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Reduced Extraction of *l*-Propranolol by Perfused Rat Liver in the Presence of Uremic Blood[1]

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ABSTRACT

Previous *in vivo* studies have shown that the presystemic clearance of p.o.-administered *levo*-isomer of propranolol is inhibited in rats with uranyl nitrate-induced acute renal failure. A series of steady-state single-pass rat liver perfusion studies were performed to explore the probable mechanism of the observed metabolic inhibition. When livers from normal rats were perfused with blood perfusate prepared from normal donor animals, a high extraction ratio (E_h) of 0.974 ± 0.005 (mean ± S.D.) was observed at an influent *l*-propranolol concentration of 400 ng/ml, *i.e.*, only 2.6% of drug entering the liver escaped single-pass extraction. The extraction of *l*-propranolol was significantly lower (*i.e.*, $E_h = 0.906 \pm 0.017$) when livers isolated from uranyl nitrate-induced renal failure rats were perfused with uremic blood; such that there was an approximate 3-fold increase in the amount of drug escaping single-pass extraction (*i.e.*, from 2.6 to 9.4%). This difference in hepatic extraction is quantitatively consistent with the increase in p.o. systemic availability of *l*-propranolol observed in our previous *in vivo* study with the uranyl nitrate-induced renal failure rat model. When livers from normal rats were cross-perfused with uremic blood, extraction of *l*-propranolol was depressed to almost the same level (*i.e.*, $E_h = 0.927 \pm 0.009$) as when livers from renal failure animals were perfused with uremic blood. In contrast, livers from renal failure rats cross-perfused with normal blood exhibited comparable extraction for *l*-propranolol ($E_h = 0.970 \pm 0.010$) as normal livers perfused with normal blood. These results indicate that the diminution in presystemic hepatic extraction of *l*-propranolol in the uranyl nitrate renal failure rat model is due to the presence of an inhibitory factor in the uremic blood. No apparent alterations in the intrinsic activities of the hepatic transport and/or drug-metabolizing enzyme systems were observed.

There is mounting evidence that alterations in the biotransformation of drugs can occur in renal failure (Reidenberg, 1977). Studies in our laboratory with the uranyl nitrate-induced acute renal failure rat model demonstrated that the presystemic hepatic clearance of the levorotatory or *S*-(−)-isomer of propranolol is inhibited during uremia after both single and repetitive p.o. administration of the *beta* blocker (Terao and Shen, 1983, 1984). Similar results with racemic propranolol were reported recently by Katayama *et al.* (1984) using the same nephrotoxic acute renal failure rat model. These animal data are consistent with the results of two early clinical studies (Lowenthal *et al.*, 1974; Bianchetti *et al.*, 1976), which showed a 3- to 7-fold higher blood or plasma concentration of racemic propranolol after a single p.o. administration of the drug in end-stage renal failure patients as compared to patients or volunteers with normal renal function. However, two more recent studies (Stone and Walle, 1980; Wood *et al.*, 1980) showed either a small or negligible increase in circulating propranolol concentrations in chronic renal failure patients receiving regular propranolol therapy. As was pointed out in our earlier reports (Terao and Shen, 1983, 1984), the apparent conflicts may be attributed to differences in experimental design and patient selection between studies. Furthermore, these findings suggest that the clinical factors affecting the metabolism of propranolol in uremia are complex and multifactorial in nature. Hence, there is a need for experimental studies in animal models to elucidate the probable mechanism(s) responsible for the decreased first-pass clearance of p.o. administered propranolol during uremia, which in turn may provide some insights as to when and how the metabolism and disposition of propranolol could be affected in chronic renal failure patients.

In the present study, an *in situ* perfused rat liver preparation was used to assess the extraction of *l*-propranolol by livers isolated from normal and renal failure animals. These experiments were designed specifically to explore two plausible mechanisms for the apparent inhibitory effect of renal failure on the hepatic extraction of *l*-propranolol: 1) the presence of endogenous inhibitors in the uremic blood or 2) an intrinsic alteration in the hepatic uptake and/or metabolic processes during renal failure. The two causal mechanisms can readily be distinguished by performing a series of "cross" perfusion experiments, which entail the perfusion of livers isolated from normal animals with blood perfusate prepared from uremic donor animals and the counter-perfusion of livers isolated from renal failure animals with blood perfusate prepared from normal donor.

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Methods

Animals. Male Sprague-Dawley rats weighing between 300 to 350 g were used in all of the perfusion experiments. The animals were allowed food and water *ad libitum* until the time of sacrifice. Following the procedure established by Giacomini *et al.* (1981), renal failure was induced by a single i.v. injection of uranyl nitrate (5 mg/kg) 5 days before the perfusion experiments. Control animals received an equal volume injection of saline. To assess the degree of uremia, plasma urea nitrogen concentration was determined by a colorimetric procedure using a commercial assay kit (Sigma Chemical Co., St. Louis, MO).

Perfusion apparatus. A schematic representation of the liver perfusion apparatus is shown in figure 1. The whole assembly of the perfusion apparatus, except for two variable speed roller pumps (Masterflex, models K-7565 and K-7553, Cole-Parmer Instrument Co., Chicago, IL), was housed in a temperature-controlled Plexiglas cabinet (MRA Corp., Clearwater, FL). The temperature inside the cabinet was maintained at 37°C.

The apparatus was made up of two independent circuits of perfusion. One circuit, referred to as the preperfusion circuit, was used in the initial part of the experiment to clear the liver of all residual blood by perfusing with a blank buffer medium. The preperfusion step also allowed the stabilization of liver temperature at 37°C. The other circuit was used for the actual perfusion with drug containing rat blood perfusate.

The preperfusion circuit consisted of a perfusate reservoir, a Lucite filter equipped with a disc of fine-meshed (120–140 threads per inch) 100% Japanese silk and an air bubble trap. The various parts of the assembly were connected with silicone tubing. The buffer medium was aerated continuously with a prewarmed humidified gas mixture of 95% oxygen and 5% carbon dioxide (Carbogen, Airco, Inc., Murray Hill, NJ).

In the blood perfusion circuit, the perfusate was oxygenated by passage through a 20-ft coil of Silastic tubing (0.058″ inside diameter, 0.077″ outside diameter; Dow Corning Co., Midland, MI), which was enclosed in a glass chamber flushed continuously with prewarmed humidified carbogen gas. The blood perfusate was stirred continuously and gently in the reservoir by a magnetic stirrer.

Surgical procedure. The liver donor animal was anesthetized with ether and placed on a movable operating platform. An abdominal midline incision was made to expose the region of the *porta hepatis* without disturbing the liver. The bile duct was located and cannulated with a PE-10 tubing (Clay Adams, Parsippany, NJ) filled with heparinized saline (100 U/ml).

A loose ligature was placed around the inferior vena cava proximal to the merger of the right renal vein. A bolus dose of heparin (300 U) in isotonic saline was injected into the inferior vena cava at a site 5 to 10 mm distal to the loose ligature. The loose ligature was left in place until the conclusion of the portal and hepatic vein cannulation procedure.

The hepato-portal vein was exposed and the pyloric branch of the vein was tied off. A polyethylene cannula (fabricated from a 1.5″ length of PE-160 tubing), filled with heparinized saline (100 U/ml) and closed in the outflow end with a metal plug (to prevent air from intruding into the cannula), was inserted into the portal vein. The tip of the cannula was placed about 5 mm away from the first bifurcation of the portal vein before its entry into the various lobes of the liver.

The thorax was cut open exposing the right atrium. The thoracic inferior vena cava was isolated and a heparinized polyethylene cannula (PE-160) was inserted into the cava quickly. The tip of the caval cannula was advanced to the junction of the hepatic vein. The preplaced ligature around the inferior vena cava was then tightened. As a final step, the hepatic artery was ligated to prevent fluid leakage during perfusion. The entire surgical procedure was usually completed in 5 to 7 min.

Perfusate preparation. The perfusate medium for both preperfusion and blood perfusion were prepared fresh daily. Blood-donor animals (between 13 to 15 normal or renal failure rats per perfusion experiment) were anesthetized with ether and blood was withdrawn from the abdominal aorta into a heparinized glass syringe. The pooled heparinized blood (at a final heparin concentration of 10 U/ml) was poured through a 3″ × 3″ surgical gauze sponge, into a 250-ml volumetric glass cylinder to remove any large blood clots that may be present.

Krebs-bicarbonate buffer solution (millimolar) (NaCl, 118.5; $NaHCO_3$, 25.0; KCl, 4.74; $CaCl_2$, 2.54; $MgSO_4$, 1.04; and KH_2PO_4, 1.19;

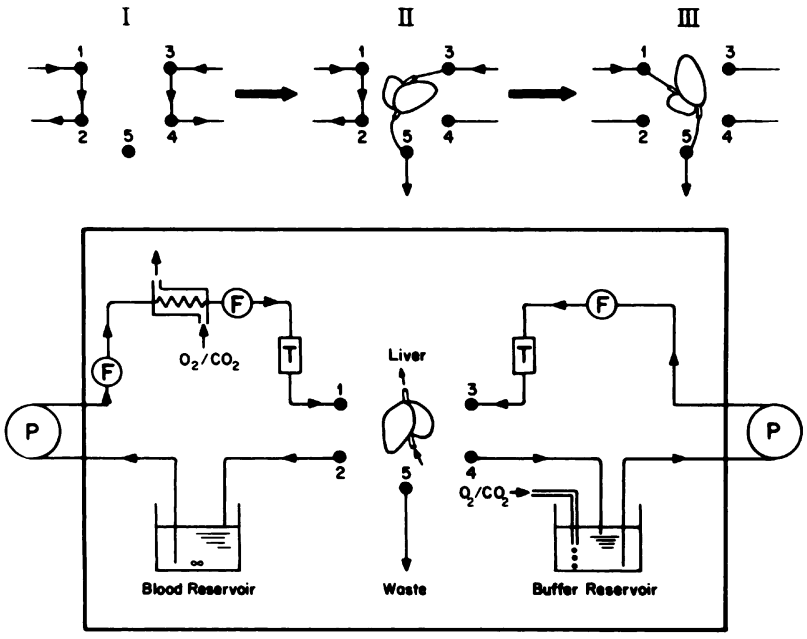

Fig. 1. Schematic representation of the rat liver perfusion system. The portal and caval connections of the liver to the individual perfusion circuits are shown for each phase of the experiment: warm-up (I), preperfusion (II) and blood perfusion (III). Key: P, pump; F, filter; T, bubble trap.

pH = 7.4) was equilibrated with Carbogen gas for 10 min. Bovine serum albumin (Fraction V, Sigma Chemical Co.) and glucose were dissolved in the buffer to yield concentrations of 5 and 0.1%, respectively. Two hundred milliliter of the buffer solution (*i.e.*, preperfusion medium) was placed in the reservoir of the preperfusion circuit where it was warmed to 37°C and kept oxygenated.

Another 100 ml of the Krebs-bicarbonate buffer solution was added to 200 ml of the pooled heparinized rat blood to make a one-third diluted whole blood perfusate. *l*-Propranolol hydrochloride (Ayerst Laboratories, New York, NY) was added to the final blood perfusate to a concentration of 500 ng/ml. Sodium taurocholate (Sigma Chemical Co.) dissolved in saline was also added to the blood perfusate to obtain a concentration of 30 μM. The perfusion medium (300 ml) was mixed thoroughly and placed in the perfusate reservoir. The blood perfusate was then recirculated through all the components of the perfusion circuit (without the liver) at a constant flow rate of 10 ml/min for 30 to 40 min (see schema I in fig. 1). The equilibration period was necessary to allow the blood perfusate to warm up to 37°C and be fully equilibrated with carbogen gas. Preliminary experiments also indicated that there was an unavoidable loss of drug from the perfusate (~20%) due to adsorption onto glasswares and silicone tubing during the initial period of recirculation.

Liver perfusion. *1) Preperfusion.* After the cannulation procedure the animal was transferred into the perfusion cabinet. Preperfusion with drug-free buffer medium was initiated immediately by connecting the portal cannula to the outflow port (No. 3, schema II in fig. 1) in the preperfusion circuit. The effluent from the liver was discarded by connecting the caval cannula to the drainage port (No. 5). The liver was preperfused for 10 min at a flow rate of 15 ml/min.

During the preperfusion the position of the liver and the inlet and outlet tubes were adjusted so that there was no obstruction to the perfusate flow. The surface of the liver was irrigated with 5 to 10 ml of warm saline. A saline-moistened gauze sponge was gently placed over the liver to avoid dehydration of the organ. The temperature of the liver was monitored by placing a thermistor probe (Model 43-TD, Yellow Springs Instrument Co., Yellow Springs, OH) between two lobes of the liver. The position of the bile duct was adjusted to obtain a constant flow of bile.

2) Single-pass extraction study. The steady-state extraction of *l*-propranolol was measured during single-pass perfusion of livers from control and renal failure animals with both normal and uremic blood. The four following perfusion arrangements were performed: i) normal liver perfused with normal blood; (ii) uremic liver perfused with uremic blood; (iii) normal liver perfused with uremic blood; and (iv) uremic liver perfused with normal blood.

At the end of the preperfusion period, the portal cannula was disconnected from the preperfusion circuit and connected quickly to the outflow port (No. 1) of the blood perfusion circuit. Care was taken not to disturb the position of the liver and the caval cannula connection. The perfusate flow rate through the liver was set at 10 ml/min.

The effluent (venous) blood perfusate was withdrawn from an in-line septum-type sampling port situated at the caval cannula outlet, at 2, 5, 8, 12, 15, 18 and 21 min after the commencement of blood perfusion. The influent (arterial) blood perfusate was collected from a sampling port placed at the inlet to the portal cannula, at 0, 7, 13 and 22 min after the start of blood perfusion. Bile was collected during the steady-state interval, *i.e.*, 14 to 24 min, in a tared polypropylene tube (Walter Sarstedt, Inc., Princeton, NJ). Bile volume was estimated gravimetrically, assuming a density of unity. At the completion of the perfusion experiment, the liver was excised, rinsed with warm saline, blotted dry and weighed.

The perfusate blood samples were split in halves. One portion was centrifuged to obtain the "plasma" fraction. The perfusate blood and plasma along with the bile and liver specimens were stored frozen at −20°C pending drug metabolite assay. Only the results on the perfusate blood and plasma will be presented in this report.

The concentration of *l*-propranolol in perfusate blood and plasma was measured by a high-performance liquid chromatographic method described earlier (Terao and Shen, 1982). The assay had a coefficient of variation of less than 4% (at the concentration of 10 ng/ml) and a sensitivity limit of 0.5 to 1 ng/ml (with a sample volume of 0.1 ml).

Protein binding of *l*-propranolol in arterial perfusate plasma collected at 13 min of perfusion and in venous perfusate plasma at 18 min were determined by equilibrium dialysis according to a procedure described previously (Terao and Shen, 1983).

3) Viability assessment. Livers prepared from control and renal failure animals were compared with respect to their viability during perfusion with normal rat blood perfusate. In these experiments perfusion was performed in the recirculating mode to minimize the volume of rat blood required. The preperfusion was the same as described earlier. Sixty milliliters of the blood perfusate was recirculated (by connecting the caval cannula to port No. 2 shown in fig. 1) at a flow rate of 10 ml/min for a period of 3 hr. Bile salt was replenished by infusing 18 μmol of sodium taurocholate per hr into the reservoir.

Bile flow rate was monitored throughout the experiment. Arterial (portal) and venous (caval) blood P_{O_2} and P_{CO_2} and arterial blood pH were determined before and after 1, 2 and 3 hr of perfusion (IL Micro 13/123 pH/Blood Gas Analyzer, Instrumentation Laboratory, Lexington, MA). Perfusate samples were also collected at hourly intervals for the following biochemical measurements in perfusate plasma: glucose, albumin, total protein, urea nitrogen, creatinine and glutamic pyruvic transaminase. All of the biochemical measurements were performed on a centrifugal chemistry analyzer (Centrifichem Analyzer 400, Union Carbide Corporation, Clinical Diagnostics, Rye, NY).

Protein binding and blood to plasma concentration ratio. *In vitro* binding of *l*-propranolol to proteins in the rat perfusate plasma and drug partitioning between blood cells and perfusate plasma were determined. Blank blood perfusate was prepared by diluting freshly collected rat blood (at a hematocrit of 0.45) from normal animals with one-third volume of Krebs-bicarbonate buffer. Earlier work (Terao and Shen, 1983) has shown that uremia does not affect serum protein binding and blood to serum partitioning of *l*-propranolol. Therefore, all subsequent experiments were conducted with perfusate prepared with blood from normal donor rats. To measure the partitioning of drug between red blood cells and perfusate plasma, a tracer amount (3 × 10^6 dpm) of *l*-[^{3}H]propranolol (specific activity, 22.4 Ci/mmol; New England Nuclear, Boston, MA) and varying amounts of cold carrier were added to 3 ml of blank blood perfusate to achieve a wide range of pre-equilibrium perfusate blood concentrations from 8 ng/ml to 300 μg/ml. The blood samples were gently mixed at 37°C for 60 min. Preliminary experiments showed that partition equilibrium is reached within an hour of incubation at 37°C. At the end of incubation, an aliquot of the blood was removed and the remaining portion was centrifuged to obtain the plasma fraction. Concentration of radioactive *l*-propranolol in the perfusate blood and plasma (10-μl aliquots) were determined by direct liquid scintillation counting in premixed commercial scintiflor (Aquosol, New England Nuclear). Quenching was corrected by external standard channel ratio method. For perfusate plasma protein binding determinations, blank perfusate plasma was dialyzed against 0.134 M phosphate buffer (pH 7.4), spiked with varying concentrations of cold *l*-propranolol (ranging from 10 ng/ml to 500 μg/ml) and a tracer quantity of *l*-[^{3}H]propranolol (10^5 dpm/ml), for a period of 6 hr at 37°C. After dialysis, aliquots (50 to 100 μl) of the perfusate plasma and buffer were assayed for total radioactivity.

Pharmacokinetic calculations. The single-pass hepatic extraction ratio (E_h) of *l*-propranolol was calculated from the steady-state arterial (C_a) and venous (C_v) perfusate blood concentration as follows:

$$E_h = \frac{C_a - C_v}{C_a} \quad (1)$$

All of the measured arterial concentrations were pooled to provide an average steady-state estimate of C_a. The steady-state venous perfusate blood concentration was calculated by averaging the measurements at 15, 18 and 21 min of perfusion.

Assuming that the liver behaves like a well-stirred compartment (*i.e.*, with drug in the venous blood being in instantaneous equilibrium

with that in the liver), it has been shown that the hepatic extraction ratio can be mathematically related to hepatic blood flow rate (Q_h) and intrinsic clearance (Cl_{int}) (Rowland *et al.*, 1973; Wilkinson and Shand, 1975). The latter parameter expresses the capability of the liver to remove drug from the perfusate in the absence of any blood flow rate limitation and is governed by the rate of uptake and/or metabolic transformation of *l*-propranolol by the hepatocytes. Assuming that the kinetics of intrahepatic distribution and metabolic removal are linear processes, this relationship is expressed by the following equation:

$$E_h = \frac{Cl_{int}}{Q_h + Cl_{int}} \quad (2)$$

Upon rearrangement of the equation, an expression for Cl_{int} can be obtained:

$$Cl_{int} = \frac{E_h \cdot Q_h}{1 - E_h} \quad (3)$$

Because the perfusion flow rate is controlled in the perfusion preparation, the intrinsic metabolic clearance of *l*-propranolol in the perfused rat liver can be estimated from the extraction ratio.

Results

Viability of perfused uremic liver. The results of the perfusate blood pH, P_{o_2} and P_{co_2} measurements from the viability experiment are presented in figure 2. No significant differences in these physiological parameters were observed between the perfused livers from the normal control and renal failure animals. Both the perfusate blood pH and the arterial and venous P_{co_2} remained constant during the entire period of perfusion. Adequate oxygenation of the blood perfusate was achieved as evident by a steady and high arterial oxygen tension (@ 280 mm Hg). One indicator of the viability of the perfused liver preparation was an effective oxygen utilization as reflected in the large arterial-venous difference in P_{o_2}. There was, however, a trend toward a higher venous P_{o_2} with time, indicating a very small (<4%) but gradual decline in oxygen consumption over the 3 hr of perfusion.

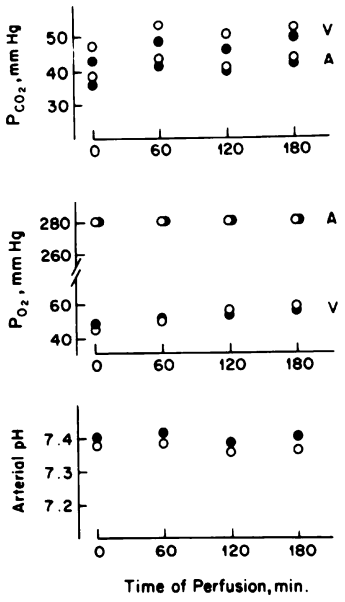

Fig. 2. Mean blood gas/pH measurements from the viability experiment for normal control liver (○) and uremic liver (●). A and V represent arterial and venous data, respectively (n = 3).

The plasma biochemical measurements from the viability experiment are presented in table 1. There was no remarkable difference in any of the biochemical indices between livers prepared from normal control and renal failure animals. Plasma glucose showed a gradual decline during recirculating perfusion reflecting a steady metabolic consumption. On the other hand, albumin and total protein concentrations increased (~20%) over the 3-hr period, most likely due to continuous net production of plasma proteins by the liver. As expected, serum creatinine and urea nitrogen concentrations (of blood samples taken before sacrifice or surgery) were elevated markedly in the renal failure donor animals as compared to the normal donor animals. However, normal concentrations of creatinine and urea nitrogen were observed during perfusion of uremic liver with blood perfusate prepared from normal rats, indicating that preperfusion with the buffer medium was effective in removing these uremic substances from the hepatic vasculature and tissue. The perfusate plasma transaminase activities remained normal throughout the experiment, suggesting that hepatocellular integrity was maintained at all times.

For both control and uremic livers, a constant bile flow was maintained for the first 2 hr (control, 282 ± 48 μl/30 min; uremic, 286 ± 62 μl/30 min). Thereafter, bile flow decreased by approximately 15% over the last hour of perfusion.

Overall, the results of the viability experiment indicate no apparent difference in the physiologic integrity of the perfused livers prepared from normal control and renal failure animals under the present experimental conditions.

Protein binding and partitioning in blood perfusate. The relationship of percentage of bound and blood to plasma concentration ratio of *l*-propranolol to the total drug concentration in perfusate plasma is graphically displayed in figure 3. Both the percentage of bound and perfusate blood to plasma concentration ratios were relatively constant at total (*i.e.*, bound and unbound) perfusate plasma concentrations below 1 μg/ml. At higher concentrations, a gradual decrease in the perfusate plasma protein binding of *l*-propranolol was observed. There was also a concurrent increase in the blood to plasma ratio, which is likely due to enhanced partitioning of *l*-propranolol into the cellular fraction as a result of the increase in the unbound fraction of drug in perfusate plasma. In order to avoid the complexities of concentration-dependent changes in the distribution of *l*-propranolol within the blood perfusate during passage across the hepatic sinusoid, the arterial perfusate drug concentration was kept below 1 μg/ml. Considerations based on these observations and the detection limitation of drug assay in the perfusate effluent led to the decision of setting the influent *l*-propranolol concentration at 500 ng/ml for the subsequent single-pass extraction study.

Single-pass perfusion experiments. The mean results of the four sets of perfusion experiments (*i.e.*, normal liver with normal blood, uremic liver with uremic blood, normal liver with uremic blood and uremic liver with normal blood) are presented in table 2. Four perfusion runs were performed per experiment. The time course of mean arterial and venous blood perfusate concentrations for each set of perfusion experiment is presented in figure 4.

There were no significant differences in the mean body weight (of the donor animals), liver weight, hematocrit and

TABLE 1

Biochemical measurements on perfusate plasma obtained from the viability experiment

All livers were perfused with blood perfusate prepared from normal animals.

Measurements	Donor	Base line[a]	Perfusion Time			
			0 min	60 min	120 min	180 min
Glucose, mg/dl	NC[b]	110 ± 40[c]	275 ± 66	231 ± 40	195 ± 33	196 ± 29
	RF[d]	95 ± 23	286 ± 25	233 ± 56	193 ± 36	185 ± 30
Albumin, g/dl	NC	3.2 ± 0.6	3.8 ± 0.4	3.8 ± 0.2	4.0 ± 0.2	4.0 ± 0.2
	RF	3.1 ± 0.5	3.7 ± 0.2	3.7 ± 0.1	3.8 ± 0.1	3.9 ± 0.1
Total protein, g/dl	NC	5.8 ± 1.2	4.5 ± 0.2	4.7 ± 0.2	4.9 ± 0.2	5.3 ± 0.2
	RF	5.5 ± 1.0	4.4 ± 0.2	4.7 ± 0.1	4.9 ± 0.2	5.0 ± 0.1
Urea nitrogen, mg/dl	NC	16 ± 4	10 ± 3	11 ± 2	14 ± 3	17 ± 3
	RF	155 ± 10	10 ± 1	15 ± 2	19 ± 2	23 ± 2
Creatinine, mg/dl	NC	1.1 ± 0.3	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.2
	RF	4.9 ± 0.6	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.2
GPT,[e] IU/ml	NC	27 ± 7	18 ± 1	19 ± 3	23 ± 1	27 ± 6
	RF	27 ± 5	17 ± 6	19 ± 10	21 ± 7	25 ± 3

[a] Measurements on serum obtained from liver-donor animals before surgery.
[b] NC, normal control rats, $n = 3$.
[c] Mean ± S.D.
[d] RF, renal failure rats, $n = 3$.
[e] GPT, glutamic pyruvic transaminase.

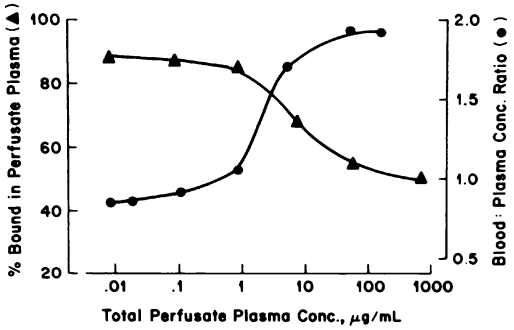

Fig. 3. Variations in plasma protein binding and blood-plasma partitioning of *l*-propranolol in rat blood perfusate medium as a function of plasma perfusate drug concentration. Conc, concentration.

steady-state bile flow between the four groups of experiments. A relatively uniform degree of uremia was induced in all renal failure donor animals as indicated by the consistency in plasma urea concentrations (*Ca.* 140 to 160 mg/dl) measured before the time of sacrifice or surgery.

The arterial blood perfusate concentration measured during the perfusion was 10 to 25% less than the anticipated concentration of 500 ng/ml. This apparent loss of drug is probably due to adsorption of *l*-propranolol onto surfaces of glasswares and silicone tubing. Although the actual arterial drug concentration varied from run to run (range 301 to 442 ng/ml), the input concentration remained constant (within ±5%) during each course of perfusion. The small between-run variation in the influent perfusate concentrations is not expected to affect the extraction ratio of *l*-propranolol.

As expected, binding to perfusate plasma proteins and the blood to plasma concentration ratio of *l*-propranolol did not differ between the four perfusion arrangements. This confirms our previous *in vivo* finding that uremia has no effect on the distribution of *l*-propranolol within the various blood elements. *l*-Propranolol concentration in the perfusate blood was always slightly lower than in the perfusate plasma. The blood to plasma concentration ratio was slightly higher for the arterial as compared to the venous perfusate blood (0.912 ± 0.048 *vs.* 0.903 ± 0.043). In addition, the unbound fraction of *l*-propranolol was consistently higher in the arterial perfusate plasma than the venous plasma perfusate by about 5%. Considering the rather large arterial-venous drug concentration gradient, these differences can probably be attributed to a small degree of concentration dependency in the protein binding and blood cell to plasma partitioning of *l*-propranolol in the blood perfusate. More critical is the observation that there was no change in blood to plasma concentration ratio with time. Therefore, all subsequent discussion will refer only to the perfusate blood data.

In all cases, steady-state concentration of *l*-propranolol in the venous perfusate blood, *i.e.* steady-state extraction, was reached within 15 min after the start of blood perfusion (see fig. 4). A small peak in venous concentration was observed consistently during the first 5 min of blood perfusion. The reason for this fluctuation in effluent perfusate concentration during the equilibration period is not clear.

Steady-state hepatic extraction ratio and estimates of intrinsic hepatic clearance of *l*-propranolol for the four sets of perfusion experiments are summarized in table 3. *l*-Propranolol was removed effectively from normal blood perfusate during a single passage through the liver isolated from normal control animals. The high hepatic extraction ratio (0.974 ± 0.005) is consistent with data from our earlier *in vivo* studies (Terao and Shen, 1983) which showed that the average systemic availability of *l*-propranolol after single p.o. administration (6 mg/kg) is only 7% in the normal rat. A much lower extraction ratio (0.906 ± 0.017) was observed in the uremic liver perfused with uremic blood, such that the mean venous to arterial concentration ratio (*i.e.*, fraction of drug escaping hepatic extraction) is 3 to 4 times higher in the uremic liver perfused with uremic blood as compared to normal liver perfused with normal blood (0.094 *vs.* 0.026). The magnitude of increase in the effluent drug concentration is similar to the relative difference in the *in vivo* p.o. systemic availability of *l*-propranolol between normal and renal failure animals (7 *vs.* 18%) reported earlier (Terao and Shen, 1983). The data from these homologous perfusion experiments confirm our hypothesis that the change in systemic availability of *l*-propranolol induced by renal failure is attributed to an inhibition of first-pass hepatic clearance.

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