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Disposition of Morphine in Tissues of the Pregnant Rat and Foetus Following Single and Continuous Intraperitoneal Administration to the Mother

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Abstract

Foetal exposure to maternally administered opiates such as morphine represent a serious human health problem but disposition studies in man are difficult to perform. Morphine disposition was therefore investigated in pregnant rats and their foetuses near term as a model. Disposition was examined either following intraperitoneal dosing as a single dose or continuous infusion. A high-pressure liquid chromatography assay for morphine in plasma and tissue was developed and validated.

Following the single morphine dose, foetal distribution was rapid and concentrations in foetal and placental tissue were from 2·6 (whole foetus) to 27·6 (placenta) times higher compared with maternal plasma. The rank order of the area under the concentration vs time curve (AUC) of morphine in tissues was: placenta $\geq$ foetal liver > foetal brain > whole foetus > maternal brain. The foetal brain to maternal brain AUC ratio for morphine was 9·5, suggesting large differences in their blood–brain barrier permeability. Following continuous administration of morphine there were significant linear relationships between maternal plasma and tissue concentrations with the same rank order as the single dose study. However, following continuous administration the relative amount of morphine in placenta and foetal liver was reduced by half and one-third, respectively, compared with the single dose study.

These results document why the rat foetus is particularly susceptible to the pharmacodynamic effects of morphine following maternal administration.

Foetal exposure to maternally administered opiates such as morphine represent a serious human health problem, particularly the neonatal abstinence syndrome (Fabris et al 1998). Whilst the maternal–foetal distribution of morphine is difficult to examine in man, such studies in rats have shown foetal tissue concentrations exceed those of maternal plasma (Mullis et al 1979; Bolander et al 1983; Gabrielsson & Paalzow 1983; Cicero et al 1997). These observations are consistent with the relatively hydrophobic nature of many centrally acting drugs (Tsuji 1998). This property also tends to lead to an extensive transplacental distribution of these agents (Garland 1998). For this reason, and the fact that in many cases foetal metabolic pathways are often immature (low capacity) or may even be different altogether from the adult, foetal drug exposure following maternal administration is frequently relatively larger than that of the mother (Rane & Tomson 1980; Pacifici & Rane 1982).

Due to morphine's central site of action, it is of particular interest that there is an apparent persistence of morphine in the brain of the adult rats following a single dose or short infusion (Hahn et al 1976; Mullis et al 1979; Gabrielsson & Paalzow 1983). The nature of this apparent slow elimination from brain and various tissues, the relatively higher foetal tissue concentrations, potential persistence in foetal and adult brain tissues and the consequences

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on maternal–foetal pharmacokinetics of morphine have not been extensively examined over an extended time course. This situation is likely to be due to limitations in specific and sensitive assay methodology for morphine in plasma and tissue. Furthermore, there is little information regarding the effect of continuous administration of morphine over a period of days on its pharmacokinetics in the pregnant rat and the foetus.

We have investigated the disposition of morphine in rats and its distribution into foetal tissues compared with their corresponding maternal plasma and brain concentrations, following both a single dose and continuous administration. This was facilitated through the development of a sensitive and specific high-pressure liquid chromatography (HPLC) assay for morphine in both plasma and tissue samples. Disposition of morphine in maternal plasma and brain, placenta, the whole foetus, foetal liver and foetal brain was then examined following a single dose administered to the mother. The distribution of morphine from maternal plasma into maternal brain and foetal tissues in animals to which different doses of morphine had been administered continuously was also examined.

Materials and Methods

Animal preparation

All animal experiments were approved by the local Institutional Animal Care and Usage Committee. Pregnant female Sprague–Dawley rats, weighing 200–300 g at term whose pregnancies were accurately timed by the observation of sperm in the vaginal lumen, were used in this study (day of insemination = day 0).

Single dose study

On day 18 or 19 post-insemination each mother was administered 20 mg kg^{-1} (calculated as free base) of morphine sulphate by the intraperitoneal route as a single dose dissolved in 1 mL physiological saline. At eight timed intervals from 15 min to 10 h following morphine administration, five or six mothers were anaesthetized with ether. Blood from each mother was immediately collected by cardiac puncture into heparinized tubes. These samples were immediately centrifuged and the plasma fraction aspirated. The animals were then dissected. Maternal brains and placental tissue were collected and the foetuses excised. One foetus from each mother was retained for whole body analysis while a second foetus from each mother was immediately dissected for collection of whole brains and livers. Following collection, all plasma, tissues and whole bodies were immediately frozen on dry ice and stored at −80°C until analysis.

Continuous administration study

Twenty-four pregnant rats were divided into four groups and surgically prepared on day 16 of pregnancy for intraperitoneal implantation of mini-osmotic pumps. Mothers were anaesthetized with ether and their abdominal cavity opened with a mid-line incision. Previously calibrated mini-osmotic pumps (Alzet Osmotic Pump, model 2ML1, Alza Corp., Palo Alto, CA) were primed to deliver doses of either 1·1, 2·2, 4·4 or 8·8 mg kg^{-1} per day (calculated as free base) of morphine sulphate. Six mothers in each dosage group had a mini-osmotic pump inserted into the abdominal cavity. Wounds were closed with silk sutures and surgical staples. On day 19 post-insemination the animals were killed and processed as described above for the single dose study.

Assay of plasma and tissue

An extraction procedure and an HPLC method with electrochemical detection were developed for the determination of morphine in plasma and tissue homogenates. Stock solutions of morphine and nalorphine (the internal standard) in methanol (1 mg mL^{-1}, calculated as free base) were obtained from Sigma Chemical Co. (St Louis, MO). Other chemicals were of analytical or HPLC grade, as appropriate.

Tissues and whole rat foetuses were homogenized in 2–10 mL of 1 M perchloric acid. Samples were made basic with potassium phosphate and the internal standard (250 ng nalorphine) was added. Morphine was extracted by liquid–liquid extraction into ether, back-extraction into aqueous acid followed by a second extraction into ether following addition of potassium phosphate to the acid layer. The aspirated ether layer was evaporated and the residue reconstituted in 0·1 mL mobile phase for HPLC analysis. The recoveries of morphine (200 ng mL^{-1}) and nalorphine from tissues and plasma ranged from 46 and 36% (foetal liver) to 71 and 60% (placenta), respectively.

The HPLC system consisted of a 4·6 × 250 mm Zorbax TMS analytical column (Hewlett Packard Analytical, Wilmington, DE) and an electrochemical detector (model LC-4B, Bioanalytical Systems, West Lafayette, IN) with a glassy carbon electrode with a detector potential of +0·80 V vs a Ag/AgCl reference electrode. The mobile phase consisted of 30% acetonitrile in 0·1 M ammonium

hydrogen phosphate buffer, pH 6·7. The mobile phase flow rate was set at 1·1 mL min^{-1} and the system was operated at ambient temperature.

Calibration curves were prepared daily by adding diluted stock solutions of morphine to blank samples (drug-free tissue homogenate or plasma) to give standards ranging from 10 to 2000 ng mL^{-1}, which was the linear range for the assay. The intraday and interday coefficients of variation in plasma and tissues for morphine (200 ng mL^{-1}) were $< 4{\cdot}6\%$ ($n = 6$) and $< 15\%$ ($n = 10$), respectively. All samples of each tissue type were analysed in the same HPLC run in order to minimize interday variability.

Data analysis

Plasma or tissue concentration vs time data were analysed using a computerized pharmacokinetic modelling program (P-Pharm, MicroPharm International, Champs-sur-Marne, France) in order to determine the apparent distribution and terminal phase half-lives ($t_{½\alpha}$ and $t_{½\beta}$, respectively). Other pharmacokinetic parameters were noted directly from the data or calculated by standard methods (Rowland & Tozer 1989).

For the single dose study, the relative amount of morphine in each tissue was assessed by determining the ratio of area under the concentration vs time profile ($AUC_{0-\infty}$) for the tissue and that of maternal plasma. Following continuous administration, relative amounts of morphine in tissues were assessed by determining the slope of the linear regression lines of best fit for maternal plasma concentration vs tissue concentration at the four doses. Statistical tests used are stated in the text and the level of significance was set at $P < 0{\cdot}05$.

Results

Single dose study

Plasma and tissue concentrations of morphine following intraperitoneal injection are shown in Figure 1. The pharmacokinetic parameters for morphine are summarized in Table 1. Morphine was rapidly absorbed and distributed to all tissues

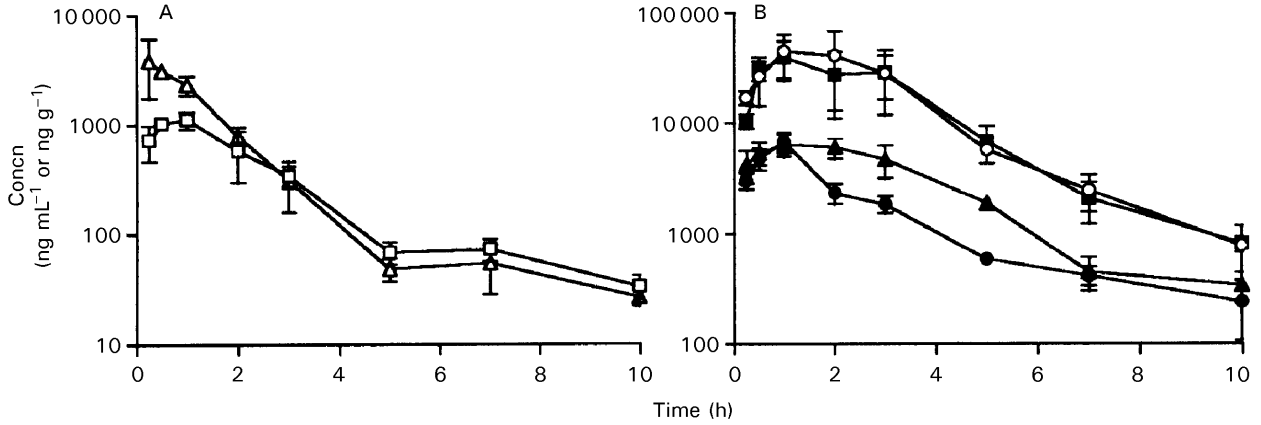

Figure 1. Concentration vs time profile of morphine following a maternal intraperitoneal dose of 20 mg kg^{-1} in (A) maternal plasma (△) and maternal brain (□), and in (B) placenta (○), whole foetus (●), foetal brain (▲) and foetal liver (■). Data are mean ± s.e.m. of five or six rats at each time point, some error bars are obscured by their symbols.

Table 1. Pharmacokinetic parameters obtained for morphine in maternal plasma and various tissues in the pregnant rat and foetus following an intraperitoneal morphine dose of 20 mg kg^{-1}.

Tissue	$t_{½\alpha}$ (h)	$t_{½\beta}$ (h)	C_{max} (ng mL^{-1} or ng g^{-1})	t_{max} (h)	$AUC_{0-\infty}$ (ng mL^{-1} h or ng g^{-1} h)
Maternal plasma	0·06	4·51	3888	0·25	5381
Maternal brain	0·37	6·95	1116	1	2884
Placenta	0·92	11·48	44974	1	143564
Foetal liver	0·83	9·05	40137	1	129675
Foetal brain	0·63	12·23	7434	1	27336
Whole foetus	0·30	7·69	6787	1	14029

$t_{½\alpha}$, apparent distribution phase half-life; $t_{½\beta}$, apparent terminal phase half-life; C_{max}, maximum concentration; t_{max}, time of C_{max}; AUC, area under the concentration time profile.

examined. The time to reach the maximum plasma concentration of morphine (C_{max}) was 1 h for all tissues and at the first measured sample at 15 min for maternal plasma. The rank order of maximal concentrations and $AUC_{0-\infty}$ for morphine in the tissues examined was: placenta ≥ foetal liver > foetal brain > whole foetus > maternal brain.

Morphine concentrations in all tissues examined were best described by a two-compartment extravascular model. Morphine concentrations in foetal tissues examined and placenta declined in parallel as demonstrated by their similar overall apparent terminal elimination half-lives ($t_{½\beta}$) which ranged from 7·69 to 11·48 h. The values obtained for $t_{½\beta}$ were not statistically different (Grubb's test, $P > 0.05$), confirming that elimination of morphine occurred in parallel from the tissues examined. Maternal brain elimination of morphine was somewhat slower than foetal or placental tissues with a $t_{½\beta}$ of 6·95 h. Maternal plasma morphine concentration vs time data were also best described by a two-compartment model. Elimination from maternal plasma was more rapid compared with elimination from all tissues, which was demonstrated by its significantly shorter $t_{½\beta}$ of 4·51 h ($P < 0.05$, Grubb's test).

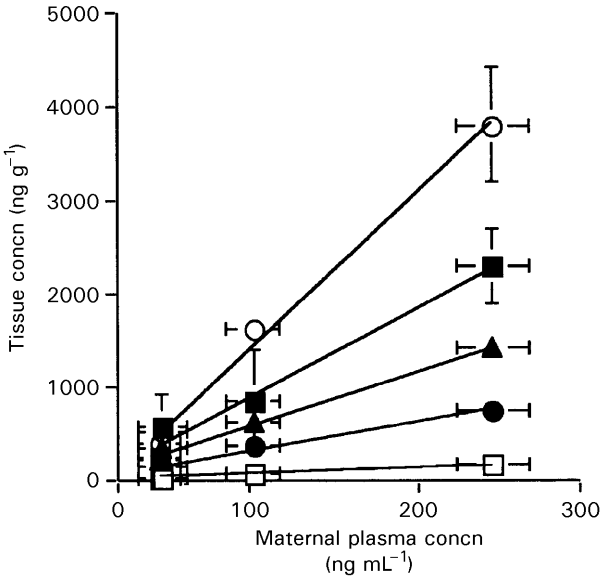

Figure 2. Relationship between maternal plasma and tissue morphine concentrations following continuous dosing of morphine administered by an implanted mini-osmotic pump at four doses (1·1, 2·2, 4·4 or 8·8 mg kg^{-1}): maternal brain (□), placenta (○), whole foetus (●), foetal brain (▲) and foetal liver (■) morphine concentrations in the pregnant rat. Lines represent the linear regression lines of best fit to the raw data. Data are presented as mean ± s.e.m. of five or six rats at each dose, some error bars are obscured by their symbols.

Table 2. Relative distribution of morphine from maternal plasma into tissues following single dosing (maternal plasma to tissue $AUC_{0-\infty}$ ratio) and continuous administration (slopes of maternal plasma vs tissue concentration data at four different doses).

Tissue	Single dose	Continuous administration
Maternal brain	0·5	0·6
Placenta	26·7	13·3
Foetal liver	24·1	7·5
Foetal brain	5·1	4·5
Whole foetus	2·6	2·4

Continuous administration study

Morphine was detected in all tissues examined following continuous administration via mini-osmotic pumps. Figure 2 shows the relationships between maternal plasma and tissue concentrations of morphine at the four doses used in the study. All tissues showed a significant linear relationship between their morphine concentration and the corresponding plasma concentration. The correlation coefficients (r^2) for these linear relationships were > 0·98. The rank order of concentrations of morphine in each tissue was the same as that observed in the single dose study. However, the relative amount of morphine in placental and liver tissue following continuous administration was found to be approximately one-half and one-third, respectively, of the amount observed following a single dose (see Table 2).

Discussion

Following single and continuous maternal dosing, all morphine concentrations in the rat foetus were higher than those observed in maternal plasma and brain tissue (Figures 1 and 2). An approximately 2·5-fold difference in morphine concentrations was found in the whole foetus compared with maternal plasma following both a single and continuous doses to the mother, demonstrating this high relative foetal drug exposure to morphine (Table 2). As with previous shorter time-course studies (Hahn et al 1976; Mullis et al 1979; Gabrielsson & Paalzow 1983), our foetal tissues : maternal plasma ratios also show that morphine distribution in the foetus was not homogenous but regional in nature with the placenta and foetal liver showing a large accumulation or binding of morphine (Table 2). The reservoir effect of this foetal and maternal tissue binding causes a long secondary elimination phase in maternal plasma with persistent low concentrations of morphine being found at times greater than

5 h post-administration (Figure 1). Most previous studies have not adequately characterized morphine disposition in both mother and foetus in this terminal phase due to inadequate sampling times, or assay sensitivity limitations, or have not examined disposition following continuous administration (Hahn et al 1976; Mullis et al 1979; Gabrielsson & Paalzow 1983). An interesting finding of our study is the change in the relative amounts of morphine found in the placenta and in the foetal liver, which dropped to approximately one-half and one-third, respectively, of the values found in the single dose study. This indicates morphine binding to these organs is reduced in a steady-state or chronic dosing situation, perhaps due to a down-regulation of receptor-mediated tissue binding.

An important observation in terms of the pharmacodynamic effects of morphine is the nearly 10-fold greater proportion of morphine distributed into foetal brain compared with the maternal brain (AUC ratio of 9·5). This phenomenon may arise from intrinsic differences in the permeability of the blood–brain barrier in the rat foetus compared with that of the mother. The rat foetus tends to have an intermediate permeability for most of the gestational term but markedly tightens perinatally (Stewart & Hayakawa 1994). It may also be possible that there are differences in tissue binding in the developing foetal brain compared with maternal brain tissue due to differing contents of lipophilic substances (Kupferberg & Way 1963). These findings may explain, at least in part, the unexpectedly high pharmacological response of rat pups to maternally administered morphine (Kupferberg & Way 1963; Johannesson & Becker 1972).

Hahn et al (1976) have previously shown dose proportionality of tissue morphine concentration in non-pregnant rats between doses of 0·07 and $10\,mg\,kg^{-1}$, although they used a non-specific analytical method (radiolabelling) which also measured metabolites. Cicero et al (1997) described also a linear relationship for morphine between adult brain concentrations and dose. We have used our HPLC method to not only confirm these observations specifically for morphine, but also to extend it to show dose proportionality also exists between maternal plasma and rat foetal tissues with a linear relationship at the doses given. Thus, the greater the maternal dose of morphine, the greater the extent of foetal drug exposure.

In summary, our study provides evidence of multiple tissue compartments for morphine in both mother and foetus. These compartments therefore need to be considered in both pharmacokinetic and pharmacodynamic analysis following the administration of morphine. Overall, foetal exposure to morphine is higher in the foetus than in the mother. Morphine shows dose proportionality in both mother and foetus confirming that foetal exposure increases in parallel with maternal dose. The apparent high permeability of morphine across the foetal blood–brain barrier leads to relatively higher foetal brain concentrations than were found in the mother. This suggests the central pharmacodynamic effects of morphine will be relatively greater in the foetus than in the mother.

Acknowledgements

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