Renal Tubular Handling of *p*-Aminohippurate and Epidermal Growth Factor (EGF) in Filtering and Nonfiltering Perfused Rat Kidneys

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We examined the integrity of renal tubular function in filtering and nonfiltering isolated perfused rat kidneys by using *p*-amino-^{3}H-hippurate (^{3}H-PAH) and the multiple indicator dilution method with ^{14}C-creatinine as a reference. The influx clearance ($PS_{u,I}$) of unbound ^{3}H-PAH was 0.37 and 0.38 ml/sec in the filtering and nonfiltering kidneys, respectively. The efflux rate constants were comparable between filtering and nonfiltering kidneys, while the sequestration rate constant in the filtering kidney was approximately three times larger than that in the nonfiltering kidney. These data suggest that the nonfiltering kidney maintains ^{3}H-PAH transporting ability through the antiluminal plasma membrane. The renal handling of epidermal growth factor (EGF) by filtering and nonfiltering kidneys was compared. The ratio of the total uptake of tracer ^{125}I-EGF over 20 min in the nonfiltering kidney to that in the filtering kidney was 0.8. This ratio was reduced to 0.2 when the kidneys were perfused with tracer ^{125}I-EGF plus 20 n*M* EGF. Furthermore, the total uptake of tracer ^{125}I-EGF in the nonfiltering kidney was reduced 20-fold in the presence of 20 n*M* unlabeled EGF. These findings suggest that the tubular uptake of tracer ^{125}I-EGF by filtering kidney takes place mainly via the antiluminal plasma membrane and that this uptake is a saturable process.

KEY WORDS: epidermal growth factor; filtering and nonfiltering perfused rat kidneys; antiluminal uptake; *p*-aminohippurate; multiple indicator dilution method.

INTRODUCTION

Previous *in vivo* studies have shown that the kidney is one of the main organs responsible for the elimination of epidermal growth factor (EGF) (1,2) via uptake through receptor-mediated endocytosis (1). The existence of specific binding sites for EGF in the antiluminal plasma membrane in several nephron segments (3,4) has been demonstrated, whereas EGF binding by the brush border membrane is minimal. However, the contribution of antiluminal EGF receptors to the uptake of plasma EGF has not been quantitated.

Nonfiltering perfused rat kidneys, prepared by raising perfusate oncotic pressure to counterbalance filtration forces, have been used to investigate the antiluminal transport of proteins (5), since luminal reabsorption and catabolism of proteins after filtration need not be considered. The integrity of these nonfiltering preparations has been poorly characterized and few indices of normality have been identified. Therefore, the present study initially characterized the biological integrity of the nonfiltering perfused rat kidney by analyzing the renal uptake of *p*-amino-^{3}H-hippurate (6) using the multiple indicator dilution method (7). The uptake of ^{125}I-EGF in filtering and nonfiltering isolated perfused rat kidneys was then determined.

MATERIALS AND METHODS

Materials

Carbonyl ^{14}C-creatinine hydrochloride (80 μCi/mg) and *p*-amino-^{3}H-hippurate (^{3}H-PAH) were purchased from Amersham International U.K. Biosynthetic human epidermal growth factor (EGF), obtained from *Escherichia coli* (8), was used in all experiments. Sodium iodide-125 (100 mCi/ml) was purchased from the Radiochemical Center (Amersham Corp., Arlington Heights, IL). EGF was radiolabeled with ^{125}I-Na by the chloramine-T method (9). The ^{125}I-EGF had a specific activity of 0.5 to 1.0 mCi/mol. ^{14}C-Inulin (2.6 μCi/mg) was purchased from ICN Radiochemicals (Division of ICN Biomedicals, Irvine, CA). Bovine serum albumin (BSA) fraction V was purchased from Sigma, l-amino acids were from Wako Chemical, and all other chemicals were obtained from commercial sources and were of analytical grade.

Kidney Isolation and Perfusion

The isolated perfused rat kidney technique previously developed in our laboratory (10) has been slightly modified. After male Wistar rats weighing 340–390 g were anesthetized with ether, the right renal artery was cannulated via the mesentric artery. The renal vein and the ureter were also cannulated. The perfusate composition has previously been described (5). Immediately after the operation, the kidney was removed from the animal and perfused in a closed-circuit system. Unless otherwise noted, kidneys were perfused at 37°C with 100 ml of perfusate. The perfusate albumin concentrations were 5 and 10 g/dl for filtering and nonfiltering modes, respectively.

Multiple Indicator Dilution Method (MID)

Filtering Kidney. The multiple indicator dilution technique (10,11) was used. The isolated rat kidneys were perfused in a filtering mode with drug free perfusate. Perfusate samples were collected every 5 min; urine was also collected during a 20-min predose period. After 20 min, 200 μl (2.0 μCi) of ^{3}H-PAH and 0.2 μCi of ^{14}C-creatinine [extracellular reference (10,12)] were administered by a pulse injection through the cannula in the right renal artery. The outflow from the renal vein cannula was collected every 0.5 sec for 10 sec. The kidneys were separated from the system 20 sec postdose and ^{14}C and ^{3}H remaining in the kidney were determined.

Nonfiltering Kidney. The perfusate samples were collected every 5 min for 20 min during the predose period. ^{3}H-PAH and ^{14}C-creatinine were administered, and samples from the renal vein were collected as described above.

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Analysis of MID Data

Model-Independent Analysis (13). The mean transit times ($\bar{t}$) were calculated based on the following equation:

$$\bar{t} = \int_0^\infty t \cdot C\, dt \Big/ \int_0^\infty C\, dt \quad (1)$$

where t is time and C is the outflow concentration. The availabilities (F) of ^{14}C-creatinine and ^{3}H-PAH were calculated by the following equations:

$$F_{cre} = Q \cdot AUC_{cre}/D_{cre} \quad (2)$$

$$F_{PAH} = Q \cdot AUC_{PAH}/D_{PAH} \quad (3)$$

where Q is the total flow, AUC is the area under the outflow concentration time curve to infinite time, and D is the administered dose. The distribution volumes were then calculated as follows:

$$V_{d_{cre}} = Q \cdot (\bar{t}_{cre} - t_o)/F_{cre} \quad (4)$$

$$V_{d_{PAH}} = Q \cdot (\bar{t}_{PAH} - t_o)/F_{PAH} \quad (5)$$

where t_o is the catheter transit time (13).

Model-Dependent Analysis (7). The distributed model (11) can be used to analyze tubular secretion (14). By simultaneously fitting the outflow dilution curves of ^{3}H-PAH and ^{14}C-creatinine to a previously described model (Eq. A6 in Ref. 10) using a nonlinear least-squares method (15), three parameters were determined for ^{3}H-PAH: K_1 (influx rate constant, K_2 (efflux rate constant), and K_3 (sequestration rate constant). Immediately after the bolus injection, the model equation can be simplified to

$$\ln [C_{cre}(t)/C_{PAH}(t)] = K_1 \cdot t \quad (6)$$

where $C_{cre}(t)$ and $C_{PAH}(t)$ are the outflow concentrations of creatinine and PAH. A semilogarithmic plot of the ^{14}C-creatinine/^{3}H-PAH ratio [ratio plot (10)] provides an estimate for K_1 as the slope of initial rising phase. The influx clearance of ^{3}H-PAH was calculated by (7)

$$PS_1 = K_1 \cdot V_{d_{cre}} \quad (7)$$

and corrected for the ^{3}H-PAH unbound fraction (f_u) in the perfusate using:

$$PS_{u,1} = PS_1/f_u \quad (8)$$

Assay

Radioactivities of ^{3}H-PAH and ^{14}C-creatinine were determined in a Tri-Carb liquid scintillation spectrometer (model 3255, Packard Instruments Corp., Downers Grove, IL). An appropriate crossover correction was given to separate the contributions of ^{3}H and ^{14}C. The concentrations of creatinine and glucose were determined using commercial kits (Creatinine-Test Wako and Glucose-B-Test Wako (Wako pure Chemical industries, Ltd., Osaka, Japan). The concentration of Na^+ in the perfusate and urine were determined by atomic absorption.

Protein Binding of *p*-Amino-^{3}H-hippurate

Perfusate samples (2 ml) containing 5 or 10% BSA were incubated at 37°C for 5 min in the presence of 0.1 μCi ^{3}H-PAH. Forty microliters of incubation medium was transferred to an Amicon tube (MPS-3) and centrifuged for 5 min at 1000g. The f_u was calculated by dividing the unbound by total drug concentrations.

Comparison of Uptake of ^{125}I-EGF Between Nonfiltering and Filtering Isolated Perfused Rat Kidneys

The nonfiltering and filtering rat kidneys were perfused in a recirculating mode with tracer ^{125}I-EGF or tracer ^{125}I-EGF plus 20 nM unlabeled EGF. After 20 min, the kidneys were switched to a EGF free perfusate and washed for 6 min to remove ^{125}I-EGF in the extracellular and luminal spaces. Kidneys were counted for ^{125}I radioactivity after the wash. The fractional uptake of EGF by the perfused kidney was calculated by dividing ^{125}I radioactivity associated with the kidney by the dose of ^{125}I-EGF (the amount of ^{125}I-EGF added to the perfusate).

RESULTS

Characterization of Perfused Rat Kidneys

Table I shows the functional properties of the filtering and nonfiltering isolated perfused rat kidneys during the 20-min perfusion period. The glomerular filtration rates (determined by creatinine clearance), the urine flow, and the reabsorption of both glucose and Na^+ were within the ranges seen for normal kidneys (16).

Handling of ^{3}H-PAH by Filtering and Nonfiltering Kidneys

Figure 1 shows the ratio plots of the dilution curves of ^{3}H-PAH and the ^{14}C-creatinine in the nonfiltering and filtering kidneys. Table II summarizes the kinetic parameters obtained. Both the mean transit time and the distribution volume of ^{14}C-creatinine in the filtering kidney were comparable to those observed in the nonfiltering kidney. The distribution volume of ^{3}H-PAH was significantly larger than that of ^{14}C-creatinine in both the filtering and the nonfiltering kidneys ($P < 0.05$). The availabilities of ^{3}H-PAH in the perfused kidneys demonstrated that 40–45% of injected ^{3}H-PAH was extracted during the single pass through the kidney. The f_u for ^{3}H-PAH in the perfusate was 0.73 ± 0.01 for

Table I. Overall Functional Properties of the Filtering and Nonfiltering Isolated Perfused Rat Kidneys[a]

	Filtering kidney	Nonfiltering kidney
Rat body weight (g)	361 ± 15	360 ± 10
Perfusate flow rate (ml/min/kidney)	17.0 ± 1.0	16.3 ± 0.3
Pressure (mm Hg)	101 ± 2	85 ± 4
GFR (μl/min/kidney)	413 ± 181	NA[b]
Urine flow (μl/min/kidney)	25 ± 7	NA
Glucose reabsorption (%)	98.3 ± 1.7	NA
Na^+ reabsorption (%)	96.5 ± 1.9	NA

[a] See details under Materials and Methods.
[b] Not applicable because no urine was formed.

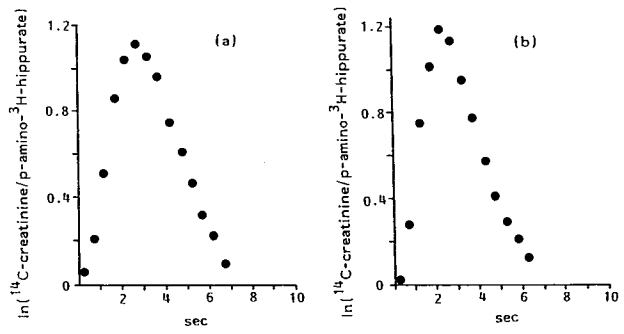

Fig. 1. Representative ratio plots of ^{3}H-PAH in the nonfiltering (a) and filtering (b) isolated perfused rat kidneys. ^{14}C-Creatinine was used as the extracellular reference.

the filtering kidney (5% BSA) and 0.50 ± 0.04 in the nonfiltering kidney. The model analysis of dilution curves showed that the values of influx clearances of unbound ^{3}H-PAH ($PS_{u,1}$) and the efflux rate constant (K_2) were comparable between filtering and nonfiltering kidneys, though the influx rate constant (K_1) for total PAH showed some differences (Table II). The value of the sequestration rate constant (K_3) in the filtering kidney was approximately three times as large as that in the nonfiltering kidney.

Uptake of ^{125}I-EGF by Filtering and Nonfiltering Kidneys

Table III shows the total uptake of ^{125}I-EGF in the presence and absence of unlabeled EGF (20 n*M*). The kidney-associated radioactivity in the nonfiltering kidney was almost completely (~95%) displaced by the presence of 20 n*M* unlabeled EGF in the perfusate, suggesting that EGF uptake via the antiluminal membrane is saturable and that the nonspecific uptake is very small.

Approximately 20% of the ^{125}I-EGF was trapped by the filtering kidney even when 20 n*M* unlabeled EGF was present. The difference in ^{125}I-EGF total uptake between the nonfiltering and the filtering kidneys was 1.81% of dose/kidney; this difference corresponds well with the total uptake of ^{125}I-EGF (1.84% of dose/kidney) in the filtering kidney when the kidney was perfused with 20 nM unlabeled EGF.

DISCUSSION

In the present study, we used ^{3}H-PAH to evaluate the transport activity of the antiluminal membrane in both nonfiltering and filtering kidneys. The influx rate constant (K_1) of ^{3}H-PAH in the filtering kidney is significantly higher than that in the nonfiltering kidney. However, considering that only the unbound ^{3}H-PAH can be transported and that the perfusate albumin concentrations were not the same between filtering (5%) and nonfiltering (10%) kidneys, the higher K_1 in the filtering kidney may be due to the higher unbound fraction of ^{3}H-PAH in the perfusate. Normalization of the K_1 value by the unbound fraction of ^{3}H-PAH estimates the true transport ability of the antiluminal membrane. The corrected influx clearance ($PS_{u,1}$) was almost the same between filtering and nonfiltering kidneys. The similarity of the influx clearance for unbound PAH and efflux rate constant in filtering and nonfiltering kidneys suggested that the organic anion transport activity through the antiluminal membrane is well preserved in the nonfiltering kidney. However, the sequestration rate constant (K_3) in the filtering kidney was about three times larger than that in the nonfiltering kidney. The decrease in tubular fluid flow in the nonfiltering kidney may lead to a decrease in the concentration gradient of ^{3}H-PAH and may explain the decreased K_3 value in the nonfiltering kidney.

The results shown in Table III suggested that the tubular uptake of tracer ^{125}I-EGF via antiluminal membrane is much larger than that via luminal membrane in the filtering kidney. It is also suggested that the renal uptake of ^{125}I-EGF via antiluminal membrane is a saturable process. The saturable

Table II. Renal Handling of *p*-Amino-^{3}H-hippurate and ^{14}C-Creatinine[a]

Expt No.	Perfusion rate (ml/min)	(a) $\bar{t}_{cre}$ (sec)	(a) $\bar{t}_{PAH}$ (sec)	(b) $V_{d_{cre}}$ (ml/kidney)	(b) $V_{d_{PAH}}$ (ml/kidney)	(c) F_{cre}	(c) F_{PAH}	Derived value K_1 (L/sec)	K_2 (L/sec)	K_3 (L/sec)	CV	(d) PS_1 (ml/sec/kidney)	(d) $PS_{u,1}$ (ml/sec/kidney)
FK-1	16.4	1.52	2.33	0.415	1.20	0.984	0.535	0.618	0.105	0.064	0.0241	0.256	0.353
FK-2	17.0	1.56	2.74	0.442	1.37	0.976	0.568	0.604	0.119	0.066	0.0242	0.267	0.368
FK-3	19.0	1.42	2.59	0.450	1.51	0.982	0.542	0.634	0.112	0.077	0.0162	0.285	0.393
Mean	17.0	1.50	2.55	0.436	1.36	0.981	0.548	0.619	0.112	0.069	0.022	0.269	0.371
	± 1.0	± 0.07	± 0.21	± 0.018	± 0.16	± 0.004	± 0.017	± 0.015	± 0.007	± 0.007	± 0.004	± 0.015	± 0.020
NFK-1	16.5	1.87	2.90	0.514	1.28	0.986	0.624	0.508	0.115	0.035	0.0155	0.261	0.508
NFK^{-2}	16.0	1.70	2.14	0.453	0.97	0.980	0.586	0.405	0.084	0.027	0.0293	0.183	0.364
NFK^{-3}	16.4	1.52	1.85	0.415	0.83	0.983	0.606	0.317	0.055	0.010	0.0173	0.132	0.262
Mean	16.3	1.69	2.30	0.461	1.03	0.983	0.605	0.410	0.085	0.024	0.021	0.192	0.378
	± 0.3	± 0.18	± 0.54	± 0.050	± 0.23	± 0.003	± 0.019	± 0.096*	± 0.030	± 0.013*	± 0.008	± 0.065	± 0.124

[a] FK and NFK represent the filtering and nonfiltering isolated perfused rat kidneys, respectively. (a) Mean transit times for ^{14}C-creatinine, and ^{3}H-PAH ($\bar{t}_{cre}$ and $\bar{t}_{PAH}$, respectively) corrected for the inflow and outflow catheter transit times. (b) Distribution volumes of ^{14}C-creatinine, and ^{3}H-PAH ($V_{d_{cre}}$ and $V_{d_{PAH}}$, respectively) calculated using Eqs. (4) and (5). (c) Availability of ^{14}C-creatinine, and ^{3}H-PAH (F_{cre} and F_{PAH}, respectively) determined using Eqs. (2) and (3). (d) The influx clearance of ^{3}H-PAH (PS_1) calculated by Eq. (7) was corrected for the unbound fraction of ^{3}H-PAH in the perfusate to yield $PS_{u,1}$ using Eq. (8). The unbound fractions of ^{3}H-PAH were 0.726 ± 0.013, and 0.503 ± 0.041 in the filtering and nonfiltering isolated perfused rat kidneys, respectively.

* Significantly different from filtering kidney ($P < 0.05$).

Table III. Comparison of EGF Total Uptake Between the Nonfiltering and the Filtering Isolated Perfused Rat Kidneys[a]

Total binding (% of dose/kidney)	Tracer ^{125}I-EGF	Tracer ^{125}I-EGF plus 20 n*M* unlabeled EGF
NFK-1	7.13	0.302
NFK-2	7.29	0.390
NFK-3	6.70	0.368
Mean ± SE	7.04 ± 0.31	0.353 ± 0.046
FK-1	9.36	1.55
FK-2	8.45	2.21
FK-3	8.73	1.76
Mean ± SE	8.85 ± 0.47	1.84 ± 0.34
NFK/FK ratio	0.80	0.19

[a] NFK and FK represent the nonfiltering and filtering isolated perfused rat kidneys, respectively. NFK and FK were perfused with tracer ^{125}I-EGF or tracer ^{125}I-EGF plus 20 n*M* unlabeled EGF in a recirculatory mode for 20 min, and then the kidney-associated ^{125}I radioactivities were counted. See details in the text.

uptake may be considered to reflect the receptor-mediated uptake specific to EGF, if the following information in the literature is taken into consideration. That is, specific and high-affinity EGF receptors have been identified in cultured cells from the kidney, including renal messangial and proximal tubule cells (17,18). Also the binding of tracer ^{125}I-EGF to basolateral membrane vesicles obtained from rat proximal tubules was not inhibited by the addition of excess unlabeled insulin, parathyroid hormone, and angiotensin II (18). It was also reported that excess unlabeled bradykinin, antidiuretic hormone, and atrial natriuretic peptide were not capable of inhibiting the binding of tracer ^{125}I-EGF in the rat inner medullary collecting duct (19).

EGF is excreted in the urine at nanomolar concentrations (20,21). This probably results from glomerular filtration of EGF and secretion of EGF by the kidney (20,22). Nielsen *et al.* (4) showed that ^{125}I-EGF was poorly absorbed from lumen to bath using isolated perfused rabbit proximal tubules. They also observed a small but significant transtubular transport of ^{125}I-EGF from bath to lumen. These results are consistent with the results of the present study. The urinary excretory clearance of endogenous rabbit EGF was determined to be comparable to that of creatinine (4). On the other hand, renal clearance of EGF exceeds creatinine clearance in the rat (20) and mice (21). These results suggest a secretion into the lumen of EGF molecules, which are synthesized in the kidney and/or taken up from the circulating plasma and may partly account for the urinary EGF in these animal species.

In conclusion, by use of the nonfiltering kidney preserving organic anion transport activity, the specific uptake of tracer ^{125}I-EGF by the filtering kidney was demonstrated to occur mainly from the antiluminal side.

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