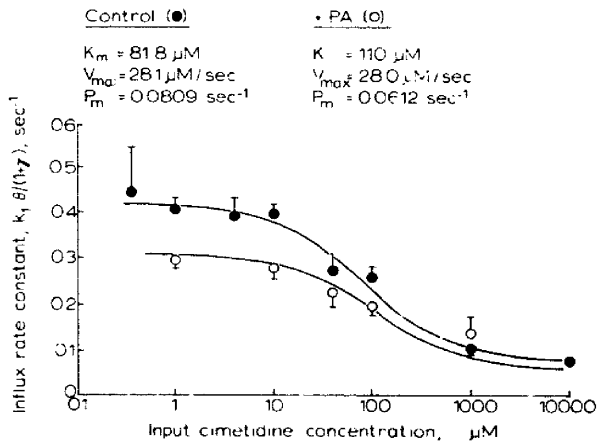


Fig. 4. Effect of PA on the influx rate constant of cimetidine. Each point and vertical bar represents the mean and S.E. of three to five experiments. Equation in the inset was used for fitting the points. $K_m$, $V_{max}$ and $P_m$ are the Michaelis constant, the maximum velocity ($\mu M \cdot s^{-1}$) and the permeability coefficient for the passive transport, respectively. Lines were calculated by the 'MULTI' method [19] using a microcomputer. Key: ●, control; and ○, with PA. $k_1 \cdot \theta/(1+\gamma) = [V_{max}/(K_m + C)] + P_m$.

TABLE I

*Experimental results*

The mean transit times were corrected for the inflow and outflow catheter transit times. $K_1$, $K_2$ and $K_3$ represent the influx, efflux and sequestration rate constants, respectively, and C.V. represents the coefficient of variation. Abbreviations: alb: albumin, cre: creatinine, CMD: cimetidine, PA: procainamide and PAH: *p*-aminohippurate.

| Exp. No. | Kidney weight (g) | Perfusion rate ($ml \cdot min^{-1}$) | Input CMD concn. ($\mu M$) | Inhibitor concn. ($\mu M$) | $t_{alb}$ ($s^{-1}$) | $t_{cre}$ ($s^{-1}$) | $t_{CMD}$ ($s^{-1}$) | Derived values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $K_1$ ($s^{-1}$) | $K_2$ ($s^{-1}$) | $K_3$ ($s^{-1}$) | C.V. |
| 1–1 | 1.9 | 15.0 | 1 | PA(100) | 2.29 | 3.09 | 3.39 | 0.292 | 0.101 | 0.0922 | 0.0127 |
| 2 | | 12.0 | 10 | PA(100) | 2.97 | 4.22 | 4.13 | 0.264 | 0.110 | 0.0718 | 0.0105 |
| 3 | | 12.0 | 100 | PA(100) | 3.22 | 4.74 | 4.43 | 0.175 | 0.132 | 0.0598 | 0.00650 |
| 4 | | 10.5 | 1000 | PA(100) | 3.32 | 4.79 | 4.31 | 0.0788 | 0.103 | 0.0366 | 0.00430 |
| 2–1 | 1.9 | 11.0 | 1 | – | 2.56 | 3.91 | 4.16 | 0.433 | 0.0721 | 0.0574 | 0.0128 |
| 2 | | 11.0 | 1 | PA(100) | 2.31 | 3.19 | 3.38 | 0.325 | 0.114 | 0.0763 | 0.0131 |
| 3 | | 11.0 | 10 | PA(100) | 2.32 | 3.17 | 3.39 | 0.325 | 0.106 | 0.0643 | 0.0112 |
| 4 | | 11.0 | 100 | PA(100) | 1.93 | 2.99 | 3.19 | 0.231 | 0.143 | 0.0474 | 0.00950 |
| 3–1 | 2.0 | 13.2 | 1 | – | 2.10 | 3.00 | 3.27 | 0.402 | 0.0647 | 0.0860 | 0.0152 |
| 2 | | 13.0 | 1 | PA(100) | 2.30 | 3.02 | 2.90 | 0.273 | 0.102 | 0.0862 | 0.0112 |
| 3 | | 12.0 | 10 | PA(100) | 2.31 | 3.65 | 3.44 | 0.252 | 0.0979 | 0.0576 | 0.00570 |
| 4 | | 12.0 | 100 | PA(100) | 2.49 | 3.42 | 3.19 | 0.187 | 0.116 | 0.0621 | 0.00470 |
| 4–1 | 1.5 | 12.0 | 40 | – | 3.60 | 5.16 | 5.36 | 0.175 | 0.101 | 0.0489 | 0.00540 |
| 2 | | 12.0 | 40 | PA(100) | 3.40 | 4.85 | 4.81 | 0.136 | 0.108 | 0.0563 | 0.00500 |
| 5–1 | 1.9 | 14.0 | 1 | – | 2.09 | 3.37 | 3.69 | 0.355 | 0.116 | 0.0649 | 0.0145 |
| 2 | | 14.0 | 40 | – | 2.10 | 3.60 | 3.67 | 0.293 | 0.125 | 0.0553 | 0.0114 |
| 3 | | 11.2 | 40 | PA(100) | 2.68 | 4.22 | 4.12 | 0.205 | 0.141 | 0.0552 | 0.00820 |
| 6–1 | 1.5 | 13.2 | 1 | – | 1.90 | 2.32 | 3.81 | 0.308 | 0.101 | 0.0580 | 0.0149 |
| 2 | | 15.0 | 4 | – | 2.39 | 4.02 | 4.53 | 0.361 | 0.126 | 0.0527 | 0.0141 |
| 3 | | 12.0 | 40 | – | 2.41 | 3.88 | 3.77 | 0.283 | 0.142 | 0.0658 | 0.0216 |
| 4 | | 12.0 | 40 | PA(100) | 2.50 | 3.73 | 3.51 | 0.266 | 0.202 | 0.0891 | 0.0119 |
| 7–1 | 1.9 | 16.0 | 1 | – | 2.39 | 3.49 | 3.37 | 0.240 | 0.102 | 0.106 | 0.00770 |
| 2 | | 16.0 | 4 | – | 2.50 | 3.75 | 3.78 | 0.247 | 0.102 | 0.0959 | 0.00960 |
| 3 | | 14.4 | 40 | PA(100) | 2.33 | 3.51 | 3.51 | 0.190 | 0.0989 | 0.0656 | 0.00940 |
| 4 | | 14.0 | 000 | PA(100) | 1.63 | 2.86 | 2.82 | 0.0743 | 0.191 | 0.0536 | 0.00610 |
| 8–1 | 1.8 | 15.0 | 1 | – | 1.30 | 2.61 | 3.93 | 0.467 | 0.0900 | 0.0357 | 0.0140 |
| 2 | | 17.0 | 40 | PA(100) | 2.23 | 3.80 | 4.55 | 0.348 | 0.153 | 0.0563 | 0.00460 |
| 3 | | 16.0 | 1000 | PA(100) | 2.22 | 3.50 | 3.44 | 0.179 | 0.330 | 0.0512 | 0.00660 |
| 9–1 | 1.6 | 16.0 | 1 | – | 2.14 | 3.38 | 4.25 | 0.534 | 0.0876 | 0.0594 | 0.0166 |
| 2 | | 16.0 | 1 | PAH(100) | 3.21 | 4.43 | 4.42 | 0.288 | 0.0874 | 0.0760 | 0.00820 |
| 10–1 | 1.8 | 14.0 | 1 | – | 1.90 | 3.34 | 3.43 | 0.391 | 0.0700 | 0.0728 | 0.0118 |
| 2 | | 11.2 | 1 | PAH(100) | 1.97 | 3.35 | 3.02 | 0.334 | 0.0889 | 0.0772 | 0.00800 |
| 11–1 | 1.9 | 15.0 | 1 | – | 2.35 | 3.70 | 3.74 | 0.458 | 0.103 | 0.0642 | 0.0190 |
| 2 | | 14.0 | 1 | PAH(100) | 2.43 | 4.00 | 3.92 | 0.406 | 0.143 | 0.0704 | 0.0118 |

TABLE II

*Effect of PA and PAH on the secretory parameters of cimetidine*

Results are the means ± S.E.

| | $k_1\theta/(1+\gamma)$ [a] ($s^{-1}$) | $K_2$ [b] ($s^{-1}$) | $K_3$ [c] ($s^{-1}$) |
|---|---|---|---|
| Control ($N = 5$) | 0.443 ± 0.0255 | 0.0794 ± 0.00700 | 0.0679 ± 0.0523 |
| + PA ($N = 3$) | 0.297 ± 0.0149 [d] | 0.105 ± 0.00414 [e] | 0.0849 ± 0.00464 |
| + PAH ($N = 3$) | 0.343 ± 0.0343 | 0.106 ± 0.0181 | 0.0745 ± 0.00210 |

[a] Influx rate constant.
[b] Efflux rate constant.
[c] Sequestration rate constant.
[d] Significantly different ($P < 0.02$) from the control.
[e] Significantly different ($P < 0.01$) from control.

TABLE III

*Effect of PA and PAH on the binding of cimetidine to rat kidney cortex homogenate*

Results are the means ± S.E. The unbound fraction of tissue ($f_T$) was determined by ultrafiltration.

| | $f_T$ |
|---|---|
| Control ($N = 6$) | 0.139 ± 0.0184 |
| + PA ($N = 3$) | 0.187 ± 0.0042 [a] |
| + PAH ($N = 3$) | 0.143 ± 0.0045 |

[a] Significantly different ($P < 0.01$) from control.

tion and one passive diffusion, the values of $V_{max}$ (maximum influx rate per unit volume of extracellular substance), $K_m$ (Michaelis-Menten constant) and $P_m$ (permeability rate constant) were calculated to be 28.1 $\mu M \cdot s^{-1}$, 81.8 $\mu M$ and 0.0809 $s^{-1}$ for the control study, respectively, and 28.0 $\mu M \cdot s^{-1}$, 110 $\mu M$ and 0.0612 $s^{-1}$ for the PA treatment, respectively (Fig. 4). Table II also shows the effects of PA and PAH on the efflux and sequestration rate constants at the cimetidine concentration of 1 $\mu M$. The efflux rate constant increased in the presence of PA. On addition of 1 mM PA, the unbound fraction of cimetidine at the total concentration of 1 mM significantly increased from 0.14 to 0.19 (Table III). PAH also decreased the influx rate constants tended to increase in the presence of PAH, but the increase was not significant.

## Discussion

The merits of applying the MID method for analyzing renal secretion process are (1) the rates of influx and efflux processes across the basolateral membranes and the sequestration process can be determined separately, and the rate-limiting step in the tubular secretion can be defined; (2) by using the intact kidney, it is possible to compare the kinetic parameters obtained by the MID method with that obtained using in vivo kidney.

In our previous report [7], we have determined the influx, efflux and sequestration rate constants of cimitidine by the MID method and reported that the influx and sequestration processes of cimetidine consist of the passive diffusion and Michaelis-Menten-type saturable transport system, whereas the efflux rate constants for the unbound cimetidine were independent of the intracellular cimetidine concentrations. The $K_m$, $V_{max}$ and $P_m$ values for the influx process based on the extracellular unbound cimetidine and for the sequestration process based on the intracellular unbound cimetidine were 58.2 $\mu M$, 27.3 $\mu M \cdot s^{-1}$ and 0.108 $s^{-1}$, and 0.0448 $\mu M$, 0.0832 $\mu M \cdot s^{-1}$ and 0.268 $s^{-1}$, respectively [8].

In the presence of PA, and organic base, the influx rate of cimetidine was decreased. Kinetic analysis based

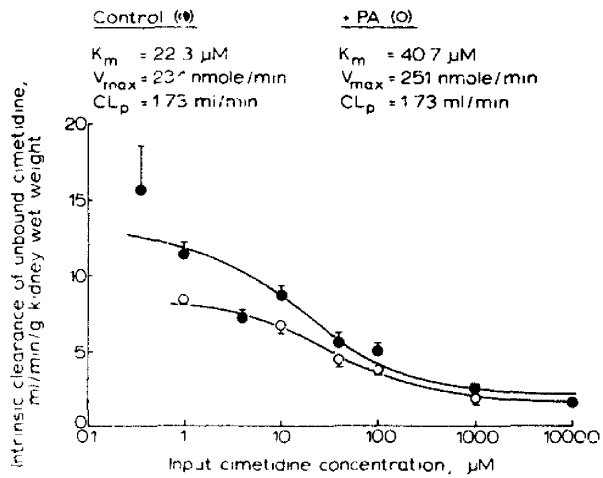


Fig. 5. Effect of PA on the intrinsic clearance of unbound cimetidine. Each point and vertical bar represents the mean and S.E. of three to five experiments. Equation in the inset was used for fitting the points. The $K_m$, $V_{max}$ and $P_m$ are the Michaelis constant, the maximum velocity ($\mu M \cdot s^{-1}$) and the permeability coefficient for the passive transport, respectively. Lines were calculated by the 'MULTI' method [19] using a microcomputer. Key: ●, control; and ○, with PA. $Clu_{int} = [V_{max}/(K_m + C)] + CL_p$.

on the input concentration of cimetidine (Fig. 4) showed that the effect of PA on the influx process was a competitive inhibition for the carrier-mediated transport of cimetidine, but the passive diffusion was not changed. This finding indicates that cimetidine is transported into the renal proximal tubular cells through this cation-transport system.

1 mM PA displaced cimetidine bound to the kidney tissue (Table III). The increase in the values of $K_2$ and $K_3$ by addition of PA may be explained by the increase in the tissue binding of cimetidine.

We have calculated the apparent intrinsic clearance ($CL_{int,app}$) using the influx, efflux and sequestration rate constants according to the equation previously described in Ref. 7 (see Fig. 5). We can compare the $CL_{int,app}$ with that obtained by other experimental methods. The $K_m$ value for apparent intrinsic clearance, 82 $\mu M$, in the absence of PA was comparable with the value, 39 $\mu M$, reported by McKinney [3,4]. Addition of PA increased only the $K_m$ value, whereas the values of $V_{max}$ and $CL_p$ (clearance for the passive diffusion) did not change, indicating a competitive inhibition of cimetidine secretion by PA. McKinney has also reported that cimetidine transport was competitively inhibited by PA using isolated perfused rabbit renal tubules [3,4].

PAH tended to inhibit the influx process of cimetidine, although this effect was not significant. These data agree with the findings of McKinney [4], that is, PAH also slightly inhibited cimetidine secretion in the isolated perfused rabbit tubules. From these findings, it is suggested that cimetidine is transported mainly by the

organic cation and partly by the organic anion-transport system.

In conclusion, we have estimated the influx, efflux and sequestration rate constants of cimetidine and revealed the inhibitory effect of PA on cimetidine transport using a multiple indicator dilution technique in the isolated perfused rat kidney, in which the spatial organization and cell polarity of the kidney are maintained.

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