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Research Article

Pharmacokinetics of Methylergometrine in the Rat: Evidence for Enterohepatic Recirculation by a Linked-Rat Model

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The pharmacokinetics of methylergometrine were investigated in the rat, with emphasis on the role of biliary excretion and enterohepatic recirculation in the overall disposition of the drug. A linked-rat model, where the bile from a rat receiving a constant rate of iv infusion of methylergometrine was allowed to flow into the duodenum of another rat, was used for the estimation of the degree of enterohepatic recirculation (EHC). The excretion of unchanged methylergometrine in the bile was estimated separately. Plasma protein binding and plasma-to-whole blood partitioning were also determined. Plasma clearance in control rats was 17.4 ± 0.7 ml/min × kg for iv bolus and 15.4 ± 0.7 ml/min × kg for iv infusion. The corresponding values in the bile-cannulated rats were significantly lower, 7.7 ± 0.4 and 8.7 ± 0.1 ml/min × kg, respectively. The lower clearance in the bile-cannulated rats was caused mainly by a lower free fraction in plasma, f_u (0.11 ± 0.01), in this group compared with the control group (0.19 ± 0.0.03). The unbound volume of distribution at steady state ($V_{ss}u$) was only 6.5 liters/kg in the bile-cannulated rats, compared to 14.7 liters/kg in control rats, suggesting that under steady-state conditions, more than 50% of the methylergometrine is conjugated or resides in the hepatobiliary loop, either as a conjugate or unchanged. The fraction of unchanged methylergometrine excreted in the bile was less than 0.3% of the given dose, while the fraction of the dose being reabsorbed during one cycle (f_{reabs}) was 8.4 ± 6.3%. Thus, recirculation is due mainly to excretion of conjugates subsequently hydrolzyed in the GI tract prior to absorption. In spite of this relatively low degree of EHC, the terminal half-life was reduced from 186 ± 22 min in control rats to 79 ± 5 min in rats with an interrupted EHC. The data presented emphasize the role of enterohepatic recirculation in the disposition of a drug, i.e., even if the extent of EHC is relatively low, it may markedly effect the apparent volume of distribution, hence the terminal half-life of a drug.

KEY WORDS: methylergometrine; pharmacokinetics; rat; enterohepatic recirculation; protein binding; volume of distribution.

INTRODUCTION

Biliary excretion followed by reabsorption in the gastrointestinal tract, usually referred to as enterohepatic recirculation (EHC), is a relatively common phenomenon for xenobiotics and endogenous compounds. Drugs reported to undergo EHC in rats include diflunisal (1), morphine, phenolphtalein, lysergic acid diethylamide (LSD), diphenylacetic acid (2), felodipine (3), glycyrrhizin (4), and many others. Most drugs subject to EHC are excreted into the bile as conjugates, i.e., glucuronides, glycosides, sulfamates, etc., that are subsequently hydrolyzed in the gastrointestinal tract, before being reabsorbed. This recirculation process retains the drug in the body and may result in secondary peaks and erratic plasma concentration–time profiles. It may also result in a relatively long terminal half-life in spite of a relatively high metabolic clearance and a low tissue distribution of the drug. If a drug has an extensive EHC, biliary obstruction or disturbances in the intestinal flora, by, e.g., concomitant intake of antibiotics, may dramatically alter the disposition of a drug (2).

Methylergometrine is a semisynthetic ergot alkaloid and an LSD derivative, used clinically in obstetrics because of its very potent uterotonic effect (5). Methylergometrine has also, in several studies *in vivo,* been shown to be formed from methysergide, the antimigraine prophylactic agent, in many species, including humans (6), rats (7), and dogs (8). Methylergometrine is formed through an N_1 demethylation. In a pharmacokinetic study of methysergide and methylergometrine in the rat, the plasma concentration–time profile of methylergometrine, after iv bolus administration of methysergide, displayed an erratic plasma concentration–time profile. When methylergometrine was administered as an iv dose, a smoother profile was obtained but a second peak in the plasma concentration appeared at 120–200 min in a large number of rats, and following this second peak the elimination shifted into a slower phase (7). These observations taken together indicate that methylergometrine undergoes enterohepatic recirculation.

The present study was to determine if, and to what ex-

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tent, methylergometrine undergoes EHC. It was also of interest to assess the role of EHC in the overall disposition of methylergometrine and to elucidate if this recirculation is responsible for the second-peak phenomenon and the relatively slow terminal phase observed after iv administration.

METHODS

Drugs and Chemicals. Methylergometrine hydrogen-maleate and ergometrine hydrogen-maleate were gifts from Sandoz Ltd., Switzerland. All other chemicals used were of analytical grade. The doses of methylergometrine were prepared by dissolving the drug in physiological saline.

Animals. Male Sprague–Dawley rats (ALAB, Sweden), weighing 175–225 g, were used throughout the study. The rats were housed in pairs under controlled environmental conditions and fed on commercial food pellets. Surgery was performed under ether anesthesia the day before the study. A silastic catheter (o.d., 0.94 mm) was inserted in the left jugular vein, for drug administration, and a polyethylene catheter (PE-50; o.d., 0.965 mm) into the left carotid artery for blood sampling. The catheters were passed under the skin and exteriorized at the back of the neck. The rats were then allowed to recover until the following day. During this recovery period the animals had no access to food but were allowed water *ad libitum*. The rats were not anesthetized or restrained at any time during the experiments and the total blood volume collected never exceeded 2.0 ml.

Intravenous Infusions of Methylergometrine to Controls. Two groups of rats received iv infusions of methylergometrine of 0.125 ($N = 6$) and 3.5 nmol/min × kg ($N = 6$). The infusions were conducted over 6 hr to reach steady state, and plasma samples were collected at 10, 20, 30, 40, 60, 90, 120, 150, 180, 240, 300, and 360 min after the start of the infusion.

Intravenous Infusion and iv Bolus to Bile-Cannulated Rats. The jugular vein and the carotid artery were cannulated as described above. In addition, the bile duct was cannulated with a polyethylene catheter (PE-10) and another catheter (PE-50) was inserted in the duodenum approximately 5 mm distally to sphincter oddi. All catheters were passed under the skin and exteriorized at the back of the neck, where the biliary and the duodenum catheters were connected to each other to allow for normal enterohepatic recirculation of bile during the postoperative period. One group of rats ($N = 7$) was given an iv infusion of 3.5 nmol/min × kg and plasma samples were taken using the same sampling scheme as for the controls (see above). Bile was collected at 15-min intervals for the first hour and at 30-min intervals for the subsequent 5 hr.

A second group of rats ($N = 4$), also with bile cannulae, received an iv dose of 2.95 μmol/kg, with blood sampling at 2, 10, 20, 40, 60, 90, 120, 180, 300, and 480 min and bile sampling at 0–10, 10–20, 20–40, 40–60, 60–90, 90–120, 120–150, 150–180, 180–240, 240–300, 300–360, 360–420, and 420–480 min.

Enterohepatic Recirculation. The degree of recirculation was estimated using a linked-rat model (9) ($N = 6$). All rats had their bile duct cannulated and were treated postoperatively in the manner described above. Two rats were linked, where the first rat received an iv infusion of 3.5 nmol/min × kg (donor) and the bile from this rat was allowed to flow into the duodenum of the second rat (recipient). Plasma samples were collected from both rats and bile was collected from the recipient rat. The same sampling schemes for plasma and bile as above (iv infusion to bile-cannulated rats) were used.

Protein Binding. Plasma protein binding studies were performed in separate groups of rats undergoing the "normal" catheterization (jugular vein and carotid artery) and in bile-cannulated rats. The free fraction was determined by ultrafiltration. Plasma from four rats of each group was collected the day after surgery and pooled. The plasma was adjusted to pH 7.40 with HCl and spiked with known amounts of methylergometrine to obtain final concentrations of 5, 20, 100, 1000, and 5000 n*M* in the control plasma and 20, 100, and 1000 n*M* in the plasma from bile-cannulated rats. Each concentration was in quadruplicate. The samples were thoroughly mixed and allowed to equilibrate for 5 min, after which 0.7 ml of each sample was transferred to an ultrafiltration tube (AMICON) and centrifuged at 1000*g* for 4 min through the membrane (AMICON YMT, membrane 40420 A), yielding a filtrate volume of approximately 125 μl. The whole procedure was performed at 37°C. The filtrates were then injected directly onto the HPLC column, where peak heights were compared to those of standard solutions prepared in phosphate buffer (0.039 *M*, pH 7.40). Adsorption to the filtration membrane was tested in advance by filtering various concentrations of methylergometrine prepared in phosphate buffer (pH 7.40). The adsorption was found to be insignificant and no correction for this was required.

The free fraction in plasma, f_u, was calculated as

$$f_u = (C_f/C_{tot}) \times 0.93 \quad (1)$$

where C_f is the concentration in the filtrate, C_{tot} is the concentration in the plasma, and 0.93 is the factor correcting for the plasma volume occupied by proteins in the plasma.

Plasma/Blood Partitioning. The partitioning of methylergometrine between erythrocytes and plasma was determined *in vivo* by the simultaneous sampling of whole blood and plasma from rats receiving a 2.95-μmol/kg iv dose of methylergometrine. The blood samples were, immediately after collection, divided into two portions and plasma was separated from one of them. The sampling times were chosen in such a manner that total plasma concentrations ranging from 5 to 6000 n*M* were obtained. The partitioning was calculated as the ratio of plasma concentration, C_p, to the whole blood concentration, C_b.

Sample Treatment and HPLC Assay. The plasma and whole-blood samples were frozen immediately after collection and stored at −20°C until analyzed. The concentration of methylergometrine was measured using an HPLC method with fluorescence detection, as described previously (7). The bile samples were protected from light during the collection period and the volume was determined by weighing, assuming a density of 1.0 mg/μl. The samples were then stored at −20°C until analyzed. The concentration of methylergometrine in the bile samples was determined by taking 100-μl aliquots and adding 200 μl of internal standard (ergometrine, 100 n*M*), followed by dilution with mobile phase (30% acetonitrile in 0.01 *M* ammonium carbonate) to appropriate con-

centrations. The diluted samples were then injected onto the column using the same chromatographic system as for plasma and whole-blood analyses (7).

Pharmacokinetic Analysis. The mean plasma concentration–time data from the rats receiving the iv infusion and the iv bolus doses were analyzed by nonlinear regression, using PCNONLIN (10). Mono-, bi-, and triexponential functions according to:

$$C = \frac{\text{dose}}{V_1} \times \sum_{i=1}^{n} C'_i \times e^{-\lambda_i \times t} \quad (2)$$

were fitted to the iv bolus data and

$$C = \frac{R_{\text{inf}}}{V_1} \times \sum_{i=1}^{n} [C'_i/\lambda_i \times (1 - e^{-\lambda_i \times t})] \quad (3)$$

to the iv infusion data, where R_{inf} is the rate of infusion, V_1 is the initial dilution space, C'_i is the fractional intercept of the ith phase, and λ_i is the corresponding rate constant.

Other parameters of interest were set as secondary parameters (10) and expressed according to the following.

Plasma clearance:

$$\text{CL} = \frac{V_1}{\sum_{i=1}^{n} C'_i/\lambda_i} \quad (4)$$

Volume of distribution at steady state:

$$V_{\text{ss}} = \frac{V_1 \times \sum_{i=1}^{n} C'_i/\lambda_i^2}{\sum_{i=1}^{n} (C'_i/\lambda_i)^2} \quad (5)$$

Volume of distribution in the terminal phase.

$$V_{\lambda_n} = \text{CL}/\lambda_n \quad (6)$$

where λ_n is the terminal rate constant.

The data points were given weights according to $N/(\text{CV} \times C_{\text{calc}})^2$, where N is the number of data points observed at time t, C_{calc} is the calculated plasma concentration, and CV is the observed coefficient of variation at that particular time. This weighting scheme was chosen rather than $N/$(observed variance) on the same grounds that weighting by $1/C_{\text{calc}}^2$ is preferred over $1/C_{\text{obs}}^2$ for individual data (11). However, weighting by $N/$(observed variance) was also applied and yielded parameter estimates very close to those who are presented in the results. The models were fitted to the data from the different infusion rates, modes of administration (iv bolus and iv infusion), and the different groups (control and bile cannulated) both simultaneously (all parameters set common) and separately. Discrimination between different disposition models and between simultaneous or separate fittings was based on visual inspection of the fits, standard errors of the computer-estimated parameter values, weighted sum of squares (partial F test), and residual analysis.

The parameters related to unbound drug, CL_u, $V_{ss,u}$ and $V_{1,u}$, were calculated by dividing the parameter based on total concentration by the average free fraction, f_u, determined in the protein binding study.

Bile clearance was calculated as

$$\text{CL}_{\text{bile}} = \text{biliary excretion rate}/C_{t\text{mid}} \quad (7)$$

where $C_{t\text{mid}}$ is the plasma concentration at the midpoint of the collection interval.

The fraction excreted unchanged in the bile, f_{bile}, was calculated as

$$f_{\text{bile}} = \text{CL}_{\text{bile}}/\text{CL} \quad (8)$$

The fraction of the dose being reabsorbed during one cycle, estimated in the linked-rat experiments and assuming equal plasma clearance in the two groups, was calculated as

$$f_{\text{reabs}} = \frac{C_{\text{ss}}(\text{rec})}{C_{\text{ss}}(\text{don})} \quad (9)$$

where C_{ss} (rec) and C_{ss} (don) are the steady-state plasma concentrations for the bile recipient and donor rat, respectively. The term f_{reabs} is here the product of several steps including excretion of unchanged drug, formation of metabolite(s), excretion of metabolite(s), hydrolysis of the metabolite(s) back to the parent drug, and finally, absorption of methylergometrine to the general recirculation.

Tests of significance for differences in the parameter values obtained in the disposition studies were executed using the t test applicable to parameters and their standard errors obtained from nonlinear regression (12). A difference in protein binding between the control and the bile-cannulated rats was tested with the usual Student's t test.

RESULTS

Mean plasma concentration time profiles from the 0.125- and the 3.5-nmol/min × kg iv infusion to the control rats and the 3.5 nmol/min × kg infusion, given to the bile-cannulated rats, are shown in Fig. 1. A biexponential function gave the best fit for all three groups. The two rates of infusion to the control group were fitted simultaneously, while the bile-cannulated rats were fitted separately. The pharmacokinetic parameters for these three groups are shown in Table I. Plasma clearance in the control group was constant for the two rates of infusion. However, plasma clearance in the bile-cannulated group was significantly ($P < 0.001$) reduced compared to control rats.

The free fraction in plasma was constant over the range of plasma concentrations observed for both groups (Fig. 2) but significantly lower ($P < 0.001$) in the bile-cannulated rats. The free fractions were 0.19 ± 0.032 (mean ± SD, N = 20) and 0.11 ± 0.012 (N = 12) in the control and bile-cannulated groups, respectively.

The distribution of methylergometrine to the erythrocytes was low and independent of the plasma concentration, and the plasma–whole-blood ratio, C_p/C_b, averaged 1.45 ± 0.13 (N = 16).

The total excretion of unchanged methylergometrine in

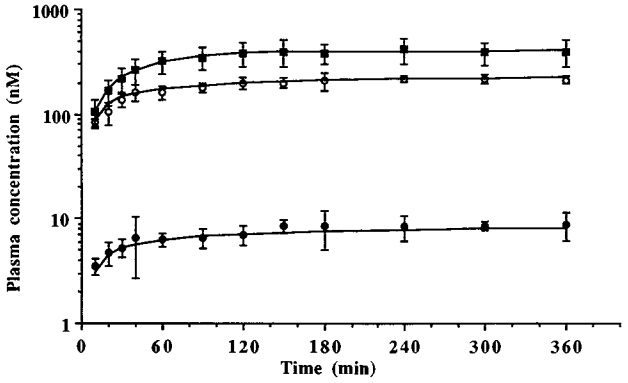

Fig. 1. Plasma concentration–time profiles of methylergometrine after iv infusion to control rats, 0.125 nmol/min × kg (●) and 3.5 nmol/min × kg (○), and to bile-cannulated rats, 3.5 nmol/min × kg (■). Each point represents the mean ± SE of 5 or 6 points for the control rats and 10–13 points for the bile-cannulated rats. The solid lines are the predicted concentrations obtained by fitting biexponential functions to the mean data. The two control groups were fitted simultaneously, while the bile-cannulated group was fitted separately.

bile (f_{bile}) was very low and averaged 0.26 ± 0.10% (N = 11) of the given dose. No sign of nonlinearity in the biliary excretion of unchanged methylergometrine was detected (Fig. 3). The bile flow rate was in agreement with previous reports (13) but decreased somewhat over the experimental time after both the iv bolus dose and the iv infusion, and there was a weak but significant (r = 0.50, P < 0.01) correlation between bile clearance and bile flow.

Figure 4 shows the mean plasma concentration–time profile of the donor and the recipient rats in the linked-rat experiment. The plasma concentrations of methylergometrine in the recipient rats well exceeded what could be explained by the amounts of unchanged methylergometrine

Table I. Pharmacokinetic Parameters of Methylergometrine (Estimate ± SE) from iv Infusion to Control and Bile-Cannulated Rats

Parameter	Rate of infusion (nmol/min · kg) 1.25 and 3.5[a]	3.5[b]	Statistical difference[c]
CL (ml/min × kg)	15.4 ± 0.7	8.7 ± 0.1	P < 0.001
CL_u[d] (ml/min × kg)	81.0	79.1	
V_1 (liters/kg)	0.30 ± 0.02	0.24 ± 0.04	NS
$V_{1,u}$[d] (liters/kg)	1.6	2.2	NS
V_{ss} (liters/kg)	0.77 ± 0.21	0.34 ± 0.02	NS
$V_{ss,u}$[d] (liters/kg)	4.0	3.1	
$V_{\lambda 2}$ (liters/kg)	1.6 ± 0.7	0.36 ± 0.05	NS
$t_{1/2}\lambda 1$ (min)	8.6 ± 1.6	5.8 ± 7.9	NS
$t_{1/2}\lambda 2$ (min)	71.8 ± 34.6	29.0 ± 4.6	NS

[a] Control rats where the biexponential function was fitted to the mean plasma concentrations from both rates of iv infusion simultaneously.
[b] Intravenous infusion given to bile-cannulated rats.
[c] Statistical difference tested using the t test for computer-estimated parameter values and their associated standard errors (11).
[d] Calculated by dividing the parameter based on total plasma concentration by the average free fraction in plasma, f_u.

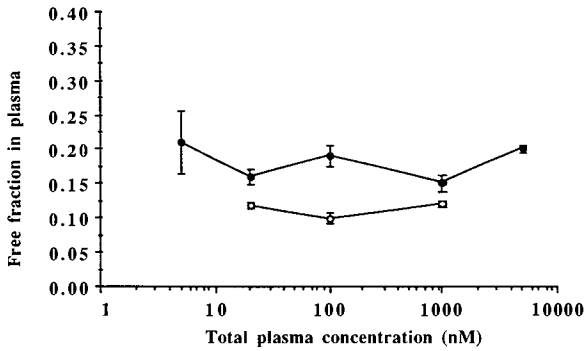

Fig. 2. The free fraction in plasma vs total plasma concentration in control (●) and bile-cannulated (○) rats. Each point represents the mean ± SD from four samples.

excreted in the bile (f_{bile} = 0.26%), found in the rats where bile was collected. The fraction reabsorbed (f_{reabs}) was 8.4 ± 6.3% (N = 6). However, the plasma concentration–time profile of the recipient rats displayed two phases, where an apparent steady-state level began to rise at approximately 200 min (Fig. 4). Since this second phase did not reach a steady-state level during the experimental time, the calculated degree of recirculation is probably underestimated.

Figure 5 depicts the unbound plasma concentrations of control and bile-cannulated rats receiving a 2.95-μmol/kg iv bolus dose. The pharmacokinetic parameters obtained from fitting biexponential functions to the mean plasma concentrations are presented in Table II. The two groups differed markedly in all the disposition parameters. The volume of the initial dilution space (V_1) and plasma clearance decreased in proportion to the decrease in the free fraction in plasma, while the volume of distribution in the terminal phase ($V_{\lambda 2}$) and at steady-state (V_{ss}) decreased more than could be expected solely from the change in protein binding. The terminal half-life was more than halved in the bile-cannulated group and no secondary peak in the plasma concentration–time profile, as observed in control rats (7), was seen in any of these rats.

DISCUSSION

The pharmacokinetics of methylergometrine in the rat

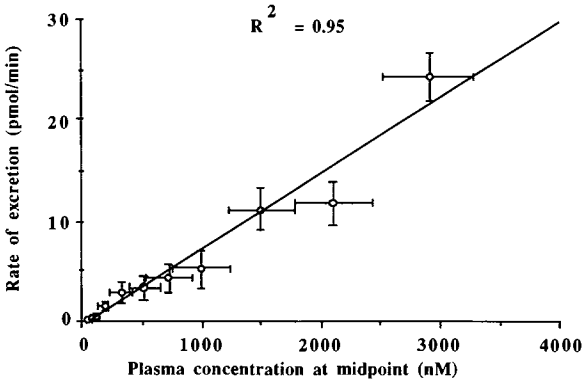

Fig. 3. The rate of excretion of unchanged methylergometrine in the bile versus plasma concentration. Each point is the mean ± SE of five to seven data points.

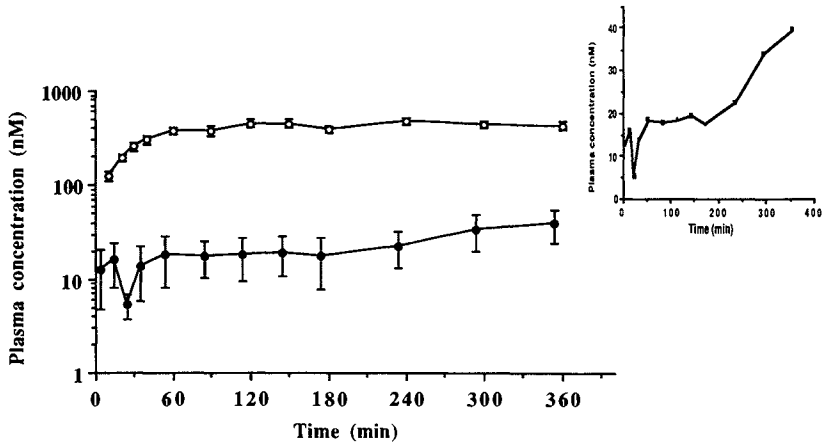

Fig. 4. Plasma concentration–time profiles of the donor (○) and the recipient (●) rats in the linked-rat experiments. Each point represents the mean ± SE of six data points. The inset shows the plasma concentration of the recipient rats with a linear concentration axis.

have previously been studied after iv bolus administration of methylergometrine and iv bolus and iv infusion of its prodrug, methysergide. That study (7) revealed several indications of the enterohepatic recirculation of methylergometrine, such as erratic plasma concentration–time profiles after iv administration of methysergide and a secondary peak following iv administration of methylergometrine.

There are several methods available for investigating whether a xenobiotic is subject to enterohepatic recirculation in rats. Comparison of plasma clearance in rats with and without a bile cannula is an indirect way of quantifying the recirculation. The underlying assumption in this method is that parameters, others than those related to bile excretion, determining the disposition of a drug, e.g., metabolic clearance, renal clearance, and plasma protein binding, remain unaltered after bile cannulation. This method also suffers from not being sensitive enough when the fraction undergoing EHC is relatively small. The determination of the amount of unchanged drug excreted in the bile is often an insufficient measurement, since for most drugs where enterohepatic recirculation has been confirmed, excretion of conjugates (glucuronides, sulfamates, etc.) followed by hydrolysis in the gastrointestinal tract is the major determinant for recirculation (14). Conjugates and other metabolites can be included in the measurement of bile excretion by using a radiolabeled drug, but this will yield information only about the potential degree of EHC.

The most appealing technique for estimating EHC is the linked-animal model (9), which we found to be the most appropriate, since it unequivocally proves the presence of EHC, and the degree of recirculation can be directly quantified from the plasma levels in the donor and recipient rats.

The biliary excretion of unchanged methylergometrine was very low (0.26% of the dose) and linear with the plasma concentration (Fig. 3). The biliary concentration of methylergometrine was only slightly higher than the calculated unbound concentration in plasma ($= C_{obs} \times f_u$), and bile clearance was slightly flow dependent ($r = 0.5$, $P < 0.05$).

The fraction reabsorbed (f_{reabs}) in the linked-rat exper-

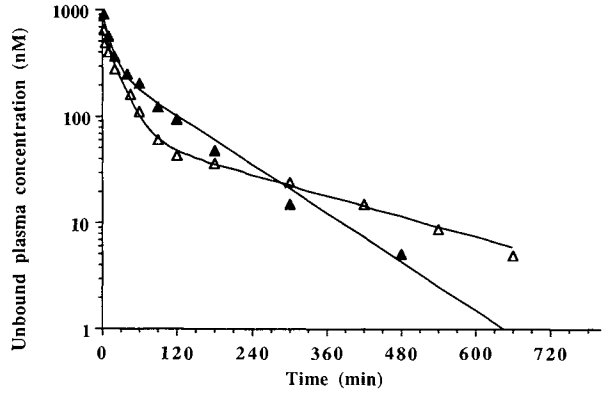

Fig. 5. Plasma concentration–time profiles of unbound methylergometrine in control (△) and bile-cannulated (▲) rats following an iv bolus dose of 2.95 μmol/kg. The unbound concentrations were obtained by multiplying the observed plasma concentration by the free fraction, f_u, obtained *in vitro*. Each point represents the mean of four to six data points. The solid lines represent the predicted concentrations obtained from fitting biexponential functions to the mean data.

Table II. Pharmacokinetic Parameters of Methylergometrine (Estimate ± SE) After 2.95-μmol/kg iv Bolus Administration to Control and Bile-Cannulated Rats

Parameter	Control[a]	Bile-cannulated	Statistical difference[b]
CL_p (ml/min × kg)	17.4 ± 0.7	7.7 ± 0.4	$P < 0.001$
Cl_u[c] (ml/min × kg)	91.6	70.0	
V_1 (liters/kg)	0.87 ± 0.04	0.33 ± 0.05	$P < 0.001$
$V_{1,u}$[c] (liters/kg)	4.6	3.0	
$V_{\lambda 2}$ (liters/kg)	4.7 ± 0.45	0.88 ± 0.07	$P < 0.001$
V_{ss} (liters/kg)	2.8 ± 0.2	0.72 ± 0.05	$P < 0.001$
$V_{ss,u}$[c] (liters/kg)	14.7	6.5	
$t_{1/2}\lambda 1$ (min)	13.2 ± 9.8	9.0 ± 2.5	NS
$t_{1/2}\lambda 2$ (min)	186.0 ± 22.0	78.9 ± 5.1	$P < 0.001$

[a] Values obtained from a previous study (7).
[b] Statistical difference tested using the t test for computer-estimated parameter values and their associated standard errors (11).
[c] Calculated by dividing the parameter based on total plasma concentration by the average free fraction in plasma, f_u.

iments averaged 8.4%. The relative amount of drug being added to the circulation after an infinite number of cycles, as proposed by Tse *et al.* (15), can be expressed as $[1/(1 - f_{reabs})] - 1$, which for the present data would be 9.2%. This value is probably an underestimate, since the plasma concentrations of the bile-recipient rats were still rising at the end of the experiment. The large discrepancy between the amount of methylergometrine excreted in the bile and the estimated fraction reabsorbed shows that the factor mainly responsible for the recirculation of methylergometrine in the rat is bile excretion of metabolites (e.g., glucuronides) followed by hydrolysis in the gastrointestinal tract. There are no data available on the *in vivo* metabolism of methylergometrine, but an N^1-hydroxy metabolite and normethylergometrine have been identified in rat microsomes (16). There are no reports of any conjugates of methylergometrine so far, but glucuronides of a closely related compound, lysergic acid diethylamide (LSD), have been found in relatively large amounts in rat bile (17).

The occurrence of a second phase in the plasma concentration–time profile in the bile-recipient rats (Fig. 4) might be caused by a higher rate of hydrolysis in the lower than in the upper part of the intestine. A larger number of microorganisms and a higher β-glucuronidase activity in cecum and colon than in the small intestine are well known (14). The time lag to this second phase (3 to 4 hr) and the second peak observed after iv administration (7) are in close agreement with the transit time of fluid from the duodenum to the colon (18).

Plasma clearance in rats receiving an iv infusion was significantly reduced, from 15.4 ± 0.66 ml/min × kg, in the control group, to 8.7 ± 0.14 ml/min × kg in the bile-cannulated group. A similar decrease was observed after iv bolus administration (Table II). One would expect an increase in total clearance in bile-cannulated rats in the same range as the fraction reabsorbed. However, the plasma protein binding was significantly elevated in rats undergoing bile cannulation, with a free fraction (f_u) of 11%, compared to 19% in the control group. This increase in binding capacity can probably be ascribed to an elevation in the plasma levels of α-1-acid glycoprotein, as a consequence of the major surgery (19) required for bile cannulation. Since methylergometrine has a total blood clearance ($= CLp \times C_p/C_b$) ranging from 22 to 25 ml/min × kg (control rats) in this study, well below reported values of liver blood flow in rat (20), clearance is expected to be sensitive to changes in protein binding (21). Thus, the difference in plasma protein binding between bile-cannulated and control rats is probably the major source to the observed difference in clearance. Some of the difference may be due to a lowered metabolic capacity as a consequence of the surgery.

A decrease in the volume of distribution at steady state (V_{ss}), from 2.4 liters/kg in the control group to 0.75 liter/kg in the bile-cannulated group, was noted in the iv bolus study. The decrease in V_{ss} in the bile-cannulated rats can be explained partly by the lower free fraction in plasma (f_u), but the interrupted EHC also contributes to a lower volume of distribution. The steady-state volume of distribution based on unbound drug ($V_{ss,u}$) was only 6.5 liters/kg compared to 14.7 liters/kg in the control group (Table II). The difference between these values represents the contribution of the EHC to the volume of distribution. This would mean that, under steady-state conditions, more than 50% of the methylergometrine in the rat is in a conjugated form in the systemic circulation or in the hepatobiliary loop and/or unchanged in the hepatobiliary loop.

The parameter values, estimated by nonlinear regression, related to distribution (V_1, V_{ss}, $V_{\lambda 2}$) obtained in the infusion studies were lower than the corresponding values obtained in the iv bolus studies, while the estimated plasma clearance agreed well between the two modes of administration (Tables I and II). The discrepancies in the distribution parameters were observed in both control and bile-cannulated rats. It is hard to tell whether these differences really reflect a concentration dependency in the distribution (higher plasma concentrations in the iv bolus study) or if they are artifactual, due to the uncertainty of estimating distribution parameters from infusion data, especially when postinfusion data are lacking. Saturable protein binding would be a natural explanation for this observation but can be ruled out, as the free fraction in plasma was found to be independent of concentration, in both control and bile-cannulated rats. Interestingly though, a similar concentration dependency has been noted in the distribution of the prodrug of methylergometrine, methysergide (7).

The most striking observation in the iv bolus study was that the terminal half-life ($t_{1/2}\lambda_2$) decreased from 186 min in the control to 79 min in the bile-cannulated group. Another important finding was that no secondary peak in the plasma concentration–time profile, which was observed in control rats (7), appeared in any of the bile-cannulated rats. Both these observations are most likely due to the interrupted EHC.

In conclusion, the results presented provide firm evidence that methylergometrine is subject to EHC in the rat and that this recirculation is responsible for the secondary peak phenomenon observed after iv administration. It is also very likely that this recirculation is responsible for the erratic plasma concentration–time profile of methylergometrine, when methysergide is administered (7). The data presented also demonstrate the impact of a relatively low degree of enterohepatic recirculation on the disposition of a drug, especially on the apparent volume of distribution and the terminal half-life.

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