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PHARMACOKINETICS AND PHARMACODYNAMICS OF FUROSEMIDE AFTER INTRAVENOUS AND ORAL ADMINISTRATION TO SPONTANEOUSLY HYPERTENSIVE RATS AND DOCA-SALT-INDUCED HYPERTENSIVE RATS

SEONG H. JANG, MYUNG G. LEE* AND NAK D. KIM

College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Korea

ABSTRACT

The pharmacokinetics and pharmacodynamics of furosemide were investigated after intravenous (i.v.), 1 mg/100 g body weight, and oral administration, 2 mg per 100 g body weight, to spontaneously hypertensive rats (SHRs) and deoxycorticosterone acetate-salt-induced hypertensive rats (DOCA-salt rats). After i.v. administration, the 8 h urinary excretion of furosemide/g kidney (397 versus 572 μg) was significantly lower and the non-renal clearance (5·78 versus 3·94 ml min^{-1} kg^{-1}) was significantly faster in SHRs of 16 weeks of age than in age-matched control Wistar rats. This suggested that the non-renal metabolism of furosemide could be faster in SHRs of 16 weeks of age than in age-matched control Wistar rats, and this could be supported by the significantly greater amount of 4-chloro-5-sulphamoyl anthranilic acid, a metabolite of furosemide, excreted in 8 h urine as expressed in terms of furosemide (11·1 versus 4·79% of the i.v. dose) in SHRs. It could also be supported at least in part by a study of liver homogenate; the amount of furosemide remaining per gram of liver after 30 min incubation of 50μg of furosemide with the 9000*g* supernatant fraction of liver homogenate was significantly smaller (40·4 versus 43·7μg) in SHRs of 16 weeks of age than in age-matched Wistar rats. The greater metabolic activity of furosemide in liver may also be supported by the result that the amount of hepatic cytochrome P-450 (0·7013 versus 0·5186 nmol/mg protein) and the weights of liver (3·52 versus 2·93% of body weight) were significantly greater in SHRs of 16 weeks of age than in age-matched Wistar rats. After i.v. administration of furosemide, the 8 h urine output (9·93 versus 16·5 ml) and 8 h urinary excretion of sodium (1·21 versus 2·05 mmol) and chloride (1·37 versus 2·17 mmol) per gram of kidney in SHRs of 16 weeks of age were lower than those in age-matched Wistar rats, this could be due to the significantly smaller amount of furosemide excreted in 8 h urine per gram of kidney. After oral administration, the pharmacokinetics and pharmacodynamics of furosemide were not significantly different between SHRs and the control Wistar rats of 16 weeks of age. After i.v. and oral administration of furosemide, there were no significant differences in the pharmacokinetics and pharmacodynamics between DOCA-salt rats and control SD rats of 16 weeks of age except for the significantly lower urinary excretion of potassium per gram of kidney in DOCA-salt rats. On the other hand, the 8 h urinary excretion of furosemide and

*Addressee for correspondence.

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non-renal clearance were not significantly different between SHRs of six weeks of age and age-matched control Wistar rats after i.v. administration of furosemide. Since the non-renal metabolism of furosemide was not faster in either DOCA-salt rats of 16 weeks of age or SHRs of six weeks of age than that in the respective age-matched control group, the faster non-renal metabolism of furosemide in SHRs of 16 weeks of age could be due to the physiological factor from the chronic phase of hypertension in SHRs, and could not be due solely to the heredity of SHRs or the hypertensive state itself.

KEY WORDS Pharmacokinetics Pharmacodynamics Furosemide Spontaneously hypertensive rats (SHRs) Deoxycorticosterone acetate-salt-induced hypertensive rats (DOCA-salt rats)

INTRODUCTION

Furosemide, a loop diuretic, is used for treating ascites and oedema of cardiac, renal and hepatic origin, and also hypertension.[1,2] In the treatment of hypertension, furosemide is usually administered with antihypertensive agents for the prevention of secondary salt and fluid retention. Although the pharmacokinetics and/or pharmacodynamics of furosemide in both volunteers and patients with specific diseases have been well documented,[1,2 and references therein] it appears that the detailed studies on hypertensive patients are rare to date.[3]

In many other studies, spontaneously hypertensive rats (SHRs)[4–8] and deoxycorticosterone acetate-salt-induced hypertensive rats (DOCA-salt rats)[9,10] have been employed as animal models for human primary (essential) and secondary hypertension, respectively. The antihypertensive effect of furosemide in SHRs is still controversial; an antihypertensive action of furosemide in SHRs was reported in some studies;[5,8,11] however, it was not found in other reports.[6,7] While the antihypertensive effect of furosemide in SHRs has been extensively studied,[5,8,11] the comparison of the pharmacokinetics and pharmacodynamics of furosemide between SHRs and normotensive Wistar rats (as a control group) seems not have been published.

The purpose of this study is to report the pharmacokinetics and pharmacodynamics of furosemide after intravenous (i.v.) and oral administration to SHRs of 16 weeks of age, following chronic exposure to the hypertension,[12] and to age-matched control normotensive Wistar rats. A similar study in DOCA-salt rats of 16 weeks of age and age-matched control Sprague–Dawley (SD) rats was also performed in order to investigate whether some differences in pharmacokinetics and pharmacodynamics of furosemide between SHRs and normotensive Wistar rats of 16 weeks of age are caused by either heredity of SHRs or the hypertensive state itself. Furthermore, the pharmacokinetics and pharmacodynamics of furosemide were also reported after i.v. administration to SHRs of six weeks of age, corresponding to the early phase of development of the hypertension at which time blood pressure remains in the normotensive range,[12] and age-matched control Wistar rats.

MATERIALS AND METHODS

Chemicals

Furosemide i.v. solution (Lasix, 20 mg/2 ml) was kindly supplied by Han Dok Pharmaceutical (Seoul, Korea), and 4-chloro-5-sulphamoyl anthranilic acid (CSA) was purchased from U.S. Pharmacopoeia (Rockville, MD, U.S.A.). DOCA, *p*-aminohippuric acid (PAH), β-glucuronidase, sodium dithionite, tris base, nicotinamide adenosine dinucleotide phosphate (NADP), glucose-6-phosphate (G6P), magnesium chloride, glucose-6-phosphate dehydrogenase (G6PD), and uridine diphosphoglucuronic acid (UDPGA) were products of Sigma Chemical (St Louis, MO, U.S.A.). The other chemicals were reagent grade and used without further purification.

Animals

SHRs of five weeks of age were kindly supplied by Yuhan Research Centre (Kunpo, Korea). At 16 weeks of age, systolic blood pressure was measured using tail cuff plethysmography (Narcotrace 40, NBS, Houston, TX, U.S.A.). The rats having systolic blood pressure higher than 170 mm Hg were employed for the study. Wistar rats used as control group for SHRs were purchased from the Laboratory Animal Centre, Seoul National University (Seoul, Korea). The rats having systolic blood pressure lower than 110 mm Hg were employed for the study. The SD rats of five weeks of age were also purchased from the Laboratory Animal Centre. The SD rats were randomly divided into two groups, DOCA-salt rats and control rats. DOCA-salt rats were given subcutaneous injections of 12·5 mg/kg body weight of DOCA (5 mg ml^{-1}) dissolved in cotton seed oil every three days and 1% NaCl as drinking water *ad libitum* from 12 to 16 weeks of age. The control SD rats were given equivalent volumes of subcutaneous injections of cotton seed oil and the tap water as drinking water *ad libitum*. Systolic blood pressure was similarly determined at 16 weeks of age. The DOCA-salt rats and the control SD rats having systolic blood pressure higher than 150 mm Hg and lower than 110 mm Hg, respectively, were employed for the study. Approximately two days before the experiment, 24 h urine output and urinary excretion of sodium, potassium, and chloride were measured with food and drinking water *ad libitum*.

Intravenous study

On the early morning at the end of 16 weeks (after overnight fasting with drinking water *ad libitum*), the carotid artery and jugular vein were catheterized with polyethylene tubing (Clay Adams, Parsippany, NJ, U.S.A.) under light ether anaesthesia. Both cannulas were exteriorized to the dorsal side of the neck, where each cannula terminated with long Silastic tubing (Dow Corning, Midland,

MI, U.S.A.). The Silastic tubings were covered with wire to allow free movement of the rat. Each rat was housed in a rat metabolic cage (Daejong Scientific, Seoul, Korea) and allowed to recover from anaesthesia for 4–5 h before study. They were not restrained at any time during the study. The Lasix solution was freshly diluted with 0.9% NaCl injectable solution (Choong Wae Pharmaceutical, Seoul, Korea) before use. Water and electrolyte losses in urine induced by furosemide were replaced volume by volume by i.v. infusion of Ringer's lactate solution (Dai Han Pharmaceutical, Seoul, Korea) via the jugular vein, because it has been reported[13] that the pharmacodynamic effects of i.v. furosemide are dependent on the rates and compositions of fluid replacement. Food and drinking water were restrained during the experimental period.

Furosemide, 1 mg/100 g body weight, was administered by i.v. infusion in 1 min via the jugular vein (the total injection volume was approximately 0·6 ml) of SHRs ($n=10$), Wistar rats ($n=10$), DOCA-salt rats ($n=9$), and SD rats ($n=8$). PAH, 80 mg/kg body weight, was also administered by i.v. infusion in 1 min via the jugular vein (the total injection volume was approximately 0·3 ml) of each rat at 10 min before i.v. administration of furosemide. Blood samples (0·12 ml) were collected via the carotid artery before (to serve as a control) and at the end of infusion of PAH and 1 (at the end of infusion of furosemide), 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min after i.v. administration of furosemide. Heparinized 0·9% NaCl injectable solution (10 units ml^{-1}), 0·25 ml, was used to flush the cannula after each blood sampling. Blood was centrifuged immediately to minimize the 'blood storage effect' of the plasma concentrations of furosemide,[14] and 0·05 ml of plasma was stored at −20 °C until HPLC analysis of furosemide and PAH. 8 h after i.v. administration of furosemide, a large volume of blood was collected through the carotid artery and the rat was sacrificed by cervical dislocation. The plasma was stored in the freezer prior to the analysis of creatinine, and the measurement of plasma protein binding. After measuring the exact volume of urine, the metabolic cage was rinsed with 20 ml of distilled water. The rinsings were combined with the urine, and the urinary bladder was cut and washed into the combined urine. After measuring the exact volume of the combined urine, an aliquot of the combined 8 h urine was collected and frozen prior to the analysis for PAH, furosemide, CSA, sodium, potassium, chloride, and creatinine. A portion (0·5 ml) of the combined urine was also incubated for 24 h with 1 ml of pH 5·0 phosphate buffer,[1] containing 10 000 units of β-glucuronidase, in a water-bath shaker that was kept at 37 °C and 50 oscillations min^{-1} (opm). The liver, kidney, and stomach of each rat were excised and weighed. At the end of the experiment, the whole gastrointestinal (GI) tract (including its contents) was removed, transferred to a 500 ml beaker filled with 200 ml of 0·01 M NaOH (to facilitate extraction of furosemide), and cut into small pieces using scissors. After vigorously shaking for 10 min, two 0·1 ml aliquots of the supernatant were collected from each beaker and stored in the freezer prior to the analysis of furosemide. Similar

experiments were also performed using normotensive SHRs ($n = 7$) and Wistar rats ($n = 5$) of six weeks of age.

Oral study

Furosemide, 2 mg/100 g body weight, was administered orally (the total oral volume was approximately 1·0 ml) to SHRs ($n = 14$), Wistar rats ($n = 9$), DOCA-salt rats ($n = 6$), and SD rats ($n = 9$) of 16 weeks of age, using a feeding tubing after overnight fasting with drinking water *ad libitum*. PAH, 80 mg/kg body weight, was also administered by i.v. infusion in 1 min via the jugular vein of each rat 14 min after oral administration of furosemide. Blood samples were collected via the carotid artery 0 (to serve as a control), 15 (at the end of infusion of PAH), 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min after oral administration of furosemide. Urine was collected for 24 h after oral administration of furosemide. The other procedures were similar to those of the i.v. study.

Plasma protein binding studies

The plasma protein binding of furosemide was determined by the equilibrium dialysis technique using plasma from SHRs ($n = 5$), Wistar rats ($n = 5$), DOCA-salt rats ($n = 5$), and SD rats ($n = 5$) of 16 weeks of age. 1 ml of the plasma was dialysed against 1 ml of isotonic phosphate buffer of pH 7·4 containing 3% dextran in order to minimize volume shifts,[15,16] using 1 ml dialysis cells (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and Spectra/Por membrane 2 (m.w. cut-off of 12 000–14 000, Spectrum Medical, Los Angeles, CA, U.S.A.). In order to reduce equilibration time, furosemide was spiked into the plasma side[17] with an initial plasma concentration of 10 μg ml^{-1}. The spiked dialysis cells were incubated for 24 h in a water bath shaker kept at 37 °C and at the rate of 50 opm. The plasma protein binding of furosemide has been reported[18] to be relatively constant for furosemide concentrations of up to 36 μg ml^{-1} using the equilibrium dialysis method. The plasma protein binding of furosemide is not influenced by CSA at CSA concentrations up to 2·6 μg ml^{-1}.[18]

Metabolism in homogenate of liver and kidney

The procedures were similar to the method[19] reported by Litterst *et al.*[20] In the early morning at the end of 16 weeks, SHRs ($n = 5$) and Wistar rats ($n = 5$) of 16 weeks of age were sacrificed by cervical dislocation. 1 g of liver or kidney was excised, rinsed in 50 mM of tris-HCl buffer (pH 7·4), blotted dry with tissue paper and weighed. All subsequent procedures were conducted at 4 °C. Each tissue was minced into small pieces with scissors and then homogenized with four volumes of cold 0·25 M sucrose in a tissue homogenizer (Ultra-Turrax T25, Janke & Kunkel, IKA-Labortechnik, Federal Republic of Germany). The

homogenates were then centrifuged (Beckman Model J2-21, Palo Alto, CA, U.S.A.) at 9000 *g* for 20 min. After discarding the floating fat layer, the 9000 *g* supernatant fraction was collected.

Metabolic activity was initiated by adding 1 ml of the above supernatant to a glass test tube containing 0·05 ml of diluted Lasix solution (50 μg of furosemide), 2·045 ml of the NADPH-generating system (1 mM NADP, 10 mM G6P, 5 mM magnesium chloride, and two units G6PD), 100 mM of pH 7·4 tris-HCl buffer, and 3·3 mM of UDPGA. The mixture was thoroughly mixed by hand and shaken in a water bath shaker kept at 37 °C and at the rate of 50 opm. After 30 min of incubation, 1 ml of 1 M NaOH was added in order to terminate the enzyme activity. After centrifugation, two 0·1 ml aliquots of the supernatant were collected and stored in the freezer prior to the analysis of furosemide.

Analytical procedure

The concentrations of PAH,[21] and furosemide and CSA[19] were determined by the reported sensitive HPLC methods. The sample preparation for PAH, furosemide, and CSA was simple; 2·5 volumes of acetonitrile was added to the biological samples. For the analysis of furosemide in tissue homogenate, 0·1 ml of 0·3% HCl was added after addition of the acetonitrile.[22] After vortex mixing and centrifugation, an aliquot of supernatant was injected directly onto the column. Therefore, the formation of CSA in the present urine sample was not due to an artifact in the process of acid extraction for the sample preparation.[23]

Concentrations of creatinine in plasma and urine, and of chloride in urine were determined using a chemical analyser (Gilford SBA-300, Corning Laboratory, Oberlin, OH, U.S.A.), and sodium and potassium in urine were determined using flame photometry (Model IL-943, Instrumentation Laboratory SpA, Milano, Italy).

Measurement of cytochrome P-450 contents

The microsomal fractions of liver from SHRs ($n = 5$) and Wistar rats ($n = 5$) of 16 weeks of age were prepared by differential centrifugation as previously described,[24] and the protein content of liver microsomes was assayed by the method of Lowry *et al.*[25] The contents of cytochrome P-450 in liver microsomes were determined by its carbon monoxide difference spectrum after reduction with sodium dithionite according to the reported procedure[26] using a spectrophotometer (Shimazu UV-260, Japan). An extinction coefficient of 91 cm^{-1} $mmol^{-1}$ was used to calculate the hepatic cytochrome P-450 contents of liver.

Pharmacokinetic analysis

The area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method;[19,22]

this method employed the logarithmic trapezoidal rule recommended by Chiou[27] for the calculation of area during the declining plasma level phase and the linear trapezoidal rule for the rising plasma level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

The standard method[28] was used to calculate the following pharmacokinetic parameters after i.v. administration: the time-averaged total body clearance (CL), area under the first moment of the plasma concentration–time curve (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and time-averaged renal (CL_R) and non-renal (CL_{NR}) clearances:

$$CL = \text{Dose}/AUC \tag{1}$$

$$AUMC = \int_0^\infty tC_P \, dt \tag{2}$$

$$MRT = AUMC/AUC \tag{3}$$

$$V_{ss} = CL \; MRT \tag{4}$$

$$CL_R = X_{U,Fu}/AUC \tag{5}$$

$$CL_{NR} = CL - CL_R \tag{6}$$

where C_P is the plasma concentration of furosemide at time t, and $X_{U,Fu}$ is the amount of furosemide excreted in urine up to time infinity. In the estimation of CL_R, the amount of furosemide excreted in urine up to time infinity was assumed to equal the total amount excreted in 8 and 24 h after i.v. infusion and oral administration, respectively, since negligible amounts of furosemide were found in urine collected later. The total AUC instead of AUC from time zero to the last blood sampling time was employed for the calculation of CL_R after i.v. studies, since the mean contributions of AUC from the last blood sampling time to time infinity to the total AUC were lower than 1·06% for each group of rats. Only AUC and CL_R were estimated after oral administration.

The extent of the dose absorbed into the general circulation after oral administration (F) was determined by the following plasma area-clearance method;[19,29]

$$F = \frac{CL_{oral} \; AUC_{oral}}{\text{Dose}_{oral}} \tag{7}$$

where CL_{oral}, the CL after the oral dose, was calculated by the summation of CL_R obtained from the oral study ($CL_{R,oral}$) and CL_{NR} obtained from the i.v. study ($CL_{NR,i.v.}$) as illustrated below:

$$CL_{NR,i.v.} = CL_{i.v.} - CL_{R,i.v.} \quad (8)$$

$$CL_{oral} = CL_{NR,i.v.} + CL_{R,oral}. \quad (9)$$

Creatinine clearance (CL_{CR}) was estimated assuming that the kidney function was stable during the study.

The mean values of each clearance, V_{ss}, and half life were calculated by the harmonic mean method.[30]

Pharmacodynamic analysis

The diuretic, natriuretic, kaluretic, and chloruretic efficiencies were calculated by dividing the total urine output (ml) and total amount (mmol) of sodium, potassium, and chloride excreted in the urine by the total amount (mg) of furosemide excreted in the urine, respectively.

Statistical analysis

Levels of statistical significance were assessed using the *t* test between two means for unpaired data. Significant differences were judged as $p<0{\cdot}05$. All results are expressed as mean $\pm$ standard deviation (S.D.).

RESULTS AND DISCUSSION

The mean arterial plasma concentration–time curves of furosemide after i.v. administration to SHRs and Wistar rats of 16 weeks of age are shown in Figure 1, and the relevant pharmacokinetic parameters are listed in Table 1. After i.v. administration, the plasma levels of furosemide declined rapidly with lower levels in SHRs. The mean terminal half life was significantly shorter in SHRs (49·0 versus 84·8 min) and resulted in a significantly shorter MRT (16·7 versus 22·2 min) in SHRs than in Wistar rats. Since it has been reported that the CL of furosemide is dose[31] or concentration[32] dependent in rats, and that CL_R of furosemide is urine flow dependent in rabbits,[33,34] the values of CL, CL_R, and CL_{NR} in the present studies were time-averaged values. There was no significant difference in the time-averaged CL and CL_R between SHRs and Wistar rats; however, the CL_{NR} was significantly faster (5·78 versus 3·94 ml min^{-1} kg^{-1}) in SHRs. The amount of furosemide excreted in 8 h urine ($X_{u,Fu,\,0-8\,h}$) was significantly smaller (31·2 versus 42·8% of the i.v. dose) in SHRs, and the smaller urinary excretion of furosemide has also been reported in hypertensive patients; the 24 h urinary excretion of furosemide was 14·7 and 25·4 mg for patients with severe arterial hypertension ($n=6$) and an age-matched control group ($n=6$), respectively, when furosemide, 40 mg, was administered intravenously.[3] The smaller $X_{u\,Fu\,0-8\,h}$ in SHRs could be due to the faster

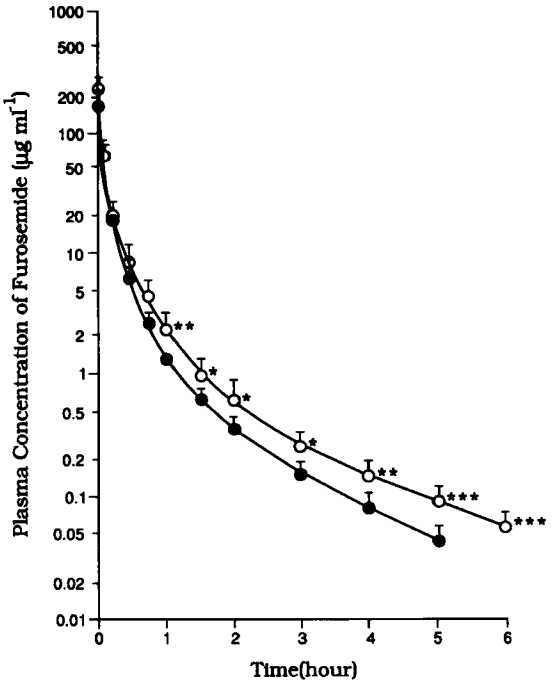

Figure 1. Mean arterial plasma concentration–time profiles of furosemide after i.v. administration of furosemide, 1 mg/100 g body weight, to SHRs (●) and Wistar rats (○) of 16 weeks of age. The bars represent S.D. *, $p<0{\cdot}05$; **, $p<0{\cdot}01$; ***, $p<0{\cdot}001$

metabolism of furosemide in SHRs as will be discussed later; however, it was not due to the lower plasma protein binding or glomerular filtration rate (GFR) in SHRs; the unbound fraction of furosemide in plasma, and the GFR values estimated by creatinine renal clearance (CL_{CR}), were not significantly different between SHRs and Wistar rats (Table 1). It has been reported[2] that the majority of furosemide available for excretion in the urine is delivered by active secretion rather than passive filtration when considering the high plasma protein binding of furosemide. The values of V_{ss} and plasma protein binding were not significantly different between SHRs and Wistar rats (Table 1), and similar results were also reported between patients with severe arterial hypertension and an age-matched control group.[3] The glucuronide formation of furosemide reported in humans,[35] dogs,[36] and rabbits (unpublished data) was less than 3% of the i.v. dose (as expressed in terms of furosemide) in SHRs, Wistar rats, DOCA-salt rats and SD rats. A negligible amount of glucuronide formation of furosemide was also reported in SD rats.[19,22] The exact reason for the negligible formation of the glucoronide of furosemide in rats is unknown; however, it may be due to the inhibition of UDP-glucuronyltransferase activity caused by furosemide in rats.[37]

Table 1. Pharmacokinetic and pharmacodynamic parameters of furosemide after i.v. administration of furosemide, 1 mg/100 g body weight, to SHRs and Wistar rats and DOCA-salt rats and SD rats of 16 weeks of age

	SHRs ($n=10$) Mean ± S.D.[a]	Wistar rats ($n=10$) Mean ± S.D.	DOCA-salt rats ($n=9$) Mean ± S.D.	SD rats ($n=8$) Mean ± S.D.
Body weight (g)	287 ± 26·8**	359 ± 24·2	386 ± 22·2	380 ± 36·4
$t_{½}$ (min)	49·0 ± 14·6***	84·8 ± 23·5	95·2 ± 32·6	99·7 ± 32·2
AUC (μg min ml^{-1})	1180 ± 371	1410 ± 353	1110 ± 405	963 ± 276
MRT (min)	16·7 ± 2·53***	22·2 ± 2·19	25·9 ± 6·6	26·5 ± 6·04
V_{ss} (ml kg^{-1})	134 ± 56·1	157 ± 38·8	226 ± 98·3	262 ± 134
CL (ml min^{-1} kg^{-1})	8·24 ± 2·58	7·08 ± 1·66	9·00 ± 3·56	10·4 ± 3·40
CL_R (ml min^{-1} kg^{-1})	2·51 ± 0·846	2·83 ± 1·24	4·03 ± 2·39	4·01 ± 3·40
CL_{NR} (ml min^{-1} kg^{-1})	5·78 ± 2·21*	3·94 ± 1·17	4·59 ± 2·39	6·02 ± 2·83
$X_{u,Fu,\ 0-8\ h}$(μg)	896 ± 205***	1530 ± 365	1410 ± 323	1510 ± 388
$X_{u,Fu\ 0-8\ h}$(μg/g kidney)	397 ± 89·5**	572 ± 138	412 ± 81·3	553 ± 152
$X^{b}_{u,CSA,\ 0-8\ h}$(μg)	230 ± 93·0*	124 ± 52·5	157 ± 87·1	157 ± 79·9
% of dose recovered from GI tract	0·901 ± 0·280	1·47 ± 0·747	1·01 ± 0·575	0·371 ± 0·319
Unbound fraction in plasma (%)	7·52 ± 4·18	5·42 ± 1·93	9·20 ± 2·72	8·32 ± 3·44
CL_{CR}[c](ml min^{-1} kg^{-1})	4·22 ± 1·20	4·54 ± 2·41	6·23 ± 1·87	6·87 ± 3·38
CL_{PAH}[d](ml min^{-1} kg^{-1})	10·6 ± 4·34	9·31 ± 3·74	9·54 ± 2·41	8·17 ± 3·76
$UO_{0-8\ h}$(ml/g kidney)	9·93 ± 3·32**	16·5 ± 5·42	11·9 ± 3·65	17·7 ± 7·09
$X_{u,Na+,0-8\ h}$(mmol/g kidney)	1·21 ± 0·410**	2·05 ± 0·653	1·55 ± 0·507	2·20 ± 0·821

$X_{u,K^+,0-8\ h}$(mmol/g kidney)	0·222±0·0642	0·258±0·0717	0·119±0·0456***	0·322±0·0981
$X_{u,Cl^-,0-8\ h}$(mmol/g kidney)	1·37±0·453**	2·17±0·693	1·57±0·455	2·29±0·932
$UO_{24\ h}$[e](ml/g kidney)	9·13±5·10	7·00±3·68	22·9±6·83**	9·03±5·05
$X^e_{u,Na^+,24\ h}$(mmol/g kidney)	1·29±0·267	0·977±0·466	4·17±0·928***	0·313±0·120
$X^e_{u,K^+,24\ h}$(mmol/g kidney)	0·902±0·167	0·669±0·258	0·636±0·197	0·797±0·128
$X^e_{u,Cl^-,24\ h}$(mmol/g kidney)	1·62±0·625	1·35±0·716	4·72±1·15***	0·493±0·218
Diuretic efficiency (ml/mg of Fu[f])	25·1±7·53	29·6±8·33	28·6±6·08	31·3±6·57
Natriuretic efficiency (mmol/mg of Fu)	3·05±0·965	3·69±1·10	3·74±0·949	3·98±1·09
Kaluretic efficiency (mmol/mg of Fu)	0·575±0·190	0·467±0·149	0·300±0·124**	0·586±0·125
Chloruretic efficiency (mmol/mg of Fu)	3·47±1·04	3·90±1·13	3·82±0·897	4·14±1·23
Liver weight (% of body weight)	3·52±0·534**	2·93±0·0961	2·99±0·185	3·00±0·172
Kidney weight (% of body weight)	0·794±0·0966	0·749±0·0559	0·881±0·0492**	0·729±0·0591
Stomach weight (% of body weight)	0·559±0·0816***	0·486±0·0621	0·481±0·0841	0·428±0·0231

[a]Standard deviation.
[b]Expressed in terms of furosemide.
[c]Renal clearance of creatinine.
[d]Renal clearance of *p*-aminohippuric acid.
[e]Urine samples were collected for 24 h before i.v. administration of furosemide.
[f]Furosemide.
*, $p<0·05$; **, $p<0·01$; ***, $p<0·001$.

The contribution of biliary and/or GI excretion of furosemide to CL_{NR} after i.v. administration seemed to be minor, because 0·901 and 1·47% of the i.v. dose were recovered from the GI tract 8 h after the i.v. dose for SHRs and Wistar rats of 16 weeks of age, respectively (Table 1). Similar results have also been reported; less than 5% of the i.v. dose was excreted in bile[38] or recovered from the GI tract[19] after i.v. administration of furosemide to rats. Therefore, the significantly faster CL_{NR} in SHRs of 16 weeks of age than that in age-matched Wistar rats suggested that the non-renal metabolism of furosemide could be faster in SHRs. This could be supported by the significantly greater amount of CSA ($X_{u,\,CSA,\,0-8\,h}$, as expressed in terms of furosemide) excreted in 8 h urine (11·1 versus 4·79% of the i.v. dose) in SHRs (Table 1) than that in age-matched Wistar rats. The faster metabolism of furosemide in SHRs of 16 weeks of age could also be supported at least in part by the result of the *in vitro* incubation of 50 μg of furosemide with the 9000 *g* supernatant fraction of liver homogenate ($n=5$); the amount of furosemide remaining per gram of liver after 30 min of incubation was significantly smaller ($40·4 \pm 1·44$ versus $43·7 \pm 1·38$ μg; $p<0·006$) in SHRs than that in age-matched Wistar rats. However, the values for kidney homogenate were not significantly different between SHRs and Wistar rats ($48·8 \pm 3·22$ versus $49·5 \pm 3·45$ μg; $p<0·75$). The greater metabolic activity of furosemide in the liver may also be supported by the significantly greater amount of hepatic cytochrome P-450 in SHRs of 16 weeks of age; the mean values were $0·701 \pm 0·01$ and $0·519 \pm 0·120$ nmol/mg protein ($p<0·023$) for SHRs ($n=5$) and Wistar rats ($n=5$), respectively. The weights of liver and stomach per body weight were significantly greater in SHRs of 16 weeks of age than in age-matched Wistar rats; however, the values for kidney were not significantly different between SHRs and Wistar rats (Table 1).

The mean arterial plasma concentration–time curves of furosemide after i.v. administration to DOCA-salt rats and SD rats of 16 weeks of age are shown in Figure 2, and the relevant pharmacokinetic parameters are also listed in Table 1. After i.v. administration the plasma levels of furosemide declined rapidly with mean terminal half lives of 95·2 and 99·7 min for DOCA-salt rats and SD rats, respectively. None of the pharmacokinetic parameters of furosemide listed in Table 1 were significantly different between DOCA-salt rats and SD rats. It is of interest to note that the CL_{NR} of furosemide was not significantly different between DOCA-salt rats and SD rats, and 1·01 and 0·371% of the i.v. dose were recovered from the GI tract at 8 h after i.v. administration for DOCA-salt rats and SD rats, respectively. This suggested that the non-renal metabolism of furosemide in DOCA-salt rats of 16 weeks of age was no faster than that in age-matched SD rats. Therefore, the faster non-renal metabolism of furosemide in SHRs of 16 weeks of age than in age-matched Wistar rats might not be due to the hypertension itself. The weights of liver and stomach per body weight were not significantly different between DOCA-salt rats and SD rats of 16 weeks of age; however, the values for kidney were significantly greater in DOCA-salt rats than in SD rats (Table 1).

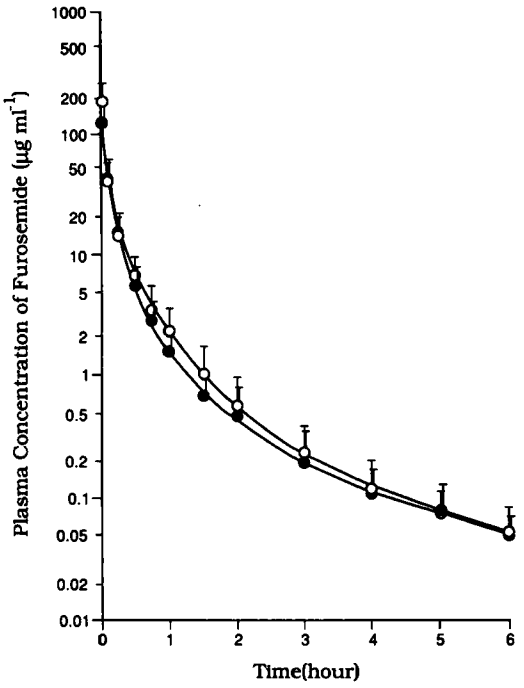

Figure 2. Mean arterial plasma concentration–time profiles of furosemide after i.v. administration of furosemide, 1 mg/100 g body weight, to DOCA-salt rats (○) and SD rats (●) of 16 weeks of age. The bars represent S.D.

The mean arterial plasma concentration–time curves of furosemide after i.v. administration to SHRs and Wistar rats of six weeks of age are shown in Figure 3, and relevant pharmacokinetic parameters are listed in Table 2. After i.v. administration, plasma concentrations of furosemide declined similarly with terminal half lives of 48·2 and 55·7 min for SHRs and Wistar rats, respectively. No pharmacokinetic parameters of furosemide listed in Table 2 were significantly different between SHRs and Wistar rats of six weeks of age except the MRT. It is to be noted that the CL_{NR} of furosemide in SHRs of six weeks of age was no faster than that in age-matched Wistar rats. Therefore, the faster CL_{NR} of furosemide in SHRs of 16 weeks of age than in age-matched Wistar rats (Table 1) could be due to the physiological factors drived from the chronic phase of hypertension in SHRs and not due solely to hereditary of SHRs itself. However, more studies are needed to prove this hypothesis.

The mean arterial plasma concentration–time curves of furosemide after oral administration to SHRs and Wistar rats, and DOCA-salt rats and SD rats of 16 weeks of age are shown in Figures 4 and 5, respectively, and the relevant pharmacokinetic parameters are listed in Table 3. None of the pharmacokinetic parameters listed in Table 3 were significantly different between SHRs and

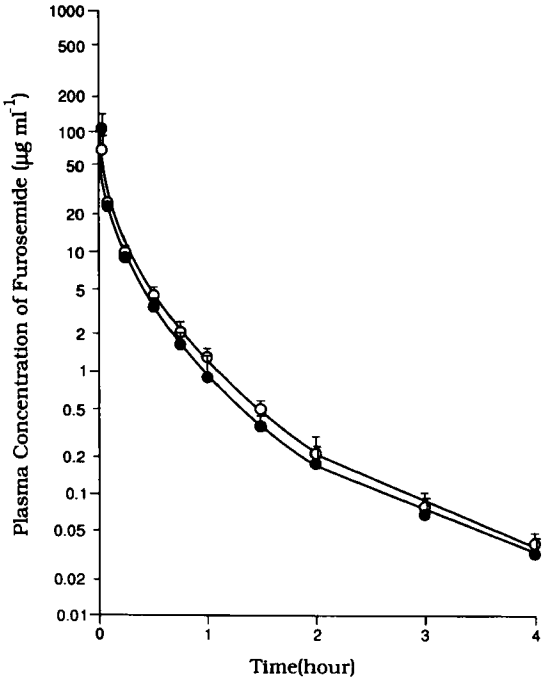

Figure 3. Mean arterial plasma concentration–time profiles of furosemide after i.v. administration of furosemide, 1 mg/100 g body weight, to SHRs (●) and Wistar rats (○) of 6 weeks of age. The bars represent S.D.

Wistar rats, and similar results were also obtained from DOCA-salt rats and SD rats. The *F* value was 25·3% in SD rats of 16 weeks of age and similar *F* values in SD rats, 30·2%[19] and 27·6%[39] have also been reported. The mean percentage of dose recovered from GI tract at 24 h after oral dose in SD rats of 16 weeks of age was 13·3%, this value was lower than the values of 40·1[39] and 40·3%[19] where the values were estimated at 8 h after oral administration of furosemide to SD rats. This suggests that some of the orally administered furosemide may be absorbed from the GI tract between 8 and 24 h after oral administration. Furosemide has been reported to be fairly stable in human gastric juice.[19,35]

The pharmacodynamic parameters of furosemide after i.v. administration to SHRs and Wistar rats of 16 weeks of age are also listed in Table 1. The 8 h urine output per gram of kidney ($UO_{0-8\,h}$) was significantly lower (9·93 versus 16·5 ml) in SHRs, and could be due to the significantly smaller amount of furosemide excreted in 8 h urine per gram of kidney (397 versus 572 μg) in SHRs (Table 1). The lower value of $UO_{0-8\,h}$ in SHRs was not due to the difference in protein binding, GFR, or effective renal plasma flow (ERPF) as listed in Table 1; the value of ERPF estimated by PAH renal clearance (CL_{PAH}) was

Table 2. Pharmacokinetic and pharmacodynamic parameters of furosemide after i.v. administration of furosemide, 1 mg/100 g of body weight to SHRs and Wistar rats of six weeks of age

	SHRs ($n=7$) Mean ± S.D.[a]	Wistar rats ($n=5$) Mean ± S.D.
Body weight (g)	114 ± 9·76	125 ± 7·07
$t_{½}$ (min)	48·2 ± 7·68	55·7 ± 25·6
AUC (μg min ml^{-1})	665 ± 107	664 ± 68·0
MRT (min)	16·4 ± 2·98*	21·2 ± 2·62
V_{ss}(ml kg^{-1})	243 ± 45·0	306 ± 56·3
CL (ml min^{-1} kg^{-1})	15·0 ± 2·37	15·1 ± 1·48
CL_R(ml min^{-1} kg^{-1})	2·78 ± 1·20	3·37 ± 0·927
CL_{NR}(ml min^{-1} kg^{-1})	12·0 ± 1·42	11·5 ± 1·24
$X_{u,Fu,0-8\ h}$(μg)	228 ± 49·3	294 ± 78·7
$X_{u,Fu,0-8\ h}$(μg/g kidney)	180 ± 42·5	212 ± 44·6
CL_{CR}[b](ml min^{-1} kg^{-1})	3·27 ± 0·939	3·47 ± 0·735
$UO_{0-8\ h}$(ml/g kidney)	12·5 ± 5·37	13·3 ± 2·98
$X_{u,Na^+,0-8\ h}$(mmol/g kidney)	1·56 ± 0·272	1·97 ± 0·432
$X_{u,K^+,0-8\ h}$(mmol/g kidney)	0·322 ± 0·0920	0·348 ± 0·108
$X_{u,Cl^-,0-8\ h}$(mmol/g kidney)	1·76 ± 0·662	2·03 ± 0·576
Diuretic efficiency (ml/mg of Fu[c])	67·3 ± 17·2	64·8 ± 21·4
Natriuretic efficiency (mmol/mg of Fu)	8·30 ± 1·83	9·58 ± 2·94
Kaluretic efficiency (mmol/mg of Fu)	2·12 ± 0·374	1·77 ± 0·904
Chloruretic efficiency (mmol/mg of Fu)	9·58 ± 1·88	10·2 ± 4·44

[a]Standard deviation.
[b]Renal clearance of creatinine.
[c]Furosemide.
*, $p<0·05$.

not significantly different between SHRs and Wistar rats. The total amount of sodium ($X_{u,Na^+,0-8\ h}$, 1·21 versus 2·05 mmol) and chloride ($X_{u,Cl^-,0-8\ h}$, 1·37 versus 2·17 mmol) excreted in 8 h urine per gram of kidney were also significantly lower in SHRs than in Wistar rats. The urinary excretion of sodium has also been reported to be lower in hypertensive patients; the 24 h urinary excretion of sodium was 134 and 293 mmol for patients with severe arterial hypertension and an age-matched control group, respectively.[3] However, the values of potassium were not significantly different between SHRs and Wistar rats although their urine output and urinary excretion of sodium were significantly different. Similar results were also obtained after administration of furosemide to humans[40] and dogs.[41] This might be due to the constant rate of potassium secretion in the distal tubule.[42] It is of interest to note that the diuretic, natriuretic, kaluretic and chloruretic efficiencies were not significantly different between SHRs and Wistar rats. The 24 h urine output and urinary excretion of sodium, potassium, and chloride per gram of kidney were also measured before i.v. administration of furosemide, and they were not significantly different between SHRs and Wistar rats (Table 1).

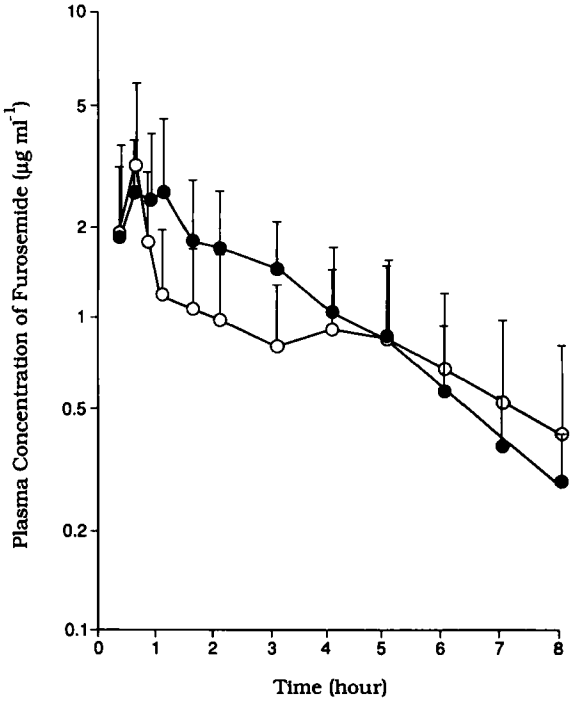

Figure 4. Mean arterial plasma concentration–time profiles of furosemide after oral administration of furosemide, 2 mg/100 g body weight, to SHRs (●) and Wistar rats (○) of 16 weeks of age. The bars represent S.D.

The pharmacodynamic parameters of furosemide after i.v. administration to DOCA-salt rats and SD rats of 16 weeks of age are also listed in Table 1. The 8 h urine output per gram of kidney was not significantly different between DOCA-salt rats and SD rats, as expected because the urinary excretion of furosemide per gram of kidney was not significantly different between DOCA-salt rats and SD rats (Table 1). The 8 h urinary excretion of sodium and chloride per gram of kidney was not significantly different either, however, the value for potassium was significantly lower in DOCA-salt rats (0·119 versus 0·322 mmol) than in SD rats. The 24 h urine was also collected before i.v. administration of furosemide; the urine output and urinary excretion of sodium and chloride per gram of kidney were significantly greater in DOCA-salt rats than in SD rats, since the water containing 1% NaCl was supplied as drinking water *ad libitum* to DOCA-salt rats during the 24 h urine collection period.

The urine output and urinary excretion of sodium, potassium, and chloride per gram of kidney were not significantly different between SHRs and Wistar rats of six weeks of age after i.v. administration of furosemide, as expected

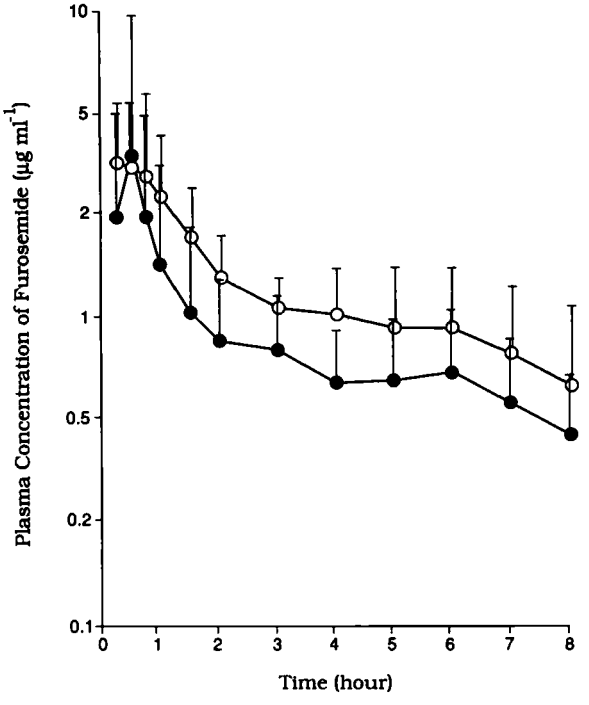

Figure 5. Mean arterial plasma concentration–time profiles of furosemide after oral administration of furosemide, 2 mg/100 g body weight, to DOCA-salt rats (○) and SD rats (●) of 16 weeks of age. The bars represent S.D.

since 8 h urinary excretion of furosemide per gram of kidney was not significantly different between SHRs and Wistar rats of six weeks of age (Table 2).

The pharmacodynamic parameters of furosemide after oral administration to SHRs and Wistar rats, and DOCA-salt rats and SD rats of 16 weeks of age are also listed in Table 3. The pharmacodynamic parameters of furosemide were not significantly different between SHRs and Wistar rats. The corresponding parameters were not significantly different between DOCA-salt rats and SD rats either, except for the significantly lower urinary excretion of potassium per gram of kidney in DOCA-salt rats. Similar results were also obtained after i.v. administration in DOCA-salt rats, and the exact reason remains to be fully elucidated. As expected, the 24 h urine output and urinary excretion of sodium and chloride per gram of kidney were significantly greater in DOCA-salt rats than in SD rats when 24 h urine was collected before oral administration of furosemide.

In conclusion, urinary excretion of furosemide (due to the significantly faster non-renal metabolism), urine output, and urinary excretion of sodium and chloride per gram of kidney in SHRs of 16 weeks of age were significantly lower

Table 3. Pharmacokinetic and pharmacodynamic parameters of furosemide after oral administration of furosemide, 2 mg/100 g body weight, to SHRs and Wistar rats and DOCA-salt rats and SD rats of 16 weeks of age

	SHRs ($n=14$) Mean ± S.D.[a]	Wistar rats ($n=9$) Mean ± S.D.	DOCA-salt rats ($n=6$) Mean ± S.D.	SD rats ($n=9$) Mean ± S.D.
Body weight (g)	293 ± 27·2	293 ± 54·5	358 ± 24·2	377 ± 41·7
$t_{½}$ (min)	137 ± 82·0	144 ± 95·4	163 ± 132	190 ± 64·0
AUC (μg min ml^{-1})	639 ± 271	595 ± 409	838 ± 464	531 ± 361
Cl_R (ml min^{-1} kg^{-1})	2·07 ± 0·962	2·81 ± 1·93	2·10 ± 2·54	3·53 ± 0·675
$X_{u,Fu,\ 0-24\ h}$(μg)	408 ± 146	507 ± 215	746 ± 268	764 ± 613
$X_{u,Fu\ 0-24\ h}$(μg/g kidney)	175 ± 61·6	213 ± 72·1	219 ± 73·2	259 ± 196
$X^{b}_{u,CSA,\ 0-24\ h}$(μg)	N.D.[c]	N.D.	N.D.	N.D.
% of dose recovered from GI tract	20·8 ± 8·52	17·7 ± 12·2	4·97 ± 2·48	13·3 ± 11·7
Unbound fraction in plasma (%)	7·78 ± 4·22	4·85 ± 1·89	12·7 ± 5·25	9·52 ± 2·33
CL_{CR}[d](ml min^{-1} kg^{-1})	6·77 ± 0·983	5·50 ± 1·57	6·66 ± 1·27	5·28 ± 1·80
CL_{PAH}[e](ml min^{-1} kg^{-1})	8·80 ± 3·73	10·1 ± 2·69	7·13 ± 4·33	10·6 ± 4·22
F[f]	25·8	20·1	28·0	25·3
$UO_{0-24\ h}$(ml/g kidney)	8·04 ± 3·17	13·4 ± 9·89	15·3 ± 6·35	17·4 ± 6·58
$X_{u,Na^+,0-24\ h}$(mmol/g kidney)	1·25 ± 0·463	1·77 ± 1·07	2·16 ± 0·820	2·16 ± 0·824

$X_{u,K^+,0-24\ h}$(mmol/g kidney)	0·307±0·0530	0·364±0·125	0·124±0·0394***	0·507±0·0704
$X_{u,Cl^-,0-24\ h}$(mmol/g kidney)	1·24±0·495	1·81±1·34	2·28±0·852	2·26±0·590
$UO_{24\ h}$[g](ml/g kidney)	6·67±2·67	8·58±4·40	18·7±7·73*	9·34±5·88
$X^{g}_{u,Na^+,24\ h}$(mmol/g kidney)	0·737±0·381	0·650±0·251	3·35±1·34***	0·377±0·272
$X^{g}_{u,K^+,24\ h}$(mmol/g kidney)	0·532±0·221	0·556±0·108	0·637±0·225	0·598±0·170
$X^{g}_{u,Cl^-,24\ h}$(mmol/g kidney)	0·961±0·527	0·694±0·466	3·88±1·44***	0·479±0·381
Diuretic efficiency (ml/mg of Fu[h])	50·0±21·5	57·6±30·5	73·9±38·7	84·8±42·1
Natriuretic efficiency (mmol/mg of Fu)	7·55±2·47	7·99±3·02	10·5±4·87	10·5±5·46
Kaluretic efficiency (mmol/mg of Fu)	1·96±0·731	1·78±0·473	0·593±0·177***	2·54±1·54
Chloruretic efficiency (mmol/mg of Fu)	7·35±2·30	8·85±5·32	11·2±5·26	11·6±5·48
Liver weight (% of body weight)	3·51±0·329	3·33±0·309	2·91±0·210	3·39±0·289
Kidney weight (% of body weight)	0·803±0·0940	0·793±0·0657	0·918±0·0733*	0·767±0·0876
Stomach weight (% of body weight)	0·547±0·0108*	0·470±0·0721	0·477±0·0506	0·437±0·0528

[a]Standard deviation.
[b]Expressed in terms of furosemide.
[c]Not detectable.
[d]Renal clearance of creatinine.
[e]Renal clearance of *p*-aminohippuric acid.
[f]The extent of dose absorbed into the general circulation.
[g]Urine samples were collected for 24 h before oral administration of furosemide.
[h]Furosemide.
*, $p<0·05$; **, $p<0·01$; ***, $p<0·001$.

than those in age-matched Wistar rats after i.v. administration of the same dose of furosemide based on body weight. However, the corresponding values were not significantly different between DOCA-salt rats and SD rats of 16 weeks of age and similar results were also obtained from SHRs of six weeks of age and age-matched Wistar rats. Therefore, the significantly faster non-renal metabolism of furosemide, and the resulting significantly lower urine output and urinary excretion of sodium and chloride in SHRs of 16 weeks of age than in age-matched Wistar rats may be due to physiological factors derived from the chronic phase of hypertension in SHRs, and not due solely to heredity of SHRs or the hypertensive state itself. Our present data might suggest (if they can be extrapolated to humans) that the i.v. dose of furosemide in essential hypertensive patients may require modifications.

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