IMPAIRED ELIMINATION OF PROPRANOLOL DUE TO RIGHT HEART FAILURE: DRUG CLEARANCE IN THE ISOLATED LIVER AND ITS RELATIONSHIP TO INTRINSIC METABOLIC CAPACITY

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(Received October 21, 1999; accepted July 6, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:

It is unclear if reduced hepatic drug elimination in congestive heart failure is primarily due to impairment of enzyme function as a result of tissue hypoxia, to the direct effects of hepatic congestion, or to changes intrinsic to the liver, such as reductions in enzyme content and activity. We therefore compared propranolol clearance in perfused rat livers from animals with right ventricular failure (RVF) with that from control animals. Despite the fact that both groups were perfused at comparable flow rates, perfusion pressures, and levels of oxygen delivery, hepatic extraction of propranolol was significantly reduced in RVF livers (0.688 ± 0.122 versus 0.991 ± 0.006 ml/min/g of liver in controls, P < .001). This effect was reflected in a 97% reduction in propranolol intrinsic clearance in RVF livers (5 ± 4 versus 172 ± 82 ml/min/g of liver in controls, P < .01). In RVF livers, total hepatic CYP expression was reduced by 19% compared with controls, whereas cytochrome P450 isoenzymes 1A1/2 and 2D1 were reduced by 41 and 26%, respectively. Despite the 97% reduction in propranolol intrinsic clearance in perfused RVF liver, intrinsic clearance in microsomal preparations from the same livers was reduced by only 48% compared with controls (P < .05). These findings suggest that impaired propranolol clearance in RVF is not primarily accounted for by reduced hepatic oxygen delivery or by changes in hepatic content and activity of drug-metabolizing enzymes.

A number of studies have provided evidence that hepatic elimination of drugs via oxidative metabolism is impaired in patients with congestive heart failure (Hepner et al., 1978; Ueda and Drindzio, 1978; Baughman et al., 1980; Rissam et al., 1983; Jenne, 1986; Patel et al., 1990; Huber et al., 1992). However, the mechanisms responsible have not been clearly defined. There is considerable evidence from in vitro studies that oxidative drug metabolism is likely to be sensitive to relatively mild reductions in hepatic oxygen supply (Jones, 1981; Angus et al., 1995). Using an experimental model of right ventricular failure (RVF), we have previously examined whether in congestive heart failure, there were significant changes in the metabolic capacity of the liver, and these changes appeared to correlate with the degree of impairment of hepatic drug elimination (Ng et al., 1995). However, other factors, including reduced enzyme content, reduced enzyme activity, or direct physical effects of liver congestion on the hepatocyte, may also be important.

If reduced hepatic oxygen delivery is largely responsible for impaired hepatic drug clearance in RVF, it would be predicted that reduced hepatic drug clearance in vivo would be restored when livers from animals with RVF were perfused with normal amounts of oxygen. Similarly, if some direct effects of hepatic congestion were the most important factor, drug clearance should be returned to normal when the liver is perfused in the absence of obstruction of hepatic venous outflow. In contrast, if drug metabolism remained abnormal when the liver was perfused with normal amounts of oxygen and at normal outflow pressures, changes in drug metabolism in vivo are likely to reflect changes intrinsic to the liver, such as a reduction of hepatic drug-metabolizing enzyme expression and/or activity. Therefore, in the present study, we have removed and perfused livers from animals with RVF and examined the ability of these livers to metabolize drugs under controlled flow and oxygen delivery conditions. Propranolol was chosen as the marker of oxidative drug elimination because the drug is eliminated almost entirely via oxidation by CYP enzymes (Fujita et al., 1993; Masubuchi et al., 1993), is highly extracted by the liver (Fenyves et al., 1993), and has a relatively short half-life, thus allowing quick attainment of steady state.

To determine the contribution of changes in hepatic enzyme content and activity to changes in hepatic propranolol clearance, we compared intrinsic hepatic clearance calculated from data obtained in the iso-
lated perfused rat liver (IPRL) with intrinsic clearance \( (V_{\text{max}}/K_{\text{m}}) \) in microsomes prepared from the same livers. The hepatic expression of total CYP as well as those isoenzymes directly involved in propranolol metabolism (CYP 1A1/2 and 2D1) were also measured.

**Materials and Methods**

**Chemicals.** The hydrochloride salt of \( \beta \)-propranolol was purchased from Imperial Chemical Industries Ltd (Cheshire, England). Rat CYP 1A1/2 antibody was a gift kindly obtained by Dr. Michael Owens (Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR). Glucose-6-phosphate dehydrogenase and NADP were purchased from Boehringer Mannheim (Mannheim, Germany). \( \beta \)-[4-\(^3\)H]Propranolol (784 GBq/mmol) was purchased from NEN DuPont (Boston, MA), and scintillation cocktail (Ready Organic) was from Beckman Instrument, Inc. (Fullerton, CA). All other chemicals used were analytical grade.

**Right Heart Failure Model.** Sprague-Dawley rats weighing between 90 and 110 g and aged between 4 and 5 weeks were randomized into sham or pulmonary artery-constricted (PAC) groups. Rats in the PAC group (n = 9) underwent constriction of the pulmonary artery via a left-sided thoracotomy as previously described (Ng et al., 1995). Rats in the sham group (n = 8) underwent the same surgery but without the pulmonary artery constriction. There was no significant difference \( (P > 0.5) \) between the initial body weight of the sham (n = 8, mean weight 104 ± 8 g) and the PAC groups (n = 9, mean weight 101 ± 9 g).

**IPRL. Experimental preparation.** Fifteen to 17 weeks after pulmonary artery constriction or sham operation, rats of the two experimental groups were anesthetized with pentobarbital (60 mg/kg). Before the removal of the liver, the left jugular vein was cannulated, and the mean central venous pressure was measured by an electronic pressure monitor unit (78205A; Hewlett Packard, Palo Alto, CA). Using standard surgical techniques, the common bile duct, the portal vein, and the inferior vena cava were cannulated. The liver was then dissected free from the abdominal cavity and weighed.

**Experimental design.** Livers were perfused at a constant flow rate, via the portal vein, with a pump that was calibrated on the day of the experiment. Similar flow rates were used in both groups with an average of 1.5 ml/min/g of liver (Table 1). The perfusion was initially in a recirculating mode, which lasted for about 20 min to allow the liver to stabilize and its viability to be assessed, and was followed by 1 h of perfusion using a single-pass design. Both the single-pass and the recirculating perfusate consisted of Krebs-Henseleit buffer (pH = 7.4) containing 20\% (v/v) washed human red blood cells, 1\% (w/v) bovine serum albumin, 0.1\% (w/v) glucose, and 30 \( \mu \)M sodium taurocholate and were maintained at 37°C in a humidified cabinet. This composition of perfusate resulted in a free fraction \( (f_{0}) \) of 0.66. Single-pass perfusate was spiked with \( \beta \)-propranolol HCl to a concentration of 2 \( \mu \)g/ml (6.76 \( \mu \)M). Bile was continually collected into preweighed tubes at 30-min intervals.

The viability of liver preparations was assessed by macroscopic appearance, oxygen consumption, and perfusion pressure. All livers were homogeneously perfused as indicated by the even color of the liver lobes during the perfusion, had oxygen consumption of greater then 4 \( \mu \)mol/min/g of liver, and had perfusion pressure of no greater than 12 cm of H\(_2\)O at the end of the perfusion and less than 2 cm of H\(_2\)O difference in the perfusion pressure between the beginning and the end of the experiment. Inflow perfusate samples \( (C_{\text{in}}) \) were collected at 15, 30, 45, and 60 min. Outflow perfusate samples \( (C_{\text{out}}) \) were collected at 5-min intervals from 15 min onward. \( C_{\text{in}} \) and \( C_{\text{out}} \) samples were stored at −20°C until drug concentrations were analyzed by HPLC. Per fusate drug concentrations were used to calculate steady-state extraction \( (E) \) and hepatic clearance of propranolol as follows (Wilkinson 1987):

\[
E = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}}
\]

\[
\text{Clearance} = E \cdot Q
\]

where \( E \) is the hepatic extraction of the drug, \( C_{\text{in}} \) is the inflow drug concentration (\( \mu \)M), \( C_{\text{out}} \) is the outflow drug concentration (\( \mu \)M), and \( Q \) is the perfusate flow rate.

Intrinsic clearance of propranolol in the isolated perfused liver has been shown to be best described by the venous equilibrium model by our group (Smallwood et al., 1988) and others (Ishida et al., 1992) using the following equation:

\[
CL_{\text{int}} = \frac{E \cdot Q}{f_{d}(1 - E)}
\]

At the completion of the perfusion experiment, the livers were weighed, and a small section of the liver was kept separately in buffered Formalin (10\%) for histological study. Histological analysis was performed in the absence of knowledge of hemodynamic and pharmacokinetic parameters. The remaining liver tissue was snap-frozen in liquid nitrogen, stored at −70°C, and later used to prepare hepatic microsomes. The heart was also removed from the thoracic cavity of the rat, and the right ventricle (including the septum) was isolated and weighed.

**Measurement of Hepatic CYP Enzyme(s) Expression and Activity.** Preparation of hepatic microsomes and measurement of total CYP expression. Microsomes were prepared (Aitio and Vainio, 1976) from five livers (2 g each) randomly chosen from the sham group and livers of the PAC rats that had evidence of RVF \( (n = 5) \). Microsomal protein content was measured by a standard Lowry assay (Lowry et al., 1951) using bovine serum albumin as the protein standard. Using this method, the mean microsomal protein recovery per gram of liver was 10.8 ± 1.4 mg in the sham group and 14.6 ± 2.7 mg in the RVF group \( (P < 0.05) \). Total CYP concentration in the microsomes was measured by dithionite-difference spectroscopy as previously described (Matsumura et al., 1976). Total CYP content of the RVF group was then expressed as a percentage of the content in the sham group.

**Measurement of hepatic CYP isoenzyme 1A1/2 and 2D1 expression.** Hepatic CYP isoenzyme expression (five sham and five RVF) was assessed by the Western blot technique as described previously (Hickey et al., 1996). Intensities of the bands in the blots were analyzed using an image analysis system equipped with the Molecular Analyst Software (v2.1; Bio-Rad Laboratories, Hercules, CA). The mean intensity of bands for each isoenzyme from the livers of the RVF group was expressed as a percentage of the mean intensity for that isoenzyme from the sham group.

The linearity of the band intensity with protein concentration was checked with a pooled sample of liver microsomes from sham-operated rats. The intensity of the band was found to be linearly related to the amount of protein loaded in each lane over the range of 2.5 and 6 \( \mu \)g of microsomal protein loaded in each lane (standard amount 3 \( \mu \)g).

**Measurement of Propranolol Intrinsic Clearance in the Microsomes.** The decline in propranolol concentration in the microsomal mix was described by a monoeponential decline:

\[
[S] = [S]_{0} \cdot e^{-kt}
\]

where \([S]_{0}\) and \([S]\) is the propranolol concentration at time 0 and time \( t \), respectively, and \( k \) is the exponential rate constant describing the decline in concentration.

Intrinsic clearance of propranolol in the microsomes was measured by dividing the amount of drug in the tube at the initiation of the reaction \( ([S]_{0} \cdot V) \), where \( V \) is the volume) by the area under the curve from zero to infinity. Because the decline in concentration was monoexponential, the area under the curve was calculated by dividing the initial concentration by the slope \( ([S]_{0}/k) \), thus

\[
CL_{\text{int}} = \frac{[S]_{0} \cdot V}{[S]_{0}/k} = V \cdot k
\]

The incubation reaction mixture (1-ml reaction volume) consisted of 50 \( \mu \)l of microsomes (final protein concentration range, 6–20 \( \mu \)g/ml), 0.505 ml of 1.15\% KCl, and 0.4 ml of 0.2 M potassium phosphate buffer (pH 7.4) containing the NADPH-generating system (10 \( \mu \)mol of glucose 6-phosphate, 0.5 \( \mu \)mol of NADP, 2 units of glucose-6-phosphate dehydrogenase, and 8 \( \mu \)mol of MgCl\(_2\)). The rate of propranolol elimination was found to be linearly related with microsomal protein concentration from 5 to 25 \( \mu \)g/ml. After incubating the mixture under air at 37°C for 3 min, the reaction was commenced by the addition of 45 \( \mu \)l of the 1:1000 diluted \( \beta \)-[4-\(^3\)H]propranolol.
(\(^{1}^{[3]H}\)propranolol) (1.85kBq), giving a final concentration of \(^{1}^{[3]H}\)propranolol in the incubation medium of 0.5 ng/ml (1.69 nM). The rate of disappearance of \(^{1}^{[3]H}\)propranolol from the incubation mixture was determined over 6 min at 1-min intervals, with each time point determined in duplicate in separate tubes. The reaction was stopped with 0.5 ml of NaOH (2.5 M), and the concentration of nonmetabolized \(^{1}^{[3]H}\)propranolol was measured as described below.

**Assays.** Propranolol perfusate concentration was measured by a specific and sensitive HPLC method as previously described (Mihaly et al., 1982). The concentration of nonmetabolized \(^{1}^{[3]H}\)propranolol in the incubation mixture was measured using a previously published extraction procedure (Fenyves et al., 1993). Five milliliters of \(n\)-heptane containing 1.5% isoamyl alcohol was added to the glass tube containing a 1.5-ml mixture of the reaction medium. The resultant mixture was vortexed and centrifuged at 1500 rpm for 10 min. Four milliliters of the supernatant was added to 10 ml of the organic liquid scintillation cocktail, and the radioactivity due to tritium was determined in a liquid scintillation analyzer (Packard Instrument Co., Meriden, CT).

Radioactivity counts were corrected for quenching and converted to propranolol concentrations determined from a six-point standard curve. The coefficient of variation of the assay for repeated measures \((n = 6)\) of \(^{1}^{[3]H}\)propranolol was 7% for 0.11 ng/ml and 2% for 0.49 ng/ml. HPLC radiochromatograms of this extract showed that only propranolol was extracted.

Before the \(^{1}^{[3]H}\)propranolol stock solution (specific activity 784 GBq/mmoll) was used, its purity was checked by HPLC (Nand et al., 1996). Mobile phase effluents from the HPLC system were collected by a fraction collector (0.6-ml fractions) and added to 5 ml of scintillation cocktail (Ultima Gold; Packard Instrument Co.), and radioactivity was counted. Approximately 90% of the radioactivity collected was recovered in the retention window of propranolol; the remaining radioactivity eluted in the solvent front. The concentration of \(^{1}^{[3]H}\)propranolol in a 1:1000 dilution was found to be 10.8 ng/ml.

The amount of microsomal protein present per gram of the rat liver was measured by the Lowry assay (Lowry et al., 1951) using bovine serum albumin as the standard. Coefficients of variation of repeated measures \((n = 6)\) of albumin in the Lowry assay were 3, 2, and 2% at 0.05, 0.15, and 0.25 mg/ml, respectively. Inaccuracies of the assay for these concentrations were 5, 2, and 2%, respectively. Between-day coefficient of variation and inaccuracy of the assay \((n = 5)\) at 0.075 mg/ml were 4 and 5%, respectively, and at 0.2 mg/ml were 1 and 2%, respectively.

**Statistical Analysis.** Data in the tables are presented as mean ± S.D. Data in the graphs are presented as mean ± S.E.M. Statistical comparisons between the sham and the RVF groups in the IPRL and the microsomal studies were made using the Student’s unpaired t test. All statistical tests were performed using the Statview SE package (v1.4; Abacus Concepts Inc., Berkeley, CA), and a P value of less than .05 was considered significant.

**Results**

**Right Heart Failure Model.** At 15 weeks, five of the nine rats that underwent the pulmonary artery constriction developed RVF as evidenced by a 10-fold increase in the mean central venous pressure (Table 1) and an engorged liver at laparotomy. In these animals, there was evidence of cardiac hypertrophy with a mean increase in right ventricular weight of 57%. Although the other four rats that underwent the pulmonary artery constriction also developed cardiac hypertrophy (mean right ventricular weight, 1.18 ± 0.13 g), there was no evidence of RVF as indicated by the near normal mean central venous pressure (3 ± 1 mm Hg) and the absence of hepatic congestion. The RVF rats did not have increased lung weight to suggest pulmonary congestion or edema due to reduction of left ventricular function (Table 1). There was no significant difference in the mean body weight between the sham and the RVF groups.

**Evidence of Hepatic Congestion.** All livers from the five animals with RVF showed macroscopic evidence of hepatic congestion. When examined under the light microscope, livers from these animals showed sinusoidal dilation and congestion. There were features of hepatocyte degeneration around the pericentral congested regions, but hepatic fibrosis was absent. None of the livers from the sham group showed hepatic congestion under light microscopy. The mean liver weight of the RVF rats was not significantly different from those of the sham group (Table 1).

**Viability of the Isolated Liver Preparation.** Physiological parameters of the isolated perfused livers from the sham and the five RVF rats are summarized in Table 1. The data illustrate that liver preparations in both groups were perfused at similar flow rate and oxygen delivery. All preparations were viable, with no difference in oxygen extraction and consumption between the two groups.

**Extraction and Hepatic Clearance of Propranolol.** The outflow concentration had reached steady state by 20 min in all liver preparations. RVF significantly reduced hepatic extraction of propranolol, as illustrated by markedly elevated outflow perfusate concentrations and reduction of extraction ratio of the drug, from a mean of 0.991 in the sham group to 0.688 in the RVF group, \(P < .001\) (Table 1). In parallel with the reduced extraction ratio, hepatic clearance of the drug in RVF was reduced \((1.07 ± 0.21 \text{ ml/min/g of liver in RVF versus } 1.52 ± 0.25 \text{ ml/min/g of liver in sham, } P < .01)\) (Table 1).

**Total CYP and Isoenzyme Expression in the Perfused Livers.** The mean total CYP expression in livers of the RVF group was 19.1 ± 3.9% less than that in the sham group \((P < .05)\). The mean CYP 1A1/2 and 2D1 contents were reduced by 41.5 ± 15.5 and 25.8 ± 3.1%, respectively \((P < .01)\).

**Propranolol Intrinsic Clearance.** The mean \(C_{\text{Lum}}\) of propranolol in the IPRL and in the microsomes is summarized in Fig. 1. The fall in mean hepatic clearance of the drug from the control value of 1.52 ml/min/g of liver to the value of 1.07 ml/min/g of liver in the IPRLs from animals with RVF (Table 1) was reflected in a 97% fall in intrinsic clearance \((257 ± 123 \text{ ml/min/g of liver in sham to } 7.0 ± 5.9 \text{ ml/min/g of liver in RVF; } P < .01)\) when calculated by the venous equilibrium model. However, in microsomes from animals with RVF, the mean \(C_{\text{Lum}}\) (microsomes) was only 48% less than that in shams \((6.9 ± 1.2 \text{ versus } 13.1 ± 2.5 \text{ ml/min/mg of microsomal protein; } P < .05)\).

**Discussion**

The present study demonstrates that in the isolated liver, hepatic elimination of propranolol is impaired in livers from animals with right ventricular failure. Hepatic elimination of propranolol is considered to be flow-dependent because the extraction ratio of the drug is very high \((>0.9)\) (Wilkinson and Shand, 1975). Thus, if perfusion of the liver was reduced in heart failure (Ng et al., 1995), it might be expected to result in impairment of hepatic clearance of the drug. The present finding that hepatic clearance of propranolol was significantly

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**TABLE 1**

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<thead>
<tr>
<th>Physiological parameters and propranolol pharmacokinetics in isolated perfused livers of sham and RVF rats</th>
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<tr>
<td>Values are means ± S.D.</td>
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<tr>
<td><strong>Sham</strong> ((n = 8))</td>
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<tr>
<td><strong>Body weight (g)</strong></td>
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<td><strong>Right ventricle weight (g)</strong></td>
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<td><strong>Liver weight (g)</strong></td>
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<td><strong>Lung weight (g)</strong></td>
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<td><strong>Central venous pressure (mm Hg)</strong></td>
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<td><strong>Perfusion flow rate (ml/min of liver)</strong></td>
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<td><strong>QO delivery (μmol/min of liver)</strong></td>
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<td><strong>O2 consumption (μmol of O2/min of liver)</strong></td>
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<td><strong>Perfusion pressure (cm H2O)</strong></td>
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<td><strong>Perfusion pH</strong></td>
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<tr>
<td><strong>Bile flow (ml/h)</strong></td>
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<tr>
<td><strong>Propranolol extraction ratio</strong></td>
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<tr>
<td><strong>Propranolol clearance (ml/min/g of liver)</strong></td>
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Significantly different from the sham group, \(*P < .01, **P < .001\).
Metabolic capacity of the enzymes involved in propranolol metabolism. Propranolol in RVF cannot be fully accounted for by a reduction in the intrinsic extent in the IPRL than in microsomes. This suggests that reduced clearance of propranolol in RVF cannot be fully accounted for by a reduction in the intrinsic metabolic capacity of the enzymes involved in propranolol metabolism.

Reduced in perfused livers from animals with RVF when perfusate flow rates and oxygen delivery were identical with those in controls, indicates that there is reduced intrinsic clearance of the drug in RVF. This suggests that RVF may affect hepatic clearance of drugs independent of changes in blood flow.

Propranolol is metabolized through four different pathways: ring oxidation, side chain oxidation, glucuronidation, and O-dealkylation (Bargar et al., 1983). Side chain oxidation, which forms N-desisopropylationpropranolol, is mainly responsible for propranolol elimination in rats (Fujita et al., 1993; Masubuchi et al., 1993). In humans, 4- and 5-hydroxylation and N-desisopropylation are the major propranolol elimination pathways (Masubuchi et al., 1994; Yoshimoto et al., 1995). Propranolol metabolism may be sensitive to reductions in hepatic oxygenation that occur in heart failure (Ng et al., 1995) because oxygen is utilized directly as a substrate in drug oxidation (Jones, 1981; Angus et al., 1995) and CYP isozymes are predominantly localized in the acinar zone 3 hepatocytes (Baron et al., 1973; Gooding et al., 1978; Ratnasavanh et al., 1991) where hepatic oxygen concentrations are lowest (Lemasters et al., 1981; Matsumura et al., 1986). Indeed, previous studies have shown that hepatic elimination of propranolol is impaired with minor reductions in hepatic oxygen supply that are likely to occur in vivo (Elliott et al., 1993). However, the present finding of markedly reduced hepatic clearance of propranolol in isolated livers from animals with RVF in the face of normal levels of hepatic perfusion and oxygenation, supports the view that reduction in hepatic oxygen delivery that may occur in heart failure (Ng et al., 1995) is unlikely to be the most important determinant of impaired elimination of propranolol.

The level of expression and the activity of the enzymes determine the intrinsic metabolic capacity of the liver. Total expression of CYP concentrations are lowest (Lemasters et al., 1981; Matsumura et al., 1986). Indeed, previous studies have shown that hepatic elimination of propranolol is impaired with minor reductions in hepatic oxygen supply that are likely to occur in vivo (Elliott et al., 1993). However, the present finding of markedly reduced hepatic clearance of propranolol in isolated livers from animals with RVF in the face of normal levels of hepatic perfusion and oxygenation, supports the view that reduction in hepatic oxygen delivery that may occur in heart failure (Ng et al., 1995) is unlikely to be the most important determinant of impaired elimination of propranolol.

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