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Evidence for Reversible Sequestration of Morphine in Rat Liver

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ABSTRACT. The residence of morphine in the systemic circulation is prolonged despite a high systemic clearance, suggestive of significant extravascular sequestration. The present study was conducted to test the hypothesis that morphine binds significantly in tissues, and that the liver plays an important role in morphine binding. [^{14}C]Morphine was administered to male Sprague–Dawley rats 55 min before unlabeled morphine or saline. Blood ^{14}C increased immediately after injection of unlabeled morphine; the area under the blood concentration–time curve (AUC) for ^{14}C increased ~2-fold after morphine compared with saline injection. Residual radioactivity in the liver was lower in morphine-treated rats than in controls, suggesting that unlabeled drug displaced [^{14}C]morphine (or a metabolite) from binding sites. To examine this phenomenon more directly, a recirculating isolated perfused liver system was employed. [^{14}C]Morphine was added to the perfusate reservoir 15 min before unlabeled morphine or saline; perfusate and bile samples were collected for 120 min. Upon termination of perfusion, the liver was fractionated to identify the hepatic subcellular fraction(s) in which morphine was sequestered. The perfusate AUC for [^{14}C]morphine was increased ~2-fold in response to unlabeled drug, consistent with the *in vivo* experiment. Morphine was associated preferentially with the cytosolic fraction, and [^{14}C]morphine in all relevant fractions was reduced after administration of unlabeled morphine. In contrast, unlabeled drug had no influence on derived [^{14}C]morphine-3-β,D-glucuronide. These data are consistent with significant, reversible binding of morphine in hepatic tissue. BIOCHEM PHARMACOL 52;4:535–541, 1996.

KEY WORDS. morphine; morphine-3-β,D-glucuronide; extravascular binding; hepatic uptake; isolated perfused liver; subcellular distribution

Morphine disposition in the rat is characterized by a relatively long terminal half-life, despite the fact that the systemic clearance of morphine is high [1]. This slow terminal elimination phase is the result of a large apparent volume of distribution associated with a peripheral pharmacokinetic compartment. Large peripheral volumes of distribution typically are due to extravascular partitioning (e.g. between blood and adipose tissue, as has been observed for lipophilic compounds such as chlorpromazine [2]), extravascular sequestration (e.g. due to specific binding to tissue proteins for compounds such as digoxin [3]), or reversible metabolic processes in which the derived metabolite serves as an apparent "distributional compartment" (e.g. enterohepatic recirculation for compounds such as valproic acid [4]).

One or more of these distributional or metabolic mechanisms may be important in the disposition of morphine. On the basis of lipophilicity (log octanol/water partition coefficient of 0.75), morphine would not be expected to partition avidly into adipose or other tissues with a high lipid content [2]. However, morphine undergoes enterohepatic recirculation in rats [5] via formation, biliary excretion, and intestinal hydrolysis of the primary metabolite M3G.† The majority (>75%) of radiolabeled morphine administered to rats with exteriorized bile flow was recovered in bile and urine as M3G or unchanged morphine; the remaining radioactivity was in the form of unidentified metabolites [6]. Morphine-6-β,D-glucuronide is not formed to a measurable extent in the rat [7]. Enterohepatic recirculation prolongs the residence of morphine in the systemic circulation, and it may contribute to the relatively long terminal half-life of the parent compound [5]. Although the influence of specific extravascular binding on the systemic disposition of morphine is unknown, the potential for significant binding exists since opioid receptors are present in a variety of tissues, including the liver [8, 9].

The present project was undertaken to test the hypothesis that morphine is bound reversibly in extravascular tissue, and that the liver plays a significant role in this process. *In vivo* experiments were performed to document the presence of apparent extravascular binding of morphine in the

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† *Abbreviations:* AUC, area under the concentration–time curve; and M3G, morphine-3-β,D-glucuronide.

rat. The experimental approach selected, which is based on the ability of a large dose of unlabeled drug to displace a tracer dose of labeled substrate from binding sites in tissues, has been used previously for examination of *in vivo* receptor binding [10]. Additional experiments in the isolated perfused rat liver were conducted to determine whether intrahepatic binding appeared to be a significant contributor to this phenomenon, and to identify the subcellular compartment(s) that plays a role in hepatic sequestration of morphine.

MATERIALS AND METHODS

Chemicals

Morphine sulfate was purchased from Research Biochemicals, Inc. (Natick, MA). [^{14}C]Morphine was synthesized by DuPont New England Nuclear (Boston, MA), and was greater than 99% pure by HPLC. All other chemicals were obtained from commercial sources, and were of the highest purity available.

Animals

Male Sprague–Dawley rats (Hilltop Laboratory Animals, Scottdale, PA) were used in all experiments. Rats were housed individually in hanging wire cages, and were maintained on a 12-hr light/dark cycle with free access to food and water prior to experimentation.

Disposition of [^{14}C]Morphine In vivo

Silicone rubber cannulae were implanted in the right jugular and right femoral veins of ether-anesthetized rats (275–350 g; N = 4 per group) 24 hr prior to the experiment. [^{14}C]Morphine (20 μCi/kg; 0.5 mg/kg) was injected as a bolus through the femoral vein cannula. Unlabeled morphine (10 mg/kg; 1 mL) or saline (1 mL/kg) as a control was injected through the femoral vein cannula at 55, 65, 75, and 85 min after administration of the labeled compound. A divided administration protocol was used to avoid severe respiratory depression associated with morphine bolus doses exceeding 10 mg/kg. Blood samples (0.1 mL each, N = 22 per animal) were withdrawn through the jugular vein cannula at timed intervals for 6 hr. The liver was perfused with saline and harvested at the end of the experiment for determination of residual radioactivity.

Disposition of [^{14}C]Morphine in the Isolated Perfused Rat Liver

Livers obtained from male rats (220–250 g) were isolated and perfused according to standard techniques [11]. Perfusate [Krebs–Ringer bicarbonate buffer (pH 7.4), 1% (w/v) dextrose, and 20% male rat donor blood containing heparin, 1000 U/mL; 80 mL total volume] was oxygenated with O_2:CO_2 (95:5) and was delivered to the organ at a rate of 20 mL/min. Perfusate pH was monitored continuously throughout the experiment, and was adjusted by addition of saturated sodium bicarbonate to maintain pH = 7.4 ± 0.1. Viability of the liver was assessed by examining bile flow, inflow perfusion pressure, and the gross appearance of the organ. The liver was allowed to equilibrate for 15 min prior to experimentation. [^{14}C]Morphine (50 μCi; 0.2 mg) was administered to the perfusate reservoir as a bolus at time 0, and perfusate samples (0.3 mL) were obtained from the reservoir at timed intervals for 105 min. Bile was collected continuously at 15-min intervals. A bolus dose of 2 mg unlabeled morphine (in 1 mL saline) or 1 mL saline as a control (N = 4 livers per treatment group) was added to the reservoir 15 min after administration of the label. At the end of the experiment, the liver was perfused with ice-cold sucrose (0.25 M), homogenized in 0.25 M sucrose (1:2, w/v), and fractionated into subcellular components (nuclear, mitochondrial, lysosomal, cytosolic, microsomal) by standard methods [12].

Morphine Analysis

Total ^{14}C in whole blood and liver tissue obtained in the *in vivo* experiment was determined by liquid scintillation spectroscopy after decolorization with an equal volume of H_2O_2 (blood) or digestion in 10 M KOH (2:1, w/v; liver). In samples obtained from the perfused liver experiments, morphine and its metabolites were quantitated by a specific HPLC assay with flow-through radiochemical detection. Proteins in biologic samples (100 μL) were precipitated by sequential addition of 50 μL each of zinc sulfate (0.1 M) and ammonium acetate (0.1 M); 75 μL of the resultant supernatant was injected on the column for analysis. Reverse-phase chromatographic separation was achieved on a C_6 column (150 × 4.6 mm, 5 μm packing material; Phase Separations, Norwalk, CT) with a mobile phase of 0.1% trifluoroacetic acid (92.5%) and acetonitrile (7.5%) delivered at 1 mL/min. Column effluent was mixed with scintillant (Flo-Scint III, Packard, Meriden, CT; 3 mL/min) for quantitation of ^{14}C. Drug and metabolite concentrations were estimated from a standard curve relating total counts in a peak to the radioactivity injected on-column.

Data Analysis

The partial AUC in whole blood or perfusate was estimated over selected intervals with the linear trapezoidal method. The AUC from time 0 through the time of administration of unlabeled morphine or saline ($AUC_{0\rightarrow50}$ or $AUC_{0\rightarrow15}$ for the *in vivo* and isolated perfused liver experiments, respectively) served as a baseline condition for both experimental groups. The AUC after administration of unlabeled morphine or saline ($AUC_{50\rightarrow370}$ and $AUC_{15\rightarrow105}$ for the *in vivo* and isolated perfused liver experiments, respectively) demonstrated the effect of the experimental treatment on the disposition of total radioactivity (intact animal) or the

pharmacokinetics of [^{14}C]morphine (isolated perfused liver). The influence of unlabeled morphine on the disposition of the labeled drug was examined by comparing the partial AUC from the time at which the unlabeled drug was administered through the end of the experiment across treatment groups. Mass balance analysis of data obtained in the isolated perfused liver experiment was performed to assess the effect of unlabeled morphine on the hepatobiliary disposition of [^{14}C]morphine and its derived metabolites. Mean parameter values for control and treated groups in both experiments were compared with the unpaired Student's *t*-test. Orthogonal linear least-squares regression analysis (i.e. not assuming error-free values of the independent variable [13]) was used to characterize the relationship between systemic morphine concentrations and residual radioactivity in the liver at the end of the *in vivo* experiment. In all cases, $P < 0.05$ was considered to be statistically significant.

RESULTS

Disposition of [^{14}C]Morphine In vivo

Representative blood ^{14}C concentration–time profiles after administration of [^{14}C]morphine with or without subsequent administration of unlabeled morphine are shown in Fig. 1. The mean difference in blood ^{14}C between groups at each time point, presumably representing ^{14}C that was displaced by unlabeled morphine, also is shown. Blood radioactivity was elevated after administration of unlabeled morphine as compared with saline; a statistically significant ($P < 0.05$) increase in blood ^{14}C was observed within 5 min after administration of the first bolus of unlabeled drug. The difference in circulating ^{14}C between groups was greater after the last dose of unlabeled morphine than after the preceding doses, and was sustained throughout the duration of sampling. Blood ^{14}C remained statistically higher in the unlabeled morphine group at the end of the experiment (275 min after the final dose of unlabeled drug). However, the difference in circulating ^{14}C between groups (i.e. the concentration of displaced ^{14}C) decreased with time, suggesting that the terminal rate of elimination of ^{14}C was more rapid in the unlabeled morphine group as compared with saline-treated controls. The AUC for blood ^{14}C after administration of unlabeled morphine or saline ($AUC_{50\rightarrow370}$) was approximately 60% higher than in the control group ($P < 0.05$; Fig. 2). No difference in the partial AUC prior to unlabeled morphine administration ($AUC_{0\rightarrow50}$) was noted between groups, indicating that the difference in $AUC_{50\rightarrow370}$ was due to treatment and not due to differences in the pre-treatment handling of [^{14}C]morphine between groups. At the end of the *in vivo* experiment, livers were harvested for determination of residual radioactivity. A statistically significant inverse relationship was observed between the partial AUC after administration of unlabeled morphine or saline and residual hepatic radioactivity (Fig. 3).

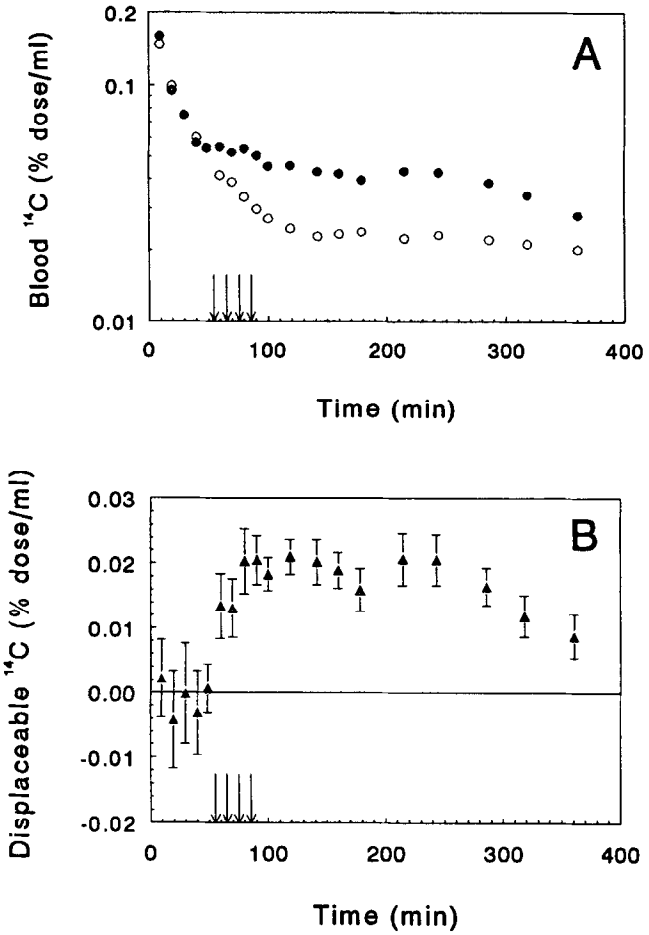

FIG. 1. Disposition of total ^{14}C in blood (panel A) after administration of [^{14}C]morphine to representative rats with unlabeled morphine (●) or saline (○) administered at 55, 65, 75, and 85 min (arrows). Panel B displays the difference in mean blood ^{14}C between experimental groups (with the estimated standard deviation, calculated from the variances for each group at each time point; N = 4 per group), representing displaceable ^{14}C.

Disposition of [^{14}C]Morphine in the Isolated Perfused Rat Liver

The apparent relationship between systemic and hepatic radioactivity was consistent with the hypothesis that morphine was sequestered in the liver and was displaced after administration of unlabeled morphine. To test this hypothesis more directly, the disposition of [^{14}C]morphine and the effect of unlabeled morphine on the disposition of labeled drug were examined in the isolated perfused rat liver with an assay procedure capable of separating [^{14}C]morphine and derived metabolites. This experiment allowed assessment of potential hepatic sequestration of morphine in the absence of extrahepatic effects and without ambiguity concerning the chemical specie(s) involved. The disposition of [^{14}C]morphine in perfusate was similar to that of total ^{14}C in blood *in vivo*; the [^{14}C]morphine concentration–time profile was biphasic, and perfusate concentrations of

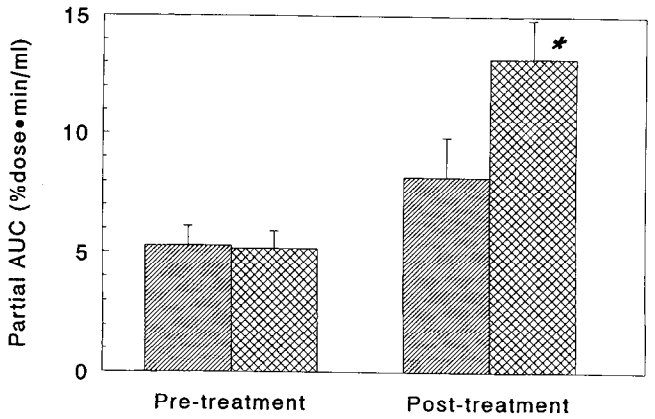

FIG. 2. Area under the blood ^{14}C versus time profile from 0–50 min (pre-treatment) and 50–370 min (post-treatment) after administration of [^{14}C]morphine in rats receiving co-administration of either saline (single-hatched) or unlabeled morphine (cross-hatched) beginning at 55 min after [^{14}C]morphine (see Fig. 1). Error bars indicate SD (N = 4 per group). Key: *$P < 0.05$, morphine versus saline.

[^{14}C]morphine were elevated in livers receiving unlabeled morphine as compared with saline-treated controls (Fig. 4). Parameters associated with concentrations of morphine and M3G in perfusate are compiled in Table 1. Addition of unlabeled morphine into the perfusate reservoir 15 min after administration of [^{14}C]morphine resulted in a nearly 2-fold increase in the $AUC_{15\rightarrow105}$ for [^{14}C]morphine. [^{14}C]Morphine concentrations 25 min after treatment with unlabeled morphine were approximately 2-fold higher as compared with the saline control group; the difference increased to >5-fold by 90 min. In contrast to the parent drug, administration of unlabeled morphine did not affect significantly the perfusate concentrations of [^{14}C]M3G at any time point.

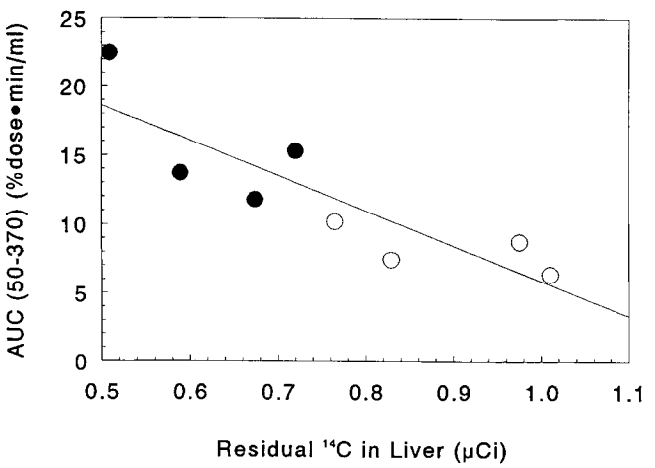

FIG. 3. Relationship between area under the curve after administration of saline (○) or unlabeled morphine (●) and residual ^{14}C in the liver 370 min after administration of [^{14}C]morphine. Line indicates the results of orthogonal linear least-squares regression ($y = 31.3 - 25.4x$, $r^2 = 0.731$, $P < 0.01$).

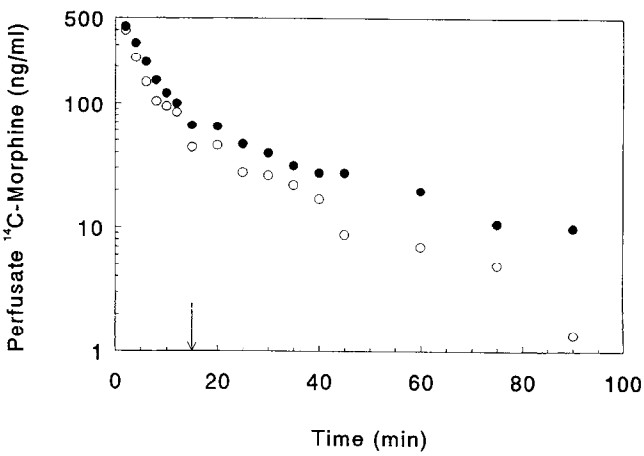

FIG. 4. Representative perfusate [^{14}C]morphine concentration versus time profile in isolated rat livers receiving unlabeled morphine (●) or saline (○) 15 min (see arrow) after administration of [^{14}C]morphine.

Mass balance analysis for the isolated perfused liver experiment is shown in Table 2. Administration of unlabeled morphine did not affect significantly the fraction of the dose remaining in perfusate (primarily as [^{14}C]M3G) at the end of the experiment. Similarly, total biliary excretion of ^{14}C (undifferentiated by chemical entity) and total recovery of label during the experiment were unaffected by administration of unlabeled morphine as compared with saline. However, consistent with the previous *in vivo* experiment, residual radioactivity in the liver at the end of the perfusion period was statistically lower ($P < 0.05$) in the unlabeled morphine group.

The distribution of [^{14}C]morphine and [^{14}C]M3G among subcellular fractions in the two treatment groups is shown in Fig. 5. Localization of [^{14}C]M3G exceeded that of [^{14}C]morphine in all fractions. The parent drug and the metabolite were associated primarily with the cytosolic fraction in both groups. Nuclear and mitochondrial localization of [^{14}C]morphine and [^{14}C]M3G was moderate in saline-treated livers; significant quantities of [^{14}C]M3G were observed in the lysosomal and microsomal fractions, while the labeled parent was not detected in these fractions. With the exception of the lysosomal and microsomal fractions, administration of unlabeled morphine resulted in a significant decrease in [^{14}C]morphine content throughout the subcellular fractions. In contrast, no statistically significant changes were observed in [^{14}C]M3G localization after administration of unlabeled morphine as compared with saline in any fraction examined.

DISCUSSION

The initial *in vivo* experiment performed as a part of this study suggested that the large apparent distributional space for morphine may be a function, at least in part, of specific binding of morphine at extravascular sites. The increase in circulating concentrations of ^{14}C observed after adminis-

TABLE 1. Influence of unlabeled morphine on the disposition of [^{14}C]morphine in the isolated perfused rat liver

Parameter	Saline		Unlabeled morphine	
	Morphine	M3G	Morphine	M3G
AUC$|_0^{15}$ (nCi · min/mL)	2847 ± 569	512 ± 302	3268 ± 379	525 ± 165
AUC$|_{15}^{105}$ (nCi · min/mL)	1553 ± 392	7186 ± 2330	2740 ± 460*	8970 ± 3576
AUC$|_0^{105}$ (nCi · min/mL)	4400 ± 769	7698 ± 2627	6007 ± 733*	9496 ± 3725
$C_{25\ min}$† (nCi/mL)	38.9 ± 9.4	112 ± 20	80.8 ± 12.9*	141 ± 21
$C_{90\ min}$† (nCi/mL)	<1.5	92.3 ± 32.7	7.32 ± 2.39*	93.7 ± 33.7

Data are presented as means ± SD for N = 4 per experimental group.
* Statistically different from the saline group, $P < 0.01$.
† Perfusate concentration at 25 or 90 min.

tration of a large dose of unlabeled drug (Fig. 1) is characteristic of a reduced apparent volume of distribution, and consistent with influx of substrate from extravascular sites into circulating blood. The fact that circulating concentrations of ^{14}C declined more rapidly after administration of unlabeled morphine than after saline (Fig. 1B) also is consistent with a reduced volume of distribution [14]. Furthermore, the liver appeared to play an important role in the retention of total radioactivity after an intravenous dose of [^{14}C]morphine. The strong statistical relationship between ^{14}C $AUC_{50\rightarrow370}$ and residual ^{14}C in the liver 370 min after administration of [^{14}C]morphine *in vivo* (Fig. 3) suggests significant hepatic sequestration of morphine-derived radioactivity.

Although the results of the *in vivo* experiment led to the hypothesis that specific binding of morphine occurs in the liver, other phenomena could explain these observations. The apparent influx of ^{14}C into the systemic circulation after a large dose of unlabeled morphine could be due to displacement from non-hepatic tissues; the relationship between systemic ^{14}C and residual hepatic ^{14}C could merely be fortuitous. More importantly, the analytical method used in this experiment was non-specific, and could not differentiate between morphine, M3G, and other metabolic products. To address these deficiencies, a parallel experiment was performed in the isolated perfused rat liver preparation, therefore removing extrahepatic influences from consideration. An HPLC assay employing radiochemical detection to separate and quantitate morphine, M3G, and other derived metabolites was utilized in the perfused liver experiments.

TABLE 2. Mass balance (% of dose) for [^{14}C]morphine in the isolated perfused rat liver

Component	Saline	Morphine
[^{14}C]Morphine in perfusate (105 min post-dose)	ND*	0.85 ± 0.64
[^{14}C]M3G in perfusate (105 min post-dose)	13.2 ± 5.1	17.4 ± 8.8
Total ^{14}C in bile (0–105 min post-dose)	37.9 ± 7.0	38.2 ± 13.7
Total ^{14}C in liver (105 min post-dose)	36.0 ± 4.1	23.9 ± 8.8†
Total recovery of ^{14}C	87.1 ± 9.6	80.4 ± 18.5

Data are presented as means ± SD for N = 4 per experimental group.
* Below limit of detection of the assay.
† Statistically different from the saline group, $P < 0.05$.

In the isolated perfused rat liver, administration of unlabeled morphine resulted in a decrease in hepatic radioactivity (Table 2) and a concomitant increase in perfusate $AUC_{15\rightarrow105}$ of morphine, but not that of the primary metabolite M3G (Table 1). [^{14}C]Morphine was localized predominantly in the hepatic cytosolic fraction (Fig. 5); the fact that the cytosolic content of [^{14}C]morphine was significantly lower after treatment with unlabeled morphine as compared with saline suggests that cytosolic [^{14}C]morphine may have been displaced from binding sites by unlabeled morphine. Content of [^{14}C]morphine in the nuclear and mitochondrial fractions, although low compared with the cytosolic fraction, also was decreased after administration of unlabeled morphine. [^{14}C]M3G also was localized predomi-

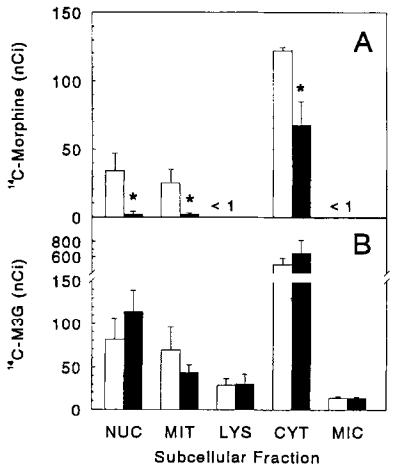

FIG. 5. Distribution of [^{14}C]morphine (panel A) and [^{14}C]M3G (panel B) in subcellular fractions prepared from isolated perfused rat livers (NUC = nuclear, MIT = mitochondrial, LYS = lysosomal, CYT = cytoplasmic, MIC = microsomal). Livers were fractionated 120 min after a dose of [^{14}C]morphine to the perfusate reservoir; saline (hatched bars) or unlabeled morphine (closed bars) was added to the reservoir 15 min after [^{14}C]morphine (N = 4 livers per experimental group). Error bars indicate SD. Key: *$P < 0.05$, unlabeled morphine versus saline.

nantly in the cytosolic fraction. However, no effect of unlabeled morphine on the metabolite was evident in any fraction (Fig. 5). Similar observations have been reported for the gastric acid pump inhibitor omeprazole [15] which, like morphine, is a weak base. Omeprazole is taken up rapidly into the isolated perfused rat liver ($T_{1/2}$ < 5 min). At distribution equilibrium, the partition coefficient between liver and perfusate is approximately 10, suggestive of significant sequestration in the organ. Subcellular fractionation revealed that the majority of the drug is localized in hepatic cytosol, and suggested that the rapid net removal of omeprazole from perfusate is due to binding to cytosolic proteins.

Comparison of the partial AUC after administration of unlabeled morphine or saline, corrected for the pre-treatment difference in partial AUC between treatment groups, allows assessment of the degree of displacement of [^{14}C]morphine by the unlabeled drug. This calculation, which can only be performed on group-averaged data, also allows comparison of the degree of displacement *in vivo* with that in the isolated perfused liver. In the *in vivo* experiment (Fig. 2), the 50- to 370-min AUC was 61.1% higher in the unlabeled morphine group than in the saline-treated controls (13.2 vs 8.19% dose · min/mL, respectively). When corrected for differences in the pre-treatment (0–50 min) partial AUC (5.17 vs 5.28% dose · min/mL, respectively), the post-treatment partial AUC was 62.4% higher in the unlabeled morphine group than in the controls. Similar calculations performed for the isolated perfused liver experiment, based on the mean partial AUC data presented in Table 1, revealed that the post-treatment partial AUC (15–105 min) was 66.6% higher in the unlabeled morphine group than in the saline controls. While the similarity in the extent of apparent [^{14}C]morphine displacement between the two experiments does not prove that the liver is the only site of extravascular binding, it does lend further support to the hypothesis that the liver plays a significant role in this process.

The uptake of morphine into isolated rat hepatocytes has been shown to proceed against a significant concentration gradient and to be saturable [16]. Saturable substrate flux against a concentration gradient may be due to either an active transport process or significant intracellular binding. Under most circumstances, it is the unbound substrate on either side of the cellular membrane that dictates the kinetics of translocation. When only total (bound plus unbound) intracellular concentrations are considered, passive diffusion across the membrane might appear to both proceed against a concentration gradient and be saturable if intracellular binding is significant and concentration dependent. The previous observations regarding morphine uptake into hepatocytes *in vitro*, therefore, do not contradict the proposed mechanism of hepatic morphine sequestration; indeed, such behavior in isolated cells would be predicted based upon the presence of a capacity-limited intracellular binding process.

Several studies have indicated that the liver may serve as a depot for a variety of xenobiotics; in some cases, hepatic sequestration may affect significantly the systemic disposition of the compound. In the isolated perfused rat liver, uptake of the tricyclic antidepressant imipramine is rapid and concentration dependent; release of imipramine into perfusate from pre-loaded livers is slow despite a large concentration gradient [17]. The kinetics of hepatic uptake and release of imipramine are consistent with the presence of both saturable and non-saturable binding within the organ. The liver appears to account for a significant fraction of the total distributional space for the antifungal agents ketoconazole [18] and fluconazole [19]; in the case of ketoconazole, the contribution of the liver to the apparent volume of distribution decreases with increasing dose, suggestive of saturable sequestration. In intact rats, the hepatic content of trospectomycin accounts for approximately 10% of the total body load of the drug; in the isolated perfused rat liver, hepatic trospectomycin is eliminated slowly into bile with little sinusoidal translocation back into perfusate [20]. In contrast to the antifungals and morphine, the apparent binding of trospectomycin does not appear to be saturable.

Significant binding of morphine in hepatic tissue may not be surprising in light of the biologic activity of many opioid compounds in the liver. For example, opioid peptides have been shown to increase glycogenolysis and gluconeogenesis in isolated rat hepatocytes [21–23]. Moreover, endogenous peptides with opioid activity, including (met)-enkephalin and β-endorphin, have been identified in hepatic tissue [9]. While the physiologic role of these peptides has yet to be determined, they may be involved in glucose homeostasis. Opioid activity in hepatocytes suggests the presence of opioid receptors; such receptors may be responsible, at least in part, for the apparent binding of morphine in the liver.

Although the present study indicates that specific binding of morphine occurs in the liver, other tissues contribute significantly to extravascular sequestration of morphine. For example, physiologically based pharmacokinetic modeling of morphine distribution in the pregnant rat has shown that a significant fraction of the total morphine body load is associated with the maternal skeletal muscle [24]. Whether this association reflects non-specific partitioning (therefore resulting in non-displaceable morphine in skeletal muscle) or specific binding to proteins or other macromolecular components is unknown.

In summary, results of the present experiments suggest that a specific binding site for morphine, possibly due to a cytosolic protein, exists in rat liver. The nature of this binding site and the contribution of intrahepatic binding to overall systemic disposition and first-pass loss of morphine require further investigation.

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