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Renal Excretion and Accumulation Kinetics of 2-Methylbenzoylglycine in the Isolated Perfused Rat Kidney

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Abstract

The effect of protein binding on kidney function has been studied by investigating the renal accumulation and secretion of the hippurate analogue 2-methylbenzoylglycine in the isolated perfused rat kidney in the absence and presence of bovine serum albumin (BSA).

Experiments were performed with either 2·5% pluronic or a combination of 2·2% pluronic and 2% BSA as oncotic agents; a wide concentration range (1–190 $\mu g\ mL^{-1}$) of 2-methylbenzoylglycine was studied. Tubular secretion appeared to be a function of the amount of unbound drug in the perfusate and was best described by a model consisting of a high and low affinity Michaelis–Menten term. Parameters obtained after the analysis of renal excretion data were maximum transport velocity for the high affinity site ($T_{M,H}$) = 3·0 ± 2·8 $\mu g\ min^{-1}$, Michaelis-Menten constant for tubular transport for the high affinity site ($K_{T,H}$) = 0·5 ± 0·8 $\mu g\ mL^{-1}$, maximum transport velocity for the low affinity site ($T_{M,L}$) = 250 ± 36 $\mu g\ min^{-1}$, and Michaelis–Menten constant for tubular transport for the low affinity site ($K_{T,L}$) = 62 ± 17 $\mu g\ mL^{-1}$. The compound accumulated extensively in kidney tissue, ratios up to 175 times the perfusate concentration were reached. Accumulation data were best analysed by a two-site model similar to the model used to describe renal excretion. Calculated parameters were theoretical maximum capacity of the high affinity site ($R_{M,H}$) = 26 ± 23 $\mu g\ g^{-1}$, affinity constant for renal accumulation at the high affinity site ($K_{A,H}$) = 0·2 ± 0·4 $\mu g\ mL^{-1}$, theoretical maximum capacity of the low affinity site ($R_{M,L}$) = 1640 ± 1100 $\mu g\ g^{-1}$ and affinity constant for renal accumulation at the low affinity site ($K_{A,L}$) = 60 ± 58 $\mu g\ mL^{-1}$.

The very high accumulation in kidney tissue could be explained by active tubular uptake, mediated by the secretory mechanisms involved, and dependent on the amount of free drug in the perfusate. This study shows that anionic drugs, subject to active secretion, may reach high concentrations in tubular cells even at low plasma concentrations.

The kidney is an important organ in the elimination of exogenous compounds from the body. Drugs may be secreted efficiently by various transport systems for organic anions and cations in the proximal tubules. During the process of secretion, the drug has to be transported across the proximal tubular cells. For organic anions, cellular uptake can be actively mediated by at least three different carrier systems with overlapping specificities at the basolateral membrane—the *p*-aminohippurate transporter and the related dicarboxylate and sulphate systems. Once inside the cell, the drug will enter the tubular lumen down its electrochemical gradient via an anion exchanger or a potential-driven pathway (Besseghir & Roch-Ramel 1987; Grantham & Chonko 1991; Pritchard & Miller 1993; Ullrich 1994). As a result of the sequence of these events, drugs can achieve high intracellular concentrations. In addition to a difference in basolateral and brush-border membrane transport, accumulation can be also caused by extensive intracellular binding or sequestration in certain cell organelles. The intracellular disposition of drugs can affect proximal tubular function or lead to tubular toxicity (Goldstein 1993; Koren 1989).

If the extent of drug accumulation in proximal tubular cells depends on the efficiency of the secretory mechanism involved, factors influencing tubular secretion will also have an effect on the intracellular concentration. In this regard, the degree of plasma protein binding could be an important determinant in tubular accumulation (Brater et al 1992). Hippurates (benzoylglycines) are known to be cleared very rapidly by the kidney, mainly via tubular secretion. *p*-Aminohippurate is commonly used as a model compound for testing kidney function and for investigation of the anionic secretory system in the proximal tubule. Because of the efficiency of the secretory process, the renal extraction ratio practically equals 1, indicating that renal clearance and accumulation are insensitive to changes in protein binding and only influenced by changes in renal blood flow (Grantham & Chonko 1991; Levy 1980). The presence of serum protein, on the other hand, may even facilitate *p*-aminohippurate secretion (Besseghir et al 1989). Plasma protein binding of this compound is, however, low (Russel et al 1989b). For this study we have chosen a hippurate analogue with relatively high plasma protein binding. In-vivo experiments in the dog showed that the high protein binding of 2-methylbenzoylglycine possibly limits tubular secretion, despite its high intrinsic clearance (Russel et al 1989a). It is, therefore, supposed that changes in protein binding of this compound will result in an altered accumulation, although nothing is known about the extent of renal accumulation.

This investigation was designed to examine the renal secretion and accumulation kinetics of 2-methylbenzoylglycine in the presence and absence of protein, by using the isolated perfused rat kidney. We have previously shown that the isolated perfused rat kidney is a useful means of investigation of renal secretion and accumulation of drugs (Cox et al 1989, 1990; Boom et al 1994). An advantage of this technique is that it

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enables accurate determination of renal drug clearance over a wide concentration range, under controlled protein-binding conditions and in the absence of non-renal effects.

Materials and Methods

Materials

Pluronic F-108 was obtained from BASF (Arnhem, The Netherlands), bovine serum albumin (BSA) from Boehringer (Mannheim, Germany), inulin from Sigma (St Louis, MO, USA), and salicyluric acid from Merck AG (Darmstadt, Germany). 2-Methylbenzoylglycine was prepared as described previously (Russel et al 1989a). All other chemicals were of analytical grade and obtained either from Sigma (St Louis, MO, USA) or from Merck.

Experimental procedure

The isolation and perfusion of the rat kidney have been described in detail elsewhere (Cox et al 1990). Studies were performed with perfusate containing either 2·5% pluronic or a combination of 2% BSA and 2·2% pluronic. In presence of 2% BSA, the addition of 2.2% pluronic was necessary to obtain an oncotic pressure comparable with that of the protein-free perfusate containing only 2·5% pluronic. To maintain a perfusate pressure of approximately 90 mmHg, a low glomerular filtration rate (GFR) resulted (between 230 and 350). For the determination of GFR, cyanocobalamin (20 μg mL^{-1}) was added to the perfusion fluid. GFR was monitored on-line using a micro flow-through cuvette in which the cyanocobalamin concentration was measured colourimetrically (Brink & Slegers 1979). Because cyanocobalamin binds to BSA, inulin (100 μg mL^{-1} in perfusion fluid) was used to determine the GFR in the experiments with 2·2% pluronic and 2% BSA, as described below in Analysis. Before the experiment the perfusion fluid was filtered through a 0·22 μm pore-size cellulose acetate-cellulose nitrate membrane filter (Millipore, Bedford, MA, USA). The perfusion fluid containing BSA was filtered through a similar 3·0 μm pore-size membrane filter (Schleicher & Schuell, Dassel, Germany).

Experimental design

The experimental period was 120 min and the experiment was started after 30 min of control. During the control period, the volume of the circulating perfusate was 500 mL, from which a sample of 5 mL was drawn. After the control period, the kidney was connected to perfusion fluid with a total volume of 250 mL, in which 2-methylbenzoylglycine was already dissolved. Doses added to the perfused kidneys were: 0·625, 1·875, 6·25, 18·75, 37·5 and 62·5 mg 2-methylbenzoylglycine. Urine samples were collected during control and experimental periods over 10-min intervals. Perfusate samples (300 μL) were drawn at the midpoint of the urine collection intervals. Two additional perfusate samples were taken, one at the beginning of the experiment and one at the end. At the end of the experiment the kidney was removed from the system, blotted and weighed. Urine, perfusate and kidney samples were stored at -20°C until analysis.

Analysis

Urine and perfusate samples were analysed for glucose and various electrolytes as described previously (Cox et al 1990). In the presence of 2% BSA, perfusate and urine samples were analysed also for inulin and protein content. The Bio-Rad Protein Assay from Bio-Rad (Munich, Germany) was used for the determination of protein. Inulin was determined according to a previously published method (Heyrovski 1956). The concentration of 2-methylbenzoylglycine in perfusate, urine and kidney samples was determined by means of reversed-phase high-performance liquid chromatography (HPLC), as described below.

HPLC assay

HPLC was performed with a 1084B liquid chromatograph (Hewlett-Packard, Böblingen, Germany) equipped with an HP 79841A auto-injector, HP 79850B LC terminal and a Spectroflow 773 UV absorbance detector (Kratos analytical instruments, Ramsey, NJ, USA) at an operating wavelength of 228 nm. Chromatography was performed on a stainless steel column (10 cm × 4·6 mm i.d.) packed with LiChrosorb RP-18 (particle size 5 μm). The mobile phase consisted of 15% methanol and 85% 0·01 M phosphate buffer, pH 2·6. The flow rate was 1 mL min^{-1}. Salicyluric acid solution (0·1 mg mL^{-1}; 10 μL) was added as internal standard to all samples (100 μL). After addition of diethyl ether (1·2 mL) the samples were vortex-mixed for 10 s and centrifuged for 10 min at 2000 *g*. When BSA was present in the perfusion fluid, the samples were first treated with HCl (6 M; 50 μL), vortex-mixed for 10 s and centrifuged at 2000 *g*. Supernatant (100 μL) was subsequently removed and diethyl ether (1·2 mL) was added. The samples were again vortex-mixed and centrifuged. The diethyl ether layer was then transferred to glass tubes on ice and evaporated to dryness with air. The residue was dissolved in mobile phase (200 μL), and 10 or 20 μL was injected into the HPLC system. Concentrations were determined by comparing the peak area ratios of 2-methylbenzoylglycine and internal standard with the ratios of a calibration curve, prepared by adding various amounts of 2-methylbenzoylglycine to blank perfusate. The concentration of 2-methylbenzoylglycine in kidney tissue was determined in a similar manner. The kidney was homogenized in distilled water (5 or 10 mL) with a Polytron homogenizer (Braun, Melsungen, Germany) on setting 10 for 2 × 60 s. Internal standard (0·1 mg mL^{-1}; 40 μL) and HCl (6 M; 100 μL) were added to this homogenate (450 μL). After protein precipitation and centrifugation at 2000 *g*, supernatant (200 mL) was extracted twice with diethyl ether (2 × 1·2 mL), vortex-mixed for 10 s and centrifuged for 10 min at 2000 *g*. The diethyl ether layer was added to ice-cold glass tubes and evaporated to dryness. The residue was dissolved in mobile phase (500 μL) and 10 or 20 μL was injected into the HPLC system. Concentrations were calculated by comparing peak area ratios of 2-methylbenzoylglycine and internal standard with a calibration curve of spiked samples of blank kidney homogenates with internal standard and various amounts of 2-methylbenzoylglycine. Linear correlations were obtained in all cases ($R^2 > 0{\cdot}98$).

Protein binding

Protein binding was determined by ultrafiltration as described previously (Russel et al 1987). The ultrafiltrates were treated and analysed in the same way as the urine samples. Perfusate protein binding was calculated according to the following equations, assuming one class of binding site:

Table 1. Functional parameters of the isolated perfused rat kidney in control experiments and after administration of 62·5 mg 2-methylbenzoylglycine (2-MBG).

Parameter	2·5% Pluronic		2·2% Pluronic/2% BSA	
	Control[a]	2-MBG[b]	Control[a]	2-MBG[b]
FE sodium (%)	2·9 ± 0·6	2·2 ± 0·9	3·1 ± 1·7	4·0 ± 1·8
FE potassium (%)	38 ± 14	73 ± 11*	14 ± 6†	51 ± 5*
FE glucose (%)	7·3 ± 2·1	7·3 ± 1·2	4·1 ± 2·0†	7·0 ± 2·1
FE magnesium (%)	42 ± 10	47 ± 6	17 ± 12†	37 ± 5*
FE calcium (%)	3·5 ± 1·3	4·0 ± 0·7	2·4 ± 1·3	4·5 ± 1·1*
H_2O re-absorption (%)	94 ± 1	93 ± 1	93 ± 1	92 ± 1
Urine flow (μL min^{-1})	18 ± 3	23 ± 2*	16 ± 3	24 ± 2*
GFR (μL min^{-1})	277 ± 35	327 ± 24*	232 ± 53†	298 ± 25*
Urinary pH	5·8 ± 0·3	6·0 ± 0·1	6·2 ± 0·2†	6·3 ± 0·1
Perfusate flow (mL min^{-1})	15 ± 2	14·3 ± 0·3	23 ± 2†	23 ± 3
Perfusate pressure (mmHg)	89 ± 8	81 ± 4	93 ± 8	102 ± 7

Mean values ± s.d. over the period 30–120 min are given. FE, fractional excretion. GFR, glomerular filtration rate. *$P < 0.05$ compared with control experiments; †$P < 0.05$ compared with the corresponding experiments without BSA; [a]n = 12; [b]n = 4.

$$C = Cu + P \cdot Cu/(K_d + Cu) \quad (1)$$

$$Cu = fu \cdot C \quad (2)$$

in which C is the total perfusate concentration (μg mL^{-1}), Cu is the unbound drug concentration (μg mL^{-1}), fu is the fraction of unbound drug in perfusate, P is the total concentration of protein binding sites (μg mL^{-1}) and K_d is the dissociation constant of the drug-protein complex (μg mL^{-1}).

Renal excretion model

For an adequate description of the renal clearance of 2-methylbenzoylglycine by the isolated perfused rat kidney, a model was necessary in which tubular secretion was expressed by two Michaelis–Menten terms. Assuming that renal excretion of 2-methylbenzoylglycine is dependent on unbound perfusate concentrations, the renal clearance of this compound can be expressed as:

$$CL_R = (Q_{GF} \cdot fu + (T_{M,H} \cdot fu/(K_{T,H} + Cu)) + (T_{M,L} \cdot fu/(K_{T,L} + Cu))) \cdot (1 - F) \quad (3)$$

The renal excretion rate (R_R) is:

$$R_R = CL_R \cdot C \quad (4)$$

where CL_R is the renal clearance (mL min^{-1}), R_R is the renal excretion rate (μg min^{-1}), Q_{GF} is the glomerular filtration rate (mL min^{-1}), C is the total drug concentration in perfusate (μg mL^{-1}), Cu is the unbound drug concentration in perfusate (μg mL^{-1}), fu is the fraction of unbound drug, T_M is the maximum transport velocity (μg min^{-1}), K_T is the Michaelis–Menten constant for tubular transport (μg mL^{-1}) for the high (H) and low (L) affinity site, and F is the fraction of excreted drug reabsorbed.

Renal accumulation

The concentration of 2-methylbenzoylglycine in the kidney (C_T) was expressed as the amount of drug per unit weight of tissue. The concentration in kidney tissue divided by the concentration in perfusate at the end of the experiment (Cu,z), resulted in an accumulation ratio (kidney:perfusate ratio). If accumulation in kidney tissue is considered to be a result of both active and passive transport processes, the renal accumulation ratio can be described as follows:

$$C_T/Cu, z = (R_{M,H}/(K_{A,H} + Cu, z)) + (R_{M,L}/(K_{A,L} + Cu, z)) + a \quad (5)$$

where R_M is the theoretical maximum capacity (μg g^{-1}) and K_A is the affinity constant for renal accumulation (μg mL^{-1}), for the high (H) and low (L) affinity site, and a is the ratio C_T/Cu,z due to passive transport.

Data analysis

Renal excretion rate and accumulation data were analysed according to equations 4 and 5 by the nonlinear least squares regression program PCNONLIN (Metzler & Weiner 1986). The goodness of fit was evaluated as the deviation between the observed and model predicted values as $R^2 = 1 - \Sigma(Dev)^2/\Sigma(Obs)^2$, where $\Sigma(Obs)^2$ is the observed sum of squared observations and $\Sigma(Dev)^2$ the sum of squared deviations. The weighted residual sums of squares of the renal excretion and accumulation models with two Michaelis–Menten terms were compared with the residual sums of squares of the corresponding models with only one term, and significance was determined with an F-test ($P < 0.05$). All data are expressed as mean ± s.d. Statistical differences between means were determined with Student's *t*-test, in which the level of significance was set to $P < 0.05$.

Results

Effects on kidney function

The renal functional parameters obtained from 12 control experiments in the absence and presence of BSA, and after administration of the highest dose of 2-methylbenzoylglycine, are listed in Table 1. The control isolated perfused rat kidney experiments with 2% BSA in perfusate showed a significantly lower GFR and fractional excretion of potassium, glucose and magnesium, and a significant increase in urinary pH, in comparison with the control experiments without BSA. To maintain a perfusate pressure of 90 mmHg, it was, furthermore, necessary to increase the perfusate flow significantly.

No negative effects on kidney function were observed with 2-methylbenzoylglycine. Only the highest dose altered renal function, as is shown in Table 1. Under both experimental conditions, a significant increase in GFR, urine flow and

fractional excretion of potassium were found for this compound, as compared with the controls. In the experiments with BSA, the fractional excretions of magnesium and calcium were also significantly increased.

Protein binding

Protein binding was determined for each dose of 2-methylbenzoylglycine administered, and all data were pooled and analysed according to equation 2. Parameters obtained were a dissociation constant, K_d, of 58 ± 16 $\mu g\ mL^{-1}$ and a total concentration of BSA binding sites, P, of 70 ± 16 $\mu g\ mL^{-1}$ ($n = 36$). The binding of 2-methylbenzoylglycine decreased gradually, with fractions unbound between 0·4 and 0·7 over a concentration range of 0·5–250 $\mu g\ mL^{-1}$. The experiments with 6·25 and 37·5 mg 2-methylbenzoylglycine were performed with another batch of BSA. This resulted in different binding, with fractions unbound of 0·38 and 0·35, respectively.

Renal excretion and accumulation

2-Methylbenzoylglycine was slowly eliminated from perfusate. Fig. 1 shows the mean unbound perfusate concentration and corresponding excretion rate data as functions of time under both experimental conditions. All doses gave log-linear concentration–time curves. The urinary excretion rate increased rapidly after addition of 2-methylbenzoylglycine, followed by a gradual decrease. The presence of protein in perfusate resulted in a lower renal excretion rate. Renal handling data for 2-methylbenzoylglycine are presented in Table 2. The renal clearance was higher than the clearance by glomerular filtration corrected for the fraction unbound ($CL_R/GF > 1$), indicating active tubular secretion. A decrease in CL_R/GF at higher perfusate concentrations was in accordance with saturable tubular secretion. The CL_R/GF values in the presence of BSA surpassed those obtained from the experiments with 2·5% pluronic, supporting the view that the renal excretion of 2-methylbenzoylglycine is dependent on unbound drug concentrations. A linear plot of the renal excretion rate against unbound perfusate concentration, a so-called tubular titration curve, is presented in Fig. 2. A two-site model dependent on unbound drug concentration in perfusate fitted the data best (F-test, $P < 0.05$). The line through the data points was obtained after analysing renal excretion data over the period 30–90 min, according to equation 4. The results show that renal excretion was composed

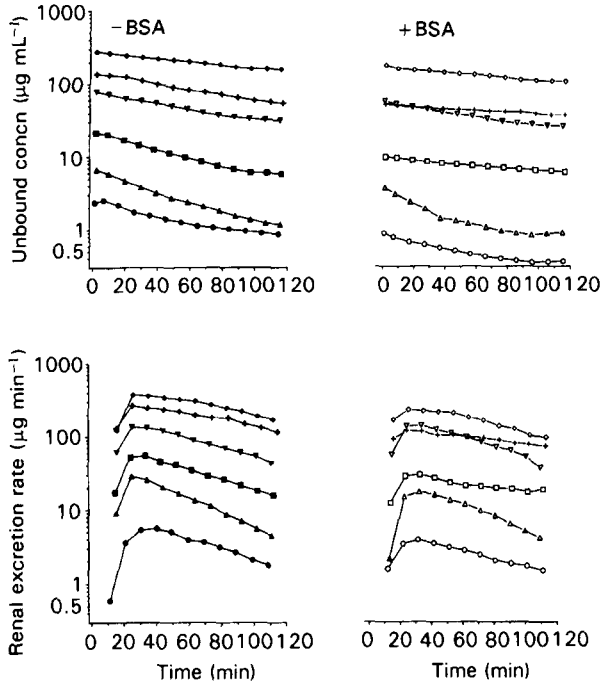

FIG. 1. 2-Methylbenzoylglycine perfusate concentration and urinary excretion rate as a function of time. Closed and open symbols represent, respectively, experiments without and with BSA in the perfusate. ● ○ 0·625, ▲ △ 1·875, ■ □ 6·25, ▽ ▼ 18·75, + + 37·5, ◆ ◇ 62·5 mg 2-methylbenzoylglycine. All data points are means of four experiments. For the sake of clarity standard deviations were omitted from this figure; they varied between 2 and 30% for perfusate concentration, and between 10 and 35% for renal excretion rate.

of glomerular filtration and active tubular secretion. At high perfusate concentration the titration curves paralleled the glomerular filtration line, indicating that tubular secretion was saturated and reabsorption was negligible ($F = 0$ in equation 3). Because renal excretion was dependent on unbound drug concentration in perfusate, clearance data from both experimental conditions could be pooled and analysed simultaneously. The kinetic parameters of the two transport systems involved in tubular secretion of 2-methylbenzoylglycine are listed in Table 3. The 2-methylbenzoylglycine concentration in kidney tissue was determined at the end of each experiment (Table 2). A plot

Table 2. Renal handling of 2-methylbenzoylglycine in the isolated perfused rat kidney.

Dose (μg)	BSA	Perfusate concn ($\mu g\ mL^{-1}$)	fu	Excretion rate ($\mu g\ min^{-1}$)	CL_R/GF	Amount in kidney ($\mu g\ g^{-1}$)
625	–	1·2 ± 0·2	–	5·3 ± 1·3	12·2 ± 1·2	24 ± 2
	+	1·1 ± 0·1	0·42	3·1 ± 0·5	22·9 ± 1·5	26 ± 5
1875	–	2·5 ± 0·8	–	19 ± 6	17·5 ± 0·5	44 ± 10
	+	2·1 ± 0·3	0·42	15 ± 3	44·2 ± 2·7	77 ± 32
6250	–	10 ± 3	–	44 ± 8	11·6 ± 0·8	220 ± 50
	+*	19 ± 1	0·38	27 ± 3	10·5 ± 0·5	200 ± 10
18750	–	44 ± 8	–	119 ± 15	7·0 ± 0·3	650 ± 130
	+	50 ± 8	0·52	115 ± 16	14·8 ± 0·6	590 ± 80
37500	–	85 ± 16	–	203 ± 12	7·3 ± 0·6	910 ± 240
	+*	120 ± 6	0·35	116 ± 8	9·0 ± 0·5	710 ± 140
62500	–	189 ± 20	–	282 ± 22	4·3 ± 0·3	1410 ± 180
	+	176 ± 16	0·73	194 ± 22	4·4 ± 0·3	1120 ± 140

Mean data over the period 30–90 min are given for both experimental conditions ($n = 4$). fu, fraction unbound. CL_R/GF, renal clearance of 2-methylbenzoylglycine corrected for glomerular filtration rate and fraction unbound in perfusate. *Another batch of BSA was used.

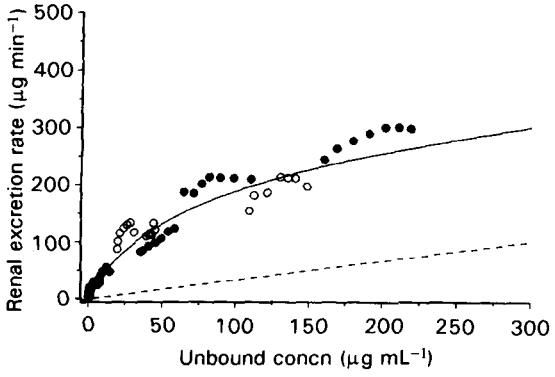

FIG. 2. Tubular titration curve of 2-methylbenzoylglycine. Renal excretion rate and rate of filtration as a function of concentration unbound in perfusate. Data obtained over the period 30–90 min of both experimental conditions were pooled and analysed simultaneously. The solid line represents the fit according to equation 4, the dashed line corresponds to the clearance by glomerular filtration only. ● Without BSA, ○ with BSA. All data points are means of four experiments.

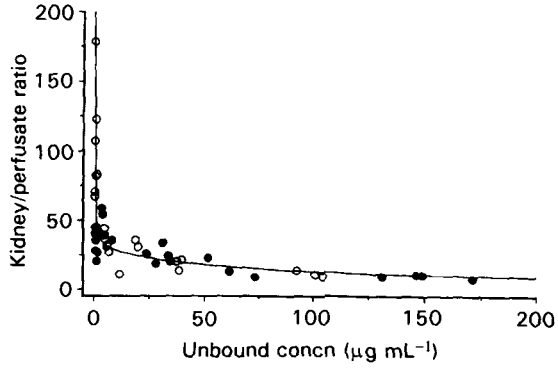

FIG. 3. Accumulation of 2-methylbenzoylglycine in kidney tissue. The kidney:perfusate ratio is plotted against the unbound perfusate concentration. ● Without BSA, ○ with BSA. All data points are for individual kidneys; n = 24 for both experimental conditions.

Table 3. Kinetic parameters for renal handling of 2-methylbenzoylglycine.

Parameter	High affinity site	Low affinity site
T_M ($\mu g\ min^{-1}$)	3·0 ± 2·8	250 ± 36
K_T ($\mu g\ mL^{-1}$)	0·5 ± 0·8	62 ± 17
R_M ($\mu g\ g^{-1}$)	26 ± 23	1640 ± 1100
K_A ($\mu g\ mL^{-1}$)	0·2 ± 0·4	60 ± 58

Parameters were obtained after fitting equations 4 and 5 to the data. Ratio of concentration in kidney tissue over unbound perfusate concentration (C_T/C_U) due to passive transport (a in equation 5) was determined to be 2·4 ± 2·2.

of kidney:perfusate ratio vs unbound perfusate concentration is illustrated in Fig. 3. At low perfusate concentrations very high accumulation ratios were observed; these decreased at increasing perfusate concentrations, indicating that a saturable component is involved in cellular uptake. Kinetic parameters for renal accumulation were obtained after fitting equation 5 to the accumulation data of 2-methylbenzoylglycine (Table 3).

Discussion

The kidneys used in this study showed a good renal functioning, and were stable for at least 2 h. Changes in kidney function on addition of BSA to the perfusion fluid are in accordance with those reported by Maack (1980) and Bekersky (1983). The fractional excretion (%) of potassium, glucose and magnesium decreased, indicating that kidney viability was increased in the presence of 2% BSA in control experiments.

Although the colloid osmotic pressure was comparable in both experimental perfusion fluids, a higher perfusate flow was necessary with albumin in the perfusion fluid, to maintain the perfusate pressure at approximately 90 mmHg. GFR decreased in the presence of 2% BSA, owing to enhanced oncotic pressure within the glomerulus and peritubular capillaries; this is in agreement with the concept of Starling (Maack 1980; Schurek & Alt 1981).

CL_R/GF for 2-methylbenzoylglycine was many times higher than 1 (Table 2), indicating that clearance proceeded via glomerular filtration and pronounced active secretion. As a result of the large perfusate volume (250 mL) in proportion to the renal clearance (1–7 mL min^{-1}), the decline in perfusate concentration was slow. Tubular secretion followed Michaelis–Menten kinetics and, interestingly, a model with two affinity sites was necessary to describe the renal excretion rate. A low and high affinity site of the basolateral *p*-aminohippurate transporter seems very unlikely, as numerous membrane studies have never provided evidence indicating more than one site.

A two-site model is consistent with high affinity, low capacity uptake across the basolateral membrane and subsequent low affinity, high capacity transport across the brush-border membrane into the tubular lumen, as was proposed previously for the renal excretion of frusemide in the isolated perfused rat kidney (Lee et al 1986). The similarity of the kinetic parameters for secretion and accumulation strongly suggest, however, that uptake takes place at the basolateral membrane of the proximal tubule, and that both saturable steps are connected with transport across this membrane. An explanation that could fit in this view may be that 2-methylbenzoylglycine is transported with high affinity via the *p*-aminohippurate carrier and that it has also a low affinity for one of the other basolateral organic anion transporters, i.e. dicarboxylate or sulphate system.

The results of the experiments in the presence of BSA show that renal excretion and accumulation of 2-methylbenzoylglycine was dependent on unbound drug concentration; in contrast with in-vivo studies in the dog, however (Russel et al 1989a), the compound is not efficiently cleared by the kidney. This is most probably a consequence of the high perfusion flow, resulting from a lack of red blood cells and maximum dilation of the vessels of the perfused kidney. In presence of 2% BSA, an even higher flow (23 vs 15 mL min^{-1}) was necessary to sustain normal perfusate pressure, because of lower perfusate viscosity. Similar to the in-vivo situation, the intrinsic secretion clearance of 2-methylbenzoylglycine in the isolated perfused rat kidney ($CL_{int} = T_{M,H}/K_{T,H} + T_{M,L}/K_{T,L}$) was high: 10 mL min^{-1}. As a result of the high perfusion flow, however, the renal extraction ratio, calculated as CL_{int} divided by the sum of CL_{int} and perfusate flow, is low. Depending on the experimental conditions the extraction ratio varied between 0·3 and 0·4, and as a result, 2-methylbenzoylglycine behaved as a low clearance drug. Lee et al (1988) have reported similar

findings for the diuretic chlorothiazide, which also shows high renal extraction in-vivo. In the isolated perfused rat kidney, however, this diuretic is cleared as a low clearance drug as a result of the high perfusate flow.

In contrast with what was found in rabbit renal proximal tubules for *p*-aminohippurate (Besseghir et al 1989), BSA did not enhance tubular secretion of this hippurate analogue. Our results show that kidney:perfusate ratios did increase in presence of BSA at low perfusate concentration, however; this could be explained by a strongly reduced free concentration of 2-methylbenzoylglycine in perfusate rather than a stimulatory effect of BSA. At low perfusate concentrations, accumulation ratios up to 175 times the perfusate concentration were observed. Such high accumulation ratios have never yet been reported for an in-vitro renal perfusion system. It is obvious that anionic drugs, pharmacologically less inert than hippurates, that are subject to tubular secretion, may have harmful effects on kidney functioning even at low plasma concentrations.

In conclusion, 2-methylbenzoylglycine is handled as a low-clearance drug in the isolated perfused rat kidney and, as a consequence, renal clearance appeared to be a function of the unbound drug concentration in perfusion medium. Renal tubular excretion data were described best by a model in which tubular secretion is composed of a high and a low affinity Michaelis–Menten term. The very high accumulation in kidney tissue could be explained by active tubular uptake, mediated by the secretory mechanisms involved. This study shows that anionic drugs, subject to active secretion, may reach high concentrations in tubular cells even at low plasma concentration.

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References

Bekersky, I. (1983) Use of the isolated perfused kidney as a tool in drug disposition studies. Drug Metab. Rev. 14: 931–960

Besseghir, K., Roch-Ramel, F. (1987) Renal excretion of drugs and other xenobiotics. Renal Physiol. 10: 221–241

Besseghir, K., Mosig, D., Roch-Ramel, F. (1989) Facilitation by serum albumin of renal tubular secretion of organic anions. Am. J. Physiol. 256: F475–F484

Boom, S. P. A., Moons, M. M., Russel, F. G. M. (1994) Renal tubular transport of cimetidine in the isolated perfused kidney of the rat. Drug Metab. Dispos. 22: 148–153

Brater, D. C., Sokol, P. P., Hall, S. D., McKinney, T. D. (1992) Renal elimination of drugs: methods and determinants. In: Seldin, D. W., Giebisch, G., (eds) The Kidney: Physiology and Pathophysiology. Raven Press Ltd, New York, pp 3597–3628

Brink, H. M., Slegers, J. F. G. (1979) Instantaneous measurement of glomerular filtration rate in the isolated perfused rat kidney. Pflügers Arch. 383: 71–73

Cox, P. G. F., Moons, M. M., Russel, F. G. M., Van Ginneken, C. A. M. (1989) Renal handling of salicyluric acid in the isolated perfused rat kidney: evidence for accumulation in tubular cells. J. Pharmacol. Exp. Ther. 251: 750–755

Cox, P. G. F., Moons, M. M., Slegers, J. F. G., Russel, F. G. M., Van Ginneken, C. A. M. (1990) The isolated perfused rat kidney as a tool in the investigation of renal handling and effects of nonsteroidal anti-inflammatory drugs. J. Pharmacol. Methods 24: 89–103

Goldstein, R. S. (1993) Biochemical heterogeneity and site-specific tubular injury. In: Hook, J. B., Goldstein, R. S. (eds) Toxicology of the Kidney. Raven Press Ltd, New York, pp 201–247

Grantham, J. J., Chonko, A. M. (1991) Renal handling of organic anions and cations; excretion of uric acid. In: Brenner, B. M., Rector, F. C. (eds) The Kidney. W. B. Saunders Company, Philadelphia, pp 483–509

Heyrovski, A. (1956) A new method for the determination of inulin in plasma and urine. Clin. Chim. Acta 1: 470–474

Koren, G. (1989) The nephrotoxic potential of drugs and chemicals. Pharmacological basis and clinical relevance. Med. Toxicol. 4: 59–72

Lee, L. J., Cook, J. A., Smith, D. E. (1986) Renal transport kinetics of furosemide in the isolated perfused rat kidney. J. Pharmacokin. Biopharm. 14: 157–174

Lee, L. J., Cook, J. A., Smith, D. E. (1988) Renal transport kinetics of chlorothiazide in the isolated perfused rat kidney. J. Pharmacol. Exp. Ther. 247: 203–208

Levy, G. (1980) Effect of plasma protein binding on renal clearance of drugs. J. Pharm. Sci. 69: 482–483

Maack, T. (1980) Physiological evaluation of the perfused rat kidney. Am. J. Physiol. 238: F71–F78

Metzler, C. M., Weiner, D. L. (1986) Statistical Consultants, Inc. PCNONLIN and NONLIN84: Software for the statistical analysis of nonlinear models. Am. Statist. 40: 52

Pritchard, J. B., Miller, D. S. (1993) Mechanisms mediating renal secretion of organic anions and cations. Physiol. Rev. 73: 765–796

Russel, F. G. M., Wouterse, A. C., Hekman, P., Grutters, G. J., Van Ginneken, C. A. M. (1987) Quantitative urine collection in renal clearance studies in the dog. J. Pharmacol. Methods 17: 125–136

Russel, F. G. M., Wouterse, A. C., Van Ginneken, C. A. M. (1989a) Renal clearance of substituted hippurates in the dog. I. Benzoylglycine (hippurate) and methyl-substituted benzoylglycines. J. Pharmacol. Exp. Ther. 248: 428–435

Russel, F. G. M., Wouterse, A. C., Van Ginneken, C. A. M. (1989b) Renal clearance of substituted hippurates in the dog. II. 4-Amino-, hydroxy- and methoxy-substituted benzoylglycines. J. Pharmacol. Exp. Ther. 248: 436–446

Schurek, H. J., Alt, J. M. (1981) Effect of albumin on the function of perfused rat kidney. Am. J. Physiol. 240: F569–F576

Ullrich, K. J. (1994) Specificity of transporters for 'organic anions' and 'organic cations' in the kidney. Biochim. Biophys. Acta 1197: 45–62