PHYSIOLOGICAL DISPOSITION OF I.V. MORPHINE IN SHEEP†


SUMMARY

In a crossover design study we have measured the total body and regional clearances of morphine. Thirteen experiments were performed in four conscious sheep that had been prepared previously with appropriate intravascular cannulae. Morphine (as sulphate pentahydrate) was infused i.v. at 2.5, 5, 10 and 20 mg h⁻¹ to produce constant blood concentrations. Morphine (base) concentrations were measured in blood, urine and tissues with a specific HPLC method. The mean (SEM) total body clearance of morphine was 1.63 (0.21) litre min⁻¹; this comprised 1.01 (0.10) litre min⁻¹ clearance by the liver and 0.55 (0.06) litre min⁻¹ by the kidneys. There was no evidence of dose-dependent clearance or significant extraction of morphine by the lungs, brain, heart, gut or hindquarters at any dose. The kidney clearance of morphine was greater than the 0.21 (0.06) litre min⁻¹ renal clearance determined from the product of the mean total body clearance and the 12.3 (2.4)% of the administered dose recovered as unmetabolized morphine from 48 h urine collection (P < 0.05). It was concluded that the liver and kidneys account for the majority of morphine clearance, and that the kidneys both excrete and metabolize morphine.

KEY WORDS


The physiological disposition of morphine is poorly understood, despite its long history and widespread clinical use. As little morphine is excreted unmetabolized into urine (approximately 10% of the dose [1]) it has been taught that the liver is the primary organ for elimination of morphine from the body. Biotransformation of morphine by conjugation with glucuronic acid, producing morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), is known to comprise the principal metabolic pathway before excretion of these metabolites into urine [2]. However, over the past 6 years, the role of the kidney in morphine clearance has been the subject of much contention. The early report of a decreased rate of morphine elimination in renal transplant patients that drew attention to this association [3] is now considered inaccurate because of unintentional simultaneous measurement in plasma of both morphine and M6G [4]. Nevertheless, there is indirect evidence of extrahepatic clearance of morphine. In some human pharmacokinetic studies, it has been found that morphine total body clearance may approach or even exceed the putative liver blood flow [5-8]. This is supported by observation of the similarity in morphine clearances in healthy subjects and in patients with hepatic cirrhosis [9]. Moreover, it is

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known that human kidney microsomes [10] are capable of metabolizing morphine to M3G and M6G, although the relevance of this finding to the disposition of morphine in vivo is not clear.

In most reports to date, the pharmacokinetics of morphine have been determined from peripheral or central venous plasma concentrations. This method of pharmacokinetic analysis, however, does not permit simultaneous investigation of the involvement of individual body regions. To examine the involvement of individual regions in the clearance of morphine, we carried out experiments in sheep with multiple intravascular cannulae so that the systemic and regional clearances could be determined simultaneously.

MATERIALS AND METHODS

Experimental design

Approval was given by the institutional Ethics Review Committee for this study to be performed in sheep prepared previously with chronic intravascular catheters to enable drug infusion, cardiovascular monitoring and regional blood sampling for blood flow indicator and morphine analyses. From these data, the systemic and regional clearances of morphine were measured. Adult merino ewes of mean (SD) weight 48 (8) kg were studied.

Animal preparation

The general methods and materials used for animal preparation have been described in detail in previous publications from this laboratory [11, 12]; the methods specific to the present study are described here briefly. Under general anaesthesia, a left-sided thoracotomy was performed to ligate the hemi-azygos vein which, in the sheep, contributes systemic venous blood to the coronary sinus. Intra-abdominal catheters were placed directly into the abdominal aorta (distal to the renal artery and proximal to the iliac bifurcation), mesenteric and portal veins via, respectively, left paravertebral and right subcostal skin incisions. After surgical exposure of the vessels in the neck, two arterial catheters were placed in the right carotid artery with their tips positioned approximately 2 cm above the aortic valve. Catheters were placed in the right jugular vein with their tips in the coronary sinus, left renal vein, posterior vena cava, right hepatic vein and right atrium (two catheters), and a flow directed thermodilution catheter was placed with its tip in the pulmonary artery. An additional catheter was placed in the sagittal sinus via a frontal incision and a trephine hole in the parietal bone. At least 1 week was allowed for the sheep to recover from surgery before the first experiment was performed.

Blood flow measurements

Cardiac output was measured using the pulmonary artery thermodilution catheter after injection of ice cold 0.9% saline 10 ml into a right atrial catheter. Gut and hindquarter blood flows were measured by indicator dilution using infusion into the mesenteric vein and abdominal aorta, respectively, of the indicators sodium para-aminohippurate (PAH) and sodium bromosulphophthalein (BSP) [12]. Liver and kidney blood flows were measured at times similar to those for gut and hindquarter blood flow determinations by the Fick method using, respectively, BSP and PAH. Steady state blood concentrations of the indicators were achieved with two-stage infusions using a constant rate infusion pump with individually calibrated glass syringes: a 15-min loading dose of BSP 32 mg min⁻¹ and PAH 8 mg min⁻¹ was followed by maintenance infusions of BSP 16 mg min⁻¹ and PAH 4 mg min⁻¹. After 45 min of maintenance infusions, simultaneous ascending aortic and appropriate regional venous blood sample sets (0.4 ml) were obtained at 15-min intervals for a further 60 min. Gut and hindquarter blood flows were calculated from the quotients, respectively, of the PAH and BSP maintenance dose rates and the appropriate venous–arterial concentration difference. Liver and kidney blood flows were calculated from the quotients, respectively, of the BSP and PAH maintenance dose rates and the appropriate arterial–regional venous concentration differences [12].

Morphine administration and sample collection

Two-stage infusions of morphine were made into a right atrial catheter: loading infusions of 8 times the maintenance rate were made over 15 min and maintenance doses of morphine (as sulphate pentahydrate) 2.5, 5, 10 and 20 mg h⁻¹ were infused for 345 min. This regimen was determined from computer simulation from preliminary data obtained by right atrial infusion of morphine 10 mg/10 min in one animal. Traditional three-compartment open model analysis of the resultant arterial blood morphine concen-
Concentration-time data collected for 4 h indicated a mean total body clearance of 2.16 litre min⁻¹, a steady state volume of distribution of 86 litre and a slow half-life of 82 min. Four experiments, performed in random order at least 3 days apart at each of the four morphine infusion rates, were planned in each of four animals to assess the potential dose-dependence of total body and regional clearances of morphine but three experiments were not completed because of catheter failure.

The last 60 min of the morphine maintenance infusion was regarded as the "steady state" period. During this period, five sets of 1.0-ml blood samples were obtained simultaneously at 15-min intervals from the aortic, pulmonary artery, sagittal sinus, coronary sinus, hepatic, portal, renal and posterior vena cava catheters for measurement of blood concentrations of morphine, together with cardiac output and liver, kidney, gut and hindquarter blood flows by methods described above. Urine was collected from the start of morphine infusion by indwelling catheter from 0-6 h and from 6-24 and 24-48 h by free flow for measurement of morphine concentrations in eight experiments. In pilot studies, it had been shown that hydrolysis of morphine conjugates did not occur under the conditions of collection and storage.

Terminal experiments

The last experiment at each dose rate was a terminal experiment for the measurement of morphine tissue:blood partition coefficients. After completion of the blood sampling period, the animal was killed immediately with a bolus of saturated potassium chloride solution 30 ml, at which time the morphine infusion was stopped. Tissue samples were obtained within 10 min from the whole brain, lung, left ventricle, liver, small bowel, kidney, hindquarter muscle and fat for morphine analysis.

Morphine concentration analysis

Blood, urine and tissue samples were stored at -20 °C until batch assay to measure morphine concentrations (as base) by normal phase high performance liquid chromatography (HPLC) after solvent extraction [13]. The method, which is selective for morphine, involves internal standardization with hydromorphone, selective pH extraction and elution followed by optimized electrochemical detection to provide high sensitivity (to 0.5 ng ml⁻¹), linearity ($r^2 > 0.998$ from 5 to 500 ng ml⁻¹) and reproducibility (coefficients of variation, respectively, 2.4 and 2.7% for 20 and 200 ng ml⁻¹, $n = 15$ each).

Data analysis

The total body clearance ($Cl$) of morphine was obtained from the ratio of the maintenance infusion rate to the mean pulmonary artery blood concentration of morphine during the steady state period. Pulmonary arterial, rather than aortic, blood morphine concentrations were used in the calculation of $Cl$, as only this calculation remains correct should there be lung clearance. However, upon retrospective analysis, there was no systematic difference when $Cl$ was calculated from aortic blood concentration of morphine ($P > 0.05$, Student's $t$ test for paired data). The regional clearances of morphine were determined directly from the relevant products of the regional extraction ratios and regional blood flows [12]. In addition, the renal clearance of morphine was determined indirectly from the product of the mean total body clearance and the fraction of dose excreted by 48 h into urine. The results of the indicator dilution measurement of gut blood flow were considered to be unreliable because of apparent streaming of the PAH; gut blood flow was estimated therefore as 80% of liver blood flow [11, 14]. Brain and coronary blood flows were estimated, respectively, as 2% of cardiac output [14] and 1.09 ml min⁻¹/g of sheep heart [15].

Morphine tissue:blood partition coefficients were determined at steady state from the terminal experiments using, for reference, the relevant mean arterial concentration during the steady state period. The calculated partition coefficient, $kp = C_T/C_B$ (where $C_T$ and $C_B$ are the morphine concentrations in tissue and blood, respectively), assumes the lack of clearance from tissues [16]. For tissues in which clearance occurred, the partition coefficient was determined as $K_p = (1 + C_T/Q_T) C_T/C_B$ (where $C_T$ is tissue clearance, and $Q_T$ is tissue blood flow) [16]. The median partition coefficients were combined with the median blood flow and clearance data to simulate the time course of blood, liver, gut, kidney, muscle concentrations and urinary excretion of morphine [16].

The relationships between dose and morphine systemic and regional kinetics were examined using multiple regression by partitioning the dose effects into linear, quadratic and cubic trend
MORPHINE DISPOSITION IN SHEEP

Fig. 1. A: Blood concentrations of morphine during the last 60 min of a 345-min maintenance infusion in sheep No. 4. A loading infusion of 20 mg h⁻¹ was administered for 15 min followed immediately by 2.5 mg h⁻¹ for 345 min. ● = Aorta; ○ = pulmonary artery; △ = sagittal sinus; □ = coronary sinus; ▲ = hepatic vein; ▽ = portal vein; ■ = renal vein; ◐ = posterior vena cava. B: Regional extraction ratios (ER) of morphine calculated from the morphine blood concentration data. In this study, significant (*P < 0.05) extraction was found only across the liver and kidneys. C: Cardiac output (CO) and regional blood flows during the period of blood sampling described above. ○ = Cardiac output; ▲ = hepatic blood flow; ■ = renal blood flow; ◐ = hindquarter blood flow. D: Cardiovascular variables during the period described above. ○ = Mean arterial pressure (MAP); ▲ = mean heart rate (HR).

components [17]. There was no evidence of dosedependent effects so the data for the different infusion rates in each of the animals were combined. The mean regional extraction ratios and clearances were examined by the one sample t test to determine if they were significantly different from zero. Other two-way comparisons were made using t tests for paired data. For all tests, P < 0.05 was considered statistically significant.

RESULTS

Data from a representative study (at the 2.5-mg h⁻¹ dose rate) are shown in figure 1. Cardiac output, regional blood flows, mean heart rate or mean arterial pressure did not alter systematically during morphine administration at any dose rate. The total body and regional clearances of morphine were independent of dose rate across the 8-fold range of morphine maintenance infusion rates: the mean data for each animal were therefore combined. The overall mean (SEM) morphine Cl was 1.63 (0.21) litre min⁻¹. With the exception of the liver and kidneys, the mean arterial–regional venous concentration gradients were small: the mean regional extraction ratios and clearances of morphine are presented in table I. There was no systematic evidence of dose dependent extraction of morphine in any region (fig. 2). The corresponding overall regional clearances were liver 1.01 (0.10) litre min⁻¹ and kidneys 0.55 (0.06) litre min⁻¹. There was no relationship between any clearance and total body weight.
TABLE I. Mean (SEM) regional extraction ratios and clearances of morphine during i.v. infusion at steady state. In the absence of regional dose independence, the data for each of the four animals at four different infusion rates were combined.

** P < 0.01 compared with a value of 0

<table>
<thead>
<tr>
<th>Region</th>
<th>Extraction ratio</th>
<th>Regional clearance (litre min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>0.05 (0.02)</td>
<td>0.29 (0.18)</td>
</tr>
<tr>
<td>Brain</td>
<td>−0.05 (0.04)</td>
<td>−0.01 (0.01)</td>
</tr>
<tr>
<td>Heart</td>
<td>−0.13 (0.04)</td>
<td>−0.03 (0.01)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.68 (0.02)**</td>
<td>1.01 (0.10)**</td>
</tr>
<tr>
<td>Gut</td>
<td>−0.03 (0.04)</td>
<td>−0.04 (0.07)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.52 (0.05)**</td>
<td>0.55 (0.06)**</td>
</tr>
<tr>
<td>Hindquarter</td>
<td>−0.13 (0.08)</td>
<td>−0.12 (0.09)</td>
</tr>
</tbody>
</table>

Analysis of 48-h urine collection from eight animals accounted for 12.3 (2.7)% of the administered dose as unmetabolized morphine (table II). With dose independent clearance, the expected value determined from the renal percentage of Cl was 37.1 (6.1)% (P < 0.01). The renal percentage of Cl was 34.8 (5.3)% in all 13 experiments. Using the combined information another way, the renal clearance measured indirectly from unmetabolized morphine urine concentrations was 0.21 (0.06) litre min⁻¹. This value is significantly different from the value of 0.55 (0.06) litre min⁻¹ measured directly (P < 0.01).

Tissue concentrations of morphine and the calculated tissue:blood partition coefficients determined at steady state for the four terminal morphine infusions are given in table III. From these experiments, despite inter-animal variability, there was no evidence of dose dependent tissue:blood partitioning of morphine. The median partition coefficients were lung 6.3, brain 2.1,
TABLE III. Concentrations of morphine (as morphine base) in arterial blood (ng ml\(^{-1}\)) and tissue (ng g\(^{-1}\)) (Concn) at steady state, and calculated tissue-blood partition coefficients (Coeff.) after different maintenance infusions of morphine sulphate (Morphine).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Morphine 2.5 mg h(^{-1}) (Sheep No. 3)</th>
<th>Morphine 5 mg h(^{-1}) (Sheep No. 4)</th>
<th>Morphine 10 mg h(^{-1}) (Sheep No. 2)</th>
<th>Morphine 20 mg h(^{-1}) (Sheep No. 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc</td>
<td>Coeff.</td>
<td>Conc</td>
<td>Coeff.</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>31</td>
<td>6.2</td>
<td>19</td>
<td>6.9</td>
</tr>
<tr>
<td>Lung</td>
<td>191</td>
<td>11.5</td>
<td>169</td>
<td>8.9</td>
</tr>
<tr>
<td>Brain</td>
<td>48</td>
<td>1.6</td>
<td>62</td>
<td>3.2</td>
</tr>
<tr>
<td>Heart</td>
<td>65</td>
<td>2.0</td>
<td>69</td>
<td>2.6</td>
</tr>
<tr>
<td>Liver (small bowel)</td>
<td>189</td>
<td>10.4</td>
<td>186</td>
<td>15.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>73</td>
<td>3.0</td>
<td>83</td>
<td>3.4</td>
</tr>
<tr>
<td>Hindquarter muscle</td>
<td>92</td>
<td>0.2</td>
<td>64</td>
<td>0.3</td>
</tr>
<tr>
<td>Hindquarter fat</td>
<td>5</td>
<td>0.2</td>
<td>5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Fig. 3. Simulated time courses of blood (ng ml\(^{-1}\)) and tissue (ng g\(^{-1}\)) concentrations of morphine for the 2.5-mg h\(^{-1}\) infusion regimen. There is close agreement with the values found at 6 h and reported in table III.

Heart muscle 2.3, liver 9.9, small bowel 11.0, kidney 3.8, hindquarter muscle 3.2 and hindquarter fat 0.2. Simulated values of time course of blood, liver, kidney, muscle concentrations and of urinary excretion of morphine for the 2.5-mg h\(^{-1}\) regimen (fig. 3) agree closely with the measured values reported in table III and elsewhere. From the simulated blood concentrations of morphine a slow half-life of 86 min was determined and this, too, is in close agreement with the value derived from the preliminary study involving a 10-min infusion of morphine and compartment analysis.

DISCUSSION
This study would appear to be the first in which a regional pharmacokinetic-mass balance approach [18] has been applied to morphine.
Most previous studies have been based upon pharmacokinetic compartmental analysis of systemic blood samples to determine the $Cl$ of morphine. Thus the impact of specific organ dysfunction on morphine kinetics would not have been revealed by such study designs, as the balance between the sites and rates of clearance may be altered without a net change in the global pharmacokinetic parameters of $Cl$, half-life and apparent volume of distribution.

One important finding of this study was that, within the dose range used, there was no evidence for significant cardiovascular effects or for dose- or time-dependent kinetics of morphine at any region. This may not pertain for much greater doses, at which morphine itself may cause significant cardiovascular disturbances and thereby affect its own disposition [19]. The most important finding, however, was that morphine disposition is more complex than generally perceived. While this study confirmed that the liver is the principal organ for the clearance of morphine, accounting for a mean of 62% of $Cl$, the kidneys accounted for 35%. In the absence of significant dose-related kidney clearance of morphine (as has been confirmed in the dog [19]), approximately 35% of the administered morphine dose should have been recovered from the urine if the kidney clearance was solely a result of excretion of unmetabolized morphine into urine. As only 13% of the morphine dose was recovered (table II), we conclude that the kidneys are metabolizing, as well as excreting, morphine. Indeed, this would be consistent with data showing the formation of morphine glucuronide in microsomes prepared from human kidney specimens [10].

The role of the kidney in morphine clearance has been debated vigorously in recent years, with the specificity of the morphine assay being of critical importance. Early work by Moore and others [3] using a radioimmunoassay (RIA) technique reported that decreased clearance of morphine occurred during renal transplantation in man; however, when this study was repeated using a specific RIA technique, no alteration of morphine kinetics was found [4]. In the period between these two reports others, using improved analytical techniques, studied the pharmacokinetics of morphine in subjects with normal kidneys and patients with renal dysfunction. For example, studies by Aitkenhead and colleagues [20] and by Säwe and Odar-Cederlöf [21] using a specific HPLC method of morphine analysis of peripheral blood samples after a single i.v. morphine bolus found no difference in clearance of morphine between uraemic patients and control subjects, suggesting a minimal role of the kidney in the clearance of morphine. However, it is important to re-emphasize that central or peripheral venous plasma samples cannot be used to infer regional drug kinetics, as these cannot be determined except by regional sampling. The absence of a significant difference in morphine $Cl$ between uraemic patients and patients with normal kidney function may represent compensatory extrahepatic, extrarenal clearance, as has been suggested for morphine disposition in patients with diabetes mellitus [22].

Studies in humans, dogs and goats have reported systemic plasma morphine clearance in excess of liver plasma flow [5–8, 19, 23]. For example, Mazoit and colleagues [5] found in normal volunteers a total plasma clearance of morphine $33.5 \text{ ml min}^{-1} \text{ kg}^{-1}$—a value well in excess of putative hepatic plasma flow. Patwardhan and co-workers [9] found normal elimination of morphine in cirrhotic patients. Others have reported that the clearance of morphine is reduced, but still present, in hepatectomized dogs [24]. These results, taken together, are consistent with significant extrahepatic morphine clearance. Although there may be species differences, it is noted that the urinary excretion of unmetabolized morphine in humans [1] and dogs [19] is similar to that reported here in sheep. Morphine metabolism in gut wall of rats has been shown to occur [25]. Direct studies need to be performed to assess if human gut does metabolize morphine. In the absence of a significant mean arterio–portal gradient, we were unable to demonstrate any net clearance of morphine in the gut of sheep, although in some individual experiments net positive or negative mean extraction ratios were noted. This variability could have been related to the timing of the sampling period with respect to gastric emptying, recycling of morphine that was ion-trapped in gastric contents, or both. Although there was not a significant net clearance of morphine in the lungs, there was a net positive mean extraction ratio in nine of the 13 experiments (and in both of two pilot experiments [data not reported]). This should not be confused with first pass extraction which is known to occur for morphine and a variety of other drugs [26].
lungs are known to be a source of drug metabolizing enzymes and their role in the clearance of morphine still requires more detailed mass balance studies than have been performed to date. The ability of the human brain in vitro to metabolize morphine to M3G and M6G [27] has been demonstrated recently; however, the present study showed no significant net morphine clearance by the sheep brain. This may reflect a low rate of metabolism in brain compared with other tissues in vivo, resulting in an inability to demonstrate extraction across the brain.

The calculated tissue: blood morphine partition coefficients were greater than unity in all tissues except (hindquarter) fat. The partition coefficient of 0.2 in fat is consistent with the poor lipophilicity of morphine [28]. The partitioning of morphine into tissues provides morphine stores that could be mobilized with changes in regional blood flow or acid-base balance, although the magnitude of these effects is unknown. The mean brain: blood partition coefficient of 2.1 is greater than has been found in humans [29], rats [30] and dogs [31], which may be a reflection of any, or all, differences in species, morphine assay and experimental design. In other studies on the physiological disposition of morphine in the pregnant rat, tissue: plasma partition coefficients were reported to be concentration-independent, except for skeletal muscle, in which non-linear partitioning was suggested [16]. Although derived from only a small number of animals, the present study found no evidence for concentration-dependent tissue: blood partition coefficients in any tissues, including hindquarter muscle. The partition coefficients in sheep No. 2 were consistently the smallest values among sheep in all tissues, confirming the suggestion that interindividual variation in tissue: blood distribution and binding contributes a major source of interindividual pharmacokinetic differences [16].

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