Biochemical Pharmacology, Vol. 45, No. 3, pp. 573–578, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

THE EFFECT OF HYPOXIA ON PROPRANOLOL CLEARANCE DURING ANTEGRADE AND RETROGRADE FLOW IN THE ISOLATED PERFUSED RAT LIVER PREPARATION

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(*Received* 5 *October* 1992; *accepted* 17 *November* 1992)

Abstract—We investigated, using the single-pass isolated perfused rat liver preparation, whether the centrilobular location of hepatic oxidative drug metabolism could be a contributing factor to the marked sensitivity of drug oxidation to hypoxia. Livers (N = 7) were each perfused for 130 min with 2 μg/mL (+)-propranolol, a drug metabolized almost entirely by oxidation in the rat. The direction of flow was reversed after 60 min, the order of flow direction being randomized. Normal oxygenation was used during the first 30 min of antegrade and of retrograde perfusion, but in the second 30 min perfusate was equilibrated with a N_2/O_2 mixture designed to reduce hepatic oxygen delivery by half. During normal oxygenation there was no significant difference between antegrade and retrograde perfusion in hepatic oxygen delivery and physiological parameters such as oxygen consumption and extraction, perfusion pressure and bile flow. During hypoxia, mean oxygen delivery was slightly lower with retrograde perfusion (retrograde: mean = 2.37 μmol/min/g liver, range = 1.56–3.17; antegrade: mean = 2.90 μmol/min/g liver, range = 1.96–4.08; P = 0.04), but there was no significant difference in physiological parameters within each liver (P > 0.05). Propranolol clearance during normal oxygenation was similar to the perfusion rate (10 mL/min) and was the same for both directions of perfusion (antegrade 9.88 ± 0.07 mL/min, retrograde 9.88 ± 0.13 mL/min, P > 0.05). Hypoxia reduced propranolol clearance substantially, but the decrease was significantly greater with antegrade perfusion (5.65 ± 1.89 mL/min) than with retrograde perfusion (6.76 ± 1.95 mL/min, P = 0.014). Oxidative drug metabolism is located primarily in the centrilobular zone and sinusoidal oxygen concentration is lowest in the "downstream" zone with both antegrade and retrograde perfusion. These findings suggest that the centrilobular location of propranolol metabolism may influence the effect of hypoxia on propranolol elimination, but is not a major contributor to the marked sensitivity of propranolol elimination to hypoxia antegrade perfusion.

There is ample evidence in subcellular systems that drug metabolism is profoundly affected by hypoxia [1–4]. However, while studies in isolated hepatocytes and microsomes provide much information on the oxygen requirements of various metabolic processes, in considering the effect of hypoxia on metabolism *in vivo* there are additional factors which need to be taken into account. Within the hepatic acinus there are large differences in the oxygen concentration to which different hepatocytes are exposed, since there is a substantial acinar oxygen gradient from high concentrations in the periportal zone (zone 1) to much lower concentrations in the pericentral zone (zone 3) [5–9]. It is therefore possible that the acinar location of a drug metabolizing system may influence its sensitivity to a reduction in oxygen delivery to the liver. An enzyme system located predominantly in zone 1, such as sulphation [10–12] might be expected to be less affected by hypoxia than a process such as microsomal drug oxidation, which is predominantly located in zone 3 [13, 14]. Indeed, in both the isolated perfused rat liver (IPRL§) and *in vivo*, hypoxia produces structural and functional changes predominantly in the centrilobular zones [15, 16].

We and others have previously demonstrated in the IPRL that the elimination of propranolol, omeprazole and hexobarbital, which is primarily dependent on oxidation, is very sensitive to hypoxia [17–20]. Oxidation has consistently been located predominantly in the pericentral zone of the acinus [13, 14, 21, 22], therefore this study was designed to determine whether the centrilobular location of oxidative drug metabolism could contribute to its marked sensitivity to hypoxia. The effect of hypoxia on the clearance of propranolol, a compound which is more than 90% oxidized in the rat [23, 24] was determined during both antegrade and retrograde perfusion of the isolated rat liver. It would be expected that if the acinar oxygen gradient in the perfused liver did contribute to the effect of a given reduction in oxygen supply on propranolol elimination, then propranolol clearance would be

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§ Abbreviation: IPRL, isolated perfused rat liver.

less impaired by hypoxia during retrograde than antegrade flow.

MATERIALS AND METHODS

Chemicals and enzymes. (+)-Propranolol was obtained from Imperial Chemical Industries (Melbourne, Victoria, Australia) and labetolol hydrochloride was donated by Glaxo (Melbourne, Victoria, Australia). The following were also used: analytical grade triethylamine, hydrochloric acid, sodium carbonate and orthophosphoric acid (Ajax, Sydney, Australia); diethyl ether (peroxide free, May and Baker, U.K.); HPLC-grade acetonitrile (Mallinckrodt, Melbourne, Victoria, Australia); sodium metabisulphite (Merck, Sharp and Dohme, West Point, PA, U.S.A.) and double glass deionized water. The diethyl ether was washed twice with 0.15% orthophosphoric acid and once with deionized water before use.

Experimental preparation. Seven non-fasting male Sprague–Dawley rats (weight 155–170 g) were anaesthetized with intraperitoneal pentobarbitone (50 mg/kg). The livers were surgically isolated with both the portal vein and the inferior vena cava cannulated with a 14 gauge catheter (Medicut®, Sherwood Medical Industries, St Louis, MO, U.S.A.). During surgery the liver was perfused in the standard manner via the portal vein with Hartmann's solution [25, 26]. At the completion of surgery the polyethylene tubing of the perfusate circuit was connected to either the portal vein for antegrade perfusion or to the inferior vena cava for retrograde perfusion. After 60 min of constant perfusion in the direction initially chosen the tubing was disconnected from the inflow cannula and then connected to the alternate cannula, thus establishing flow in the opposite direction.

A non-recirculating design was used, with flow maintained at a constant 10 mL/min (approx. 2 mL/min/g liver), at 37°. The perfusate consisted of 20% (v/v) washed human red blood cells, 1% bovine serum albumin and 0.1% *d*-glucose in a Krebs–Henseleit electrolyte solution. To provide a stimulus to bile flow, sodium taurocholate (30 μmol/L) was added to the perfusate reservoir. Viability of the liver preparation was determined by macroscopic appearance, oxygen consumption of greater than 3 μmol/min/g liver during normal oxygenation, constant perfusion pressure of 4–8 cm/H_2O and bile flow of greater than 0.3 mL/hr.

The perfusate was oxygenated by equilibration with 100% oxygen in a Silastic membrane oxygenator. To achieve hypoxia, the perfusate was equilibrated with a mixture of oxygen and nitrogen. This proportion of oxygen to nitrogen determined the level of hypoxia.

Experimental design. At the beginning of the single pass perfusion, 1.4 mL of an aqueous solution of (+)-propranolol hydrochloride (2 mg/mL) was added to the 1400 mL perfusate reservoir so that the liver was perfused with 2 μg/mL (+)-propranolol, throughout each experiment.

The experiments were designed to allow propranolol clearance in each liver preparation to be studied under four different experimental conditions (i.e. antegrade normoxia and hypoxia, and retrograde normoxia and hypoxia). This enabled each liver to act as its own control for both direction of flow and degree of oxygenation.

The direction of flow (antegrade or retrograde) for the first phase of each experiment was randomly assigned prior to surgery and perfusion was continued for 60 min. The direction of perfusion was then reversed. After a 10 min period of stabilization, the experiment was continued for a further 60 min. The initial perfusion was antegrade in four experiments and retrograde in three. The perfusion pressure was not altered significantly by reversal of the direction of flow during normal oxygenation nor during hypoxia.

Each of the two 60 min periods of perfusion

Table 1. Propranolol clearance in the individual liver preparations

Expt. No.	Normal oxygen		Hypoxia					
	Clearance (mL/min)		Clearance (mL/min)		O_2 Delivery (μmol/min/g)		Clearance ratio§	
	Ante	Retro	Ante	Retro	Ante	Retro	Ante	Retro
1*	9.81	9.94	8.37	9.35	3.52	2.67	0.85	0.94
2	9.92	9.71	6.27	6.37	1.96	1.56	0.63	0.66
3	9.97	9.79	5.70	7.90	2.22	1.76	0.57	0.81
4	9.68	8.91	6.06	5.99	2.62	2.60	0.63	0.67
5	9.94	9.61	6.02	7.73	2.79	3.17	0.61	0.72
6*	8.69	9.81	2.04	3.14	2.54	1.99	0.23	0.32
7*	8.85	9.83	5.06	6.87	4.08	3.10	0.57	0.70
Mean	9.55	9.66	5.65	6.76†	2.90	2.41	0.59	0.69‡
SD	0.54	0.34	1.89	1.95	0.84	0.64	0.18	0.19

* In these experiments, retrograde perfusion preceded antegrade perfusion.
† Significantly greater than antegrade, P = 0.014.
‡ Significantly greater than antegrade, P = 0.007.
§ Calculated as clearance during hypoxia/clearance during normoxia.

consisted of two 30 min phases: a normal oxygenation phase and a phase of hypoxia. During the phase of normal oxygenation the perfusate was equilibrated with 100% oxygen. During the hypoxia phase the perfusate was equilibrated with a mixture of nitrogen and oxygen to obtain an oxygen delivery about half that of the control oxygenation phase. This level of hypoxia was known from previous studies using antegrade perfusion to produce substantial impairment in the propranolol clearance.

Steady-state extraction of propranolol was attained by 20 min during both antegrade and retrograde perfusion. Consequently, inflow and outflow perfusates were sampled for measurement of propranolol concentration and oxygen content at time 0, 20, 25 and 30 min in each of the four 30 min experimental phases.

Bile was collected continuously in preweighed vials in 30 min aliquots. The bile collected during the 10 min stabilization period was discarded and not included in the results.

Oxygen delivery and consumption determinations. During each experiment frequent analyses of the inflow and outflow perfusate were made for the determination of pO_2, pCO_2, pH, HCO_3^- and % saturation using an ILS pH/blood gas analyser (Instrument Laboratory, Lexington, MA, U.S.A.). Oxygen content, delivery, consumption and extraction were calculated using standard equations, previously described in detail [18].

Propranolol assay. Inflow and outflow perfusate samples were stored at −70° until the assay was performed (within 5 days of the liver perfusion). Propranolol concentrations in perfusate samples were determined by a sensitive and specific HPLC method which has been previously described in detail [27]. The minimum quantifiable concentration of propranolol in perfusate was 0.5 ng/mL.

Statistics. Data are expressed as the mean and standard deviation unless otherwise specified. The Student's *t*-test for paired observations was used to compare antegrade and retrograde perfusions, and correlations between variables were analysed using linear regression analysis [Statworks® (version 1.2), Cricket Software Inc, Philadelphia, PA, U.S.A.] performed on a Macintosh SE computer (Apple Computer Inc, Cupertino, CA, U.S.A.). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Oxygen delivery and consumption

Normal oxygenation (control phase). The mean rates of oxygen delivery during the control phases in each direction of perfusion were 5.92 ± 0.62 μmol/min/g liver for antegrade perfusion and 6.00 ± 0.63 μmol/min/g liver for retrograde perfusion (P = 0.82). Oxygen consumption was not altered by the change in direction of flow (antegrade 4.39 ± 0.61 μmol/min/g liver; retrograde 4.55 ± 0.61 μmol/min/g liver; P = 0.63). Oxygen extraction during antegrade and retrograde perfusion was 72.1 ± 10.1% and 70.4 ± 9.4%, respectively (P = 0.37).

Hypoxia phase. A range of rates of oxygen delivery was produced during the hypoxia phases (Table 1).

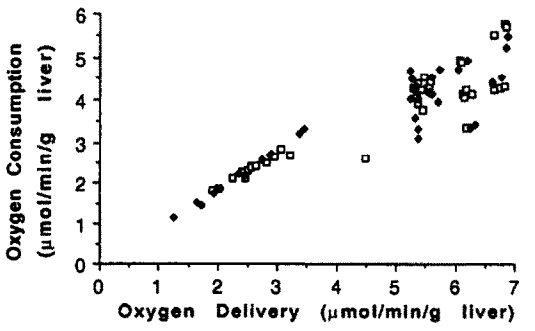

Fig. 1. Relationship between oxygen consumption and oxygen delivery during normal oxygenation and hypoxia in antegrade (□) and retrograde (◆) perfused liver preparations.

During antegrade perfusion the rates ranged from 1.96 to 4.08 μmol/min/g liver, whereas during retrograde perfusion the range was from 1.56 to 3.17 μmol/min/g liver. There was an inadvertent small difference in mean rate of oxygen delivery between the two directions of flow (antegrade 2.90 ± 0.84 μmol/min/g liver; retrograde 2.37 ± 0.66 μmol/min/g liver; P = 0.04).

Reflecting the higher oxygen delivery presented during the antegrade hypoxic perfusions, the mean oxygen consumption tended to be higher during antegrade than retrograde perfusion, but this difference did not reach statistical significance (antegrade 2.34 ± 0.30 μmol/min/g liver; retrograde 2.02 ± 0.61 μmol/min/g liver; P = 0.12). Oxygen extraction was similar in the two directions (91.3 ± 4.4%; 91.4 ± 4.1%; P = 0.95).

Oxygen consumption was highly correlated with oxygen delivery at rates of oxygen delivery less than approx. 5.0 μmol/min/g liver ($r = 0.99$, $P < 0.005$) (Fig. 1). At higher levels of oxygen delivery, consumption began to plateau, and was independent of delivery ($r = 0.47$, P = 0.060). These relationships applied to both antegrade and retrograde perfusion.

Bile flow. Bile flow was not reduced during hypoxia and was not altered by change in direction of flow during either normal oxygenation (antegrade 0.43 ± 0.17 mL/min; retrograde 0.52 ± 0.20 m/min; P = 0.46) or hypoxia (antegrade 0.42 ± 0.15 mL/min; retrograde 0.35 ± 0.17 mL/min; P = 0.21).

Propranolol clearance. The clearance data for each liver preparation are shown in Table 1. With normal oxygenation, propranolol clearance was high in both directions of perfusion (antegrade 9.88 ± 0.07 mL/min; retrograde 9.88 ± 0.13 mL/min; P = 0.96: perfusion rate 10 mL/min). Propranolol clearance was reduced by hypoxia during both antegrade and retrograde perfusion. However, in all but one liver preparation, propranolol clearance was *lower* during antegrade perfusion than during retrograde perfusion (Table 1). The mean antegrade clearance was 5.65 ± 1.89 mL/min, which was significantly less than the mean retrograde clearance (6.76 ± 1.95 mL/min; P = 0.014). The propranolol clearance ratio (propranolol clearance during

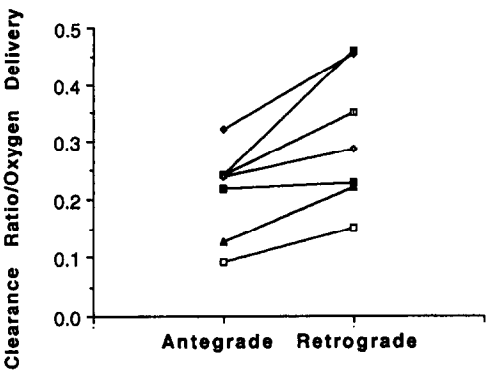

Fig. 2. Propranolol clearance ratio (i.e. ratio of propranolol clearance during hypoxia/normoxia) during antegrade and retrograde perfusion. Clearance ratio has been divided by the oxygen delivery during hypoxia to allow for slight differences in oxygen delivery with antegrade and retrograde perfusion.

hypoxia/clearance during normal oxygenation) was calculated for each liver to eliminate inter-animal variation. In every liver the clearance ratio was lower during antegrade perfusion than during retrograde perfusion (Table 1), and the mean antegrade clearance ratio was significantly lower (antegrade 0.59 ± 0.18; retrograde 0.69 ± 0.19; P = 0.007). The lower propranolol clearance found during antegrade perfusion occurred despite the somewhat higher mean rate of oxygen delivery in that direction of flow. To correct for this difference, the propranolol clearance ratio for each preparation was divided by the oxygen delivery during hypoxia in μmol/min/g liver (Fig. 2). Clearance during antegrade perfusion was again significantly lower than during retrograde perfusion.

DISCUSSION

Although it is in essence a non-physiological experimental model, retrograde liver perfusion has become an important tool for investigating acinar heterogeneity [28, 29]. During normal oxygenation, this study found that physiological parameters, such as perfusion pressure, bile flow and oxygen consumption and extraction were not altered by changing the direction of flow. The values obtained were in good agreement with those obtained by others with antegrade and retrograde perfusions [28, 30, 31]. Comparisons of these physiological parameters during antegrade and retrograde perfusion under conditions of hypoxia, which has not been performed previously, again revealed no significant differences. With both directions of perfusion, oxygen consumption was directly related to oxygen delivery when oxygen delivery was less than approx. 5 μmol/min/g liver (Fig. 1). At higher rates of oxygen delivery this relationship was not apparent (Fig. 1), in keeping with previous studies [32, 33].

Under conditions of normal oxygenation, propranolol clearance was high and comparable in both directions of perfusion (Table 1). This is in contrast to findings with several other substrates. The hepatic extraction ratio of lidocaine was found to decrease with retrograde perfusion [34] while harmol had an increased extraction ratio with retrograde perfusion [11, 12]. This difference can be explained by the acinar location of the major competing enzyme systems for each drug, because although most cytochrome P450 isoenzymes are indeed perivenous, a few are more evenly distributed within the acinus [35]. In the case of lidocaine, the low affinity, high capacity N-deethylation is proximal on antegrade flow to the competing process of high affinity, low capacity hydroxylation suggesting that the deethylation isoenzymes (CYP2C11, CYP2B1) are proximal to the hydroxylation isoenzymes (CYP1A2, CYP2B2 [36]). In contrast, for harmol the high affinity, low capacity sulphation is located in the acinus before the low affinity, high capacity glucuronidation system. Therefore, the clearance of the drug is higher in the direction of flow in which the high capacity metabolizing system (i.e. N-deethylation for lidocaine and glucuronidation for harmol) is exposed to the greater concentration of drug, i.e. the direction of flow in which it is "upstream". In the case of propranolol, which is over 90% cleared by ring and side-chain oxidation by CYP2D1 and CYP2C11, respectively [23, 24, 37] the lack of difference between antegrade and retrograde clearance during normal oxygenation suggests that either (i) the substrate concentration is below the K_m of each of the major enzymic systems so that the capacity of none is exceeded, or (ii) that the K_m of each enzymic system is exceeded but competition between the different metabolizing processes, in different locations, does not occur, or (iii) that the K_m of each enzymic system is exceeded but the enzymic systems are in the same location.

During hypoxia, the propranolol clearance ratio was significantly less with antegrade than with retrograde perfusion (Table 1, Fig. 2). The oxidation and elimination of a drug by the intact liver may depend on several factors, including uptake of the substrate by the hepatocyte, enzyme activity, competition with other metabolic processes, transport of the metabolite across the canalicular or sinusoidal membrane and the availability of essential co-factors [38]. Since the elimination of propranolol was unaltered by the change in direction of perfusion under conditions of normal oxygenation, but was reduced in the antegrade direction during hypoxia, it would appear most likely that in the antegrade direction there was a greater deprivation of a necessary co-factor, oxygen.

There is much evidence that drug oxidation is predominantly centrilobular. The essential components of the system, NADPH cytochrome P450 reductase [39–41] and the cytochrome P450 isoenzymes [22, 35, 49], as well as the NADPH generating enzymes, glucose-6-phosphate, malic acid and isocitrate dehydrogenase [6] are concentrated in the pericentral zone. This concentration of cytochrome P450 isoenzymes may be further accentuated by enzyme induction [42–44]. In addition, kinetic studies in the IPRL have strongly suggested that oxidation is a pericentral process

[10–13] during both antegrade and retrograde perfusion [14]. Using miniature oxygen electrodes on the surfaces of the perfused rat liver, Matsumura and Thurman [32] have shown that the oxygen gradient within the liver promptly reverses on reversal of the direction of perfusion. In livers perfused with a haemoglobin-free medium the gradient of 478 ± 37 μM in the periportal region to 263 ± 21 μM in the pericentral region during antegrade perfusion reversed to 565 ± 20 μM in the pericentral zone to 232 ± 18 μM in the periportal zone on retrograde perfusion [32]. Thus, with both antegrade and retrograde perfusion, the perfusate oxygen concentration in the "downstream" zone is considerably less than that entering the liver and in contact with the "upstream" zone.

If drug oxidation is located predominantly in the centrilobular zone, it will be more deprived of oxygen, for a given oxygen delivery to the liver, during antegrade perfusion than during retrograde perfusion. This acinar localization of oxidation and the reversal of the lobular oxygen gradient is the most plausible mechanism for the greater sensitivity of propranolol clearance to hypoxia when perfusion was in the antegrade direction (Table 1, Fig. 2). Retrograde liver perfusion is known to cause a marked increase in sinusoidal volume and surface area [31, 45, 46]. An increase in sinusoidal surface area might well increase hepatic clearance of a compound whose clearance is rate-limited by passive uptake into the hepatocyte [31], but this does not apply to propranolol whose clearance is flow-limited. Moreover, with normal oxygenation, propranolol clearance was the same with both directions of perfusion (Table 1). It is therefore unlikely that increased sinusoidal volume could account for the lesser effect of hypoxia on propranolol clearance during retrograde perfusion.

In conclusion, this study has shown that propranolol elimination is unaltered by change in direction of perfusion during conditions of normal oxygenation. However, clearance is more sensitive to hypoxia during antegrade perfusion than during retrograde perfusion. This finding is consistent with the centrilobular location of oxidative metabolic processes and suggest that zonal location of a metabolic process will influence its sensitivity to hypoxia. The relatively small difference in effect of hypoxia between antegrade and retrograde perfusion indicates, however, that the centrilobular location of oxidative metabolism is not a major contributor to the marked sensitivity of propranolol to hypoxia during antegrade perfusion.

Acknowledgement—This work was supported by the National Health and Medical Research Council of Australia.

REFERENCES

1. Aw TY and Jones DP, Secondary bioenergetic hypoxia. Inhibition of sulphation and glucuronidation reactions in isolated hepatocytes at low O_2 concentrations. *J Biol Chem* **257**: 8997–9004, 1982.
2. Jones DP and Mason HS, Gradients of O_2 concentration in hepatocytes. *J Biol Chem* **253**: 4874–4880, 1978.
3. Jones DP and Mason HS, Metabolic hypoxia: accumulation of tyrosine metabolites in hepatocytes at low pO_2. *Biochem Biophys Res Commun* **96**: 1128–1134, 1978.
4. Jones DP, Aw TY and Shan X, Drug metabolism and toxicity during hypoxia. *Drug Metab Rev* **20**: 247–260, 1989.
5. Kessler M, Hoper J, Lubbers DW and Ji S, Local factors affecting regulation of microflow, O_2 uptake and energy metabolism. *Adv Physiol Sci* **25**: 155–162, 1981.
6. Jungermann K and Katz N, Functional hepatocellular heterogeneity. *Hepatology* **2**: 385–395, 1982.
7. Ji S, Lemasters JJ, Christenson V and Thurman RG, Periportal and pericentral pyridine nucleotide fluorescence from the surface of the perfused liver: evaluation of the hypothesis that chronic treatment with ethanol produces pericentral hypoxia. *Proc Natl Acad Sci USA* **79**: 5415–5419, 1982.
8. Yamamoto K, Sherman I, Phillips MJ and Fisher MM, Three-dimensional observations of the hepatic arterial terminations in rat, hamster and livers by scanning electron microscopy of microvascular casts. *Hepatology* **5**: 452–456, 1985.
9. Gumucio JJ, Hepatocyte heterogeneity: the coming of age from the description of a biological curiosity to a partial understanding of its physiological meaning and regulation. *Hepatology* **9**: 154–160, 1989.
10. Pang KS, Koster H, Halsema ICM, Scholtens E and Mulder GJ, Aberrant pharmacokinetics of harmol in the perfused rat liver preparation: sulfate and glucuronide conjugations. *J Pharmacol Exp Ther* **219**: 134–140, 1981.
11. Pang KS, Koster H, Halsema ICM, Scholtens E, Mulder GJ and Stillwell RN, Normal and retrograde perfusion to probe the zonal distribution of sulphation and glucuronidation activities of harmol in the perfused rat liver preparation. *J Pharmacol Exp Ther* **224**: 647–653, 1983.
12. Dawson JR, Weitering JG, Mulder GJ, Stillwell RN and Pang KS, Alteration of transit time and direction of flow to probe the heterogeneous distribution of conjugating activities for harmol in the perfused rat liver preparation. *J Pharmacol Exp Ther* **234**: 691–697, 1985.
13. Pang KS and Terrell JA, Retrograde perfusion to probe the heterogeneous distribution of hepatic drug metabolizing enzymes in rats. *J Pharmacol Exp Ther* **216**: 339–346, 1981.
14. Thurman RG and Kauffman FC, Sublobular compartmentation of pharmacologic events (SCOPE): metabolic fluxes in periportal and pericentral regions of the liver lobule. *Hepatology* **5**: 144–151, 1985.
15. Rappaport AM and Hiraki GY, The anatomical pattern of lesions in the liver. *Acta Anat* **32**: 126, 1958.
16. Lemasters JL, Ji S and Thurman RG, Centrilobular injury following hypoxia in the isolated perfused rat liver. *Science* **213**: 661–663, 1981.
17. Roth RA and Rubin RJ, Role of blood flow in carbon monoxide- and hypoxic and hypoxia-induced alterations in hexobarbital metabolism in rats. *Drug Metab Dispos* **4**: 460–467, 1976.
18. Jones DB, Mihaly GW, Smallwood RA, Webster LK, Morgan DJ and Madsen NP, Differential effects of hypoxia on the disposition of propranolol and sodium taurocholate by the isolated perfused rat liver. *Hepatology* **4**: 461–466, 1984.
19. Webster LK, Jones DB, Mihaly GW, Morgan DJ and Smallwood RA, Effect of hypoxia on oxidative and reductive pathways of omeprazole metabolism by the isolated perfused rat liver. *Biochem Pharmacol* **34**: 1239–1245, 1985.
20. Angus PW, Mihaly GW, Morgan DJ and Smallwood RA, Oxygen dependence of omeprazole clearance and the production of its sulphone and sulphide metabolites

in the isolated perfused rat liver. *J Pharmacol Exp Ther* **250**: 1043–1047, 1989.
21. Gumucio JJ, De Mason JJ, Miller DL, Krazoski SO and Keender M, Induction of cytochrome P450 in a selective subpopulation of hepatocytes. *Am J Physiol* **234**: 102–109, 1978.
22. Tsutsumi M, Lasker JM, Shimizu M, Rosman AS and Lieber CS, The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. *Hepatology* **10**: 437–446, 1989.
23. Bargar EM, Walle UK, Bai SA and Walle T, Quantitative metabolic fate of propranolol in the dog, rat and hamster using radiotracer, high performance liquid chromatography, and gas chromatography-mass spectrometry techniques. *Drug Metab Dispos* **11**: 266–272, 1983.
24. Nelson WL and Shetty HU, Stereoselective oxidative metabolism of propranolol in the microsomal fraction from rat and human liver. Use of deuterium labelling and pseudoracemic mixtures. *Drug Metab Dispos* **14**: 506–508, 1986.
25. Gollan J, Hammaker LK, Licko V and Schmid R, Bilirubin kinetics in intact rats and isolated perfused liver. *J Clin Invest* **67**: 1003–1015, 1981.
26. Angus PW, Mihaly GW, Morgan DJ and Smallwood RA, Oxygen dependence of salbutamol elimination in the isolated perfused rat liver. *Biochem Pharmacol* **38**: 1443–1449, 1989.
27. Mihaly GW, Morgan DJ, Smallwood RA and Hardy KJ, The developing liver: the steady state disposition of propranolol in pregnant sheep. *Hepatology* **2**: 344–349, 1982.
28. Gores GJ, Kost LJ and La Russo NF, The isolated perfused rat liver: conceptual and practical considerations. *Hepatology* **6**: 511–517, 1986.
29. Javitt NB, Hepatic heterogeneity: backward perfusion—a forward step. *Lab Invest* **56**: 565–567, 1987.
30. Baumgartner U, Miyai K and Hardison WGM, Greater taurodeoxycholate biotransformation during backward perfusion of rat liver. *Am J Physiol* **251**: G431–G435, 1986.
31. Bass NM, Manning JA and Weisiger RA, Increased sinusoidal volume and solute extraction during retrograde liver perfusion. *Am J Physiol* **256**: G1041–1048, 1989.
32. Matsumura T and Thurman RG, Measuring rates of oxygen uptake in periportal and pericentral regions of the liver lobule: stop flow experiments with perfused liver. *Am J Physiol* **244**: G656–G659, 1983.
33. Keller BJ, Yamanaka H, Liang D, Kauffman FC and Thurman RG, O_2-dependent hepatotoxicity due to ethylhexanol in the perfused rat liver: mitochondria as a site of action. *J Pharmacol Exp Ther* **252**: 1355–1360, 1980.
34. Pang KS, Terrell JA, Nelson SD, Feuer KF, Clements M-J and Endrenyi L, An enzyme-distributed system for lidocaine metabolism in the perfused rat liver preparation. *J Pharmacokin Biopharm* **14**: 107—130, 1986.
35. Buhler R, Lindros KO, Nordling A, Johansson I and Ingleman-Sundberg M, Zonation of cytochrome P450 isozyme expression and induction in rat liver. *Eur J Biochem* **204**: 407–412, 1992.
36. Oda Y, Imaoka S, Nakahira Y, Asada A, Fujimori M, Fujita S and Funae Y, Metabolism of lidocaine by purified rat liver microsomal cytochrome P450 isozymes. *Biochem Pharmacol* **38**: 4439–4444, 1989.
37. Masubuchi Y, Fujita S, Chiba M, Kagimoto N, Umeda S and Suzuki T, Impairment of debrisoquine-4-hydroxylase and related monooxygenase activities in the rat following treatment with propranolol. *Biochem Pharmacol* **41**: 861–865, 1991.
38. Mitchell MC, Hamilton R, Wacker L and Branch RA, Zonal distribution of paracetamol glucuronidation in the isolated perfused rat liver. *Xenobiotica* **19**: 389–400, 1989.
39. Taira Y, Redick RA and Baron J, An immunohistochemical study of the localization and distribution of NADPH-cytochrome C (P450) reductase in rat liver. *Mol Pharmacol* **17**: 374–381, 1979.
40. Smith MT, Redick JA and Baron J, A comparison of microdensitometric analysis of unlabelled antibody peoxidase-antiperoxidase staining and of microfluorometric analysis of indirect fluorescent antibody staining for nicotanamide adenosine dinucleotide phosphate (NADPH)-cytochrome c (P450) reductase in rat liver. *J Histochem Cytochrome* **31**: 1183–1189, 1983.
41. Baron J, Kawabata TT, Knapp SA, Voigt JM, Redick JA, Jakoby WB and Guengerich FP, Intrahepatic distribution of xenobiotic-metabolising enzymes. In: *Foreign Compound Metabolism* (Eds. Caldwell J and Paulson GD), pp. 17–36. Taylor and Francis, London, 1984.
42. Baron J, Redick JA and Guengerich FP, An immunohistochemical study of the localization and distributions of phenhobarbital- and 3-methylcholanthrene-inducible cytochrome P450 within the livers of untreated rats. *J Biol Chem* **256**: 5931–5937, 1981.
43. Chianale J, Dvorak C, May M and Gumucio JJ, Heterogenous expression of phenobarbital-inducible cytochrome P450 genes within the hepatic acinus in the rat. *Hepatology* **6**: 945–951, 1986.
44. Van Sliedregt A and Van Bezooijen CFA, Effect of different doses of 3-methylcholanthrene on the localization of the 3-methylcholanthrene-inducible isoenzymes of cytochrome P450 within the centrilobular and periportal zones of the rat liver. *Biochem Pharmacol* **39**: 1703–1708, 1990.
45. St-Pierre MV, Schwab AJ, Goresky CA, Lee W-F and Pang KS, The multiple-indicator technique for characterization of normal and retrograde flow in once-through rat liver perfusions. *Hepatology* **9**: 285–296, 1989.
46. Roberts MS, Fraser S, Wagner A and McLeod L, Residence time distributions of solutes in the perfused rat liver using a dispersion model of hepatic elimination: 2. Effect of pharmacological agents, retrograde perfusions, and enzyme inhibition on Evans blue, sucrose, water, and taurocholate. *J Pharmacokin Biopharm* **18**: 235–258, 1990.