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EFFECT OF HYPOXIA ON OXIDATIVE AND REDUCTIVE PATHWAYS OF OMEPRAZOLE METABOLISM BY THE ISOLATED PERFUSED RAT LIVER

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Abstract—The effect of hypoxia on the elimination of omeprazole, a potent inhibitor of gastric acid secretion, was studied in the isolated perfused rat liver. During normal oxygenation, a 10 mg bolus dose was eliminated rapidly ($T_{\frac{1}{2}}\beta = 8.0 \pm 1.1$ min; mean ± S.E.M., N = 4), while under hypoxic conditions $T_{\frac{1}{2}}\beta$ was increased to 81.6 ± 5.4 min ($P < 0.01$). Upon reoxygenation, $T_{\frac{1}{2}}\beta$ returned to 9.6 ± 1.3 min. During hypoxia, perfusate concentrations of an oxidative metabolite (the sulphone) were reduced by 68%, while those of the reductively-generated sulphide increased 4-fold. With reoxygenation, both formation and elimination of the sulphone were increased, while the sulphide, which had accumulated during the hypoxic period, was eliminated rapidly. These findings were duplicated in steady-state experiments, in which omeprazole clearance during hypoxia fell by at least 70%, and sulphide concentrations in perfusate rose from undetectable levels to 200 ng/ml (at least a 10-fold increase). Sulphone concentrations did not change with hypoxia, consistent with a reduction in both its formation and elimination rates. We conclude that the hepatic elimination of omeprazole is severely retarded by hypoxia, but that this effect is promptly reversed by reoxygenation. The increased formation of reductive metabolite during hypoxia is not of sufficient magnitude to sustain the normal hepatic elimination of omeprazole.

Drug-metabolizing systems use oxygen both directly as a substrate for oxidases and oxygenases, and indirectly as a terminal electron acceptor for cellular redox reactions and high-energy bond formation [1]. The metabolism of a number of drugs has been shown to be very sensitive to oxygen deprivation in sub-cellular systems [2–4], but the effect of hypoxia on drug disposition by intact tissues has received little attention [1]. The impact of hypoxia on the hepatic elimination of a drug will be largely determined by the oxygen dependence of its principal metabolic pathways, although uptake and excretory mechanisms may theoretically also be important [1]. We have shown recently that, while the uptake remains normal, overall elimination of propranolol by the isolated perfused rat liver is inhibited profoundly by hypoxia [5]. In contrast, Smith *et al.* [6] demonstrated a striking *increase* in the elimination of misonidazole by the isolated perfused rat liver under hypoxic conditions, suggesting that with this drug the increase in reductively-generated metabolites outweighed any decrease in oxidative metabolism.

The substituted benzimidazole omeprazole (Fig. 1) is a potent inhibitor of gastric acid secretion which is currently undergoing clinical trials for the treatment of peptic ulceration [7]. It is metabolized extensively by the liver [8], and two metabolites which have been identified are omeprazole-sulphone, resulting from an S-oxidation, and omeprazole-sulphide, which is produced by a sulphoxide reduction. The oxygen dependence of these two types of biotransformation has not been studied previously in intact preparations. The aim of the present study, then, was to determine, in the isolated perfused rat liver, the effect of hypoxia on the disposition of omeprazole, with particular reference to an oxidative (sulphone) and a reductive (sulphide) pathway.

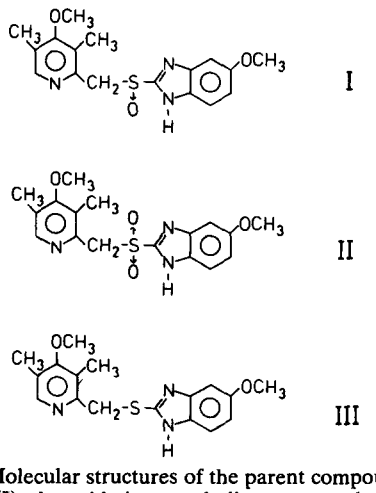

Fig. 1. Molecular structures of the parent compound omeprazole (I), the oxidative metabolite omeprazole-sulphone (II), and the reductive metabolite omeprazole-sulphide (III).

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MATERIALS AND METHODS

Chemicals

Omeprazole (H168/68), omeprazole-sulphone (H168/66), and omeprazole-sulphide (H168/22) were supplied by Astra Pharmaceuticals (Sydney, Australia). Omeprazole is poorly soluble in water, and all stock solutions were prepared using polyethylene glycol (PEG-400) as the solvent. The bovine serum albumin (BSA) was obtained from the Commonwealth Serum Laboratories (Melbourne, Australia), and sodium taurocholate was purchased from Calbiochem (San Diego, CA, U.S.A.). All other chemicals and solvents were of analytical reagent grade.

Experimental preparation

Livers of non-fasting male Sprague–Dawley rats (190–240 g) were surgically isolated [9] under ether anesthesia, and perfused via the portal vein in a constant flow (16 ml/min) recirculating system at 37° [10]. The perfusate (100 ml total volume) consisted of 10% (v/v) washed human red cells, 1% (w/v) BSA and 0.1% (w/v) glucose in a standard electrolyte solution at pH 7.4 [11]. The perfusate was oxygenated during its passage through 4 m of coiled Silastic tubing (1.47 mm i.d., 1.96 mm o.d., Dow-Corning Corp., Midland, MI, U.S.A.) in a jar into which 100% O_2 was delivered at 1.5 l/min. Hypoxia was achieved by replacing the O_2 with 100% N_2. A constant infusion of sodium taurocholate (30 μmoles/hr) into the perfusate reservoir maintained bile salt concentrations, and bile was collected in pre-weighed vials. All omeprazole doses were delivered into the reservoir in order to simulate systemic administration. The principal indices of liver viability were steady oxygen consumption (1.8 to 2.5 μmoles O_2/min/g liver), sustained bile production (0.6 to 1.2 ml/hr), constant perfusion pressure (6 to 8 cm water), and normal appearance on light microscopy.

Experimental design

Bolus dose studies. For the bolus dose hypoxia studies (N = 4 livers), perfusate concentrations of omeprazole and the sulphone and sulphide metabolites were measured in the same isolated liver preparation during three phases of oxygenation. At the start of the control phase, with the perfusate equilibrated with 100% O_2, 10 mg (29 μmoles) of omeprazole was delivered into the perfusate reservoir. After 90 min, the hypoxia phase was commenced by replacing the O_2 with 100% N_2. Following a 10-min equilibration period, the 10 mg dose was repeated. One hour later, the perfusate was reoxygenated (100% O_2), but no further omeprazole was given during this 90-min recovery phase. In two additional livers, the elimination profiles were examined after two consecutive 10 mg omeprazole doses given to the same liver at 0 and 90 min, with 100% O_2 used throughout the entire experiment. Perfusate (1 ml) was sampled from the reservoir for omeprazole and metabolite estimations pre-dose and at 5, 10, 20, 30, 45 and 60 min during each phase, with additional samples at 90 min during the control and recovery phases. An equal volume of fresh perfusate was added to replace that removed by sampling. The total amount of omeprazole lost through sampling was less than 3% of the dose. Bile was collected serially at 30-min intervals.

Steady-state studies. Using the pharmacokinetic data obtained from the bolus dose studies, a dosage regimen was calculated to achieve a steady-state perfusate omeprazole concentration of approximately 4 μg/ml. A bolus dose of 500 μg was immediately followed by a constant infusion of 2.5 mg/hr at 1 ml/hr. In three control experiments, the perfusate was equilibrated with 100% O_2 throughout a 4-hr perfusion. The steady-state hypoxia experiments (N = 3) were then designed, comprising an initial 1 hr control phase (100% O_2), followed by 1 hr of hypoxia (100% N_2) and 2 hr of recovery (100% O_2). Perfusate for drug estimation was collected pre-dose and then every 15 min. Bile was collected hourly.

Sample treatment and assays

Perfusate samples were collected on ice and centrifuged to separate the red cells. The supernatant fraction was stored at −20° until assayed. Omeprazole and its sulphone and sulphide metabolites were quantified in perfusate by a specific and sensitive high pressure liquid chromatographic plasma assay method [12]. The minimum detectable concentrations in perfusate of omeprazole, omeprazole-sulphone, and omeprazole-sulphide were 15, 15, and 20 ng/ml respectively. Partial pressures of oxygen (pO_2, mm Hg) in perfusate (portal vein inflow and hepatic vein effluent) were measured using a Corning 175 Automatic pH/blood gas system (Corning Medical, Medfield, MA, U.S.A.), utilizing a Clark electrode [13]. The coefficient of variation at both 28 and 378 mm Hg was 2.4% (N = 27 and 24 respectively). Oxygen utilization parameters were calculated with standard equations [14]. The ratios of lactate to pyruvate concentrations in hepatic venous effluent were estimated enzymatically [15] using a commercially available kit (Sigma, St. Louis, MO, U.S.A.) to assess the redox state of liver cells. The lactate:pyruvate ratio was assumed to reflect the relative amounts of lactate and pyruvate, and hence NADH and NAD^+, in the liver cell.

Calculations

Clearance equals the dose divided by the area under the concentration–time curve after a single systemic dose [16]. It was not possible to calculate this parameter for omeprazole in the reoxygenation phase, nor for any of the metabolite data, since these doses were unknown. Thus, elimination half-lives ($T_{\frac{1}{2}}\beta$) have been used for quantitative comparisons of effects resulting from hypoxia. The perfusate $T_{\frac{1}{2}}\beta$ values for omeprazole and the metabolites were calculated from the terminal elimination rate constants of the semi-logarithmic concentration–time profiles. In steady-state experiments, clearance can be calculated as infusion rate divided by steady-state concentration. However, since a new steady state was not reached during hypoxia, and since formation rates of metabolites could not be determined, average concentrations and changes in peak concentrations have been used to describe the results for the steady-state experiments. Statistical comparisons were made using two-tailed, paired Student's *t*-tests

Table 1. Effect of hypoxia on oxygen levels and utilization in the isolated perfused rat liver preparation*

Phase	Inflow pO_2 (mm Hg)	Outflow pO_2 (mm Hg)	O_2 delivery (μmoles/min/g liver)	O_2 consumption (μmoles/min/g liver)
Control	407 ± 19	39 ± 7	4.49 ± 0.51	2.19 ± 0.15
Hypoxia	17 ± 2	16 ± 1	0.57 ± 0.06	0.11 ± 0.04
Recovery	417 ± 18	43 ± 6	4.51 ± 0.51	2.51 ± 0.15

* Partial pressure of O_2 (pO_2) was measured in portal (inflow) and hepatic (outflow) vein perfusate during equilibration with either 100% O_2 (control and recovery phase) or 100% N_2 (hypoxia phase). Results are expressed as mean ± S.E.M. for five experiments.

or Bonferroni's *t*-test for multiple dependent samples. All results are expressed as mean ± S.E.M.

RESULTS

Extent of hypoxia

Table 1 shows the degree of hypoxia achieved in these experiments. The change from 100% O_2 in the control phase to 100% N_2 in the hypoxia phase caused a 96% drop in inflow pO_2, reducing O_2 delivery by 87% and O_2 consumption by 95%. Complete anoxia was not achieved due to exposure of the liver and perfusate reservoir to atmospheric oxygen. Following reoxygenation, hepatic O_2 consumption returned to control values, indicating that the hour of hypoxia had not led to irreversible loss of liver function. The lactate:pyruvate ratio in hepatic venous effluent was measured at various times during the first 3 hr of the steady-state hypoxia experiments (Fig. 2), and was within the control range (less than 20) [11] during the first hour (19 ± 2). However, during hypoxia, the lactate:pyruvate ratio increased almost 9-fold to 165 ± 19 ($P < 0.01$, $N = 5$). Following reoxygenation, the ratio quickly returned to near control values (34 ± 3), once again reflecting the ability of the liver to regain most of its normal function after a hypoxic episode.

Bolus dose studies

In the normoxic studies, the concentration–time profiles observed for omeprazole and its metabolites after two consecutive bolus doses were virtually superimposable. The changes seen during hypoxia can, therefore, be attributed to the hypoxic conditions rather than to an artifact produced by the double dosing.

The 10 mg bolus dose of omeprazole administered during normoxia underwent rapid monoexponential elimination, with a $T_{\frac{1}{2}}\beta$ of 8.0 ± 1.1 min (Fig. 3). However, when the dose was repeated during hypoxia, the $T_{\frac{1}{2}}\beta$ increased 10-fold to 81.6 ± 5.4 min ($P < 0.01$), reflecting a profound decrease in clearance of omeprazole. Upon reoxygenation, the remainder of the second dose was eliminated with similar efficiency to the control ($T_{\frac{1}{2}}\beta = 9.6 \pm 1.3$ min), indicating that the drug elimination processes had recovered from the hypoxic episode.

The sulphone and sulphide metabolites were also measured in these experiments, and the results are shown in Figs. 4 and 5. During normoxia, omeprazole-suphone, the oxidative metabolite, reached peak perfusate concentrations at 30 min, after which

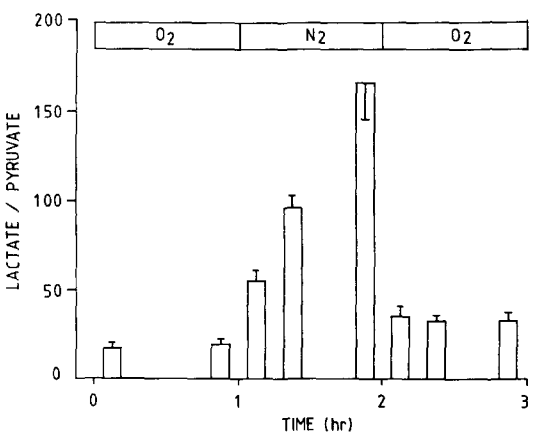

Fig. 2. Ratios of lactate to pyruvate concentrations in hepatic venous effluent during the control (100% O_2), hypoxia (100% N_2), and recovery (100% O_2) phases of steady-state omeprazole experiments. Values are expressed as mean ± S.E.M., N = 5.

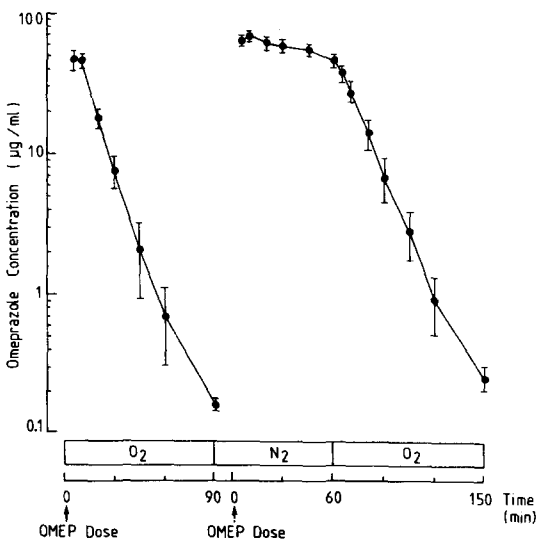

Fig. 3. Perfusate elimination of omeprazole following a 10 mg bolus omeprazole dose given during control oxygenation (100% O_2) and repeated during hypoxia (100% N_2). Values are expressed as mean ± S.E.M., N = 4.

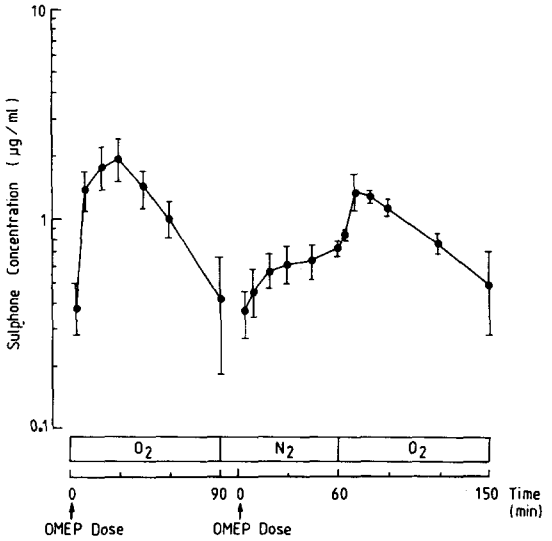

Fig. 4. Production and elimination of the oxidative metabolite omeprazole-sulphone, following a 10 mg omeprazole dose administered during control oxygenation (100% O_2), and repeated during hypoxia (100% N_2), measured in perfusate from the same experiments as in Fig. 3. Sulphone production and/or elimination appeared to decrease during hypoxia, but the recovery profile resembled that of the control phase. Values are expressed as mean ± S.E.M., N = 4.

an elimination phase with an apparent $T_{\frac{1}{2}}\beta$ of 29.5 ± 5.0 min was evident. During hypoxia, the sulphone concentrations reached a plateau which was less than one-third of the peak concentration achieved during normoxia. This finding is consistent with a decrease in both formation and elimination of the sulphone. After reoxygenation, sulphone concentrations initially increased, but then fell with an apparent $T_{\frac{1}{2}}\beta$ of 49.5 ± 20.6 min, reflecting recovery of both sulphone production and elimination processes. Omeprazole-sulphide was eliminated rapidly during normoxia ($T_{\frac{1}{2}}\beta$ = 16.0 ± 3.6 min), but in contrast to the sulphone, sulphide concentrations showed a 4-fold increase during hypoxia, rising to a peak of 2.7 μg/ml (0.7 μg/ml during normoxia). This is consistent with increased formation and decreased elimination of the sulphide metabolite. When normal oxygenation was restored, the sulphide was again eliminated rapidly, with a $T_{\frac{1}{2}}\beta$ of 13.1 ± 1.3 min.

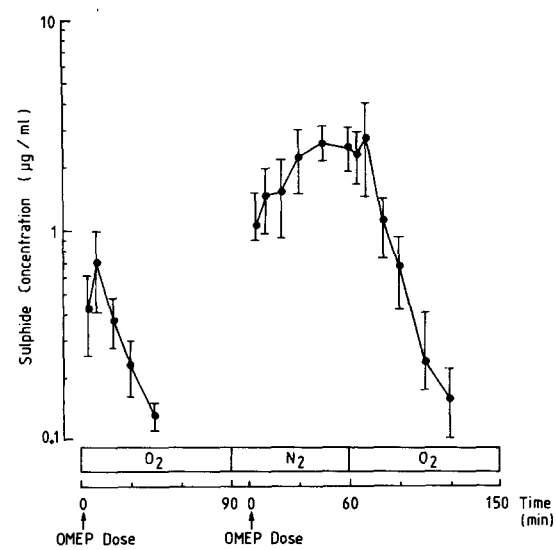

Fig. 5. Production and elimination of the reductive metabolite omeprazole-sulphide, following a 10 mg omeprazole dose given during control oxygenation (100% O_2), and repeated during hypoxia (100% N_2), measured in perfusate from the same experiments as in Figs. 3 and 4. Values are expressed as mean ± S.E.M., N = 4.

Steady-state studies

The perfusate concentrations of omeprazole and the two metabolites for the control and hypoxia steady-state experiments are illustrated in Fig. 6. In the pilot studies, omeprazole concentrations remained at an average of 3.7 ± 0.1 μg/ml from 15 to 180 min, but increased to 4.8 ± 0.6 μg/ml at 240 min. Omeprazole-sulphone concentrations tended to increase throughout the 4-hr omeprazole infusion. Omeprazole-sulphide was undetectable until the second hour, and thereafter maintained a concentration of 0.037 ± 0.004 μg/ml. In the hypoxia experiments, there were striking changes in the omeprazole and sulphide profiles. During the initial normoxic hour, perfusate concentrations of omeprazole averaged 3.3 ± 0.1 μg/ml. They began to increase at the start of hypoxia, and after 1 hr had risen almost 4-fold (14.6 ± 1.5, $P < 0.01$). This reflects a decrease in hepatic clearance of omeprazole of at least 70% during hypoxia. With the recovery of omeprazole elimination processes following reoxygenation, perfusate concentrations returned to original steady-state values within 30 min. The omeprazole-sulphide profile was similar to the parent compound. An increase in sulphide concentrations was noticeable within 15 min of hypoxia, peaking after 1 hr at 0.182 ± 0.022 μg/ml, which was markedly greater than the pre-hypoxia level of less than 0.020 μg/ml. This would result from an increase in sulphide production and/or a decrease in its elimination. As with omeprazole, the sulphide concentrations dramatically decreased following reoxygenation, reaching a sustained concentration of 0.05 μg/ml within 30 min. In contrast, the sulphone profile was unaltered by hypoxia; the concentrations continued to rise as they did in the first hour, and a comparison of the control versus hypoxia profiles over the 4 hr showed no statistically significant differences. A similar degree of inhibition of both production and elimination processes could explain this apparent contradiction of the bolus dose results.

DISCUSSION

The isolated perfused rat liver is likely to prove a most useful experimental model to assess the oxygen dependence of hepatic elimination processes. Oxygen utilization can be measured precisely, while oxygen delivery can be readily varied, and the lactate: pyruvate ratio in hepatic venous effluent provides an independent and sensitive index of the cellular redox state [1, 5, 11, 17]. This system is particularly useful

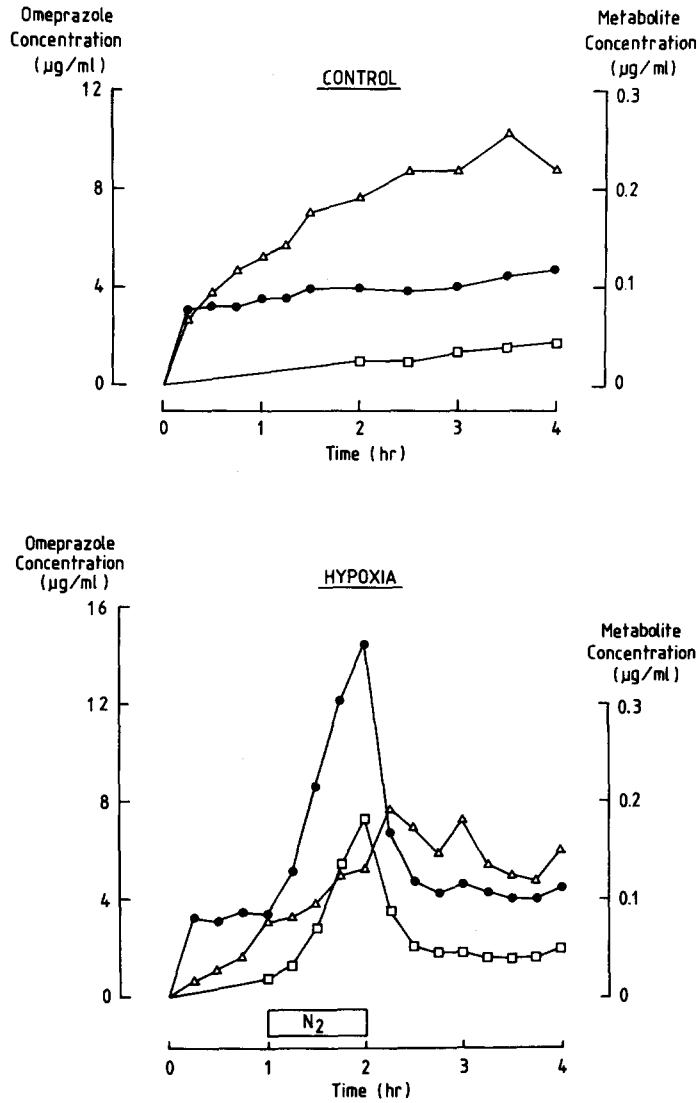

Fig. 6. Perfusate concentrations of omeprazole (●—●), the sulphone metabolite (△—△) and the sulphide metabolite (□—□) during the control (upper panel) and hypoxia (lower panel) steady-state omeprazole experiments (N = 3 livers). The perfusate was equilibrated with 100% O_2 except during the second hour of the hypoxia experiments, where 100% N_2 was used to cause hypoxia.

for drug disposition studies, since hepatic perfusion rates are controlled, the volume of the system is constant, and other potential routes of drug clearance seen in the intact animal are eliminated.

To date there has been little work done in the isolated rat liver on the effect of hypoxia on hepatic drug disposition. Studies from Anderson *et al.* [18] and our own laboratory [5] demonstrated profound inhibition of overall propranolol elimination during hypoxia, although hepatic uptake appeared to be unimpaired. This indicated that hepatic metabolism and not uptake of propranolol was the rate-limiting step in its elimination. The elimination of another highly cleared compound, sodium taurocholate, has been found to be unaffected by the same degree of hypoxia [5]. Taurocholate is not dependent on biotransformation for its hepatic clearance but rather on a secondary active transport mechanism involving $(Na^+ + K^+)$-ATPase [19]. It would therefore appear that this latter process, which has a low oxygen requirement [20], is relatively insensitive to oxygen deprivation.

In striking contrast to the studies with propranolol, Smith *et al.* [6] found that the clearance of the nitro-substituted imidazole misonidazole by the isolated rat liver is *enhanced* 3- to 4-fold by hypoxia. Misonidazole metabolism is changed qualitatively, with the appearance of three new major metabolites in bile under hypoxic conditions. In this case, hypoxia presumably potentiated reductive metabolism to a point where it greatly exceeded the capacity of the liver to metabolize misonidazole by oxidation.

The findings from the present study indicate that the hepatic elimination of omeprazole was pro-

foundly inhibited by hypoxia. This was reflected in the 8-fold increase in elimination half-life following bolus dosage (Fig. 3) and the 4-fold rise in perfusate concentration during constant infusion (Fig. 6). The effect of hypoxia was immediate in both studies, suggesting that oxygen is needed as a direct substrate for the major metabolic pathways of omeprazole. The prompt return to normal after reoxygenation of oxygen utilization, lactate:pyruvate ratio, and omeprazole metabolism indicates that the changes observed during hypoxia were not a non-specific effect of tissue damage.

The complete metabolic profile of omeprazole has not yet been established. Although the sulphone and sulphide do not appear to be major metabolites, their study provides valuable insight into an oxidative and a reductive pathway of metabolism. Preliminary experiments showed less than 0.05% accumulation of the drug in the liver, suggesting that formation of unknown metabolites accounts in part for the rapid disappearance of omeprazole during normoxia. S-oxidation reactions are known to be catalysed by cytochrome P-450 [21], and thus any effect of hypoxia on the omeprazole-sulphone metabolite concentrations may be related to an effect on the cytochrome P-450-dependent monooxygenases. The alterations in the omeprazole metabolite profiles during hypoxia are consistent with inhibition of oxidative metabolism and potentiation of reductive metabolism. In bolus dose studies (Figs. 4 and 5), the peak perfusate concentration of the oxidative metabolite (the sulphone) was reduced by two-thirds during hypoxia, while that of the reductive metabolite (the sulphide) was increased 3-fold. It is theoretically possible that such changes might be explained by enhancement of sulphone clearance together with inhibition of sulphide clearance during hypoxia, without any alteration to the rates of formation of these two metabolites. Although this latter explanation is inherently much less likely, without knowing the true formation or elimination rates of each metabolite, it is not possible to provide an unequivocal interpretation of their perfusate concentration–time curves. The apparent lack of effect of hypoxia on the sulphone profile during steady-state experiments (Fig. 6) can be explained by a similar degree of inhibition of both formation and elimination of this metabolite. This argues against enhanced sulphone clearance as the sole mechanism for the changes in sulphone concentration observed during hypoxia in bolus dose studies (Fig. 4).

It is perhaps to be expected that the hepatic elimination of different compounds will be inhibited to a different extent by any given degree of hypoxia. Oxygen tensions are not uniform throughout the liver, but follow a gradient with the highest oxygen concentrations in the periportal area (acinar zone 1) and the lowest in the pericentral area (acinar zone 3) [22]. Thus, elimination processes located primarily in zone 3, such as most oxidative drug biotransformations [23], may be affected more profoundly than zone 1 processes, such as bile salt transport. Even amongst compounds eliminated in the same zone, and which depend directly for their metabolism and elimination on the use of oxygen as a substrate, there will be a range of sensitivities to hypoxia, depending on the oxygen affinities of their respective enzymes. This has been well demonstrated with cytochrome P-450, where oxygen affinities may vary 300-fold with either the nature of the substrate or the species of P-450 [1].

The overall change in elimination rate will also reflect the capacity of an alternative route of elimination which might come into play during hypoxia. The present study suggests that hypoxia increases the production of reductive metabolites of omeprazole, but that this increase does not offset the decrease in oxidative metabolism, so that the hepatic clearance of omeprazole is reduced. With misonidazole, however, hypoxia leads to a 3- to 4-fold increase in hepatic elimination, indicating that reductive metabolism of this drug is more efficient [6]. These studies in the isolated perfused rat liver serve to emphasize that the impact of hypoxia on the hepatic disposition of different compounds is not readily predictable, even though their normal route of elimination has a direct requirement for oxygen as a substrate.

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