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Renal Handling of Aspirin in the Rat

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ABSTRACT

Aspirin (ASA), in addition to blocking platelet cyclooxygenase, thus preventing thromboxane A_2 formation, can also block renal cyclooxygenase thus inhibiting the renal synthesis of vasodilatory prostaglandins (PGs) which can induce renal function deterioration. The purpose of the present study was to clarify the pharmacological basis of the inhibitory effect of ASA on renal cell cyclooxygenase in the rat. ASA was given to rats either i.v. or p.o. at doses ranging from 10 to 200 mg/kg. After both i.v. and p.o. administration ASA was rapidly detected in plasma as intact molecule. The kinetics were of a dose-dependent type with a disproportionate increase in plasma level increasing the dose. Plasma salicylic acid (SA) concentrations peaked after ASA with a precursor product relationship. ASA levels in kidney homogenates were also determined after i.v. and p.o. ASA. Whereas after i.v. administration ASA was detected in the kidney as intact molecule, no ASA was detected in the kidney after p.o. administration. SA was measurable in the kidney after both i.v. and p.o. ASA with a time course which paralleled the plasma concentrations. Results of isolated kidneys perfused with a medium containing ASA and of kidney homogenates exposed to ASA "*in vitro*" indicate that ASA is rapidly converted to SA by kidney tissue enzymes. After ASA hydrolysis SA accumulates in the kidney and may protect renal cyclooxygenase from the inhibitor effect of ASA.

ASA beside inhibiting platelet function by irreversibly blocking the synthesis of platelet thromboxane (Tx)A_2 (Roth *et al.*, 1975) can also inhibit the formation of other cyclooxygenase derived products and particularly vasodilatory PGs in cells other than platelets. The unselective effect of ASA on various cell cyclooxygenase may induce renal function deterioration under conditions in which the renin-angiotensin axis is activated (Aiken and Vane, 1973; Oliver *et al.*, 1981) as renal PGs, i.e. PGE_2 and PGI_2, counteract the negative effect of angiotensin II on glomerular hemodynamics (Aiken and Vane, 1973; Henrich *et al.*, 1978a,b). Conditions in which vasodilatory PGs have been found to protect from renal function deterioration induced by vasoconstrictors include volume depletion, chronic renal insufficiency, systemic lupus and rheumatoid arthritis (Muther *et al.*, 1981; Berg, 1977; Kimberly *et al.*, 1978a,b). Thus, extensive investigation has been devoted in the last few years to identify an ideal ASA formulation capable of selectively suppressing platelet TxA_2 sparing the renal synthesis of PGI_2 and PGE_2. Due to its property of being deacetylated to SA by the gastrointestinal tract and by the liver (Harris and Riegelman, 1969) it has been suggested that p.o. ASA may selectively suppress platelet TxA_2 while leaving renal cyclooxygenase intact (Patrignani *et al.*, 1982). We have addressed recently whether p.o. ASA selectively spares renal cyclooxygenase by direct measurements of platelet, glomerular and medullary cyclooxygenase activity in the rat (Livio *et al.*, 1989). The results indicated that both p.o. and i.v. ASA at doses which fully suppress platelet cyclooxygenase also partially inhibit renal cyclooxygenase. However, although the inhibitory effect on platelet cyclooxygenase was long-lasting, the effect on renal cyclooxygenase was transient and rapidly reversible. The dissociation of the ASA effect on platelet and kidney cells may be attributed partly to the well-known irreversible acetylation of cyclooxygenase in platelets in contrast to nucleated cells in which cyclooxygenase activity is restored rapidly by virtue of new enzyme synthesis. Additional possibilities to explain the rapid recovery of the renal cyclooxygenase activity after p.o. or i.v. ASA are either that only a small fraction of the administered ASA reaches the kidney as intact molecule and/or that the renal tissue actively metabolizes ASA to SA thus protecting renal cell cyclooxygenase from its inhibitory effect. An answer to this question may derive from direct measurements of the renal concentrations of ASA and SA at various time intervals after different doses of ASA. So far, however, such evaluation has not been done in a systematic manner.

We have undertaken the present study with the purpose to clarify the pharmacological basis of the transient and rapidly reversible inhibitory effect of ASA on the renal cell cyclooxygenase in the rat.

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ABBREVIATIONS: ASA, aspirin, Tx, thromboxane; PG, prostaglandin; PGI_2, prostacyclin; SA, salicylic acid; GFR, glomerular filtration rate; AUC, area under the plasma concentration-time curve; C_{max}, maximum concentration.

Materials and Methods

Experimental protocol. Studies were performed with Sprague-Dawley CD-COBS male rats (Charles River Italia S.p.a., Calco, Italy) weighing 175 to 200 g. Rats received a single dose of ASA in the form of its soluble lysine salt (Flectadol, Maggioni, Milan, Italy) dissolved in isotonic saline.

In Vivo Studies

Seven groups of rats were used: four groups were injected i.v. with 10, 30, 100, or 200 mg/kg of ASA. Three other groups received a p.o. dose of 30, 100 or 200 mg/kg of ASA after an overnight fasting. At 2.5, 5, 10, 15 and 30 min, 1, 2, 4, 6, 12, 18, 24 and 30 hr after drug administration blood was collected and kidneys were removed from ether anesthetized animals. For each point of sampling five animals were used. Blood was collected by intracardiac puncture. One milliliter of blood was collected in chilled plastic tubes containing 10 μl of heparin solution (1000 U/ml) and 10 μl of 50% w/v of potassium fluoride in distilled water to prevent ASA hydrolysis (Rowland and Riegelman, 1967). The blood was gently mixed and centrifuged immediately at 0°C for 20 min at 2000 × *g*; the plasma was pipetted off and stored at −80°C until analysis (within 1 week). After removal a kidney was homogenized using an ultraturrax homogenizer (Janke and Kunkel GMBH and Co., KG, IKA-WERK Staufen, FRG) in water (1:4, w/v) containing 5 mg/ml of potassium fluoride and stored at −80°C until analysis (within 1 week).

In Vitro Studies

Isolated perfused kidney. To assess further the capability of the kidney to metabolize ASA we performed additional experiments using the isolated perfused rat kidney preparation exposed to two different ASA concentrations. For this purpose kidneys were perfused in a recirculating system at a constant pressure of 95 to 100 mm Hg with an artificial cell-free medium containing ASA. Each kidney was allowed 15 to 20 min to equilibrate after beginning the perfusion. After two 10-min control periods, new perfusion medium (300 ml) containing ASA at final concentration of either 200 (n = 5) or 6.7 (n = 5) μg/ml substituted the previous one in order to expose the kidney immediately to the selected ASA concentration. The ASA concentration of 200 μg/ml was chosen on the basis of plasma concentration of ASA found in animals 2.5 min after a bolus i.v. injection of the drug (200 mg/kg). Inasmuch as the total volume of the perfusion fluid used in the isolated perfused kidney preparation was largely in excess as compared to the rat blood volume "*in vivo*," the total amount of ASA reaching the kidney was higher than the *in vivo*. Therefore, to overcome this problem we exposed the perfused kidney to a total amount of ASA comparable to the *in vivo* situation after a 200 mg/kg ASA i.v. dose, the ASA concentration of 6.7 μg/ml in the perfusion solution was also considered. This value was estimated taking into account the mean blood volume of the rat, the plasma ASA concentration measured at the first time of sampling (2.5 min) after 200 mg/kg of ASA i.v. in *in vivo* experiments, and that only one kidney is exposed to ASA in the isolated perfused kidney preparation. The experimental period was divided into seven 10-min clearance intervals for the measurements of kidney function. In addition perfusate (300 μl) and urine samples were collected every 5 min for measurement of ASA metabolism. Each perfusate sample was collected contemporaneously, before (arterial sample) and after (venous sample) the kidney to evaluate the conversion of ASA into SA during the passage of the perfusion fluid through the renal tissue. Perfusate and urine samples were frozen and stored at −80°C until analysis for creatinine, ASA and SA concentration (within 1 week). After completion of the perfusion, kidneys were removed, homogenized immediately, frozen and stored at −80°C until measurement of renal tissue concentration of ASA and SA.

Perfusion procedure and apparatus. The perfusion technique used in these experiments was a modification of that described by Roblero *et al.* (1976) and Vio *et al.* (1983). S.D. rats were anesthetized by an i.p. injection of thiopental sodium (50 mg/kg b.w.) and placed on a heated surgical table. The right kidney and retroperitoneal structures were exposed through a midline abdominal incision. After the abdominal cavity was exposed, the following steps were performed: 1) the adrenal artery, which arises from the right renal artery, was identified and tied; 2) the vena cava tributaries below the right renal pedicle and above the iliac bifurcation were tied. Additional loose ligatures were placed around the vena cava just above the right renal pedicle and below the right renal vein; 3) after i.v. injection of 0.5 ml of 10% mannitol and 120 I.U. of heparin, the right ureter was isolated from the surrounding connective tissue and cannulated with PE-10 polyethylene tubing (Clay-Adams, Parsippany, NJ): 4) the open tip of a venous cannula (PE-240 polyethylene catheter) with its distal end closed was introduced into the vena cava below the right renal vein and secured in place; 5) the distal end of the superior mesenteric artery was tied and loose ligatures were placed around the superior mesenteric artery and the right renal artery near the aorta. Care was taken to avoid excessive manipulation of the renal pedicle; 6) the renal artery was cannulated with a short, blunted 19-gauge needle, *via* the superior mesenteric artery to avoid interruption of flow to the kidney; 7) the arterial cannula was then secured in place by the previously placed ligatures, the distal end of the venous cannula was opened and the ligature around the vena cava above the right renal pedicle was tied; and 8) the kidney was washed with 50 ml of perfusion solution until the venous effluent was free of blood, as determined by the absence of the peroxidase-like activity of hemoglobin (Hemastix strip, Miles GmbH, Sparte Ames, Frankfurt, FRG).

Venous outflow was collected into a small glass beaker and returned to a reservoir (closed system) through a two-headed Minipulse peristaltic pump (Gilson 2, Villier Le Bel, France). The perfusion apparatus consisted of a plastic reservoir bottle from which the perfusion medium passed to hollow-fiber membrane oxygenator (Bellco, Mirandola, Italy). The perfusate was drawn through the blood compartment of the oxygenator, and a gas mixture (95% O_2-5% CO_2) passed continuously through the gas compartment, oxygenating the perfusion solution and holding its pH at 7.35 to 7.40. From the oxygenator the medium passed through Tygon tubing to the second head of the peristaltic pump, an in-line 8-μm filter (Sartorius, Gottingen, FRG) and a glass bubble trap. Finally the perfusate was delivered through latex tubing to the renal artery cannula.

The perfusion medium was maintained at 37°C by a constant Haake D1 temperature circulator (Haake, Berlin, FRG). Perfusion pressure was measured continuously with a Statham transducer (Gould, Dusseldorf, FRG) connected by polyethylene tubing to the perfusion line proximally to the arterial cannula and recorded on a Battaglia Rangoni KV 135 recorder (Battaglia Rangoni, Casalecchio di Reno, Italy). Perfusion pressure was corrected by the pressure generated by the catheter alone at each flow rate, and variations in perfusion pressure were compensated for by changing the rate of the peristaltic pump. Because the system was maintained at constant pressure (95–100 mm Hg), changes in perfusate flow reflected changes in renal vascular resistance.

The perfusate consisted of Krebs-Henseleit buffer (in millimolar: NaCl, 120; $NaHCO_3$, 25; KCl, 2.8; $CaCl_2$, 2.5; KH_2PO_4, 1.2; and $MgSO_4$, 1.5) containing 3.5 g/100 ml of Ficoll 70 (Pharmacia Fine Chemicals, Uppsala, Sweden), 1.0 g/100 ml of bovine serum albumin (Pentex BSA Fraction V, Miles Laboratories, Elkhart, IN), 200 mg/100 ml of dextrose, 36 mg/100 ml of urea, 50 mg/100 ml of creatinine and the following L-amino acids in millimolar concentration: methionine, 0.5; alanine, 2.0; glycine, 2.0; serine, 2.0; arginine, 1.0; isoleucine, 1.0; aspartic acid, 3.0; and cysteine, 0.5. This perfusate was filtered through a 0.45-μm membrane filter (Sartorius, Gottingen, FRG) before use, and when equilibrated with the gas mixture at 37°C, its pH was approximately 7.4. The total volume of the perfusate was 300 ml.

Assessment of renal function. Urine flow rates were determined gravimetrically. Perfusate and urine concentrations of creatinine were measured in duplicate, using the method of Bonsnes and Taussky (1945). Creatinine clearance was calculated using the standard formula (Pitts, 1973) and was taken as GFR. This technique has been shown

to give the same GFR estimates in the isolated perfused kidney as inulin clearance (Ross, 1978). GFR values were normalized per gram of kidney weight, taking the weight of the contralateral kidney (not perfused) as the control weight for the perfused kidney. Perfusate pressure was measured proximally to the arterial cannula. Cannula tip pressures were derived by subtracting from the measured pressure the pressure drop known to occur across the arterial cannula at a given flow. Perfusate flow was determined volumetrically.

Blood and kidney incubations. Fresh samples ($n = 3$) of heparinized rat blood were incubated with ASA (initial concentrations of 200 and 10 μg/ml) at 37°C for up to 2 hr. A small volume of ASA solution was added thus allowing the study of ASA hydrolysis in virtually undiluted blood (>90 volume %). Blood samples were collected at 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 120 min in precooled test tubes containing KF and the plasma retained for analysis. The kidneys were removed from three anesthetized rats, weighed, placed in four volumes of saline (0.9% NaCl in water) and homogenized. An initial ASA concentration of 200 μg/ml, similar to that obtained in plasma of treated animals at the first time of sampling after 200 mg/kg i.v., was added to the homogenates which had been warmed to 37°C. Homogenate samples (200 μl) were collected at 0.5, 1, 2, 2.5, 5, 10, 15, 30, 60 and 120 min, placed in a precooled test tube containing 20 μl of KF solution and 50 μl of 7 M H_3PO_4 and extracted immediately for analysis.

Assay for ASA and SA. Aliquots of plasma, kidney homogenates, perfusate and urine samples (200 μl) were added to a centrifuge tube containing 15 μl of 7 M H_3PO_4, 80 mg NaCl and *p*-toluic acid as internal standard. To each tube 8 ml of dichloromethane was added and the samples were shaken for 15 min. After centrifugation the organic layer was separated and evaporated to dryness. The residue was dissolved in 200 μl of mobile phase and aliquots were injected into the liquid chromatograph. Using a model 342 liquid chromatograph equipped with a model 160 UV detector (Beckman, Fullerton, CA) operating at 229 nm and a reversed-phase column (LiChrosorb RP-8, 7 μm, 250 mm × 4 mm, E. Merck, Darmstadt, FRG) the samples were eluted in isocratic conditions with a mobile phase of 30% (v/v) acetonitrile in water at pH 2.5 ± 0.2 with H_3PO_4 at 1.0 ml/min flow rate. Internal calibration curves of ASA and SA were prepared for each set of samples. Linearity was found over the investigated concentration range. The detection limit was 20 ng/ml in plasma and 80 ng/g in kidney homogenates for both ASA and SA. Pharmacokinetic parameters for ASA and SA were calculated by noncompartmental pharmacokinetic methods according to Gibaldi and Perrier (1975).

Statistical analysis. Values from isolated perfused kidneys were analyzed by Student's *t* test. P values < .05 were considered significant.

Results

In Vivo Studies

ASA and SA levels in plasma after i.v. and p.o. ASA. Figure 1 shows plasma concentration time profiles of ASA given i.v. or p.o. to rats at doses of 10, 30, 100 and 200 mg/kg and 30, 100 and 200 mg/kg, respectively. Table 1 shows the corresponding pharmacokinetic parameters. With i.v. ASA elimination $T_{1/2}$ was similar for the two lower doses (10 and 30 mg/kg) whereas roughly doubled with the two higher doses (100 and 200 mg/kg). Total body clearance was higher with the two lower doses whereas the apparent volume of distribution was higher with the two higher doses. The AUC increased proportionally to the dose from 10 to 30 mg/kg whereas a higher AUC than expected was observed with 100 mg/kg and especially with 200 mg/kg. These findings would indicate nonlinear pharmacokinetics at the higher i.v. ASA doses. ASA absorption after p.o. ASA administration was rapid: the drug was detected in plasma within 2.5 min and the peak time was at 5 min for all the doses tested. After 30 mg/kg p.o. administration, ASA $T_{1/2}$ was longer than that observed with the corresponding i.v. dose. This finding would suggest that further absorption from duodenum or upper jejunum as well as from stomach still occurs during the decline of the plasma ASA levels. Similarly to the corresponding i.v. ASA dose, a higher AUC than expected was found after the two higher p.o. doses. Bioavailability, calculated by the ratio between the AUC of the same p.o. and i.v. dose, was low and similar for all the three ASA doses (23.7–27.5%).

Figure 2 shows that the plasma concentration-time curve of SA had a different shape depending on the route of ASA administration: after i.v. ASA (fig. 2A), SA concentrations reached the plateau immediately and remained at such levels about 1 hr before elimination occurred. After ASA p.o. (fig. 2B), SA concentrations rose relatively slowly before reaching the peak time (1 hr).

Table 2 shows pharmacokinetic parameters of SA in plasma after i.v. and p.o. ASA administration. $T_{1/2}$ values of SA after i.v. ASA increased with the dose; the C_{max} was observed at 30 min, except for 10 mg/kg of ASA (peak time, 15 min). C_{max} and AUC of SA obtained after 200 mg/kg i.v. of ASA were markedly lower than expected for a linear kinetics. This finding suggests a saturation in the metabolism of the parent drug. After p.o. ASA, $T_{1/2}$ of SA was slightly longer than after i.v. ASA. The time of C_{max} observed was 1 hr, and AUC increased proportionally with the dose.

ASA and SA levels in kidney homogenate preparations after i.v. and p.o. ASA. Table 3 shows that ASA after i.v. administration was detectable in kidney homogenates only after the two higher doses (200 and 100 mg/kg) and for corresponding plasma concentrations higher than 35 to 40 μg/ml. After 200 mg/kg at 30 min ASA was no longer detectable, whereas after 100 mg/kg ASA was not detectable already at 15 min.

ASA levels in kidney homogenates after 200 and 100 mg/kg i.v. showed a great interindividual variability and were markedly lower than in plasma. After p.o. administration ASA was not detectable in the kidney at any time independently of the dose studied. This is possibly the result of the substantial presystemic hydrolysis of p.o. ASA which occurs in the enterohepatic circulation leading to low ASA concentrations in the peripheral circulation. Another possibility is that ASA is metabolized rapidly by specific enzymes in the kidney.

Pharmacokinetic parameters and the time course of SA concentrations in kidney after i.v. ASA are shown in table 4 and in figure 3 (A). At 2.5 min we observed the highest concentration of SA which sharply decreased at 5 min, remaining approximately at this level until 30 min. This time course of SA concentrations is different from that found in plasma where the highest SA concentrations were reached at 30 min. In the kidney, SA C_{max} and AUC increased proportionally to the dose, but after 200 mg/kg of ASA i.v. both were lower than expected, in close agreement with the findings obtained for plasma. In tissue $T_{1/2}$ were slightly longer than those observed in plasma at the corresponding dose. The peculiar SA profile in kidney is probably the result of both extrarenal and renal hydrolysis of ASA to SA.

At variance with ASA, SA levels were measurable in the kidney after the three different p.o. doses. The time course of SA concentrations are given in figure 3 (B). Kidney SA levels rose slowly after ASA administration reaching the maximum concentration at 1 hr. Both SA C_{max} and AUC increased proportionally to the dose, whereas $T_{1/2}$ was longer with the highest dose. The time course of SA concentration in kidney paralleled

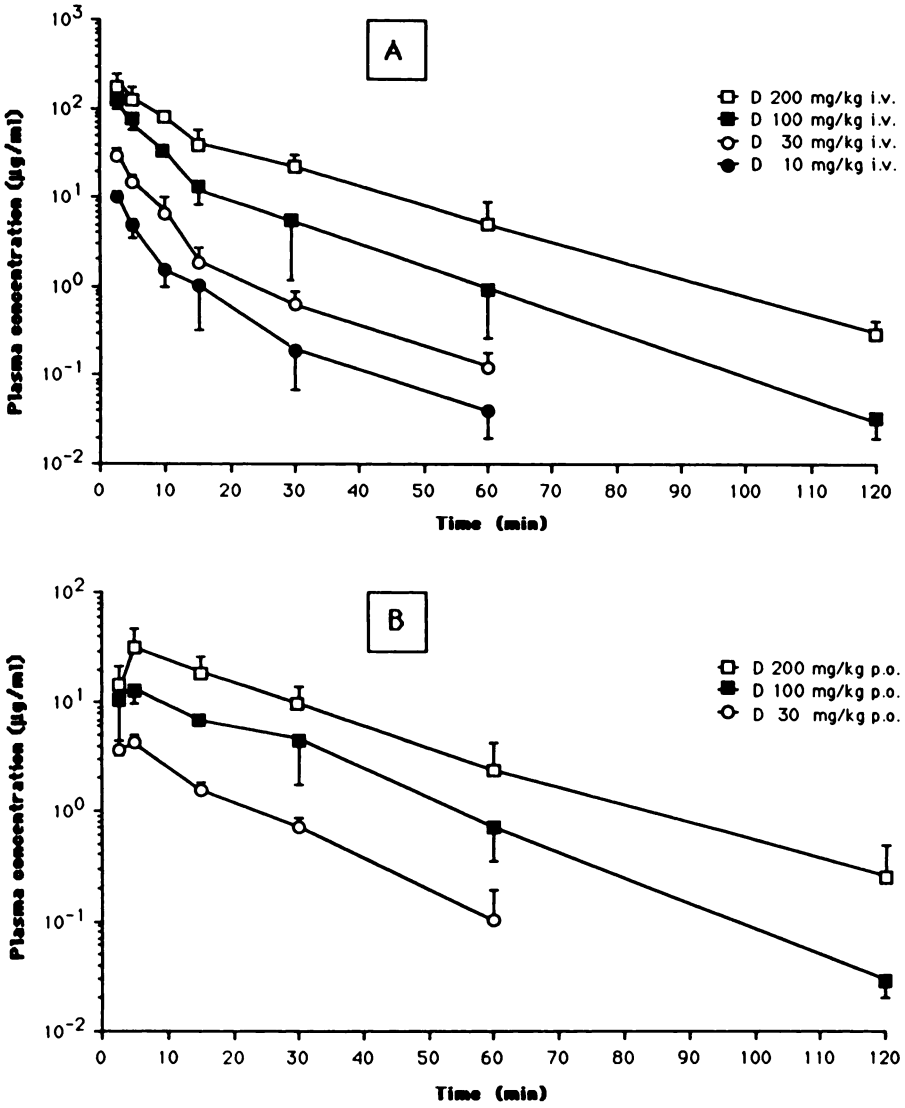

Fig. 1. Plasma concentration of ASA in rats after i.v. (A) and p.o. (B) administration. Values are mean ± S.D. D, dose.

TABLE 1

Pharmacokinetic findings in plasma after i.v. and p.o. administration of ASA

Abbreviations: T_{max}, time of maximum concentration; CL, total body clearance; V, apparent volume of distribution.

Kinetic Parameter	i.v. Dose (mg/kg)				p.o. Dose (mg/kg)		
	10	30	100	200	30	100	200
$T_{½}$ (min)	6.7	6.3	12.4	14.7	10.5	13.2	16.6
C_{max} (μg/ml)[a]	24.9[b] ±2.5	82.0 ±7.8	209.6 ±34.7	369.4 ±67.3	4.3 ±0.9	13.6 ±3.9	32.1 ±15.9
T_{max} (min)					5.0	5.0	5.0
CL (ml/min/kg)	104	97	80	68	97	80	68
V (ml/kg)	998	883	1420	1434	1469	1515	1625
AUC (μg·min/ml)	96	310	1256	2952	73	328	811
F (%)					23.7	26.1	27.5

[a] Indicates C_0 after i.v. administration.
[b] Mean ± S.D.

that observed in plasma, the ratio between kidney and plasma concentration being about constant (0.4 after i.v. or p.o. ASA) at all the sampling times but the earliest after i.v. ASA. Similarly, the ratio between plasma and kidney AUC was 0.4 for both i.v. or p.o. ASA.

In vitro Studies

Isolated perfused kidney. Addition of ASA to the perfusate at the final concentration of either 200 or 6.7 μg/ml did not influence renal function parameters of isolated rat kidneys. In particular, GFR was comparable before and after exposure to ASA (ASA, 200 μg/ml: before, 0.573 ± 0.126; after, 0.529 ± 0.140 ml/min/g of kidney; ASA, 6.7 μg/ml: before, 0.542 ± 0.084; after, 0.574 ± 0.058 ml/min/g of kidney). ASA was not hydrolized when added to the perfusion fluid alone up to 1 hr of incubation.

Perfusate concentration of ASA and SA in samples obtained before (renal artery) and after (renal vein) the kidney at different time intervals during the perfusion are given in figure 4. After a 10-min exposure of kidney to ASA, at the two concentrations, the concentration of the drug in the renal artery was higher than in the renal vein suggesting that kidney actively metabolizes and/or excretes into the urine the drug in absence of blood (ASA, 200 μg/ml: renal artery, 178.24 ± 4.93; renal vein, 162.12 ± 3.46 μg/ml, $P < .05$. ASA, 6.7 μg/ml: renal artery, 5.87 ± 0.32; renal vein, 4.95 ± 0.41 μg/ml, $P < .05$). That kidney metabolizes ASA is supported further by the observation that at the same time the renal artery concentration of SA was lower than in renal vein (ASA, 200 μg/ml: renal artery, 6.13 ± 4.84; renal vein 17.34 ± 5.20 μg/ml, $P < .01$. ASA, 6.7 μg/ml: renal artery, 0.32 ± 0.05; renal vein 1.01 ± 0.16 μg/ml, $P < .05$). ASA concentration in the perfusion fluid declined progressively during the experimental period, whereas the concentration of

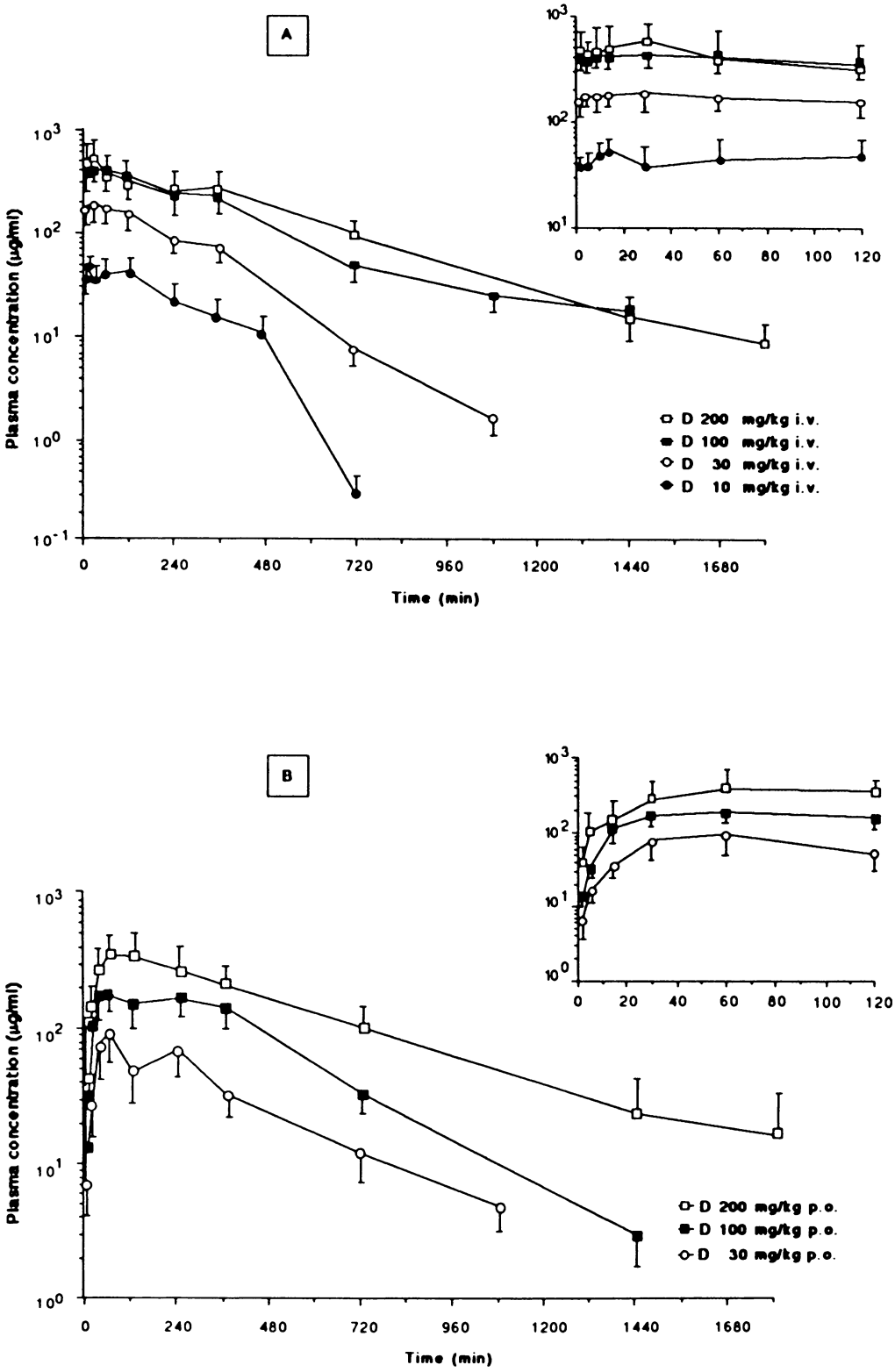

Fig. 2. Plasma concentration of SA after i.v. (A) and p.o. (B) administration of ASA to rats. SA concentrations until 120 min are shown in the insert. Values are mean ± S.D. D, dose.

SA increased. The percentage of ASA conversion to SA remained quite constant for each perfusate ASA concentration considered (ASA, 200 μg/ml: 10%; ASA, 6.7 μg/ml: 19%), as indicated by the comparable difference between the renal artery and venous ASA concentration at each time intervals. ASA as well as SA were also recovered in urine during the perfusion. The ratio between the ASA and SA concentration in urine samples showed a slight tendency to decrease with time (ASA, 200 μg/ml, ASA/SA ratio, 10 min: 3.8, 50 min: 2.2. ASA, 6.7 μg/ml, ASA/SA ratio, 10 min: 0.74; 50 min: 0.32). That kidney can metabolize ASA to SA is indicated further by the demonstration of SA in renal homogenate obtained at the end of the perfusion. Perfusing kidneys with ASA (200 μg/ml) resulted in a renal tissue concentration of SA almost 40-fold higher than that of ASA (SA, 88.73 ± 4.70 μg/g; ASA, 1.92 ± 0.68 μg/g), despite the final ASA concentration in the perfusion fluid was 2- to 3-fold higher than that of SA. Moreover, exposing kidneys to low concentration of ASA (6.7 μg/ml) in the perfusate we

TABLE 2

Pharmacokinetic parameters of SA in plasma after ASA administration

Abbreviation: T_{max}, time of maximum concentration.

Kinetic Parameter	i.v. Dose (mg/kg)				p.o. Dose (mg/kg)		
	10	30	100	200	30	100	200
$T_{½}$ (hr)	1.8	2.5	4.6	5.0	4.1	3.8	6.2
C_{max} (μg/ml)	52.9[a]	188.1	422.9	573.3	96.1	189.1	374.2
	±6.2	±51.8	±123.0	±79.2	±12.9	±28.6	±36.5
T_{max} (hr)	0.25	0.5	0.5	0.5	1.0	1.0	1.0
AUC (μg·hr/ml)	239	1010	3143	3735	574	1799	3739

[a] Mean ± S.D.

TABLE 3

ASA plasma and kidney concentrations after i.v. ASA administration

Time	10 mg/kg		30 mg/kg		100 mg/kg		200 mg/kg	
	Plasma	Kidney	Plasma	Kidney	Plasma	Kidney	Plasma	Kidney
min	μg/ml	μg/g	μg/ml	μg/g	μg/ml	μg/g	μg/ml	μg/g
2.5	10.48[a]	N.D.[b]	32.92	N.D.	118.56	4.34	207.87	20.35
	±1.06		±3.12		±19.63	±4.90	±37.88	±14.90
5	4.58	N.D.	15.73	N.D.	67.05	1.61	133.08	3.20
	±1.11		±2.13		±8.59	±1.35	±43.71	±2.93
10	1.55	N.D.	7.11	N.D.	34.33	1.45	82.79	5.61
	±0.58		±2.77		±2.05	±1.25	±2.50	±1.73
15	1.05	N.D.	1.95	N.D.	12.13	N.D.	40.97	1.14
	±0.73		±0.83		±3.78		±16.38	±1.11
30	0.20	N.D.	0.64	N.D.	5.39	N.D.	23.20	N.D.
	±0.13		±0.25		±4.67		±6.99	
60	0.04	N.D.	0.13	N.D.	0.98	N.D.	5.02	N.D.
	±0.02		±0.05		±0.71		±4.16	
120	N.D.	N.D.	N.D.	N.D.	0.03	N.D.	0.31	N.D.
					±0.01		±0.10	

[a] Mean ± S.D.
[b] N.D., not detectable (<20 ng/ml of plasma or <80 ng/ml of kidney).

were unable to detect any ASA in renal tissue, whereas SA concentration was 4.09 ± 1.09 μg/g.

Blood and kidney incubations. The disappearance of ASA from rat blood incubations (fig. 5) showed a concentration-dependent pattern. With initial blood concentration of 200 μg/ml, ASA decreased relatively slowly, with a mean $T_{1/2}$ of 21 ± 2 min ($K_{hydrolysis}$ = 0.03291 min^{-1}), until a concentration of approximately 50 to 60 μg/ml was reached (about 30 min), then the rate of disappearance increased markedly (mean $T_{1/2}$, 6 ± 1 min, $K_{hydrolysis}$ = 0.11552 min^{-1}). At lower initial concentrations, 10 μg/ml, ASA hydrolysis appeared to follow pseudo first-order kinetics, with a mean $T_{1/2}$ of 5 ± 2 min, a value similar to that obtained between 35 to 60 min of incubation in experiments performed at the highest ASA concentrations. In a control incubation with saline ASA was hydrolyzed less than 5% over a 2-hr period.

When incubated *in vitro* ASA disappeared from kidney homogenates in a first-order manner (data not shown) with the metabolism of ASA to SA accounting for all the ASA lost. After 2.5 min of incubation of ASA in homogenates, the ASA remaining to be hydrolyzed accounted for about 6%. Thus, the rate of ASA disappearance from kidney tissue was extremely fast with a mean $T_{1/2}$ of 41 ± 6 sec. Because we performed incubations using 200 μg/g as initial ASA concentration, it is possible that ASA disappearance from kidney homogenates would have been even faster at lower concentrations.

Discussion

Despite the mushrooming interest in the last few years on the pharmacology of ASA by virtue of its potential therapeutic benefit in cardiovascular diseases, information on renal handling of ASA in relation to plasma pharmacokinetics is not available so far. The issue is relevant to the purpose of identifying the ideal ASA formulation capable of selectively inhibiting platelet TxA_2 sparing the renal synthesis of vasodilatory PGs which protect the kidney from the fall in GFR in conditions of maximal activation of renin-angiotensin axis (Aiken and Vane, 1973; Patrignani *et al.*, 1982).

With the present study we compared systemic and renal metabolism of ASA in the rat after i.v. or p.o. administration.

When given i.v. ASA had a rapid distribution phase followed by a very fast elimination phase. After p.o. administration ASA was absorbed rapidly and lasted in plasma for a longer period, probably due to continued absorption during the decline phase. These data are in agreement with those of Iwamoto *et al.* (1982) in the rat, or Rowland *et al.* (1972) in healthy volunteers and of our own groups (Gaspari *et al.*, 1987) in uremic patients. The elimination $T_{1/2}$ of ASA was rapid, independently of the route of administration. This value was similar to that reported previously in rabbits (Mays *et al.*, 1984) but shorter than in sheep (25 min) (Cossum *et al.*, 1986) and humans (20 min) (Gaspari *et al.*, 1987; Rowland and Riegelman, 1968). ASA showed a relatively lower bioavailability than in dogs (Iwamoto *et al.*, 1982) and humans (Rowland *et al.*, 1972; Gaspari *et al.*, 1987), possibly due to the higher intestinal and hepatic metabolism reported in rat (Iwamoto *et al.*, 1982). SA levels in plasma

TABLE 4

Pharmacokinetic parameters of SA in kidney after ASA administration

Abbreviation: T_{max}, time of maximum concentration.

Index	i.v. Dose (mg/kg)				p.o. Dose (mg/kg)		
	10	30	100	200	30	100	200
$T_{½}$ (hr)	1.9	2.6	4.8	6.4	3.7	3.9	6.6
C_{max} (μg/g)	26.1[a]	122.3	377.5	427.6	37.4	82.1	164.6
	±4.8	±46.2	±121.4	±135.9	±2.9	±15.6	±10.9
T_{max} (hr)	0.04	0.04	0.04	0.04	1.0	1.0	1.0
AUC (μg·hr/g)	115	386	1093	1361	255	737	1623

[a] Mean ± S.D.

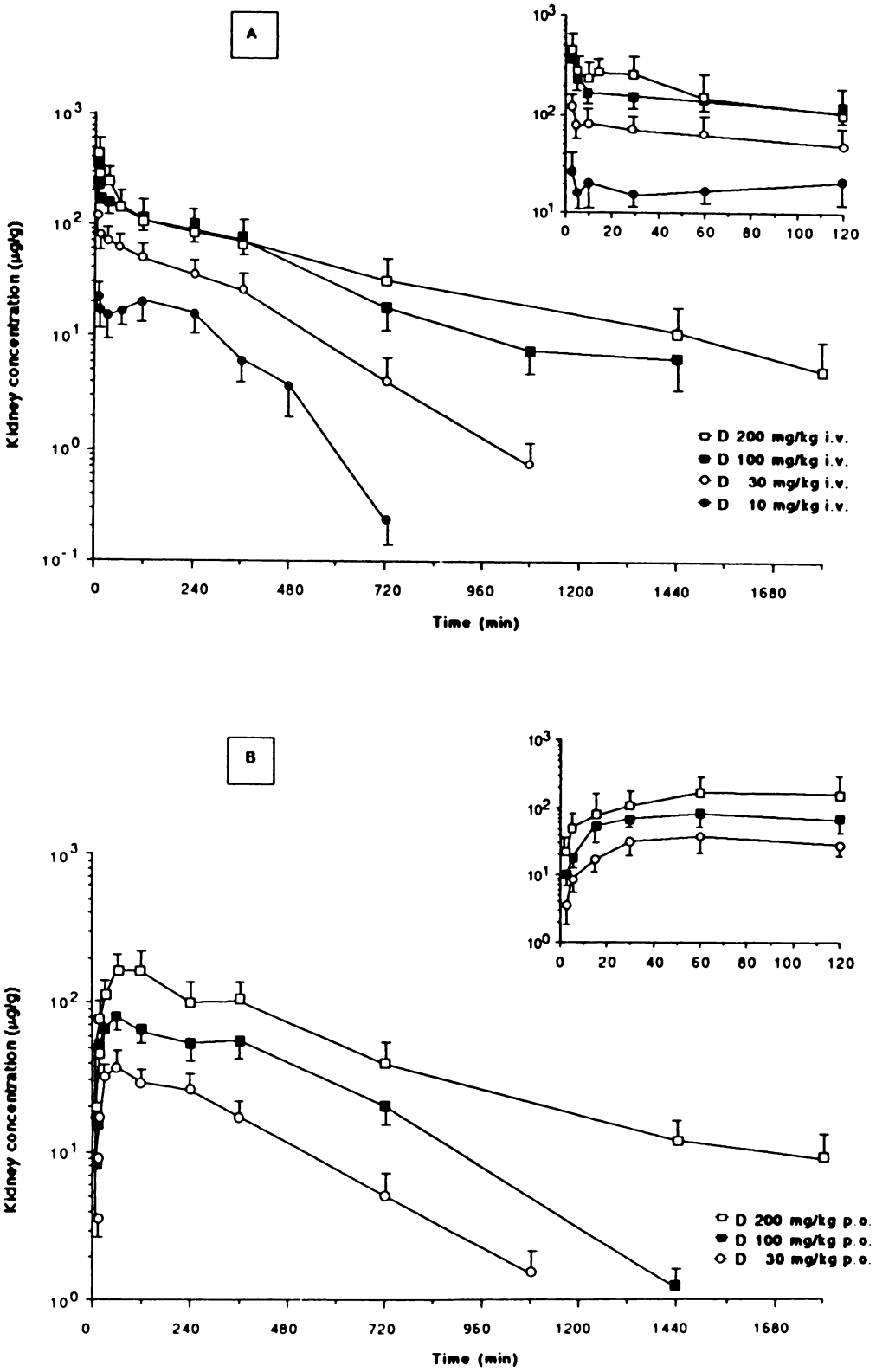

Fig. 3. Kidney concentration of SA after i.v. (A) and p.o. (B) administration of ASA to rats. SA concentrations until 120 min are shown in the insert. Values are mean ± S.D. D, dose.

rose rapidly after both i.v. or p.o. ASA and eventually exceeded those of ASA reaching the maximum concentration later than ASA which is consistent with a precursor-product relationship. The $T_{1/2}$ values of ASA and SA increased by increasing the ASA doses whereas SA C_{max} and AUC did not increase in a proportional way (at least after 100 and 200 mg/kg of ASA). The dose-dependent kinetics of ASA are likely to be the consequence of a saturation of the ASA hydrolysis process occurring mainly in the liver (Iwamoto *et al.*, 1982), a major organ for ASA metabolism, and may have important implications. In fact by increasing 20 times (from 10–200 mg/kg) the i.v. dose of ASA, the plasma exposure to ASA, based on AUC values, increased by about 30 times; similarly an increase of 6.6 times in the p.o. dose of ASA (from 30–200 mg/kg) resulted in an

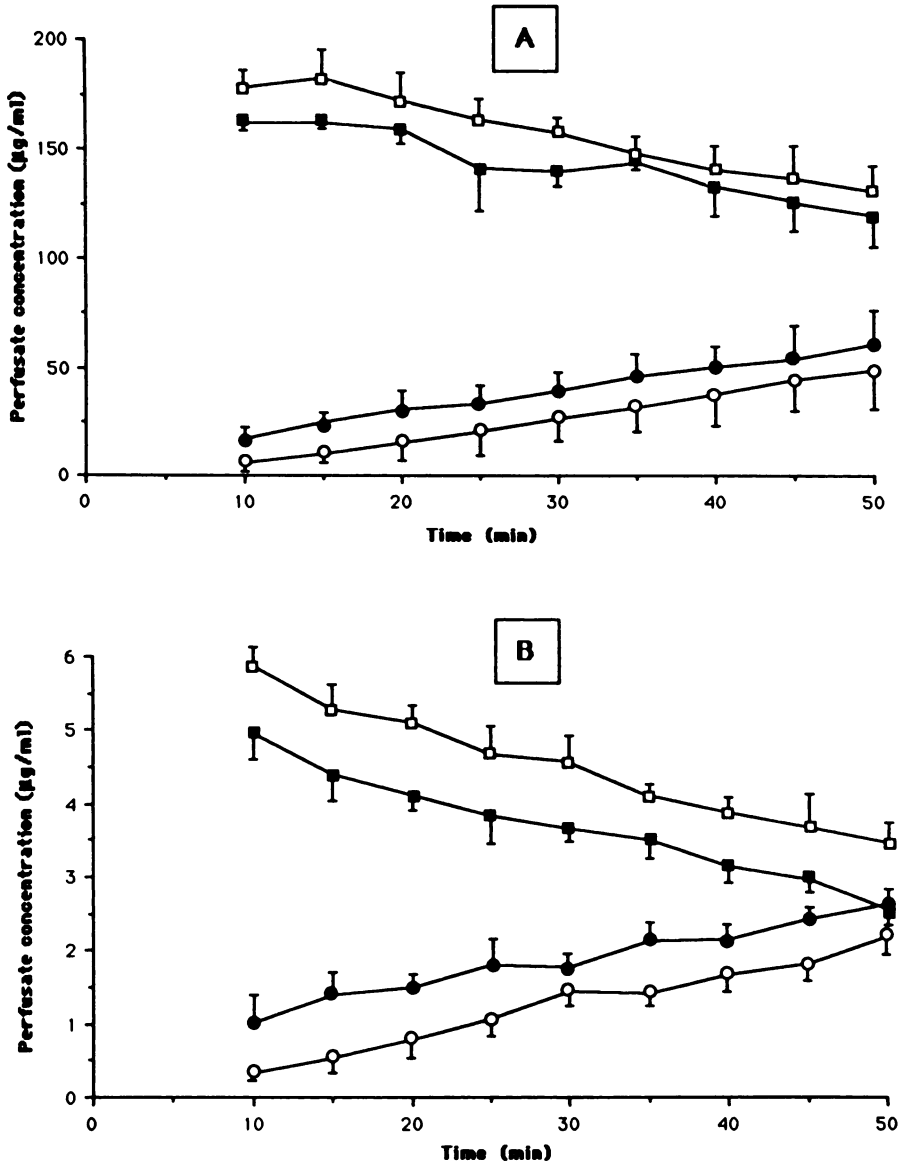

Fig. 4. Time course of perfusate concentrations of ASA and SA during the perfusion of isolated kidneys with an artificial cell-free medium containing ASA, 200 µg/ml (A) or 6.7 µg/ml (B). ASA: □, renal artery; ■, renal vein; SA: ○, renal artery; ●, renal vein. Values are mean ± S.D.

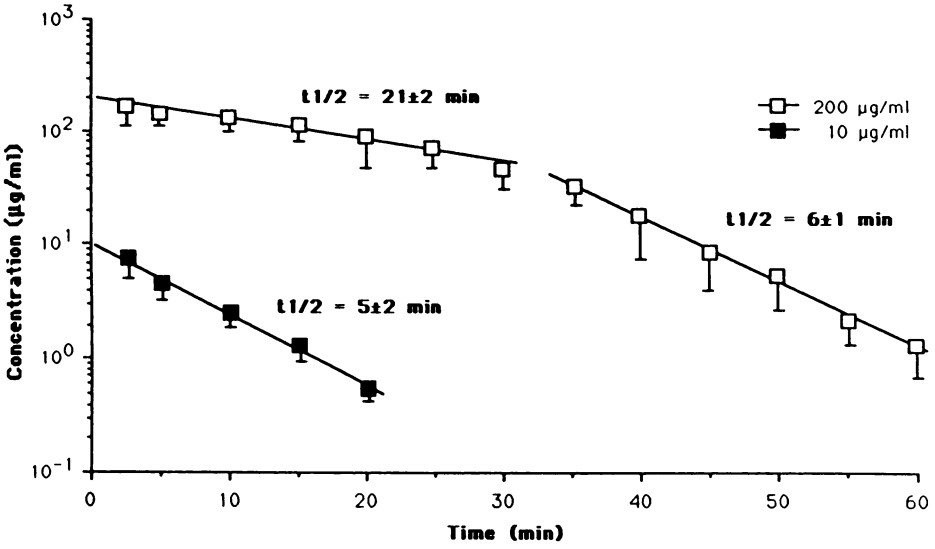

Fig. 5. Hydrolysis of ASA in fresh samples of heparinized rat blood incubated with ASA (200 and 10 µg/ml) at 37°C. Values are mean ± S.D.

increase of ASA AUC of about 11 times. Conversely, the formation of SA did not proportionally reflect the administered dose of ASA as shown by the fact that increasing 20 times the i.v. dose of ASA resulted only in about 15 times increase of plasma AUC for SA. This effect was not seen with the p.o. ASA probably because of the lower plasma levels of ASA achieved by this route in respect to the i.v. administration.

Measuring the renal concentrations of ASA and SA we have been able to detect ASA in kidney homogenates after i.v. but not after p.o. administration and only for plasma concentrations exceeding 35 to 40 μg/ml. By contrast SA levels in the kidney increased progressively after both i.v. and p.o. ASA with a time course which paralleled the plasma concentrations. Given the results of previous studies (Livio *et al.*, 1989) showing that ASA can inhibit renal PGs even at doses of 30 mg/kg p.o. the fact that ASA can be measured in the kidney only above a certain plasma level would indicate an active p.o. metabolic process for ASA (likely due to tissue esterases that hydrolyze ASA to SA) which, when saturated, results in increased plasma concentration of the drug. One could argue that blood esterases may play an important role in the hydrolysis of ASA through the kidney.

However, *in vitro* experiments indicated a rapid and concentration-dependent hydrolysis of ASA in rat whole blood the $T_{1/2}$ being 21 ± 2 min for concentrations higher than 50 to 60 μg/ml, and decreasing to 5 ± 2 min for lower concentrations. Thus, assuming a blood volume of 14 ml in an average rat weighing 200 g, the estimated ASA clearance due to hydrolysis in whole blood ranges between 0.46 and 1.72 ml/min, depending on the actual ASA blood concentration. The latter figures were derived from the product of the *in vitro* hydrolysis rate constant and the total blood volume. Assuming a plasma/whole blood concentration of 0.88 (F. Gaspari, unpublished observation) and a systemic clearance ranging from 13.5 and 20.8 ml/min (table 1), the whole blood hydrolysis accounts for only 3 to 8% of the total systemic blood clearance. The differences obtained when measuring hydrolysis $T_{1/2}$ in humans (about 32 min) (Harris and Riegelman, 1967) and sheep (about 2 hr) (Cossum *et al.*, 1986), indicate a possible species dependence of blood cell esterases activity. Independently of these discrepancies if one considers the rate of hydrolysis in the blood, it derives that although the kidney is a highly perfused organ, the contribution of ematic esterases to ASA hydrolysis cannot account for the rapid hydrolysis occurring in the kidney. These findings support further a major role for the kidney in metabolizing ASA to SA.

We investigated further the renal metabolism of ASA, using an isolated rat kidney preparation perfused with a blood-free medium. The isolated perfused kidney allows the investigation of the renal handling of a given molecule avoiding factors such as metabolism or binding to tissues other than kidney which can affect its *in vivo* disposition. Exposure of isolated perfused kidney to ASA resulted in a progressive decrease in the venous effluent ASA concentration associated with a parallel increase in SA concentration in the renal vein effluent, indicating that the kidney actually metabolizes ASA to SA. The metabolism is saturable in a concentration-dependent fashion. The isolated perfused kidney findings that ASA was measurable in the kidney only above a given concentration in the perfusion fluid are also consistent with *in vivo* findings that ASA was measurable in the kidney only for plasma concentrations exceeding 35 to 40 μg/ml. Both the *in vivo* and the isolated perfused kidney finding converge to indicate that the kidney has an active metabolic process for ASA. This is supported by the kidney homogenate finding showing that ASA is converted *in vitro* by the kidney tissue with $T_{1/2}$ of 41 sec for concentration of 200 μg/g.

Inasmuch as ASA but no SA inhibits PG and Tx synthesis by irreversibly acetylating the enzyme cyclooxygenase, the degree of suppression of renal cyclooxygenase activity is a function of the amount of ASA accessible to the renal enzymes as intact molecule. The present data showing that ASA cannot be detected in the kidney below plasma levels of 35 to 40 μg/ml is in harmony with the observed failure of 10 mg/kg of ASA to significantly suppress renal cyclooxygenase activity. On the other hand finding that ASA reaches the kidney as intact molecule but is hydrolized rapidly to SA would explain the transient rapidly reversible inhibition of renal PG synthesis observed in the rat after doses of ASA ranging from 30 to 200 mg/kg. An additional factor which must contribute to the transient inhibitory effect of ASA on renal cyclooxygenase is the competition between SA and ASA for the binding site of cyclooxygenase (Vargaftig, 1978; Dejana *et al.*, 1981). Thus, for SA concentrations as high as those detected in the kidney when plasma ASA exceeded 35 to 40 μg/ml, one can postulate a major interference with the inhibitory activity of ASA on renal cyclooxygenase.

In conclusion the present study indicates that in the rat ASA 1) has a dose-dependent type of kinetics with a disproportionate increase in plasma levels increasing the dose; 2) is detected in kidney as intact molecule only when the plasma concentrations exceed 35 to 40 μg/ml; 3) is hydrolyzed rapidly by the kidney enzymes to SA, which accumulates in the kidney and may interfere with the ASA inhibitory effect on renal cyclooxygenase.

These findings could explain the transient and rapidly reversible inhibition of renal PGs observed in rats giving i.v. or p.o. ASA at doses ranging from 10 to 200 mg/kg.

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