Biochemical Pharmacology, Vol. 45, No. 3, pp. 763–765, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

SHORT COMMUNICATIONS

The effect of hypoxia and acidosis on propranolol clearance in the isolated perfused rat liver preparation

(*Received* 15 *October* 1992; *accepted* 24 *November* 1992)

Abstract—The effect of hypoxia and acidosis on the elimination of an oxidatively metabolized drug, *S*-propranolol, was examined in the single-pass isolated perfused rat liver (IPRL). The experiments (N = 6) consisted of four consecutive 30 min phases: normal pH (pH 7.4)/normal oxygen delivery, normal pH/hypoxia, hypercapnic acidosis (pH 7.1)/normal oxygenation and hypercapnic acidosis/hypoxia. Hypoxia and acidosis were produced by equilibrating the perfusate with appropriate mixtures of O_2, N_2 and CO_2. With normal oxygen delivery there was no difference in hepatic clearance of propranolol between normal pH and acidosis (9.65 ± 0.34 and 9.78 ± 0.11 mL/min, respectively. $P < 0.05$). During hypoxia, propranolol clearance was impaired to a similar extent under both pH conditions (7.41 ± 0.97 and 8.06 ± 0.81 mL/min, respectively, $P > 0.05$). Therefore, respiratory acidosis does not affect the clearance of propranolol by the IPRL, nor does it influence the sensitivity of propranolol clearance to hypoxia. Neither acidosis nor hypoxia resulted in a significant reduction in bile flow compared with the normal pH/normal oxygen phase and there was no correlation between bile flow and perfusate bicarbonate concentration ($P > 0.05$).

Acute hypoxia has been shown to impair the elimination of drugs metabolized by hepatic oxidative metabolism [1–5]. In clinical practice, acidosis and hypercapnia ("respiratory acidosis") frequently accompany hypoxia and the increase in pCO_2 associated with respiratory acidosis is known to induce acidification of the hepatocyte cytosol [6]. Relatively little is known about the effect of acidosis on hepatic drug metabolism and few studies have examined whether the presence of acidosis modifies the effect of hypoxia on drug elimination by the liver.

We have investigated the effect on hepatic oxidative drug metabolism of respiratory acidosis and hypoxia, separately and together, in the isolated perfused rat liver (IPRL). This preparation enables the effect of pH and pCO_2 to be examined directly, without the possibility of respiratory and metabolic compensation. *S*-Propranolol was used as the test substrate as it is predominantly (greater than 90%) oxidized by the rat liver [7, 8] and is efficiently cleared by the isolated liver, re-equilibrating rapidly under different experimental conditions [9]. Moreover the marked sensitivity of propranolol clearance to hypoxia in this model has already been demonstrated [9].

Materials and Methods

Chemicals and enzymes. *S*-Propranolol was obtained from Imperial Chemical Industries (Melbourne, Australia) and labetolol hydrochloride was donated by Glaxo (Boronia, Victoria, Australia). Bovine serum albumin was obtained from the Commonwealth Serum Laboratories (Melbourne, Australia) and sodium taurocholate was purchased from Calbiochem (San Diego, CA, U.S.A.). Other chemicals were of analytical reagent grade quality.

Experimental preparation. Livers of male Sprague–Dawley rats (weight 140–170 g) were perfused via the portal vein in a constant flow (10 mL/min i.e. approximately 2 mL/g liver/min), single-pass design at 37° [4]. The perfusate consisted of 20% (v/v) washed human red blood cells, 1% bovine serum albumin, 0.1% *d*-glucose, sodium taurocholate (30 μmol/L) and propranolol (2 μg/mL) in a Krebs–Henseleit electrolyte solution. Bile was collected throughout the experiment into preweighed vials in 30 min aliquots. Viability of the liver preparation was determined by macroscopic appearance, oxygen consumption of greater than 3 μmol/min/g liver during normal oxygenation, stable perfusion pressure of 4–8 cm of H_2O and initial bile flow of greater than 0.3 mL/hr [4]. Perfusate pO_2, pCO_2, pH, HCO_3^- and per cent saturation were monitored throughout.

Experimental design. The experiments consisted of four consecutive 30 min phases: normal pH (pH 7.4)/normal oxygen delivery, normal pH/hypoxia, hypercapnic acidosis (pH 7.1)/normal oxygenation and hypercapnic acidosis/hypoxia. The sequence of oxygenation conditions was the same in all experiments: normal–hypoxia–normal–hypoxia. In two of the experiments normal pH was used in phases 1 and 2 and acidosis in phases 3 and 4, and in the other four experiments acidosis was used in phases 1 and 2 and normal pH in phases 3 and 4. A 10 min restabilization phase was allowed between phases 2 and 3. For normal oxygenation/normal pH (pH 7.35–7.45), the perfusate was equilibrated with 100% O_2 in a Silastic membrane oxygenator and for hypoxia/normal pH the perfusate was equilibrated with a mixture of O_2 and N_2. For respiratory acidosis/normal oxygen delivery, the perfusate was equilibrated with 80% O_2:20% CO_2 and for hypoxia/acidosis, the gas mixture was changed to a variable mixture of O_2 and 80% N_2:20% CO_2. Inflow and outflow perfusate samples were collected at 0, 15, 20, 25 and 30 min in each of the four 30 min phases for measurement of steady-state propranolol concentration and oxygen content.

Assays. *S*-Propranolol concentrations in perfusate were determined by a specific and sensitive HPLC method [10].

Calculations and statistics. The results are expressed as the mean and standard deviation (mean ± SD). Statistical comparisons of data were made using the Student's *t*-test for paired observations, accepting $P < 0.05$ as significant. 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.). The difference between the two slopes of the regression lines was examined using a *t*-test [11].

Results and Discussion

The physiological parameters and propranolol clearance data for the liver preparations are shown in Table 1. During normal oxygenation, there was no significant difference in oxygen consumption between the normal pH and acidosis

Table 1. Physiological parameters and propranolol clearance under conditions of normal and acid pH

	Normal pH	Acidosis	P value*
pH			
Normal O_2	7.38 ± 0.06	7.10 ± 0.02	<0.0001
Hypoxia	7.38 ± 0.04	7.09 ± 0.03	<0.0001
pCO_2 (mmHg)			
Normal O_2	21.4 ± 3.91	47.5 ± 2.37	<0.0001
Hypoxia	21.7 ± 2.60	48.7 ± 3.17	<0.0001
$pHCO_3$ (mmHg)			
Normal O_2	12.7 ± 0.93	14.7 ± 0.70	<0.0001
Hypoxia	13.1 ± 0.79	15.1 ± 0.49	<0.0001
Oxygen delivery (μmol/min/g liver)			
Normal O_2	6.76 ± 0.89	6.59 ± 0.99	0.15
Hypoxia	2.46 ± 0.90	2.80 ± 1.39	0.49
Bile flow (mL/hr)			
Normal O_2	0.61 ± 0.10	0.55 ± 0.12	0.46
Hypoxia	0.45 ± 0.12	0.62 ± 0.05	0.052
Propranolol clearance (mL/min)			
Normal O_2	9.65 ± 0.34	9.78 ± 0.11	0.43
Hypoxia	7.41 ± 2.39	8.06 ± 1.99	0.65
Clearance ratio	0.77 ± 0.25	0.83 ± 0.20	0.67

* Paired Student's *t*-test, N = 6.

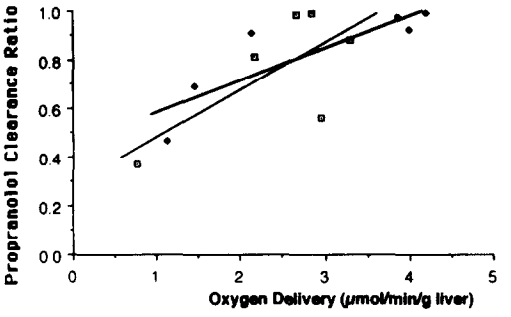

Fig. 1. Relationship between propranolol clearance ratio and oxygen delivery for normal pH (□) and acidosis (◆) phases.

phases (3.69 ± 0.07 and 3.49 ± 0.88 μmol/min/g liver, respectively, P = 0.68). Similarly, there was no significant difference in oxygen extraction between these two phases (66.5 ± 17.3% and 65.9 ± 22.8%, respectively, P = 0.76).

During hypoxia, mean oxygen delivery was similar for both the normal pH and acidosis phases (Table 1). Oxygen consumption was directly related to oxygen delivery below an oxygen delivery of approximately 5 μmol/min/g liver in both the normal pH and acidosis phases ($r = 0.97$, $P < 0.0001$). Oxygen extraction was high and similar under both normal pH and acidosis conditions (71.9 ± 20.1% and 81.5 ± 11.8%, respectively, P = 0.64).

Neither acidosis during normal oxygenation nor hypoxia during normal pH resulted in a significant reduction in bile flow compared with that during the normal oxygenation/normal pH phase (Table 1). There was no correlation between bile flow and perfusate bicarbonate concentration in any of the experimental phases ($P > 0.05$ for each phase). This is in contrast to two previous studies. Bile flow was increased under conditions simulating respiratory acidosis in the isolated perfused guinea pig liver [12] and in both that study and another in the IPRL [13], bile flow was correlated with perfusate bicarbonate concentration. This may be due to the stimulation of bile flow by taurocholate used in the perfusate of our experiments, but not used by the other authors.

The presence of respiratory acidosis had no effect on the clearance of propranolol during normal oxygenation (Table 1). During hypoxia, propranolol clearance was impaired to a similar extent under both pH conditions (normal pH: 7.41 ± 0.97 mL/min; acidosis: 8.06 ± 0.81 mL/min; P = 0.65). A *propranolol clearance ratio* was calculated for each preparation as the ratio of clearance during hypoxia to clearance during normal oxygenation. Thus each liver preparation acted as its own control. As it was not possible to produce the same oxygen delivery during hypoxia in all the experiments, propranolol clearance ratio has been plotted against oxygen delivery in Fig. 1. For both the normal pH phase and the acidosis phase there was a linear relationship between propranolol clearance ratio and oxygen delivery, with correlation coefficients of 0.70 and 0.86, respectively (Fig. 1). The slopes of the regression lines were 0.193 and 0.128 for normal pH and acidosis, respectively, and were not significantly different ($P > 0.10$). Thus the presence of acidosis did not influence the relationship between propranolol clearance and hypoxia.

A potential concern in this study is the effect that changes in pH may have on protein binding of propranolol, as the degree of protein binding of some compounds can be an important determinant of hepatic clearance [14]. Propranolol is a lipophilic base with a pK_a of 9.45 [15]. However, although the unbound fraction of propranolol has been shown to increase by approximately 30% with a pH change from 7.4 to 7.1 [16], in the single-pass IPRL the hepatic extraction and clearance of propranolol are known to be independent of the unbound fraction over an unbound fraction range of 0.1–0.8 [16]. Therefore, any effect of pH on propranolol protein binding would not have influenced the results in this study.

The few previous studies which have examined the effects of hypoxia and acidosis on drug metabolism have produced conflicting results. In rabbits theophylline non-renal clearance was decreased by hypercapnia and by hypoxia [17]. However, the combination of the two did not show an additive effect. In contrast to these findings in rabbits, respiratory acidosis has been shown to increase theophylline clearance in dogs [18, 19]. While these conflicting results reflect in part species differences in the hepatic metabolism of theophylline [20], they probably also reflect the difficulties of standardizing experimental conditions *in vivo*. The IPRL model provides an experimental system which allows one or other function of the whole liver to be studied, in this case propranolol metabolism, under controlled and reproducible conditions. However, no previous studies have examined drug metabolism and acidosis in this model.

Clinically, the combination of hypercapnia and acidosis with hypoxia is commonly seen in patients with respiratory insufficiency from a variety of causes. The results of this study would suggest that respiratory acidosis *per se* would be unlikely to magnify the already profound effects of hypoxia on oxidative drug metabolism by the liver.

In conclusion, respiratory acidosis does not affect the clearance of propranolol in the IPRL, nor does it modify the sensitivity of propranolol clearance to hypoxia.

Acknowledgements—This study was supported by the National Health and Medical Research Council of Australia.

Department of Medicine
University of Melbourne
Repatriation Hospital
Victoria, 3081, and
**Victorian College of Pharmacy*
Melbourne
Victoria 3052
Australia

SUSAN L. ELLIOTT
DENIS J. MORGAN*
PETER W. ANGUS
HANY GHABRIAL
RICHARD A. SMALLWOOD†

REFERENCES

1. Jones DP, Hypoxia and drug metabolism. *Biochem Pharmacol* **30**: 1019–1023, 1981.
2. Roth RA and Rubin RJ, Role of blood flow in carbon monoxide- and hypoxia-induced alterations in hexobarbital metabolism in rats. *Drug Metab Dispos* **4**: 460–467, 1976.
3. 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.
4. 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.
5. 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.
6. Cohen RD, Henderson RM, Iles RA and Smith JA, Metabolic interrelationships of intracellular pH measured by double-barrelled microelectrodes in perfused rat liver. *J Physiol Lond* **330**: 69–80, 1982.
7. 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.
8. Nelson WL and Shetty HU, Stereoselective oxidative metabolism of propranolol in the microsomal fraction from rat and human liver. Use of deuteriun labeling and pseudoracemic mixtures. *Drug Metab Dispos* **14**: 506–508, 1986.
9. Elliott SL, Morgan DJ, Angus PW, Ghabrial H and Smallwood RA, Sensitivity of propranolol elimination to hypoxia in the isolated perfused rat liver preparation. *Clin Exp Pharmacol Physiol*, in press.
10. 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.
11. Moore PG and Edwards DE, Correlation and regression. In: *Standard Statistical Calculations* (Eds. Moore PG and Shirley EAC), pp. 59–61. Pitman Publishing, London, 1965.
12. Blot-Chabaud M, Dumont M, Corbic M and Erlinger S, Effect of acid–base balance on biliary bicarbonate secretion in the isolated perfused guinea pig liver. *Am J Physiol* **258**: G863–G872, 1990.
13. Garcia-Marin JJ, Dumont M, Gorbic M, de Couet G and Erlinger S, Effect of acid–base balance and acetazolamide on ursodeoxycholate-induced biliary bicarbonate secretion. *Am J Physiol* **248**: G20–G27, 1985.
14. Wilkinson GR, Clearance approaches in pharmacology. *Pharmacol Rev* **39**: 1–47, 1987.
15. Sager G, Nilsen OG and Jacobsen S, Variable binding of propranolol in human serum. *Biochem Pharmacol* **28**: 905–911, 1978.
16. Jones DB, Ching MS, Smallwood RA and Morgan DJ, A carrier-protein receptor is not a prerequisite for avid hepatic elimination of highly bound compounds: a study of propranolol elimination by the isolated perfused rat liver. *Hepatology* **5**: 590–593, 1985.
17. Kolbeck RC, Speir WA, Harrison GN and Vallner JJ, Influence of respiratory acidosis and alkalosis on volume of distribution of theophylline in dogs. *Res Commun Chem Pathol Pharmacol* **23**: 643–646, 1978.
18. Clozel JP, Saunier C, Royer-Morot MJ, Royer RJ and Sadoul P, Respiratory acidemia and theophylline pharmacokinetics in the awake dog. *Chest* **80**: 631–633, 1981.
19. Letarte L and du Souich P, Influence of hypercapnia and/or hypoxemia and metabolic acidosis on theophylline kinetics in the conscious rabbit. *Am Rev Respir Dis* **129**: 762–766, 1984.
20. Saunier C, du Souich P, Hartemann D and Sautegeau A, Theophylline disposition during acute and chronic hypoxia in the conscious dog. *Res Commun Chem Pathol Pharmacol* **57**: 291–299, 1987.

† Corresponding author: Richard A. Smallwood, Department of Medicine, Heidelberg Repatriation Hospital, West Heidelberg, Victoria, Australia, 3081.