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OXYGEN DEPENDENCE OF SALBUTAMOL ELIMINATION BY THE ISOLATED PERFUSED RAT LIVER

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Abstract—Although impairment of drug metabolism by severe hypoxia is well documented in perfused liver preparations, the degree of hypoxia required to produce inhibition of drug elimination pathways in the intact liver has not been defined. In this study, in the isolated perfused rat liver, we examined the relationship between the rate of hepatic oxygen supply and the elimination rate of the drug salbutamol, which in the rat liver is eliminated largely by glucuronidation. Livers (N = 15) from male Sprague–Dawley rats were perfused in a non-recycling design with 10% human red cells in a Krebs–Henseleit electrolyte solution. Salbutamol elimination was examined during normal oxygenation (perfusate equilibrated with 100% O_2; mean O_2 delivery 3.21 μmol/min/g liver), at a given lower rate of oxygen delivery (achieved by producing different mixtures of N_2 with O_2 in the perfusate oxygenator) and after reoxygenation. In these experiments, hepatic clearance of salbutamol (perfusate concentration 50 ng/ml) was essentially independent of oxygen delivery above a rate of 2.0 μmol/min/g liver; below this level, clearance fell linearly as O_2 supply was reduced. In all livers, reoxygenation restored drug elimination to control levels. In further experiments using a recycling design (N = 22), the effect of hypoxia on salbutamol elimination was found to be very similar. In recycling normoxic experiments (N = 3), the glucuronide metabolite was detected in perfusate and bile, but no sulphate metabolite was detected. While previous studies indicate that elimination of some oxidatively metabolised substrates is very sensitive to reductions in hepatic oxygenation, the present study shows that, in the isolated liver, large reductions in hepatic oxygen supply were required to produce significant impairment of the glucuronidation-dependent elimination of salbutamol.

Studies in isolated hepatocytes and subcellular systems have shown that a number of major pathways of drug metabolism are sensitive to relatively mild reductions in oxygen availability [1]. In these studies the amount of oxygen available to different enzyme systems can be precisely controlled and measured. In the intact liver, however, there are substantial acinar oxygen gradients, and within the acini there is considerable heterogeneity in the distribution of enzyme systems and essential cofactors [2, 3]. Thus, it is not possible to predict directly the relative oxygen dependence of different drug-metabolising systems in the whole organ from work in isolated cells and cell fractions. A number of studies in the isolated perfused liver have confirmed that large reductions in hepatic oxygen supply may greatly affect the oxidative elimination of drugs by the liver [4–6]. However, these studies did not attempt to define the level of hypoxia at which these changes in drug elimination occur.

Using the substrate harmol, we recently demonstrated in the isolated perfused rat liver (IPRL) that glucuronidation-dependent drug elimination is also impaired significantly by acute, severe hypoxia [7]. However, this study did not examine the impact of lesser degrees of hypoxia on the rate of drug glucuronidation. The aim of the present study in the IPRL was to examine the relationship between the rate of hepatic oxygen supply and the rate of drug glucuronidation, and to determine the threshold level of hypoxia which is required to produce inhibition of glucuronidation.

(±)-Salbutamol, an important drug often used in states of clinical hypoxia, was selected as the substrate for this work because in the rat it is metabolised almost exclusively to the glucuronide. It does not undergo phase I metabolism and it is not conjugated with sulphate [8]. The oxygen dependence of salbutamol elimination by the IPRL was examined using a constant rate infusion of drug in a non-recycling, single-pass design and also using a bolus dose of drug in a recycling design.

METHODS

Materials

(±)-Salbutamol sulphate was supplied by Glaxo Australia (Melbourne, 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.).

Experimental preparation

Non-fasting male Sprague–Dawley rats (body weight 200–250 g) were anaesthetised with pentobarbitone. In *non-recycling experiments*, livers were surgically removed and initially perfused for 20 min via the portal vein in a standard constant flow (15 ml/min) recycling system at 37° [9]. The perfusate

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(100 ml) consisted of 10% (v/v) washed human red cells, 1% bovine serum albumin and 0.1% glucose in a Krebs–Henseleit buffer at pH 7.4 [9]. During this initial recycling period, perfusate was pumped from the perfusate reservoir, through a silastic membrane oxygenator (containing 100% oxygen), a bubble trap, and a pressure manometer into the portal vein. Perfusate drained via the inferior vena cava back into the perfusate reservoir. After this post-surgical equilibration period, perfusion was switched to a constant flow non-recycling (single-pass) mode at a flow rate of 15 ml/min. For this single-pass mode a much larger volume of perfusate containing salbutamol (50 ng/ml) was prepared (1000 ml) in a 2-litre conical flask, which was otherwise of identical composition to that used in the initial equilibration period. The perfusate was pumped from the flask through the same circuit as used initially; however, hepatic venous effluent was discarded [9].

To achieve substantial and uniform levels of perfusate deoxygenation and to normalise CO_2 content and pH (7.3–7.4), 95% N_2/5% CO_2 was bubbled into the flask for 1 hr prior to each experiment, and the perfusate in the flask was equilibrated with this gas mixture throughout the experiment. During normal liver oxygenation, the perfusate was then pumped from its container through the membrane oxygenator (*vide infra*), where it was reoxygenated by equilibration with 100% oxygen. Graded changes in oxygen delivery were achieved by mixing nitrogen with oxygen in the oxygenator. To maintain bile flow, sodium taurocholate (30 μmol) was added to perfusate (1000 ml) at the beginning of each experiment. Bile flow was monitored throughout the studies by collecting bile into pre-weighed vials at 30-min intervals.

At the start of the recycling phase, and at frequent intervals throughout the experiment, perfusate pO_2, oxygen content, pH and pCO_2 in hepatic inflow were measured. Equilibration of the perfusate in the flask with the 95% N_2/5% CO_2 mix, and in the membrane oxygenator with CO_2-free gas mixtures, enabled us to maintain pH in the range 7.3–7.4 throughout all experiments. Where necessary, minor adjustments could be made to perfusate pH and pCO_2 by altering the total gas flow in the membrane oxygenator.

In *recycling* experiments, livers were perfused for the entire duration of the experiment using the same recycling procedure as described above for the initial 20-min equilibration period in the non-recycling experiments. A constant infusion of sodium taurocholate (30 μmol/hr) was administered to maintain bile flow, and bile was collected as described above. As in the non-recycling experiments, graded changes in oxygen delivery were achieved by mixing nitrogen with oxygen in the membrane oxygenator.

Experimental design

In the *non-recycling experiments*, immediately after the initial equilibration period, a 50-μg bolus dose of salbutamol in 2 ml of perfusate was administered to the 1000 ml of non-recycling perfusate, producing a salbutamol concentration of 50 ng/ml. In preliminary experiments (N = 4), it was found that, with normal oxygenation (with 100% O_2), steady-state extraction of salbutamol, indicated by a constant hepatic venous perfusate concentration, was consistently achieved within 10 min of perfusion. Under hypoxic conditions (equilibration with 100% N_2), steady-state extraction was achieved within 15–20 min. Each of the non-recycling experiments (N = 15) was conducted over a 60-min period which was divided into three consecutive phases. The first (control) phase lasted for 10 min, and during this time perfusate was equilibrated with 100% oxygen. At 9 min and again at 10 min, inflow and outflow perfusate were sampled for measurement of salbutamol concentration, pH, pCO_2 and perfusate oxygen content. Immediately after these samples were taken (t = 10 min), the oxygen concentration in the oxygenator was reduced to a predetermined level by varying the flow rate of 100% nitrogen and 100% oxygen into the oxygenator in a reciprocal fashion, to produce a total gas flow of 4 l./min. Only one given level of hypoxia was produced in each experiment during this phase. The gas mixture ranged from 100% oxygen (producing oxygen delivery to the liver identical to the control phase) to 100% nitrogen in the "most hypoxic" experiment. The liver was perfused at this new rate of oxygen delivery for 30 min (graded hypoxia phase) to allow for equilibration of perfusate with the gas mixture in the oxygenator, and for the establishment of a new steady-state level of salbutamol extraction. Inflow and outflow perfusate were sampled for measurement of salbutamol concentration, pH and oxygen content at 35 and 40 min. Normal hepatic oxygenation was then restored (t = 40 min) by re-equilibrating perfusate with 100% oxygen (recovery phase). Portal perfusion pressure was monitored at the commencement of each experimental phase and was normally less than 7 cm of water at the flow rate used. Experiments in which normal perfusion pressures could not be established, or in which irreversible elevations of perfusion pressures developed, were abandoned. After a further 20 min of perfusion (i.e. at 60 min) two further samples of inflow and outflow perfusate were taken at 60 and 65 min for measurement of salbutamol concentration, pH and oxygen content, to determine if salbutamol extraction and oxygen consumption had recovered from the graded hypoxia phase.

In *recycling experiments*, preliminary studies (N = 3) were performed (with perfusate equilibrated with 100% oxygen) to determine the metabolic fate of salbutamol. In one of these experiments, a bolus dose of salbutamol (25 μg) was administered to the perfusate reservoir, and perfusate samples (1 ml) were collected at 5, 10, 15, 20, 25, 30, 40 and 50 min for measurement of salbutamol concentration. In the other two experiments, an initial bolus of 10 μg was administered followed by a constant infusion of 0.53 μg/min. Perfusate was sampled at 10-min intervals during the first hour and after the infusion at the same times as after the bolus doses (as described above). Bile was collected in half-hourly aliquots for 2 hr.

Further *recycling experiments* (N = 22) were carried out to determine the effect of hypoxia on salbutamol elimination. The study period was divided into three consecutive 1-hr phases. In the first hour

Table 1. Oxygen delivery, oxygen consumption and salbutamol clearance in the control and recovery phase of non-recycling experiments

Phase	Oxygen delivery (μmol/min/g liver)	Oxygen consumption (μmol/min/g liver)	Salbutamol clearance (ml/min)
Control (0–10 min)	3.17 ± 0.07	2.44 ± 0.08	9.5 ± 0.9
Recovery (40–60 min)	3.21 ± 0.09	2.47 ± 0.07	9.5 ± 0.7

Values are means ± SD, N = 15.

(control phase), the perfusate was equilibrated with 100% oxygen and a 20 μg bolus dose of salbutamol was administered to the reservoir. Perfusate was sampled as for the bolus dose experiment described above. During the second hour (graded hypoxia phase), fresh perfusate was used to ensure no carry-over of drug or metabolites from the initial phase. In this phase, the oxygen concentration in the oxygenator was reduced to a predetermined level (as in the non-recycling experiments). After the liver had been perfused for 30 min at this new level of oxygen supply, a second 20 μg bolus dose of salbutamol was administered. Perfusate samples were then taken at 5-min intervals for the remaining 30 min of this phase. Normal oxygenation, with 100% oxygen, was restored at the beginning of the third hour (recovery phase). No further drug was given and the perfusate was not replaced. Perfusate samples were collected as in the first hour. Bile was collected in half-hourly aliquots.

Assays

Salbutamol concentrations in perfusate and bile were determined by a selective and sensitive HPLC technique using fluorimetric detection [10]. Concentrations of the sulphate and glucuronide conjugates of salbutamol were determined as the difference between the concentrations of salbutamol in hydrolyzed and non-hydrolyzed samples. Details of this procedure have been described previously [11]. Oxygen and CO_2 concentrations and pH of perfusate were measured using an ILS pH/blood gas analyser (Instrument Laboratory, Lexington, MA, U.S.A.).

Calculations and statistics

Data in tables and text are expressed as mean ± standard deviation. Statistical comparisons of groups of data were made using Student's *t*-test for paired observations, accepting $P < 0.05$ as significant [12]. In the non-recycling experiments, the mean of each pair of hepatic inflow salbutamol concentrations (C_{in}) and each pair of outflow concentrations (C_{out}) were used to calculate hepatic clearance for each phase as $Q_H(C_{in} - C_{out})/C_{in}$, where Q_H is the hepatic perfusate flow rate. In recycling experiments, elimination half-life ($T_{1/2}$) was calculated by least squares regression analysis of the terminal log-linear phase of the perfusate concentration–time data.

RESULTS

Non-recycling experiments

During the control phase, mean oxygen *delivery* for the fifteen experiments was 3.17 ± 0.07 μmol/min/g liver. Mean oxygen *consumption* during this initial phase was 2.44 ± 0.08 μmol/min/g liver (Table 1).

A wide range of rates of oxygen delivery was produced during the graded hypoxia phase (t = 10–40 min). The maximum was 3.10 μmol/min/g liver in an experiment in which the perfusate was equilibrated with 100% O_2 alone (4 l./min; i.e. the same as the control phase), while the minimum value was 0.61 μmol/min/g liver in an experiment in which perfusate was equilibrated with 100% nitrogen. Even in this latter experiment complete anoxia was not produced due to exposure of the liver and the rest of the perfusion circuit to atmospheric oxygen. There was a close relationship between the rate of oxygen delivery and the rate of oxygen consumption during this phase ($r = 0.99$, $P < 0.001$; Fig. 1), so that at all rates of oxygen delivery approximately 80% of available oxygen was extracted by the liver.

In all experiments, there was a return of oxygen consumption to within 10% of the control value when normal oxygenation was restored to the liver in the recovery phase (t = 40–60 min), whatever the degree of hypoxia had been in the second phase. This finding indicates that reductions in hepatic oxygenation during the second phase did not lead to irreversible impairment of cellular respiration (Table 1).

The pH of perfusate entering the liver was virtually identical during the control and hypoxia phases (mean 7.34 and 7.35 respectively). There was also no difference in the pH of hepatic venous effluent between the control and hypoxia phases (mean 7.18 and 7.20 respectively). There was no significant difference in bile flow between the graded hypoxia phase and the control phase.

Mean salbutamol clearance during the control phase was 9.5 ± 0.9 ml/min (Table 1). Salbutamol clearance at the end of the graded hypoxia phase was divided by the clearance during the control phase (i.e. clearance ratio), to exclude the contribution of inter-animal variation. The relationship between salbutamol clearance ratio and oxygen delivery during graded hypoxia is shown in Fig. 2. Reducing oxygen delivery from control to approximately 2.0 μmol/min/g liver produced no appreciable inhibition of salbutamol elimination. As oxygen delivery was reduced below this threshold level, however, there was a progressive decline in salbutamol elimination. Below the threshold, the clearance ratio appeared to be linearly related to oxygen delivery ($r = 0.93$, $P < 0.01$).

The salbutamol clearance ratio was also correlated

Fig. 1. Oxygen consumption versus oxygen delivery in the graded hypoxia phase of the non-recycling experiments. There was a linear relationship between these two parameters ($y = 0.01 + 0.79\,X$; $r = 0.99$, $P < 0.001$).

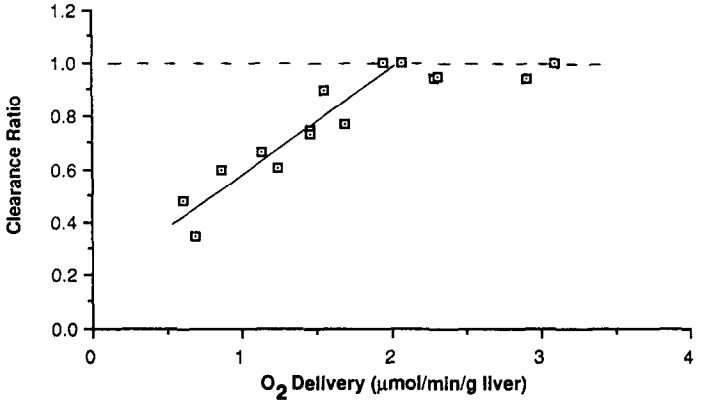

Fig. 2. Ratio of clearance during the graded hypoxia phase (10–40 min) to clearance during the control phase (0–10 min) versus oxygen delivery in the graded hypoxia phase of the non-recycling studies. There was an apparently linear decline in drug clearance ratio between an oxygen delivery threshold of approximately 2.0 μmol/min/g liver ($y = 0.18 + 0.40\,X$; $r = 0.93$, $P < 0.01$).

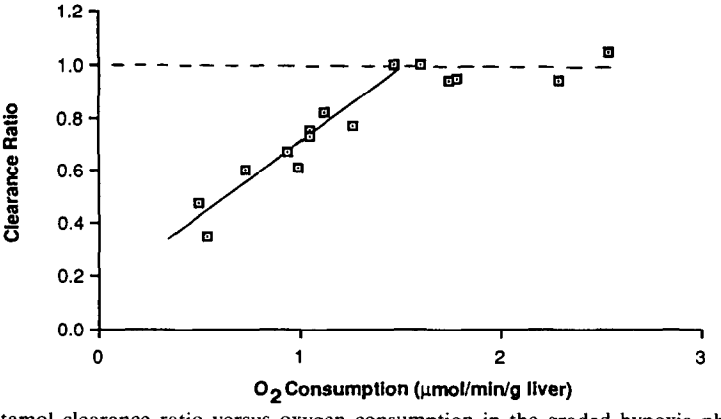

Fig. 3. Salbutamol clearance ratio versus oxygen consumption in the graded hypoxia phase of non-recycling experiments. Below an oxygen consumption threshold of 1.5 μmol/min/g liver, there appeared to be a linear decline in drug clearance ratio ($y = 0.15 + 0.56\,X$; $r = 0.94$, $P < 0.01$).

with the level of oxygen consumption by the liver during the graded hypoxia phase. From Fig. 3 it can be seen that salbutamol clearance was not impaired by hypoxia until hepatic oxygen consumption fell below approximately 1.5 μmol/min/g liver. Again, there was a linear relationship between clearance and oxygen consumption below this threshold ($r = 0.94$, $P < 0.01$). Following reoxygenation of the liver

(t = 40–60 min), salbutamol clearance returned to control values (Table 1).

The sulphate and glucuronide metabolites were undetectable in hepatic venous (outflow) perfusate.

Recycling experiments

Metabolic fate of salbutamol. In three experiments with normal oxygenation, the elimination half-life ranged from 8 to 14 min. Shortly after cessation of salbutamol administration (bolus or infusion), the ratio of perfusate glucuronide/unchanged salbutamol concentrations was very low (mean 0.07). The glucuronide accumulated in perfusate thereafter so that 1 hr later the glucuronide/unchanged salbutamol ratio had increased over 50-fold. Negligible amounts of unchanged salbutamol were excreted in bile (mean 0.12% of administered dose). The small bile volumes did not permit all samples to be assayed for both metabolites. However, at least 12% of the dose was excreted in the 2-hr bile collection as the glucuronide metabolite, whereas the sulphate was not detected in bile or perfusate.

Effect of hypoxia. The mean elimination half-life of salbutamol during the control phase (first hour) was 6.1 ± 1.2 min. A measure of the effect of reduction in oxygen supply during the graded hypoxia phase (second hour) was obtained by calculating the ratio of salbutamol half-life during the control phase to that during the graded hypoxia phase. The effect of hypoxia on half-life ratio was similar to that observed on clearance ratio in the single-pass experiments. A decline in half-life ratio occurred below an oxygen delivery threshold of 1.7 μmol/min/g liver and an oxygen consumption threshold of 1.3 μmol/min/g liver. Below these thresholds, half-life ratio was linearly related to oxygen delivery ($r = 0.92$, $P < 0.01$) and to oxygen consumption ($r = 0.92$, $P < 0.01$). In eleven experiments, sufficient salbutamol remained at the end of the graded hypoxia phase to allow the salbutamol half-life to be determined during the recovery phase (third hour). Half-life in the recovery phase was similar to that in the control phase (7.0 ± 0.5 hr, $P > 0.3$). During hypoxia, glucuronidation appeared to decrease markedly. Under the greatest degree of hypoxia, the ratio of perfusate glucuronide/unchanged salbutamol concentration fell by a factor of 30 between 1 and 2 hr, while only 0.5% of the dose was excreted in bile as the glucuronide between 1.5 and 2 hr. No sulphate metabolite was detected in bile or perfusate in any of the three phases.

DISCUSSION

Previous studies, which have examined the effect of hypoxia on the rate of drug elimination by the isolated perfused rat liver, have used a recycling perfusion system [4–7]. In the present study a non-recycling or single-pass system was developed. This design has considerable advantages over previously used approaches in that it allows for direct measurement of drug clearance from relatively few perfusate samples and, since steady-state is attained more rapidly than in recirculating experiments, clearance can be determined in a shorter time. Furthermore, in the non-recycling system the liver is always perfused with fresh perfusate and one can therefore be sure that perfusate electrolyte and glucose levels and pH are constant and that the effects of hypoxia are not partly due to the indirect effects of a fall in pH or of accumulation of potentially toxic compounds in perfusate. Notwithstanding the potential shortcomings of the recycling system, the findings were similar to those obtained with the single-pass system.

In the single-pass experiments, it was not possible to detect salbutamol metabolites in hepatic venous perfusate. Therefore, the metabolic fate of salbutamol was investigated in recycling experiments in which metabolites have the opportunity to accumulate in the perfusate reservoir. The only metabolite detected in perfusate or bile in these experiments was the glucuronide conjugate. The sulphate ester metabolite was not detected, and only negligible amounts of salbutamol were excreted as unchanged drug in bile. These findings are consistent with a previous study in the rat *in vivo*, which also found only the glucuronide and not the sulphate metabolite of salbutamol [8]. This indicates that the reduction in salbutamol elimination, resulting from reduced oxygen supply, was due primarily to a reduction in glucuronide formation. This was also reflected in the marked reduction in the ratio of perfusate glucuronide/unchanged salbutamol concentrations and low biliary output of glucuronide during hypoxia in recycling experiments. Although drug uptake by the liver may conceivably have been affected by hypoxia, this cannot explain our results, because the single-pass experiments were conducted under steady-state conditions.

Figure 2 clearly indicates that the supply of oxygen to the liver did not limit salbutamol elimination at oxygen delivery rates above 2.0 μmol/min/g liver. There was a steady, apparently linear, decline in the salbutamol elimination rate below this "hypoxic threshold". There appeared to be a similar form of relationship between drug elimination and oxygen consumption. Figure 3 shows that appreciable reduction in salbutamol elimination did not occur until oxygen consumption had fallen below a threshold value of approximately 1.5 μmol/min/g liver. As in Fig. 2, there appeared to be a linear fall in salbutamol clearance below this threshold.

The relationship between salbutamol clearance ratio and oxygen delivery below the "oxygen delivery threshold" appeared to be best described by a straight line with a slope of approximately 0.4. This relationship was also examined in terms of the Michaelis–Menten equation and an inverted exponential equation, which both predict an asymptotic relationship between salbutamol clearance and oxygen delivery/consumption. Both of these equations, however, gave a poor fit. Moreover, the decrease in salbutamol elimination with decreasing oxygen delivery seemed to begin at a discrete level or threshold of oxygen delivery, indicating that an asymptotic relationship between these two variables did not exist. Previous data showing the oxygen dependence of the metabolism of a variety of substrates in isolated hepatocyte suspensions were also inconsistent with a simple Michaelis–Menten relationship [13–15]. In these studies, oxygen depen-

dence was defined by either the P_{50} value, which is the oxygen concentration giving half-maximum activity determined visually from the graph of oxygen concentration versus rate of metabolism, and/or critical O_2, which is the oxygen concentration at which the first detectable change in function occurs as oxygen becomes rate limiting.

One might expect the oxygen dependence of the hepatic elimination of different drugs to vary greatly because, in subcellular fractions, drug metabolising systems display a wide range of oxygen affinities (K_mO_2) [1]. For cytochrome P-450 alone, K_mO_2 values have been shown to vary 200-fold depending on the species of cytochrome P-450 and the nature of the substrate. Jones [1] pointed out that for many forms of cytochrome P-450 the K_mO_2 was above 10–15 μM. Since the average oxygen tension in the liver *in vivo* is thought to be 35 μM [16], he concluded that the metabolism of many oxidatively metabolised drugs may be impaired by relatively mild reductions in hepatic oxygen supply. In isolated hepatocytes, glucuronidation is inhibited when the oxygen concentration falls below 20 μM and the P_{50} for glucuronidation lies between 2 and 5 μM. It has therefore been suggested that this pathway may also be impaired by levels of hypoxia likely to occur *in vivo* [14, 17].

In contrast to subcellular fractions and isolated hepatocytes, in the intact liver oxygen tension is not uniform throughout the lobule but follows a gradient with the highest tension in acinar zone 1 and the lowest in acinar zone 3. Thus, *in vivo*, and in the isolated perfused liver, hepatic elimination processes thought to be concentrated primarily in the centrilobular zone, such as oxidative drug biotransformation and glucuronidation [3, 18], might be expected to be affected more by reductions in hepatic oxygen supply than pathways with similar oxygen affinities which are located primarily in zone 1.

These considerations suggest that drug glucuronidation in the intact liver is likely to be sensitive to relatively mild reductions in hepatic oxygen supply. However, the current study shows that, in the isolated liver, a substantial reduction in oxygen supply (> 40%) was required before a reduction in salbutamol elimination was detected, and that oxygen delivery had to fall by 75% before elimination rate was halved (Fig. 2). *In vivo* hepatic oxygen delivery calculated from studies in rats and other experimental animals, ranges between 4 and 7 μmol/min/g liver, while normal hepatic oxygen consumption ranges from 2 to 3 μmol/min/g liver [19–21]. When one compares these values with the critical thresholds of oxygen delivery and consumption for salbutamol elimination in the isolated liver (2.0 and 1.50 μmol/min/g liver respectively), it becomes apparent that, *in vivo*, large reductions in hepatic oxygen supply may be required before significant impairment of salbutamol elimination will occur. In contrast to these findings, Roth and Rubin [22] showed that, in the isolated perfused rat liver, the critical threshold level of oxygen delivery for hexobarbital metabolism by the mixed-function oxidase system was approximately 6 μmol/min/g liver. This finding suggests that, in the intact liver, oxidative drug metabolism may be considerably more sensitive to hypoxia than is glucuronidation. It is important to note, however, that the current experiments with salbutamol were carried out using the livers of fed rats. In the isolated liver, we have shown recently that fasting substantially increases the impact of hypoxia on drug glucuronidation [23]. Thus, the oxygen requirements of salbutamol elimination in the livers of fasted rats may be considerably higher than the values reported here.

It should also be noted that a constant perfusate glucose concentration of 5 mM was used in these studies. Under certain conditions of hypoxic stress *in vivo*, the concentrations of substances which affect glucose homeostasis, such as insulin and catecholamines, may be altered, leading to changes in the availability of glucose for drug glucuronidation. Under these circumstances, the sensitivity of glucuronidation to hypoxia may be modified.

Hepatic oxygen supply is likely to decrease during episodes of respiratory and cardiac failure, and there is evidence that in patients such episodes may result in impairment of the elimination of oxidatively metabolised drugs [24–26]. The perfusate concentration of salbutamol used in our experiments (50 ng/ml) was relatively low. For drugs that achieve higher concentrations and, therefore, higher rates of glucuronidation (e.g. paracetamol), the "hypoxic threshold" may be greater due to the greater demand on UDP-glucuronic acid supplies. Nevertheless, our study demonstrates, for the first time, that changes in hepatic oxygenation may rapidly affect glucuronidation-dependent drug elimination, that this effect occurs at a distinct and, at least in the case of salbutamol, relatively low degree of hepatic oxygenation, and that glucuronidation recovers, independent of the degree of hypoxia.

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