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# BILIARY AND URINARY ELIMINATION OF METHADONE AND ITS METABOLITES IN THE RAT

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**Abstract**—The biliary and urinary elimination of $^{3}H$-methadone and its metabolites was quantitatively studied after various routes of administration of the drug to rats. The biliary excretion products consisted largely of the *N*-demethylated metabolite of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and a highly water-soluble compound which is apparently a glucuronide conjugate of a hydroxylated derivative of EDDP.

Phenobarbital pretreatment of rats was found to decrease the total excretion of EDDP in the bile, possibly through enhancement of another metabolic pathway, and at the same time to decrease the duration of narcosis resulting from the administration of methadone.

Pretreatment with methadone was observed to greatly enhance the rate of biliary EDDP excretion in the first 4 hr after methadone administration.

EARLY studies on the disposition of methadone in the rat indicated that within a 24-hr period the majority of the compound was eliminated in the feces as a result of biliary secretion, while approximately 10 per cent of the dose could be accounted for in the urine.[1–3] Since the identities of any methadone metabolites were not yet established, quantitative estimates of the excretion products were made on the basis of total radioactivity, countercurrent distribution patterns and rather nonspecific chemical methods.

Recently the major methadone metabolite in both man and the rat, a product of *N*-demethylation followed by cyclization, was identified as 2-ethylidene-1, 5-dimethyl-3,3-diphenylpyrrolidine (EDDP).[4,5] The kinetics of the renal elimination of this compound and its parent drug have been studied in tolerant and non-tolerant human subjects by these authors.[6] Additionally, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP), arising from further *N*-demethylation of EDDP has been reported present to a minor extent in rat bile.[5] Finally, methadone-*N*-oxide was detected as a product of enzymatic oxidation of methadone in guinea pig liver microsomal preparations.[7]

It is the purpose of the present investigation to quantitatively describe the elimination of methadone and its metabolites in rats after various routes of administration, and to assess the effects of phenobarbital and methadone pretreatment on the rate of excretion of the major methadone metabolite in the bile.

## MATERIALS AND METHODS

*Animals and drugs.* Male Sprague–Dawley rats (Martin Farms, E. Palo Alto, Calif.) of 375–425 g body wt. were used. The hydrobromide of *dl*-methadone-1-$^{3}H$

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(New England Nuclear Corp., Boston, Mass.) was diluted with stable *dl*-methadone HCl (Merck Chemical Co., Rahway, N.J.) to result in a total dose to each rat of 3–5 μc of radioactivity. Phenobarbital sodium was purchased from Abbott Laboratories (N. Chicago, Ill.). EMDP HCl was a gift of Eli Lilly & Co. (Indianapolis, Ind.), while EDDP and methadone-*N*-oxide were synthesized using established methods.[4,8]

*Bile duct cannulation and drug administration.* Under ether anesthesia, the common bile duct of the rat was exposed by a midline abdominal incision and cannulated with PE-10 polyethylene tubing. Upon closure of the wound and emergence of the animal from anesthesia, $^3$H-methadone was administered; intravenous injections (1 mg/kg) were given via the right femoral vein, while subcutaneous injections (5 or 16 mg/kg) were given in the right hind leg. Oral dosages (5 mg/kg) were administered via stomach tube. The animals were then placed in wire restraining cages and given unlimited water while bile and urine were collected.

Pretreated rats received either phenobarbital sodium, 80 mg/kg, i.p., once daily for 3 days, or methadone HCl, given s.c. once daily according to the following regimen: days 1–5, 5 mg/kg; days 6–10, 10 mg/kg; days 11–15, 15 mg/kg. In all cases $^3$H-methadone was administered 24 hr after the last pretreatment dose.

*Analytical method.* The extraction from biological samples and quantitation by gas–liquid chromatography (GLC) of methadone and EDDP have been described previously.[6] Retention times in minutes for the GLC assay were EMDP, 1·8; methaphenilene (internal standard), 2·0; EDDP, 2·4; methadone, 3·3.

In preparation for liquid scintillation, 0·5 ml of either urine or bile (diluted 1 : 3 with water) was combined with 0·5 ml of pH 9·3 sodium borate buffer in a 15-ml screwcap tube and extracted with 9 ml $CHCl_3$. After centrifuging at 2000 rpm for 5 min, 0·5 ml of the aqueous layer was removed for counting. A 4·5-ml aliquot of the $CHCl_3$ layer was pipetted into a separate counting vial and evaporated to dryness under a stream of nitrogen. To this vial was added 0·5 ml of a 1 : 1 mixture of borate buffer and previously extracted bile from a rat given only stable methadone, in order to duplicate the quenching conditions of the vial containing the aqueous layer. All counting was performed on a Beckman model LS-150 liquid scintillator in 10 ml of naphthalene–PPO–POPOP–dioxane (100 : 7·0 : 0·3 g : qs to 1 l.).*

Thin-layer chromatography was accomplished on Silica gel G with butanol–acetic acid–water, 4 : 1 : 5 (top layer), as solvent. After spraying with iodoplatinate reagent (1 ml 10% platinum chloride solution and 1 g potassium iodide in 100 ml $H_2O$), the following $R_f$ values were observed: EDDP, 0·30; methadone, 0·35; methadone-*N*-oxide, 0·50; EMDP, 0·62.

## RESULTS

*Methadone excretion products in bile and urine.* The 24-hr bile and urine samples collected from the rats in these studies were subjected to analysis by both gas–liquid chromatography (GLC) and liquid scintillation spectrometry (LSS). The $CHCl_3$ extract of bile at pH 9·3 contained essentially all EDDP, with only trace amounts of methadone and EMDP. The same extract of urine contained approximately equal amounts of EDDP and unchanged methadone. Quantitative GLC determinations of the EDDP removed from bile by this single $CHCl_3$ extraction agreed excellently

* PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

with the removal of radioactivity, as measured by LSS. Subsequent extractions did not improve the yield.

After $CHCl_3$ extraction of 20 ml of bile, which removed over half the radioactivity, the aqueous layer was adjusted to pH 1 with 1 N HCl. After centrifugation of the precipitated bile acids, the supernate was freeze-dried and the residue dissolved in 20 ml of pH 5·2 acetate buffer. No loss of radioactivity was observed throughout these manipulations. This solution was then incubated with β-glucuronidase (750 units/ml) for 24 hr at 37°. It was then possible to remove 63·2 per cent of the radioactivity present in the incubation mixture by $CHCl_3$ extraction (at pH 5·2), while a

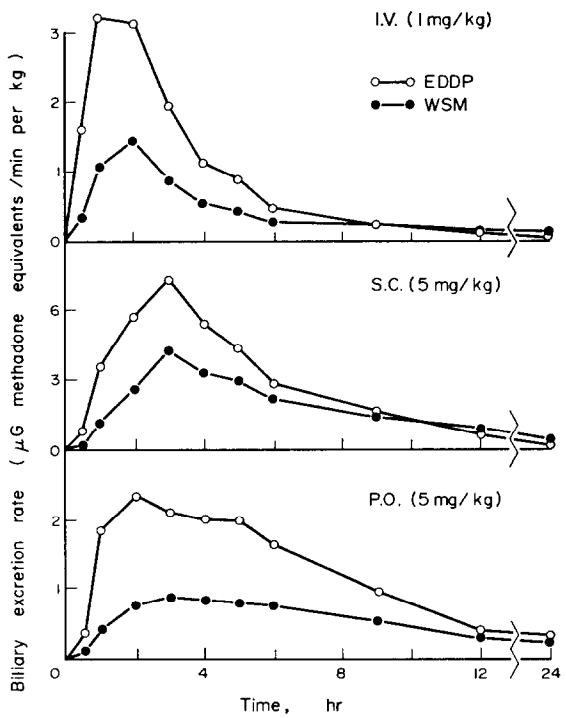


FIG. 1. Biliary excretion rates over 24 hr of EDDP and WSM in rats after three routes of administration of $^3$H-methadone. Each point represents the mean of values from at least three rats.

control incubation (without the β-glucuronidase) showed removal of only 9·8 per cent. Extraction of higher pH values removed considerably less radioactivity.

Concentration of this $CHCl_3$ extract and application to TLC yielded a single iodoplatinate spot at $R_f$ 0·62. GLC of the same extract produced a single peak at 4·8-min retention time. This compound and its conjugate, assumedly a glucuronide, shall be referred to henceforth as the water-soluble metabolite (WSM).

$^3$H-EDDP was extracted from the pooled bile of rats which had previously received $^3$H-methadone and purified by TLC. This material in aqueous solution was then administered i.v. (30 mg/kg) to a rat with a biliary fistula, and bile was collected for 4 hr. During this period, 36·4 per cent of the injected radioactivity was eliminated as unchanged EDDP and 24·8 per cent as the WSM.

Analysis of both urine and bile from rats receiving $^3$H-methadone for methadone-*N*-oxide by the method of Beckett *et al.*[7] proved negative. Analysis for the WSM showed only traces in the urine of rats either with or without the biliary fistula.

*Time course of methadone elimination.* The rate and extent of elimination of methadone and its metabolites were studied in rats with biliary fistulas after intravenous, subcutaneous and oral administration of $^3$H-methadone. Bile was sampled at various intervals over a 24-hr period and the excretion rates of EDDP and the WSM were determined (Fig. 1). After intravenous administration, the peak excretion period for both metabolites is 1–2 hr, while for the subcutaneous route it is at 3 hr, and orally, at 2–3 hr; for the most part, WSM levels are substantially lower than for EDDP. It is significant that with oral administration, although the peak EDDP excretion

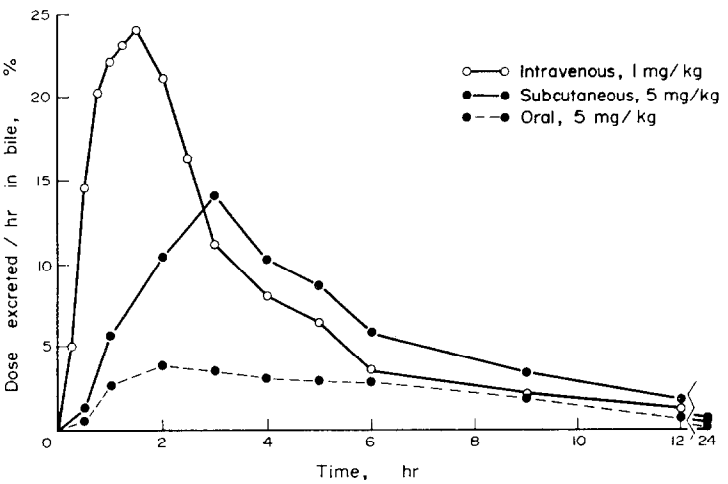


FIG. 2. Biliary excretion rate over 24 hr of the metabolites of $^3$H-methadone as percentage of the total dose after three routes of administration. Each point represents the mean of values from at least three rats.

period is reached relatively early, the levels are considerably lower than for the subcutaneous route and slightly lower than after the intravenous injection, which represents only one-fifth of the oral dosage.

Figure 2 is a representation of the total biliary metabolite excretion as a function of the total dose after i.v., s.c. and p.o. administration. Metabolism and excretion of methadone after intravenous injection are clearly efficient processes, whereas the low output of the compound after oral administration is indicative of losses through inefficient intestinal absorption or storage in body depots. The cumulative 24-hr results of the urinary and biliary elimination of methadone (Table 1) are a further indication of the importance of the route of administration on the extent of biotransformation of this drug.

*Effects of pretreatment on the biliary excretion of EDDP.* The rate of biliary excretion of EDDP in biliary fistula rats was determined after s.c. administration of $^3$H-methadone (16 mg/kg) to control rats and those pretreated with either phenobarbital or methadone (Fig. 3). It is interesting to note that the peak excretion rate in the

TABLE 1. PERCENTAGE OF DOSE (±S. E.) EXCRETED IN BILE AND URINE OVER A 24-hr PERIOD AFTER VARIOUS ROUTES OF ADMINISTRATION OF $^{3}$H-METHADONE TO RATS

| Route and dose | n* | Bile | | Urine total | Total bile and urine |
|---|---|---|---|---|---|
| | | EDDP | WSM | | |
| Intravenous (1mg/kg) | 4 | 58·0 ± 5·4 | 26·7 ± 2·6 | 7·1 ± 0·9 | 91·8 ± 3·6 |
| Subcutaneous (5 mg/kg) | 3 | 42·9 ± 3·8 | 30·9 ± 1·9 | 6·2 ± 1·9 | 80·0 ± 3·1 |
| Oral (5 mg/kg) | 3 | 22·0 ± 0·1 | 12·3 ± 2·3 | 1·5 ± 0·4 | 35·8 ± 3·4 |

* The number of animals in each experiment.

control animals was at 5–6 hr, whereas with the smaller s.c. dose (Fig. 1) the peak rate occurred considerably earlier.

The results of both phenobarbital and methadone pretreatment on the excretion of EDDP are comparable in that the peak rate was reached in one-half the control time; however, the phenobarbital rats never attained the high EDDP levels of the controls, while the methadone rats exceeded control values. In the first 4 hr after injection, the control rats excreted 12·3 ± 1·0 per cent of the dose as EDDP; the corresponding figures for the phenobarbital and methadone groups are 10·8 ± 0·3 and 18·3 ± 0·9 per cent respectively. This result for the methadone-pretreated rats is statistically significant ($P < 0·05$) and represents an increase over controls of 48·7

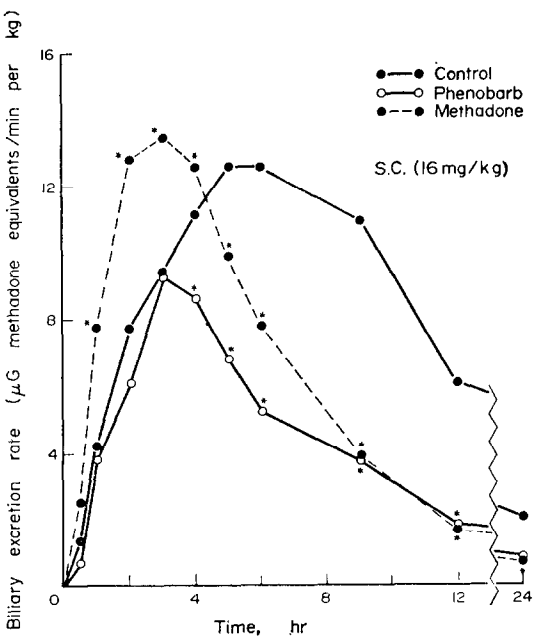


FIG. 3. Biliary excretion rate over 24 hr of EDDP after s.c. administration of methadone (16 mg/kg) to rats. Phenobarbital- and methadone-pretreatment regimens are as detailed under Materials and Methods. Each point represents the mean of values from at least three rats. Values significantly different from controls ($P < 0·05$) are designated by an asterisk.

per cent. This difference was abolished by the seventh hour, however, as the EDDP excretion rate of the methadone rats fell off much more rapidly than that of the controls (Fig. 3). It was further observed that the control animals remained in a state of apparent coma for a period of 8–10 hr after drug administration, while both of the pretreated groups demonstrated activity after only 3–4 hr. The control rats also exhibited considerable depression of the bile flow rate over an extended period of time when contrasted to the two pretreated groups (Fig. 4).

TABLE 2. PERCENTAGE OF DOSE EXCRETED IN BILE AND URINE OVER A 24-HR PERIOD AFTER S.C. ADMINISTRATION OF METHADONE (16 mg/kg) TO RATS

| Pretreatment | n* | Bile | Urine | |
|---|---|---|---|---|
| | | EDDP | EDDP | Methadone |
| None, no fistula | 4 | | 4·1 ± 0·3 | 5·1 ± 0·5 |
| None, biliary fistula | 4 | 54·6 ± 6·4 | 4·2 ± 0·5 | 6·0 ± 0·6 |
| Phenobarbital, biliary fistula | 3 | 27·3 ± 1·1† | 1·4 ± 0·3† | 4·5 ± 0·9 |
| Methadone, biliary fistula | 4 | 37·0 ± 3·0 | 4·3 ± 0·5 | 3·6 ± 0·8 |

* The number of animals in each experiment.
† Significantly different ($P < 0·05$) from controls.

Table 2 summarizes the 24-hr bile and urine outputs of EDDP and unchanged methadone for the control and phenobarbital- and methadone-pretreated groups of rats which received 16 mg/kg, s.c., $^3$H-methadone. The control rats excreted significantly more EDDP in both bile and urine than the phenobarbital-pretreated group.

Also included in Table 2 are the 24-hr urine results for four untreated rats in which biliary fistulas were not established. The urine EDDP and methadone excretion figures are not significantly different from the biliary fistula control rats.

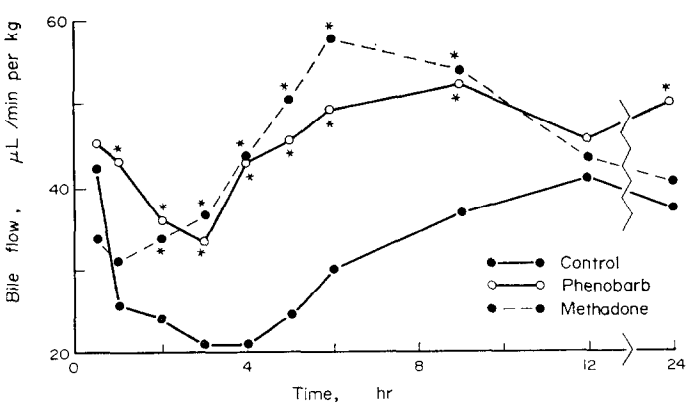


FIG. 4. Bile flow rate over 24 hr after s.c. administration of methadone (16 mg/kg) to rats. Phenobarbital- and methadone-pretreatment regimens are as detailed under Materials and Methods. Each point represents the mean of values from at least three rats. Values significantly different from controls ($P < 0·05$) are designated by an asterisk.

## DISCUSSION

It has been shown that methadone is eliminated by the rat primarily in the bile as the *N*-demethylated derivative, EDDP, and secondarily as a highly water-soluble substance which is apparently a glucuronide conjugate. Hydrolysis of this conjugate, which also arises after EDDP administration to a rat, yields a substance which is considerably more polar than either methadone or EDDP, and which is extracted by $CHCl_3$ more efficiently at acid pH than basic. It is suggested that this substance is a derivative of EDDP containing one or more phenolic hydroxyl groups, which may then undergo conjugation with glucuronic acid.

Whereas glucuronide conjugates are known to be particularly suitable for secretion into bile,[9] weakly basic lipophilic molecules such as methadone rarely exceed a bile/plasma ratio of 1. However, EDDP, with a p*K*a of 10·42[6], is considerably more polar and appears to conform to the requirements for active transport into the bile.

The highest concentrations of methadone metabolites in the bile were attained after i.v. administration of the drug, followed by the s.c. and p.o. routes in decreasing order. These results are comparable to the findings of Wolen *et al.*[10] in regard to plasma levels of propoxyphene (a congener of methadone) following these same routes of administration, and are probably a reflection of the methadone plasma values, although these were not determined.

Phenobarbital pretreatment has been shown to induce the metabolism *in vitro* of a variety of narcotic drugs,[11,12] including methadone,[13] by stimulation of liver microsomal *N*-demethylase activity. Also stimulated by phenobarbital is the bile flow rate of rats.[14] The fact that the phenobarbital-pretreated rats in this study recovered earlier from the narcotic effects of methadone than did the controls may be due to a combination of these effects. The fact that a lesser amount of EDDP was excreted by these rats could be a result of stimulation of a secondary metabolic pathway, such as hydroxylation or glucuronide conjugation, both of which are known to be enhanced by phenobarbital.[15,16]

The effects of methadone pretreatment on methadone metabolism *in vitro* have been reported to be either inhibitory[3,17] or nil.[13,18] However, Sung *et al.*[3] reported a significantly increased fecal excretion of a basic metabolite of methadone in tolerant rats over controls after 3 days of methadone administration (20 mg/kg/day, i.p.). This finding appears to correlate with our observation of enhanced biliary EDDP excretion in the first 4 hr after s.c. methadone administration to methadone-pretreated rats. The obvious conclusion that methadone is being metabolized at a greater rate by the pretreated animals is supported by a previous study showing nearly a doubling of the rate of codeine-*N*-demethylation *in vivo* in mice made tolerant to morphine.[19]

The deep coma produced in the control rats by the high (16 mg/kg) subcutaneous dose of methadone used in this study and the relative tolerance of the methadone-pretreated rats to this dose may be very significant factors in the observed biliary excretion differences. Were these two groups of rats to have been treated with equal pharmacological doses of methadone the excretion differences may not have occurred.

Hypothermia during coma could conceivably play a role in the reduction of the rate of biliary elimination of EDDP. Kalser *et al.*[20] found that hypothermia significantly reduced the biliary elimination of atropine metabolites by the isolated, perfused rat liver primarily due to a decrease in the rate of biotransformation of the drug and secondarily to a reduction in the rate of secretion of the metabolites into the bile.

The effects of drug pretreatment and the role of hypothermia on the rate of biliary elimination of methadone metabolites will be further investigated in a future communication.

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