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The Disposition of Morphine and Its Metabolites in the In-situ Rat Isolated Perfused Liver

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Abstract

A specific HPLC method with UV detection was used to investigate the disposition of morphine and its metabolites in the in-situ rat isolated perfused liver preparation.

Livers of male Sprague-Dawley rats (n = 4) were perfused under single pass conditions with protein- and erythrocyte-free perfusate, containing 2·66 μM morphine, for up to 90 min. The concentration of morphine, normorphine and morphine-3-glucuronide (M3G) in outflow perfusate, and the biliary excretion of M3G and normorphine glucuronide, all reached steady-state levels within 15–20 min after commencing perfusion. At steady-state, the mean (± s.d.) extraction ratio of morphine was 0·87 ± 0·06 and clearance (26·0 ± 1·7 mL min^{-1}) approached perfusate flow rate (30 mL min^{-1}). Although M3G was the main metabolite, accounting for 72·8 ± 12·7% of eliminated morphine, a significant proportion (21·6 ± 13·5%) was *N*-demethylated to normorphine and was recovered as unchanged normorphine in outflow perfusate and normorphine glucuronide in bile. The biliary extraction ratio of hepatically-formed M3G was 0·61 ± 0·31.

Results from an additional six experiments, in which livers were perfused with 1·33 and 2·66 μM of morphine for 30 min each in a balanced cross-over manner, indicated that the disposition of morphine and its metabolites was approximately linear within this concentration range.

In man and many other species, the disposition of morphine is characterized by extensive metabolism, intermediate to high hepatic extraction, and low bioavailability after oral administration. Although the pattern of morphine metabolism is species-dependent, the main metabolic routes commonly include glucuronidation to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), *N*-demethylation to normorphine, and sulphoconjugation to morphine-3-sulphate (Oguri et al 1970; Yeh & Woods 1970; Boerner et al 1975; Yeh et al 1977, 1979). Interest in the disposition of morphine metabolites has been heightened by the recognition that they can interact with opioid receptors and possibly contribute to the pharmacological and toxicological effects of morphine. For example, M6G, which is formed in man, but not rats, is a potent opioid agonist (Shimomura et al 1971; Christensen & Jorgensen 1987; Pasternak et al 1987; Abbott & Palmour 1988; Gong et al 1991) which provides pain relief in patients (Osborne et al 1992). In addition, respiratory depression has been associated with elevated plasma concentrations of M6G in patients with renal failure (Hasselström et al 1989; Bodd et al 1990). In contrast, M3G, which is the major metabolite of morphine in most species, including the rat, is devoid of analgesic effect but competitively antagonizes the opioid activity of morphine and M6G in animal models (Gong et al 1991; Ekblom et al 1993).

Although the liver is an important site of morphine elimination, there is evidence of extrahepatic metabolism, particularly within the gastrointestinal tract and possibly the kidney (Del Villar et al 1974; Koster et al 1985; Jacqz et al 1986; Mistry & Houston 1987; Yue et al 1988; Horton & Pollack 1991; Milne et al 1993). In addition, morphine undergoes enterohepatic recycling, whereby glucuronides excreted via bile are thought to be reabsorbed as the aglycone following cleavage within the gastrointestinal tract (Walsh & Levine 1975; Dahlström & Paalzow 1978; Hasselström & Säwe 1993; Milne et al 1993). Because of extrahepatic metabolism and enterohepatic recycling, it is difficult to assess the efficiency of morphine metabolism within the liver of the intact animal and the effects of physiological and pathological factors and other drugs on the hepatic elimination of morphine. Experimental models which have been used to investigate the hepatic metabolism of morphine include isolated perfused livers (Kreek et al 1978; Imamura & Fujimoto 1980; Auansakul & Vore 1982; Brock & Vore 1982; Sweeney et al 1984; Callaghan et al 1993), isolated hepatocytes (Nagamatsu et al 1986) and microsomal preparations (Rane et al 1984, 1985; Säwe et al 1985). A potential advantage of the isolated perfused liver model is that the hepatic elimination of morphine and of locally-generated and pre-formed metabolites can be studied under controlled conditions without the confounding influence of entero-hepatic recycling and elimination by other organs and tissues of the body. In addition, many of the physiological factors which influence the hepatic disposition of drugs and their metabolites within the intact animal, including liver blood flow and binding to plasma proteins, can be readily controlled and quantified in perfused liver systems.

In previous studies on the metabolism of morphine in the

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isolated perfused rat liver (IPRL), radiolabelled morphine was added to the perfusion medium as a tracer for non-labelled material, and radiolabelled material exiting the liver via perfusate or bile was assumed to represent either morphine or M3G (Imamura & Fujimoto 1980; Auansakul & Vore 1982; Sweeney et al 1984). Although studies in intact rats (Klutch 1974; Yeh et al 1979) and rat hepatic microsomes (Blanck et al 1990) have identified normorphine as a metabolite of morphine, the overall extent of morphine *N*-demethylation in rat liver is not known. In addition, there is no information on the extent to which hepatically-generated normorphine undergoes subsequent metabolism and biliary excretion.

In the present studies, a specific HPLC method was used to investigate the hepatic disposition of morphine and its metabolites. The specific aims were to identify the relative importance of morphine glucuronidation and *N*-demethylation by the intact liver and to determine the fate of hepatically-formed M3G and normorphine.

Materials and Methods

Chemicals

Morphine (Morphine HCl.3H_2O; McFarlane Smith, Edinburgh, UK), morphine-3β-D-glucuronide (Sigma, St Louis, MO, USA), normorphine (normorphine HCl; Makor Chemicals, Israel), and hydromorphone (hydromorphone HCl; Sigma) were all purchased commercially. β-Glucuronidase (Type B-3; from bovine liver) and sodium taurocholate were purchased from Sigma and 1-dodecylsulphate sodium salt was obtained from Regis (Morton Grove, IL, USA). Acetonitrile (UV cut-off 190 nm) and methanol were HPLC-grade (Waters Associates, Lane Cove, Australia), and all other chemicals were of analytical grade and were used as received.

Liver perfusion

Livers of male Sprague-Dawley rats (200–300 g; Gillies Plains Animal Resource Centre, Adelaide, Australia) were perfused in-situ using surgical techniques described previously (Pang & Rowland 1977; Evans et al 1991). Animals were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, Boehringer Ingelheim, NSW) before surgery. Freshly prepared and filtered (0·2 μm) albumin- and erythrocyte-free Krebs-bicarbonate buffer (pH 7·4), supplemented with glucose (3 g L^{-1}) and taurocholic acid (4·5 mg L^{-1}) and bubbled with humidified carbogen, was delivered to the portal vein catheter (18 GA Insyte IV Catheter, Becton Dickinson, Spain) via a Masterflex Model 7518-00 peristaltic pump (Cole Palmer, IL, USA) at a constant flow rate of 30 mL min^{-1}. The temperature of the perfusion cabinet and perfusion medium was thermostatically controlled at 37°C. Perfusions were conducted using the single-pass mode, and hepatic outflow was collected from a cannula (14 GA Insyte IV Catheter, Becton Dickinson, Spain) inserted into the superior vena cava. Cannulation of the common bile duct (PE 10 tubing, Paton Scientific, South Australia) permitted collection of bile, the flow rate of which was determined gravimetrically assuming a specific gravity of 1. An initial stabilization period of 15 min was allowed before adding morphine to the inflow perfusion medium. The concentration of oxygen in inflow and outflow perfusion medium was determined at regular intervals using a Model 820 Dissolved Oxygen Meter (Orion, MA, USA). The viability of the IPRL was assessed during each perfusion by monitoring oxygen consumption (> 10 μmol min^{-1}), bile flow (> 5 μL min^{-1}), percent recovery of inflow perfusate (> 95%), and by the gross appearance of the organ. Information on organ viability was also provided retrospectively by the constancy of morphine elimination and M3G excretion at steady-state. Livers were weighed at the end of each experiment.

In the first series of experiments, four IPRL preparations were perfused with a constant inflow concentration of morphine (2·66 μM) for greater than 60 min and up to 100 min. These experiments were performed to define the metabolic profile of morphine, to assess the time needed to reach steady-state with respect to the concentration of morphine and its various metabolites in outflow perfusate and bile, and to determine the maximum duration of subsequent IPRL experiments. Inflow and outflow perfusate samples (10 mL) were collected 1, 2, 5 and 10 min after the addition of morphine and at 10 min intervals thereafter. Total bile was collected at 10-min intervals throughout.

An additional six IPRL experiments were conducted in which livers were perfused with two different inflow concentrations of morphine (1·33 and 2·66 μM), each for 30 min, in a balanced cross-over manner. Perfusate samples were collected at 20, 25 and 30 min (period 1) and 50, 55 and 60 min (period 2). Bile was collected at 5-min intervals throughout each perfusate collection period.

Analytical methods

The concentration of morphine in inflow perfusate, and morphine, normorphine and M3G in outflow perfusate was determine by HPLC, using a method based on that described by Milne et al (1991) with modifications which included those needed for the determination of normorphine. A mixture of perfusate (2 mL), internal standard solution (hydromorphone HCl, 100 μL of a 0·03 mM aqueous solution), and 0·5 M pH 9·3 bicarbonate buffer (5 mL) was applied at a rate of 2 mL min^{-1} to a C18 Sep-Pak cartridge (Waters Associates), previously conditioned with 10 mL methanol, 10 mL water and 1 mL 0·5 M bicarbonate buffer at 5 mL min^{-1} (fresh cartridges were used each day, but each cartridge could be re-used on any one day up to 14 times without a loss of performance). The cartridge was rinsed successively with 5 mL 5 mM pH 9·3 bicarbonate buffer and 0·5 mL water at 2 mL min^{-1}. The analytes were eluted over a 1-min interval with 1·5 mL methanol (discarding the first 0·2 mL), which was evaporated to dryness at 50°C using a vortex evaporator (Buchler Instruments, KS, USA).

Samples were reconstituted in 120 μL HPLC mobile phase (26% acetonitrile and 0·8 mM dodecylsulphate in 10 mM pH 2·1 sodium dihydrogen phosphate) and, after brief vortex mixing, 10 μL was injected onto the HPLC system, which consisted of a Model M45 pump, a Wisp 712 autosampler, a Nova-pak C18, 4 μm Radial-Pak cartridge, and a Model 481 UV detector (210 nm), all from Waters Associates. The detector signal was monitored by a C-R6A Chromatopak integrator (Shimadzu, Tokyo,

Japan). Recirculating mobile phase was delivered to the column isocratically at a flow rate of 0·9 mL min^{-1}. Typical retention times for M3G, normorphine, morphine and hydromorphone were 7·5, 18, 20 and 29 min, respectively. M6G, which had a retention time of 12 min, was not routinely monitored. The run time for each sample was 60 min due to the presence of late eluting endogenous compounds. Calibration curves (0·05–3 μM) for each analyte were linear ($r^2 > 0·998$) and repeated analysis of low, medium and high quality control samples indicated that the accuracy and precision of the assay were less than 10%.

The concentration in bile of M3G was determined by direct injection of 10 μL diluted bile (1 : 200 with water) onto the HPLC system described above, with external standardization. Calibration curves, containing M3G (2–20 μM) in diluted blank bile, were linear ($r^2 > 0·999$) and repeated analysis of quality control samples indicated that the accuracy and precision for the determination of M3G were less than 5%. Negligible quantities of morphine and normorphine could be detected in unhydrolysed bile samples and so these compounds were not routinely monitored. A metabolite tentatively identified as normorphine glucuronide (see below) appeared in bile and was determined as normorphine equivalents after complete hydrolysis with β-glucuronidase. To assess the validity of the indirect approach of analysis, this method was also used to determine the concentration in bile of M3G, and the results were compared with the corresponding concentrations measured directly, with reference to authentic M3G. Briefly, 100 μL diluted bile sample (1 : 200 with water) was added to 100 μL of a solution of β-glucuronidase (1 mg mL^{-1} in 0·1 M sodium acetate buffer, pH 5·0). After 18 h at 37°C, 10 μL incubation medium was injected onto the HPLC system. Calibration standards and quality control samples were prepared containing 0·5–20 μM morphine and normorphine in diluted blank bile. Repeated analysis of quality control samples containing known concentrations of M3G, which were included with each analytical run to assess the performance of the hydrolysis/HPLC method, indicated an accuracy and precision of less than 10%.

Data analysis

Model-independent pharmacokinetic parameters were calculated using data recorded after steady-state was reached. Inflow and outflow molar concentrations and biliary excretion rates during the steady-state period were averaged before inclusion into the following pharmacokinetic equations:

Availability of morphine (F):

$$F = \frac{\text{Morphine concn in outflow perfusate } (M_{out})}{\text{Morphine concn in inflow perfusate } (M_{in})} \quad (1)$$

Clearance of morphine (CL):

$$CL = E \times \text{Perfusate flow rate} \quad (2)$$

where E (extraction ratio) is 1 − F.

Partial clearance of morphine to M3G ($CL_{f,M3G}$):

$$CL_{f,M3G} = \frac{\text{Rate of M3G formation}}{M_{in}} \quad (3)$$

where the rate of M3G formation was assumed to be the sum of steady-state biliary excretion rate of M3G and the rate of recovery of M3G in outflow perfusate.

Partial clearance of morphine to other metabolites ($CL_{f,other}$):

$$CL_{f,other} = CL - CL_{f,M3G} \quad (4)$$

Biliary extraction ratio of hepatically-generated M3G (E_b):

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

All data are presented as mean ± s.d. Analysis of variance was used to test for changes in the disposition of morphine and its metabolites with respect to morphine inflow concentration.

Results

Experiments in which livers were perfused with a constant morphine concentration (2·66 μM) indicated that less than 10 min was required before steady-state was reached with respect to the concentration of morphine, M3G and normorphine in outflow perfusate. Although steady-state was maintained for a further 50–60 min, thereafter the outflow concentration of morphine tended to increase in an unpredictable manner in some preparations, indicative of a decline in organ viability. Subsequent experiments were therefore limited to 60 min after the addition of morphine.

After commencing the perfusion with morphine, the biliary excretion of M3G increased progressively for 15 min, but subsequently remained constant. Diluted bile, when injected onto the HPLC system, gave rise to a chromatographic peak which eluted immediately before, but was well resolved from, M3G. The growth of this peak after commencing perfusion with morphine followed similar kinetics to that of M3G and upon incubation of bile samples with β-glucuronidase for various lengths of time, both the M3G peak and the neighbouring peak progressively decreased in height and two new peaks, at the retention times of morphine and normorphine, emerged.

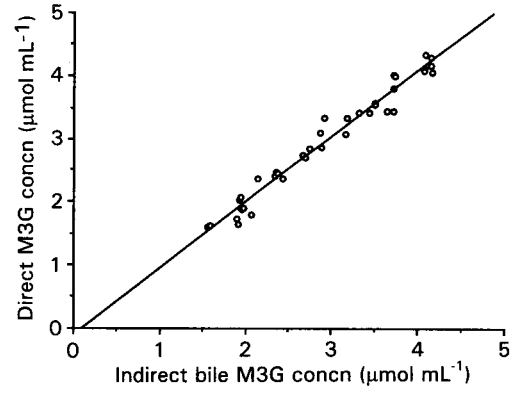

FIG. 1. Comparison of the biliary concentration of M3G determined directly with reference to authentic M3G with that determined indirectly as morphine equivalents after hydrolysis with β-glucuronidase. The slope of the linear regression line was 1·04 ($r^2 = 0·97$).

Table 1. Mean (± s.d.) kinetic parameters for morphine and M3G in the rat isolated perfused liver, calculated using data from outflow perfusate and bile samples collected between 20 and 60 min after the commencement of perfusion with morphine at a concentration of 2·66 μM. Perfusions were performed with protein- and erythrocyte-free perfusate delivered single pass at a rate of 30 mL min^{-1}.

Parameter	Rat 1	Rat 2	Rat 3	Rat 4	Mean ± s.d.
Body weight (g)	270	280	225	250	256 ± 24
Liver weight (g)	9·8	10·3	11·0	8·2	9·8 ± 1·2
Extraction ratio	0·87	0·79	0·93	0·88	0·87 ± 0·06
Availability	0·13	0·21	0·073	0·12	0·13 ± 0·06
Clearance (mL min^{-1})	26·2	23·8	27·8	26·3	26·0 ± 1·7
Partial clearance to M3G (mL min^{-1})	15·4	15·5	23·1	22·2	19·1 ± 4·2
Partial clearance to other metabolites (mL min^{-1})	10·8	8·3	4·7	4·1	7·0 ± 3·2
Mass balance (%)*	94	95	95	93	94·4 ± 0·94
Biliary extraction ratio of M3G	0·85	0·89	0·30	0·39	0·61 ± 0·31

* Mass balance was calculated as $\frac{\text{Rate of recovery of morphine metabolites}}{\text{Rate of morphine elimination}} \times 100$.

After 18 h, the two earlier peaks had disappeared completely, and the height of the peaks at the normorphine and morphine retention times had reached a plateau. The compound eluting before M3G was, therefore, tentatively identified as normorphine glucuronide. Further evidence of peak identity was obtained from the fact that the metabolite also appeared in perfusate and bile in a single experiment in which normorphine was recirculated through an IPRL system for 60 min (data not shown).

Because an authentic sample of normorphine-3-glucuronide or normorphine-6-glucuronide could not be obtained, the concentration in bile of the normorphine conjugate was determined as normorphine equivalents after complete hydrolysis with β-glucuronidase. To assess the performance of the hydrolysis conditions and of the indirect method of quantification, the concentration of M3G in bile was also determined indirectly as morphine equivalents after β-glucuronidase hydrolysis and the results compared with those obtained by direct analysis using authentic M3G. Linear regression of the data generated from these two methods gave rise to a line with a slope of 1·04 and a correlation coefficient of 0·97 (Fig. 1), indicating that both methods furnished virtually identical results. This finding also suggests that conjugated morphine excreted in bile was comprised almost exclusively of M3G.

Perfusate samples collected during perfusion with morphine gave rise to a small peak which eluted before M3G at the retention time of 'normorphine glucuronide'. However, this peak could not be completely resolved from background material present in blank perfusate, and in view of the fact that the area of the peak was small relative to that of unchanged normorphine, no attempt was made to quantify normorphine glucuronide in outflow perfusate samples.

Pharmacokinetic parameters describing the disposition of morphine in the IPRL (n = 4) at a nominal inflow concentration of 2·66 μM are presented in Table 1. The pharmacokinetic analysis was performed by standard techniques (eqns 1–5) using data from samples collected during the steady-state period (20–60 min after the commencement of infusion). Morphine was found to have a low availability (0·13 ± 0·06), and a clearance (26·0 ± 1·7 mL min^{-1}) which approached perfusion flow rate (30 mL min^{-1}). The biliary extraction ratio of hepatically-generated M3G, which is defined as the rate of recovery of M3G in bile relative to the total rate of M3G recovery via perfusate and bile, was 0·61 ± 0·31.

The steady-state recovery rate of morphine metabolites in outflow perfusate and bile was 94·4 ± 0·94% of the total rate of morphine elimination. Although M3G was the main metabolite, accounting for 72·8 ± 12·7% of eliminated morphine, a significant proportion (21·6 ± 13·5%) was *N*-demethylated to normorphine and recovered as unchanged normorphine in outflow perfusate

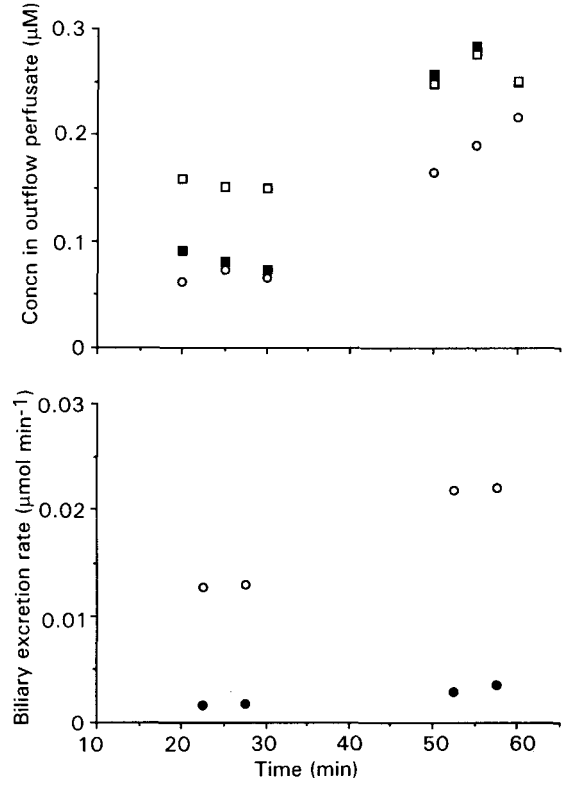

FIG. 2. Concentration of morphine (■), normorphine (□) and M3G (○) in outflow perfusate (upper panel) and biliary excretion of M3G (○) and normorphine glucuronide (●) (lower panel) during perfusion of an IPRL with perfusate (30 mL min^{-1}) containing 1·33 μM (period 1; 0–30 min) and 2·66 μM (period 2; 30–60 min) of morphine. Morphine was added to the perfusion medium at t = 0 following 15 min equilibration.

Table 2. Effect of concentration of morphine in inflow perfusate on the kinetics of morphine and M3G in rat isolated perfused liver (n = 6). Each liver was perfused at a morphine concentration of 1·33 and 2·66 μM in a balanced, cross-over manner. All data are presented as mean ± s.d.

Parameter	Morphine		Analysis of variance
	1·33 μM	2·66 μM	
Extraction ratio	0·89 ± 0·037	0·86 ± 0·037	$P < 0·05$
Clearance (mL min^{-1})	26·8 ± 1·1	25·9 ± 1·1	$P < 0·05$
Availability	0·11 ± 0·037	0·14 ± 0·037	$P < 0·05$
Partial clearance to M3G (mL min^{-1})	17·8 ± 3·8	18·0 ± 6·3	NS
Partial clearance of morphine to other metabolites (mL min^{-1})	9·58 ● 3·6	10·6 ± 3·7	NS
Biliary extraction ratio of M3G	0·52 ± 0·10	0·53 ± 0·18	NS

NS, not significantly different.

(11·0 ± 6·5%) and normorphine glucuronide in bile (10·2 ± 7·7%). Unidentified metabolites probably accounted for the remaining 5·6% of eliminated morphine.

To determine whether livers were operating under linear conditions at an inflow concentration of 2·66 μM, a further six livers were perfused with two different inflow morphine concentrations (1·33 and 2·66 μM, equivalent to 0·5 and 1·0 μg mL^{-1} morphine HCl·3H$_2$O) in a balanced, cross-over manner. Results from a representative experiment are presented in Fig. 2, and mean pharmacokinetic parameters for each of the two inflow concentrations are presented in Table 2. Availability was significantly higher at a perfusate concentration of 2·66 μM ($P < 0·05$) but the magnitude of the difference (27%) was small. Morphine inflow concentration also had a significant effect on the extraction ratio and clearance of morphine, although in these cases, the mean difference was less than 5%. The partial clearances of morphine to M3G and other metabolites, and the biliary extraction ratio of M3G, were not influenced by morphine inflow concentration (Table 2).

Discussion

In the present study, the rat isolated perfused liver was used to investigate the hepatic metabolism of morphine and the fate of hepatically-formed morphine metabolites, and, in keeping with previous reports (Imamura & Fujimoto 1980; Auansakul & Vore 1982; Sweeney et al 1984; Callaghan et al 1993), morphine was found to have a high hepatic extraction ratio and a clearance which approached perfusate flow rate (Table 1). The results are also in keeping with the high hepatic extraction ratio of morphine in man and other species (Iwamoto & Klaassen 1977; Kreek et al 1978; Rane et al 1984; Mistry & Houston 1987; Milne et al 1993).

In addition to M3G, significant quantities of normorphine and normorphine glucuronide were formed by the IPRL. Normorphine has been identified as a metabolite of morphine in several species, including rats (Klutch 1974; Yeh et al 1979) and man (Glare et al 1990). Although normorphine is less potent than morphine as an analgesic agent (Lasagna & de Kornfeldt 1958), normorphine-6-glucuronide is substantially more potent than morphine (Oguri et al 1989). In addition, normorphine-3-glucuronide, although a weak analgesic, causes convulsions in mice (Oguri et al 1989). In rats, the natural (laevorotatory) isomer of morphine undergoes glucuroconjugation exclusively at the phenolic 3-hydroxy group, forming M3G, with negligible quantities of the 6-hydroxy conjugate (M6G) being produced (Rane et al 1985; Coughtrie et al 1989). It may be expected, therefore, that the glucuronide moiety of normorphine glucuronide formed by the IPRL would be attached to the 3-position of the normorphine nucleus. However, we are not aware of any study which has characterized the isomeric composition of rat normorphine glucuronide.

The extent of morphine *N*-demethylation in the IPRL was assessed by comparing the rate of normorphine and normorphine glucuronide recovery with the rate of morphine elimination. Thus, it was found that 21·6 ± 13·5% of eliminated morphine underwent *N*-demethylation, compared with 72·8 ± 12·7% which underwent 3-glucuronidation. The estimate of *N*-demethylation is likely to be a slight underestimate because normorphine glucuronide in outflow perfusate was not quantified. In any case, the fact that 94·4% of eliminated morphine could be accounted for, indicates that any other morphine elimination routes were minor. Interestingly, the relative rates of morphine *N*-demethylation and glucuronidation in the IPRL compares well with the findings of Rane et al (1985), who reported that the rate of morphine *N*-demethylation by microsomes prepared from livers of male Sprague-Dawley rats was 20% of the rate of M3G formation.

Previous studies on morphine disposition in the IPRL made no attempt to specifically quantify morphine *N*-demethylation. Fujimoto and colleagues (Imamura & Fujimoto 1980; Sweeney et al 1984) used [*N*-^{14}C]-morphine to study the fate of morphine in IPRLs of male Sprague-Dawley rats. In order to differentiate between radiolabelled morphine and M3G in outflow perfusate and bile, [*N*-^{14}C]morphine was separated from [*N*-^{14}C]M3G by solvent extraction before radiochemical analysis. If *N*-demethylation had occurred within their IPRL experiments, stoichiometric amounts of radiolabelled formaldehyde and carbon dioxide would have been produced. These materials may have eluted from the liver and contributed to measured radioactivity. Brock & Vore (1982) also used [*N*-^{14}C]morphine to study the fate of morphine in the IPRL, except in this case livers were obtained from female Sprague-Dawley rats. It is well recognized that the *N*-demethylation of many drugs, including morphine, exhibits substantial sex differences in the rat, and recently it was found that the activity of male rat microsomal morphine-*N*-

demethylase is 22 times greater than that in female liver microsomes (Blanck et al 1990). In keeping with these findings, we have also observed significant sex differences in the oxidative metabolism of morphine in the IPRL, with female rats producing negligible quantities of normorphine or normorphine glucuronide (Shanahan & Evans 1993).

To determine whether livers were operating under linear conditions with respect to the disposition of morphine and its metabolites, six perfusions were conducted at two different morphine inflow concentrations (1·33 and 2·66 μM). Although the extraction ratio of morphine was significantly higher at an inflow morphine concentration of 1·33 μM, the magnitude of the difference was less than 5%. The partial clearance of morphine by glucuronidation and other routes, and the biliary extraction of M3G, were unaffected by substrate concentration. Hence, the extraction of morphine, the production of morphine metabolites, and the biliary excretion of M3G, were essentially concentration-independent. However, it needs to be considered that for high extraction drugs, such as morphine, extraction ratio (and clearance) is a relatively poor indicator of subtle changes in organ enzyme activity, or organ intrinsic clearance. In assessing such changes, availability is a more sensitive parameter. It is significant, therefore, that the availability of morphine increased significantly, by 27%, when the inflow concentration was increased to 1·0 $\mu g\,mL^{-1}$. This suggests partial saturation of morphine metabolism.

In conclusion, morphine was efficiently eliminated by male rat livers isolated and perfused with erythrocyte- and albumin-free medium. M3G was the main metabolite, accounting for 73% of eliminated morphine. Hepatically-generated M3G was extensively (61%) excreted in bile. About 22% of eliminated morphine was *N*-demethylated to normorphine, which appeared unchanged in outflow perfusate and as normorphine glucuronide in bile. The results suggest that the IPRL may be a useful model for examining the effects of physiological, pathological and pharmacological factors on the oxidative and conjugative metabolism of morphine and the subsequent handling of the hepatically-formed metabolites.

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