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The Disposition of Morphine and Morphine-3-glucuronide in the Isolated Perfused Rat Liver: Effects of Altered Perfusate Flow Rate

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Abstract

The rat single-pass isolated perfused liver preparation was used to study the effects of altered perfusate flow rate on the hepatic disposition of morphine and its polar metabolite morphine-3-glucuronide (M3G). Using a balanced, cross-over design, livers of female Sprague–Dawley rats (n = 6) were perfused at 15 and 30 mL min^{-1} with erythrocyte- and protein-free perfusion medium containing a constant concentration of morphine (2·7 μM). After reaching steady-state, inflow and outflow perfusate and bile samples were collected and morphine and M3G were measured by HPLC.

Doubling of perfusate flow rate was associated with a significant increase ($P < 0.05$) in the availability of morphine (mean ± s.d. of 0·19 ± 0·06 at 15 mL min^{-1} and 0·29 ± 0·08 at 30 mL min^{-1}). The magnitude of the change in morphine availability was consistent with the predictions of the well-stirred model of hepatic elimination.

The fate of hepatically generated M3G was assessed by the biliary extraction ratio of M3G; alterations in perfusate flow rate had no significant effect on this ratio (mean ± s.d. of 0·49 ± 0·14 at a perfusate flow rate of 15 mL min^{-1} and 0·47 ± 0·22 at 30 mL min^{-1}).

A physiologically-based mathematical model, in which the vascular and intracellular spaces of the liver were represented by two well-mixed compartments, was utilized to derive an equation for the biliary extraction ratio of M3G. According to the model, the value of this extraction ratio will become insensitive to changes in perfusate flow rate when the permeability for M3G of the membrane separating the intracellular and vascular compartments is low compared with perfusate flow rate. Hence, the experimental results are consistent with the concept that the hepatic sinusoidal membrane represents a diffusional barrier to M3G.

The efficiency of hepatic drug elimination is influenced by a variety of physiological factors, including blood flow rate to the liver, binding of drug to blood components and the activity of metabolic and biliary transport systems within the organ (Wilkinson 1987). In some cases, particularly for polar drugs, the permeability of the hepatocyte membranes and the activity of specific sinusoidal transport systems can also influence the overall efficiency of hepatic elimination (Meijer et al 1983; de Lannoy & Pang 1986; Tiribelli 1992). Various physiological models have been derived to describe the relationship between physiological factors and drug elimination by the liver. These models include the well-stirred model and the parallel-tube model (Pang & Rowland 1977a), and the axial dispersion model (Roberts & Rowland 1986).

For drugs with a low hepatic extraction ratio, all physiological models predict that hepatic clearance will be directly proportional to the unbound fraction of the drug in blood (fu) and the intrinsic ability of the liver to eliminate the drug, often expressed as intrinsic clearance (CL_{int}), and that clearance will be independent of liver blood-flow (Q) (Wilkinson 1987). For drugs with a high hepatic extraction ratio, liver blood-flow is the most important determinant of drug clearance. However, in this case, the fraction escaping elimination during a single pass through the liver (availability) will be influenced by alterations in Q, fu and CL_{int} (Wilkinson 1987). Much of the information on the physiological determinants of hepatic drug elimination has been obtained from the use of the isolated perfused rat liver (IPRL), where factors such as drug binding and perfusate flow can be controlled and quantified and drug availability is readily measured. For example, a number of studies have examined the influence of changes in perfusate flow rate on the availability of drugs which have a medium to high hepatic extraction ratio, including pethidine (Ahmad et al 1983), lignocaine (Pang & Rowland 1977b), propranolol (Smallwood et al 1988) and diazepam (in the absence of perfusate protein) (Diaz-Garcia et al 1992).

Relatively few studies have focused on the factors influencing the fate of drug metabolites formed in the liver from their precursors (generated metabolites). However, recent findings indicate that the fate of a generated metabolite may differ substantially, both qualitatively and quantitatively, from that of pre-formed metabolite gaining access to the liver via the portal or arterial blood supply (Pang et al 1992). Factors leading to these differences include the highly polar nature of some metabolites, which can lead to difficulties in diffusion across the hepatocyte sinusoidal membranes, and the uneven distribution of metabolic enzymes and transport systems along individual sinusoids (Pang et al 1992).

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The aim of this work was to examine the effect of alterations in perfusate flow-rate on the hepatic disposition of a drug which is converted to a polar metabolite which is likely to experience hepatocyte membranes as permeability barriers. Morphine has a high hepatic extraction ratio in the IPRL (Imamura & Fujimoto 1980a, b; Sweeney et al 1984; Evans & Shanahan 1995) and therefore an availability which should, in theory, be influenced by alterations in perfusate flow rate. In addition, in the female rat, morphine is converted almost exclusively to a single, polar metabolite, morphine-3-glucuronide (M3G). The highly polar nature of this molecule is evidenced by a negligible octanol to phoshate buffer (pH 7·4) partition coefficient (Van Crugten et al 1991). After formation, M3G is excreted across the canalicular domain of hepatocytes and undergoes efflux across the sinusoidal membrane into the vascular space (Imamura & Fujimoto 1980a, b; Shanahan & Evans 1993). The effects of perfusate flow rate on the disposition of morphine and M3G were compared with the predictions of a physiological model in which the cellular and vascular spaces of the liver were represented by two well-mixed compartments separated by a semi-permeable membrane.

Materials and Methods

Chemicals

Morphine hydrochloride (McFarlane Smith, Edingburgh, UK), morphine-3β-D-glucuronide (Sigma Chemical Co., St Louis, MO) and hydromorphone hydrochloride (Sigma Chemical Co., St Louis, MO) were all purchased commercially. Sodium taurocholate was purchased from Sigma and 1-dodecylsulphate sodium was purchased from Regis (Morton Grove, IL). Acetonitrile (UV cut-off 190 nm) and methanol were HPLC-grade (Waters Associates, Lane Cove, NSW, Australia), and all other chemicals were of analytical grade and used as received.

Liver perfusion

The protocol for the experiments was reviewed and approved by the institutional animal ethics committee. Experiments were conducted using isolated perfused livers from six female Sprague–Dawley rats (240–255 g) obtained from Gilles Plains Animal Resource Centre, South Australia. Each rat was anaesthetized with sodium pentobarbital (60 mg kg^{-1}, Boehringer Ingelheim, New South Wales, Australia) and the liver was perfused via the portal vein under single-pass conditions as described earlier (Evans & Shanahan 1995). The perfusate medium consisted of erythrocyte- and albumin-free Krebs-bicarbonate buffer (pH 7·4), containing glucose (3 g L^{-1}) and sodium taurocholate (4·5 mg L^{-1}). After perfusing the liver with drug-free medium at a flow rate of 30 mL min^{-1} for an equilibration period of 15 min, morphine hydrochloride was added to the inflow perfusate to produce a final concentration of 2·7 μM (1000 μg L^{-1}). Using a balanced, cross-over design, each liver was then perfused under single-pass conditions at flow rates of 30 and 15 mL min^{-1}. Earlier experiments indicated that morphine and M3G achieved steady-state conditions in bile and outflow perfusate after a period of 15 min (Evans & Shanahan 1995). Therefore, at each flow rate, livers were perfused for 30 min and steady-state outflow perfusate samples were collected at 20, 25 and 30 min. Bile samples were collected between 20–25 min and 25–30 min at each perfusate flow rate. Inflow perfusate samples were collected by direct sampling from the reservoir. The viability of each IPRL was assessed by measurements of pH of outflow perfusate, bile flow, recovery of perfusate and by gross liver appearance.

Analytical methods

A validated HPLC method involving UV detection was used to measure concentrations of morphine and M3G in perfusate and bile (Evans & Shanahan 1995). Calibration curves for morphine and M3G in perfusate were constructed over the range of 50–1000 μg L^{-1} and calibration curves for M3G in bile were constructed over the range of 1–10 mg L^{-1}. Repeated analysis of quality control samples indicated that the absolute percentage difference between nominal and measured concentrations, and the coefficient of variation of the assay, were less than 13%.

Data analysis

Using data from samples collected at steady state, model-independent pharmacokinetic parameters were calculated using the following equations:

$$\text{Availability of morphine } (F^M) = \frac{\text{Concn morphine in outflow perfusate } (M_{out})}{\text{concn morphine in inflow perfusate } (M_{in})} \quad (1)$$

$$\text{Clearance of morphine (CL)} = \text{perfusate flow rate (Q)} \times (1 - F^M) \quad (2)$$

$$\text{Rate of M3G formation} = \text{rate of recovery of M3G in perfusate} + \text{rate of recovery of M3G in bile} \quad (3)$$

$$\text{Partial clearance of morphine to M3G } (CL_f^{M3G}) = \frac{\text{rate of M3G formation}}{M_{in}} \quad (4)$$

Biliary extraction ratio of hepatically-generated M3G (E_b^{M3G}) (this parameter has been described previously by de Lannoy et al (1993)) =

$$\frac{\text{rate of biliary M3G excretion}}{\text{Rate of M3G formation}} \quad (5)$$

$$\text{Fraction of hepatically-eliminated morphine converted to M3G } (f_M^{M3G}) = CL_f^{M3G}/CL \quad (6)$$

$$\text{Mass balance} = \frac{\text{rate of recovery of morphine and M3G in outflow perfusate and bile}}{\text{rate of delivery of morphine to the liver via inflow perfusate}} \quad (7)$$

All data are presented as mean ± standard deviation. Analysis of variance was used to test for differences in the hepatic disposition of morphine and M3G at perfusate flow rates of 30 and 15 mL min^{-1} and $P < 0.05$ was taken to represent statistical significance.

Theoretical Section

To fully understand the influence of physiological factors on the hepatic disposition of drugs and their metabolites, it is useful to compare experimental findings with the predictions of physiologically-based mathematical models. The liver was therefore

modelled as two well-mixed homogeneous compartments, representing the vascular and cellular spaces, separated by a semi-permeable membrane (Fig. 1). In the derivation of the model equations, it was assumed that: the influx and efflux clearances (permeability) of morphine (P^M) across the sinusoidal membrane are equal; hepatically-generated M3G is either excreted into bile or appears in perfusate outflow; the efflux and influx sinusoidal membrane permeabilities of M3G (P^{M3G}) are equal; and both morphine and M3G are completely unbound in perfusate (fu = 1). Under these conditions, equations for the availability of morphine (F^M) and the biliary extraction ratio of hepatically generated M3G (E_b^{M3G}) can be derived (see Appendix).

$$F^M = Q/(Q + \rho CL_{int}^M) \quad (8)$$

where $\rho = P^M/(P^M + CL_{int}^M)$

$$E_b^{M3G} = CL_{int}^{M3G}/(CL_{int}^{M3G} + \sigma Q) \quad (9)$$

where $\sigma = P^{M3G}/(P^{M3G} + Q)$

In equations 8 and 9, Q is perfusate flow-rate, CL_{int}^M is intrinsic clearance of morphine and CL_{int}^{M3G} is the intrinsic biliary clearance of M3G. Equations 8 and 9 are equivalent to those derived by Rowland & Evans (1991) and de Lannoy et al (1993), respectively. It should be noted, however, that de Lannoy et al (1993) did not assume that the influx and efflux permeabilities across the sinusoidal membrane are equal.

Results

Bile production remained constant over the course of each perfusion and perfusate flow rate had no significant effect ($P > 0.05$) on mean bile flow rate (data not shown). In all livers, the recovery of inflow perfusate was greater than 98% at each flow rate. Fig. 2 shows a representative plot of the outflow perfusate concentrations of morphine and M3G and the biliary excretion rate of M3G vs time at perfusate flow rates of 15 and 30 mL min^{-1}. For all liver perfusions, the concentration of morphine in outflow perfusate and M3G in perfusate and bile, were within the calibration ranges. The concentration of morphine and M3G in perfusate, and the rate of M3G excretion into bile remained relatively constant during each of the two sampling periods of each liver perfusion. Unchanged morphine was undetectable in bile and in keeping with previous observations in female rats (Shanahan & Evans 1993), the *N*-demethylated metabolite of morphine, normorphine, could not be detected in bile or outflow perfusate.

Table 1 summarizes the pharmacokinetic parameters calculated using equations 1–7. In each liver, the fraction of morphine escaping hepatic extraction (availability) was less than 0·25 at 15 mL min^{-1}. Increasing the perfusate flow rate to 30 mL min^{-1} resulted in a 53% increase in mean availability and a 75% increase in mean clearance ($P < 0.05$; Table 1). The mean fraction of morphine converted to M3G was 0·71 at 15 mL min^{-1}, indicating that M3G was the predominant metabolite of morphine, and did not change with a doubling of perfusate flow rate (Table 1). As indicated by the biliary extraction ratio (Table 1), approximately half of the hepatically-generated M3G was excreted into bile and the fraction undergoing biliary excretion was not influenced by perfusate flow rate. Mass balance indicated that, at perfusate flow rates of 15 and 30 mL min^{-1}, 80% of morphine delivered to the liver via inflow perfusate was recovered as morphine and M3G in bile and outflow perfusate (Table 1).

Discussion

Previous studies involving the perfusion of rat isolated livers at a flow rate of 30 mL min^{-1}, using a perfusate composition identical to that used in the current investigation, indicated that 15 min was needed to reach steady-state with respect to morphine and M3G disposition and that the hepatic handling of morphine and M3G remained constant for 1 h (Evans & Shanahan 1995). For this reason, the present studies were limited to assessing morphine and M3G disposition at two flow rates in each IPRL experiment. The bile flow rate was not affected by changes in perfusate flow rate and was similar to previous studies in the IPRL (Schmucker et al 1975; Brock & Vore 1982) and in-vivo (Fuhrman-Lane & Fujimoto 1982; Brouwer &

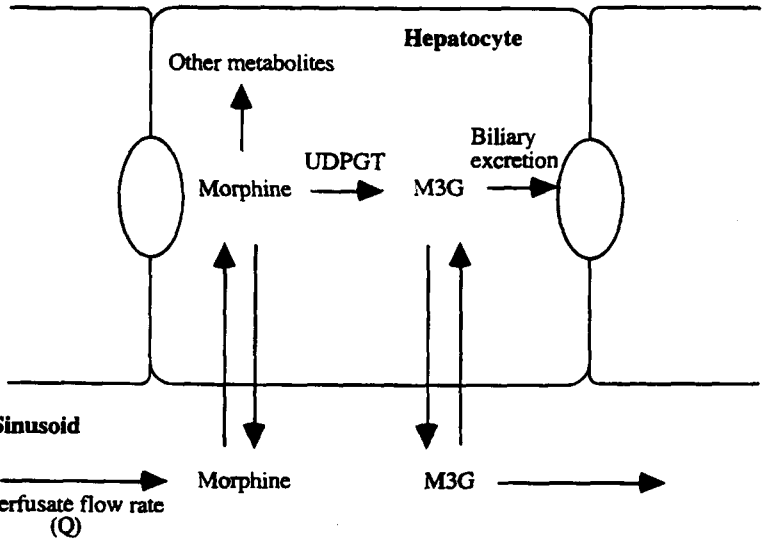

FIG. 1. Diagrammatic representation of the hepatic elimination of morphine at the level of the hepatocyte.

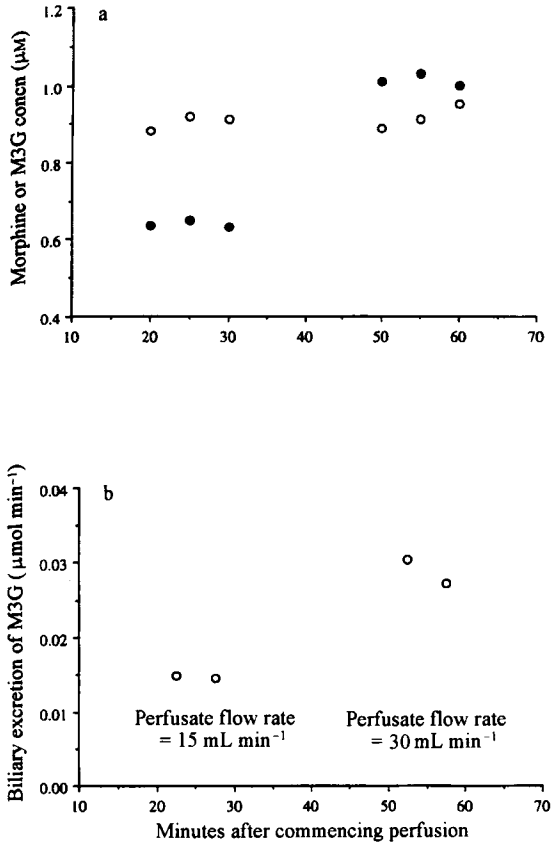

FIG. 2. Representative plots of (a) the outflow perfusate concentration of morphine (●) and M3G (○), and (b) the biliary excretion rate of M3G vs time at perfusate flow rates of 15 and 30 mL min^{-1}.

Table 1. Effects of perfusate flow rate on the hepatic disposition of morphine and hepatically generated M3G in the IPRL.

Parameter	Perfusate flow rate	
	15 mL min^{-1}	30 mL min^{-1}
F^M	0·19 ± 0·06	0·29 ± 0·08*
CL (mL min^{-1})	12·2 ± 0·9	21·4 ± 2·5*
CL_f^{M3G} (mL min^{-1})	8·5 ± 2·4	14·0 ± 1·7*
f_M^{M3G}	0·71 ± 0·22	0·66 ± 0·08
E_b^{M3G}	0·49 ± 0·14	0·47 ± 0·22
Mass balance	0·80 ± 0·12	0·80 ± 0·07

Values given are mean ± s.d. *$P < 0·05$ compared with the corresponding value at 15 mL min^{-1}.

Jones 1990). Further evidence for the stability of the IPRL preparation was the constancy of the steady-state perfusate outflow concentrations of morphine and M3G at each flow rate. Perfusion of the liver at 15 mL min^{-1} was not associated with liver injury or a decrease in liver performance as indicated by gross appearance of the IPRL and a significantly lower availability of morphine at a perfusate flow of 15 mL min^{-1} compared with that at 30 mL min^{-1}.

In man, morphine is primarily eliminated from the body by metabolism within the liver and after oral administration the fraction of the dose ultimately reaching the systemic circulation is low due to the extensive presystemic hepatic elimination (Glare & Walsh 1991). Similarly, in rats, the hepatic elimination of morphine in-vivo indicates that it is highly extracted by the liver (Iwamoto & Klaassen 1977; Mistry & Housten 1987). Furthermore, previous investigations using the IPRL have reported high hepatic extraction ratios for male and female rats (Sweeney et al 1984; Shanahan & Evans 1993; Evans & Shanahan 1995). Consistent with previous findings, the hepatic extraction ratio of morphine in the present study was high, resulting in an availability of only 0·29 ± 0·08 at 30 mL min^{-1}. As indicated by mass balance (Table 1), M3G in outflow perfusate and bile accounted for the major part of the eliminated morphine.

As expected for a drug with a high hepatic extraction ratio, doubling the perfusate flow rate led to a significant increase in morphine availability. This finding is due to the reduced transit time of morphine in the liver and therefore decreased exposure to the hepatic drug metabolizing enzymes. It is well known that for a drug with a high hepatic extraction ratio, the various models of hepatic elimination differ in the magnitude of the change in availability produced by a change in blood (perfusate) flow rate. Thus, compared with the well-stirred model, the parallel-tube model always predicts greater changes in availability with changes in blood (perfusate) flow rate (Morgan & Smallwood 1990). Using the IPRL, investigation of the effects of changes in physiological parameters (e.g. changes in perfusate flow rate) on the availability of a drug enables determination of the model which best predicts hepatic elimination events.

Equation 8 was derived on the basis that the liver is viewed as two well-mixed homogeneous compartments representing the vascular and cellular spaces. This equation is identical to that predicted by the well-stirred model of hepatic elimination when it is not assumed that distribution of drug within the liver is perfusion-rate limited (Rowland & Evans 1991). According to this model, availability is influenced by both Q and ρCL_{int}^M.

If it is assumed that ρCL_{int}^M remains constant with perturbations in Q, an equation for the relationship between availability and perfusate flow rate can be derived:

$$F_1(1 - F_2)/F_2(1 - F_1) = Q_1/Q_2 \quad (10)$$

where F_1 and F_2 are the availabilities of the drug at perfusate flow rates, Q_1 and Q_2, respectively.

The magnitude of $F_1(1 - F_2)/F_2(1 - F_1)$ was calculated for each liver. The mean value was 1·75 and the 95% confidence interval (1·46 to 2·04) included 2·0, the ratio of the two perfusate flow rates. For the parallel-tube model, the following expression relating availability and Q can be derived (Rowland & Evans 1991):

$$F = e^{-\rho CL_{int}^M/Q} \quad (11)$$

Assuming that ρCL_{int}^M remains constant with perturbations in Q, then:

$$\ln F_2/\ln F_1 = Q_1/Q_2 \quad (12)$$

For each perfusion, the magnitude of ($\ln F_2/\ln F_1$) was determined. The mean value was 1·34 and the 95% confidence interval (1·21 to 1·47) did not include 2·0. This suggests that the parallel-tube model is a poor predictor of the effects of changes in Q on morphine availability. Hence, the effect of alterations in Q on the availability of morphine was more consistent with the

predictions of the well-stirred model than the parallel-tube model. This is consistent with the findings of the effect of Q on the availability of other drugs with high hepatic extraction ratios (Pang & Rowland 1977b; Ahmad et al 1983; Smallwood et al 1988) and is in keeping with the concept that the liver is an organ of high blood flow heterogeneity (Rowland & Evans 1991).

Previous investigations have shown that M3G is a major metabolite of morphine. In man, the formation clearance of M3G accounts for approximately 60% total morphine clearance (Hasselstrom & Sawe 1993). Investigations using Sprague-Dawley rats in-vivo have determined that M3G is the major hepatic metabolite of morphine (Smith et al 1973; Horton & Pollack 1991) and investigations using the IPRL have found that approximately 55–65% of morphine delivered via the hepatic portal vein is converted to M3G during single pass through the liver (Fuhrman-Lane & Fujimoto 1982; Shanahan & Evans 1993). Consistent with previous investigations, the present study found that the majority of morphine eliminated by the liver was converted to M3G.

The effects of changes in perfusate flow rate on the disposition of hepatically-generated M3G was examined in relation to the parameter E_b^{M3G}, the biliary extraction ratio. This parameter essentially describes the fraction of hepatically-generated M3G which is excreted into bile. It should be noted that E_b^{M3G} is much greater than the extraction ratio of preformed M3G entering the perfused liver via the hepatic portal vein (Imamura & Fujimoto 1980a; Evans & Shanahan 1993). According to equation 9, the effect of changes in Q on E_b^{M3G} is dependent upon the relative magnitudes of P^{M3G} and Q. If P^{M3G} greatly exceeds Q, that is the generated M3G experiences no permeability barrier at the hepatocyte sinusoidal membrane, then equation 9 becomes:

$$E_b^{M3G} = CL_{int}^{M3G}/(CL_{int}^{M3G} + Q) \quad (13)$$

Hence, in this case, an increase in Q will result in a decrease in E_b^{M3G}. Considering that E_b^{M3G} was found to be 0·49 at a perfusate flow rate of 15 mL min^{-1}, equation 13 predicts that at a Q value of 30 mL min^{-1}, E_b^{M3G} would be approximately 0·32 (assuming that P^{M3G} and CL_{int}^{M3G} are not influenced by Q). At the other extreme, when Q greatly exceeds P^{M3G}, that is the generated M3G experiences a permeability barrier at the hepatocyte sinusoidal membrane, equation 9 simplifies to:

$$E_b^{M3G} = CL_{int}^{M3G}/(CL_{int}^{M3G} + P^{M3G}) \quad (14)$$

In this case, E_b^{M3G} will be virtually unaffected by changes in Q. Hence, the experimental finding that E_b^{M3G} was $0{\cdot}47 \pm 0{\cdot}22$ at a perfusate flow rate of 30 mL min^{-1} and $0{\cdot}49 \pm 0{\cdot}14$ at a perfusate flow rate of 15 mL min^{-1} was in keeping with the latter of these two scenarios. Therefore, the lack of effect of Q on E_b^{M3G} is consistent with the concept that hepatically-generated M3G experiences the hepatocyte sinusoidal membrane as a permeability barrier. Similarly, previous investigations have determined that other highly polar hepatically-generated metabolites experience difficulty in moving from the hepatocyte into the sinusoidal space. For example, the glucuronide metabolites of paracetamol (Brouwer & Jones 1990) and oxazepam (St Pierre et al 1990) have been found to experience diffusional barriers between the cellular and vascular spaces of the liver. Also, de Lannoy et al (1993) utilized a physiological model similar to that used in the present study to determine the existence of an influx barrier at the hepatocyte sinusoidal membrane to enalaprilat, the polar metabolite of enalapril.

The concept that hepatically-generated M3G experiences hepatocyte sinusoidal membranes as permeablilty barriers is supported by a number of experimental findings. Evans & Shanahan (1993) used the IPRL to compare the biliary extraction ratio of preformed M3G with that of M3G generated in-situ from its precursor, morphine. The proportion of the M3G recovered in bile during morphine infusion was approximately 50 times that during infusion of preformed M3G into the liver. The apparent barrier to M3G across hepatocyte membranes is consistent with the involvement of carrier-mediated transport in the hepatic handling of M3G. Several investigations utilizing the IPRL have found that specific transport mechanisms are involved in the movement of M3G across hepatocyte membranes. Imamura & Fujimoto (1980a, b) reported that the cholephilic anion, dehydrocholate, blocked the egress of M3G into bile and that SKF525-A, a cation at physiological pH, effectively trapped M3G within the hepatocyte, probably by inhibiting sinusoidal and canalicular membrane transport systems. It has been found that specific carriers are involved in the hepatocyte sinusoidal and canalicular membrane transport of the polar metabolites of other compounds. For instance, investigations by Brouwer et al (1987) indicate that the glucuronide metabolites of female sex hormones are taken up into hepatocytes by two distinct organic anion carrier-mediated transport systems. Also, there is evidence that specific transport mechanisms are involved in the movement of paracetamol-glucuronide, an anion, from the hepatocyte into bile across the canalicular membrane (Brouwer & Jones 1990; Savina & Brouwer 1992).

In conclusion, the results from this investigation demonstrate that in the rat liver, morphine is a high hepatic extraction ratio drug and its major metabolite is M3G. The availability of morphine in the IPRL is relatively low and the effect of alterations in perfusate flow rate on availability are consistent with the predictions of the well-stirred model of hepatic elimination. The lack of effect of alterations in perfusate flow rate on the biliary extraction ratio of M3G indicates that this highly polar metabolite experiences the hepatocyte sinusoidal membrane as a permeability barrier.

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Appendix

In the current investigation, the liver is viewed as two well-mixed compartments representing the vascular and cellular spaces, separated by a semi-permeable membrane (Fig. 1), similar to the model described previously by de Lannoy et al (1993). In a single pass design, a constant input of morphine is delivered via the hepatic portal vein into the liver. Morphine is metabolized within the hepatocyte to form the metabolite, morphine-3-glucuronide (M3G), which is then removed from the cell by excretion into bile or movement across the sinusoidal membrane into the vascular space. M3G reaching the vascular space is subsequently available for uptake into the cellular space.

For morphine and M3G, it is assumed that the respective influx and efflux permeabilities across the sinusoidal membrane are equal and that both morphine and M3G are completely unbound in perfusate. According to the model, the rate of change of morphine and M3G in vascular and cellular spaces may be described by the following equations:

$$dMv/dt = QC_{IN}^{M} - QC_{OUT}^{M} - P^{M}[Mv] + P^{M}[Mc] \quad (A1)$$

$$dMc/dt = P^{M}[Mv] - CL_{int}^{M}[Mc] - P^{M}[Mc] \quad (A2)$$

$$dM3Gv/dt = P^{M3G}[M3Gc] - P^{M3G}[M3Gv] - QC_{OUT}^{M3G} \quad (A3)$$

$$dM3Gc/dt = f_{M}^{M3G}QEC_{IN}^{M} + P^{M3G}[M3Gv] - P^{M3G}[M3Gc] - CL_{int}^{M3G}[M3Gc] \quad (A4)$$

where C_{IN}^{M} = concentration of morphine in inflow perfusate, C_{OUT}^{M} = concentration of morphine in outflow perfusate, C_{OUT}^{M3G} = concentration of M3G in outflow perfusate, CL_{int}^{M} = intrinsic clearance of morphine, CL_{int}^{M3G} = the biliary intrinsic clearance of M3G, E = extraction ratio of morphine, f_{M}^{M3G} = fraction of eliminated morphine which is metabolized to M3G within the liver, Mc = amount of morphine in the cellular space of the liver and [Mc] is the corresponding concentration term for the unbound species, Mv = amount of morphine in the

vascular space of the liver and [Mv] is the corresponding concentration term for the unbound species, M3Gc = amount of M3G in the cellular space of the liver and [M3Gc] is the corresponding concentration term for the unbound species, M3Gv = amount of M3G in the vascular space of the liver and [M3Gv] is the corresponding concentration term for the unbound species, P^M = permeability of the sinusoidal membrane for morphine, P^{M3G} = permeability of the sinusoidal membrane for M3G, and Q = perfusate flow-rate.

To derive expressions for the availability of morphine (F^M) and the biliary extraction ratio of M3G (E_b^{3MG}) a well-stirred model is assumed. It is also assumed that neither morphine nor M3G is bound to the protein-free perfusion medium. Thus, $C_{OUT}^M = [Mv]$ and $C_{OUT}^{M3G} = [M3Gv]$.

At steady-state, the rate of change of morphine and M3G in the vascular and cellular spaces equals zero. For morphine, at steady state, dMv/dt = dMc/dt = 0. Solving for [Mc] from equation A1:

$$[Mc] = C_{OUT}^M - (Q(C_{IN}^M - C_{OUT}^M))/P^M \tag{A5}$$

Substituting for [Mc] into equation A2:

$$C_{OUT}^M/C_{IN}^M = F^M = (Q(CL_{int}^M + P^M))/(Q(CL_{int}^M + P^M) + P^M CL_{int}^M) \tag{A6}$$

Thus, morphine availability (F^M) is given by:

$$F^M = Q/(Q + \rho CL_{int}^M) \tag{A7}$$

where $\rho = P^M/(P^M + CL_{int}^M)$.

For M3G at steady state, dM3Gv/dt = dM3Gc/dt = 0. Solving for [M3Gc] from equation A4:

$$[M3GC] = (f_M^{M3G} QEC_{IN}^M + P^{M3G} C_{OUT}^{M3G})/(P^{M3G} + CL_{int}^{M3G}) \tag{A8}$$

Substituting for [M3Gc] into equation A3:

$$C_{OUT}^{M3G} = (P^{M3G} f_M^{M3G} QEC_{IN}^M)/(CL_{int}^{M3G}(P^{M3G} + Q) + QP^{M3G}) \tag{A9}$$

The biliary extraction ratio of M3G (E_b^{M3G}), which is the ratio of the rate of M3G excreted into bile to the rate of M3G formation, is given by:

$$E_b^{M3G} = (\text{Rate of M3G formation} - \text{Rate of M3G out via perfusate})/\text{Rate of M3G formation} = (f_M^{M3G} QEC_{IN}^M - QC_{OUT}^{M3G})/(f_M^{M3G} QEC_{IN}^M) \tag{A10}$$

By substituting equation A9 for C_{OUT}^{M3G} into equation A10:

$$E_b^{M3G} = CL_{int}^{M3G}/(CL_{int}^{M3G} + \sigma Q) \tag{A11}$$

where $\sigma = P^{M3G}/(P^{M3G} + Q)$.