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EFFECTS OF CHRONIC METHADONE TREATMENT ON BILE FLOW AND ON METABOLISM AND EXCRETION OF ^{3}H-METHADONE. EXPERIMENTS WITH ISOLATED PERFUSED RAT LIVERS

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Abstract—Methadone, administered subcutaneously to rats once daily for 12 days, nearly doubled the bile flow rate in isolated perfused livers. The increase in bile flow was accompanied by an increase in the biliary excretion rate of metabolites of ^{3}H-methadone, which was added to the perfusion system at a dose of 1 mg/kg body weight. To determine whether an alteration of the rate of methadone metabolism accounted for this effect, phenol-3,6-dibromphthalein disulfonate (DBSP), an analogue of BSP which is excreted unchanged in the bile, was added (80 mg/kg) to the perfusion system. It was also excreted at an enhanced rate by the livers of the methadone pretreated rats. Neither the plasma disappearance rate of methadone or DBSP nor the pattern of biotransformation of methadone was significantly affected by the methadone pretreatment. It is concluded that chronic methadone treatment of rats stimulates bile flow *via* an unknown mechanism, resulting in increased biliary excretion of methadone metabolites and possibly of other compounds which undergo elimination in the bile.

THE NARCOTIC analgesic methadone is efficiently metabolized and eliminated by the rat following parenteral administration. After an intravenous dose of 1 mg/kg to a bile duct cannulated rat, more than 58 per cent of the dose appears in the bile within 4 hr and more than 84 per cent within 24 hr.[1,2] The biliary excretion products consist primarily of 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)[1–3] and of a *p*-hydroxylated and conjugated derivative of 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP).[4,5] EDDP and EMDP are mono- and di-*N*-demethylated metabolites of methadone, respectively. Compounds present in minor amounts in rat bile include unchanged methadone, EMDP and various polar conjugates.[5–7]

In view of the widespread use of methadone in the long-term high-dose maintenance of heroin addicts, the effects of chronic methadone administration on the pharmacokinetics of the drug are of great interest. Recently Baselt and Casarett[1] demonstrated that the *in vivo* rate of biliary excretion of EDDP by rats following subcutaneous administration of 16 mg/kg methadone was significantly enhanced by methadone pretreatment. Results of Alvares and Kappas[8] suggest that methadone induction of microsomal *N*-demethylase activity is not responsible for this effect.

A difficulty with *in vivo* experiments which measure biliary excretion of drug metabolites is that the pharmacological actions of methadone are exerted to varying degrees on the control and pretreated rats, the latter being partially tolerant to the

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drug. Some of these established pharmacological effects of methadone, including hypothermia,[9] hypoxia[10] and hypotension,[11] could appreciably influence drugs disposition. In this study we have used therefore the isolated perfused rat liver to study the effects of methadone pretreatment on methadone disposition in order to reduce the physiological variations encountered with intact animals.

MATERIALS AND METHODS

Animals and drugs. Male Sprague–Dawley rats were used. The hydrobromide of *dl*-methadone-1-^{3}H (New England Nuclear Corp., Boston, Mass.) was diluted with stable *dl*-methadone HCl (Merck Chemical Co., Rahway, N.J.), so that about 1 μCi was added to each isolated liver. EDDP and EMDP were gifts of Eli Lilly and Co. (Indianapolis, Ind.). Phenyl-3,6-dibromphthalein disulfonate (DBSP, a dibrominated analogue of BSP) was a gift of Dr. C. D. Klaassen, University of Kansas Medical Center. The doses of ^{3}H-methadone (1 mg/kg) and DBSP (80 mg/kg) administered to the perfused livers were based on the weights of the rats on the day of liver perfusion.

Pretreatment and surgery. Pretreated rats received methadone HCl once daily s.c., at a dose of 5 mg/kg on days 1–7, and 10 mg/kg on days 8–12. The liver was perfused 24 hr after the last pretreatment dose. Control rats were untreated.

The surgical techniques, performed under ether anesthesia, for cannulation of the bile duct, portal vein and inferior vena cava and isolation of the liver are essentially those of Miller *et al.*[12] The period of ischemia in no case exceeded 5 min.

Liver perfusion. The perfusion apparatus utilized has been described in detail by Bickel and Minder.[13] Briefly, the livers were perfused at 37° at a perfusion rate of 1·5 ml/min/g liver and a portal pressure of 9–12 cm H_2O. A constant pH of 7·40–7·45 was achieved by oxygenation with a mixture of 97·5% O_2 and 2·5% CO_2 at a flow rate of 180 ml/min. The 200 ml of perfusion medium consisted of Krebs-Ringer-bicarbonate solution containing washed bovine red cells (16 g hemoglobin), bovine serum albumin (4·0 g), glucose (0·2 g) and aureomycin (4 mg). The 3 hr experimental perfusion period was initiated after 45 min of perfusion. Drugs were added directly to the perfusate reservoir. At various time intervals 2 ml perfusate samples were removed for analysis and bile samples were collected and weighed.

Analytical methods. ^{3}H-methadone and metabolites. Plasma was prepared from the perfusate by centrifugation at 2000 *g* for 10 min. Bile samples were diluted with water to a volume of 2 ml. Whole livers were homogenized in equal volumes of 1·15% KCl in pH 7·4 0·01 M phosphate buffer. Aliquots of the liver homogenate were then incubated overnight at 80° with equal volumes of 10% KOH. Liquid scintillation counting of 0·1 ml samples of plasma, diluted bile and liver digest was carried out before and after extraction of the samples with $CHCl_3$ at pH 9·3. Standards prepared by the addition of a known amount of ^{3}H-methadone to control perfusate, bile and liver, were analyzed in a similar manner. Unextracted radioactivity represented conjugated methadone metabolites.[1,4] The $CHCl_3$ extracts were analyzed for methadone, EDDP and EMDP by a gas–liquid chromatographic (GLC) assay.[14]

Radioactivity was estimated with a Packard model 3320 liquid scintillation spectrometer. The scintillation medium consisted either of a dioxane–napthalene based mixture[1] (plasma and bile samples) or of 4 g BBOT per liter of toluene (liver

samples). In the latter instance 3 ml of methanol per 10 ml of medium was added to achieve solubilization of the liver digest.

DBSP. The concentration of DBSP in plasma and bile samples were determined according to the method described by Klaassen and Plaa.[15]

RESULTS

Effects of pretreatment on body weight and bile flow. As chronic methadone treatment is known to have an adverse effect upon weight gain in adult rats,[16] the rats chosen to receive the 12 day methadone pretreatment were of a larger initial size, in order that the final body weights would approximate those of controls (Table 1). Thus during the 13 days preceeding liver perfusion, control rats increased in size by about 24 per cent, whereas the pretreated animals lost 1–8 per cent of their initial weight. The livers of the pretreated rats were in all cases significantly smaller than the livers of controls. However, bile flow, expressed as an average rate over the 3-hr perfusion period, was significantly elevated in pretreated rats.

Effects of pretreatment on ^{3}H-methadone disposition. As in previous *in vivo* experiments,[1,2] the livers of the pretreated rats excreted the ^{3}H-methadone in the bile at a much greater initial rate than controls (Fig. 1). The total percentage of the dose

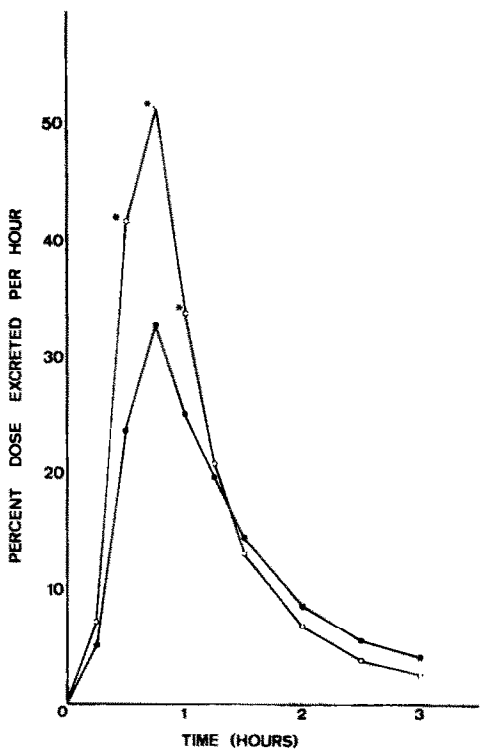

FIG. 1. Biliary excretion rate of methadone and metabolites over a 3 hr period following administration of ^{3}H-methadone (1 mg/kg) to isolated perfused livers of control (●) and methadone pretreated (○) rats. Each point represents the mean of values from at least five rats. Values significantly different from controls ($P < 0.05$) are designated by an asterisk.

TABLE 1. SELECTED CHARACTERISTICS OF THE EXPERIMENTAL GROUPS OF RATS (MEAN ± S.E.)

Group	Administered drug (per kg body wt)	n	Body wt (g)			Liver wt (g)	Mean bile flow (μl min/kg)	Drug dose (μg/g liver)
			Initial*	Final†	% change			
Control	None	4	259 ± 11	325 ± 17	+25	14·1 ± 0·3	14·5 ± 0·9	
Pretreated		4	326 ± 12‡	311 ± 10	−5	12·0 ± 0·4‡	23·8 ± 1·5‡	
Control	Methadone	8	273 ± 11	336 ± 13	+23	14·4 ± 0·4	13·8 ± 0·8	23·6 ± 1·2
Pretreated	(1 mg/kg)	5	346 ± 6‡	320 ± 4	−8	12·3 ± 0·5‡	24·0 ± 1·2‡	26·2 ± 0·9
Control	DBSP	4	258 ± 15	323 ± 18	+24	14·3 ± 0·4	21·4 ± 1·1	1800 ± 72
Pretreated	(80 mg/kg)	4	295 ± 12	292 ± 16	−1	11·8 ± 0·6‡	29·7 ± 2·7‡	2000 ± 116

* Body weight 13 days before liver perfusion.
† Body weight on day of perfusion.
‡ Significantly different from control values ($P < 0·05$).

TABLE 2. DISTRIBUTION OF METHADONE AND METABOLITES IN PLASMA, BILE AND LIVER, 1 AND 3 HOURS AFTER ADMINISTRATION OF ^{3}H-METHADONE (1 MG/KG)

Group	n	Time	Percentage of initial dose (mean ± S.E.)									
			Plasma	Bile				Liver				Total
			Methadone	Methadone	EDDP	Conjugates	Total	Methadone	EDDP	Conjugates	Total	P.B.L
Control	4	1	12·1 ± 0·4	0·7 ± 0·1	6·3 ± 1·1	14·6 ± 1·8	21·7 ± 1·4	45·1 ± 2·3	14·9 ± 1·9	<0·5	62·2 ± 2·4	96·0 ± 2·8
Pretreated	4		9·5 ± 2·2	1·0 ± 0·1	10·0 ± 1·3	19·2 ± 2·1	29·0 ± 2·2*	46·6 ± 2·1	1·5 ± 0·3*	<0·5	49·1 ± 1·5*	87·6 ± 2·3
Control	8	3	3·7 ± 0·7	1·3 ± 0·2	10·7 ± 0·9	28·6 ± 2·8	39·4 ± 3·3	1·3 ± 0·6	14·5 ± 1·8	5·0 ± 1·4	20·8 ± 2·3	63·9 ± 2·5
Pretreated	5		3·7 ± 1·4	1·5 ± 0·3	13·7 ± 1·4	29·2 ± 1·9	43·2 ± 2·5	2·9 ± 0·8	9·2 ± 1·9	4·8 ± 0·7	16·9 ± 1·9	63·8 ± 2·1

* Significantly different from control values ($P < 0·05$).

eliminated in the bile by these livers was significantly higher 1 hr after methadone administration, a difference which is accounted for by both EDDP and conjugated metabolites (Table 2). The figure of 40 per cent biliary excretion after 3 hr of perfusion is low compared with the 60 per cent excretion previously observed in *in vivo* experiments,[2] two-thirds of which was EDDP. This appears to be a function of the type of rats used rather than the liver perfusion, however, since unpublished *in vivo* trials in this laboratory agree well with the perfusion results (40·7 per cent excretion in 4 hr following a 1 mg/kg intravenous methadone dose).

The uptake of methadone from the perfusate by the perfused liver was quite rapid and was similar in the control and pretreated groups (Table 2). Unchanged methadone accounted for over 98 per cent of the radioactivity present in the perfusate plasma in all samples, as determined by the GLC assay.

After 1 hr the livers contained the majority of the methadone dose, primarily as unchanged methadone (Table 2). The important difference at this time period is that the livers of the pretreated rats contained much less EDDP than controls, whereas EDDP production (all EDDP plus conjugates in the system) was nearly equal in the 2 groups (35·8 per cent of the dose in the controls and 30·7 per cent in pretreated), an indication that excretion of this compound was occurring at a faster rate in the pretreated livers. The lack of conjugated metabolites in the livers at 1 hr suggests that these compounds are excreted immediately upon formation. After 3 hr methadone metabolism was nearly complete, both control and pretreated livers contained

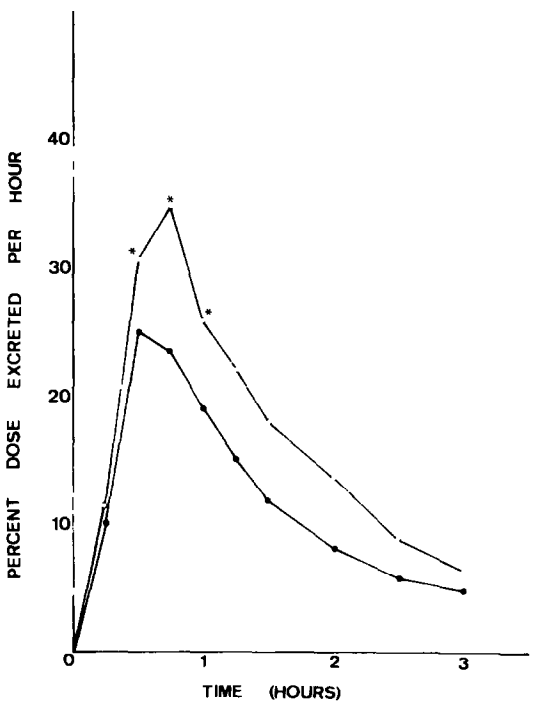

FIG. 2. Biliary excretion rate of DBSP over a 3 hr period following administration of DBSP (80 mg/kg) to isolated perfused livers of control (●) and methadone pretreated (○) rats. Each point represents the mean of values from four rats. Values significantly different from controls ($P < 0.05$) are designated by an asterisk.

substantial amounts of unexcreted metabolites, possibly as a result of a decline in liver function.

Effects of pretreatment on DBSP disposition. DBSP, a dibrominated analogue of sulfobromophthalein which is excreted unchanged in the bile,[17,18] was chosen as a test substance in this investigation to distiguish between altered biotransformation and altered biliary excretory function. The livers of the pretreated rats were able to eliminate DBSP in the bile at a significantly greater rate in the first hour after administration than controls (Fig. 2). Although the presence of the DBSP, which is a choleretic agent,[15] elevated the bile flow in both groups of rats over drug-free levels, the pretreated group still demonstrated a significantly higher flow than controls (Table 1).

While the bile of the pretreated group contained a greater percentage than controls of the DBSP dose at both 1 and 3 hr after administration, there was little difference in the rate of hepatic uptake of the compound between the 2 groups as indicated by the perfusate levels (Table 3).

TABLE 3. DISTRIBUTION OF DBSP IN PLASMA, BILE AND LIVER, 1 AND 3 HR AFTER ADMINISTRATION (80 mg/kg)

			Percentage of initial dose (mean ± S.E.)		
Group	n	Time (hr)	Plasma	Bile	Liver*
Control	4	1	60·2 ± 2·1	19·5 ± 1·9	20·3 ± 2·0
Pretreated	4		63·1 ± 0·5	25·6 ± 1·2†	11·3 ± 1·1†
Control	4	3	48·7 ± 2·9	35·7 ± 2·5	15·6 ± 3·1
Pretreated	4		50·6 ± 3·5	47·3 ± 2·8†	2·1 ± 0·6†

* Liver values = 100% (perfusate + bile).

† Significantly different from control values ($P < 0.05$).

DISCUSSION

It has been determined that chronic methadone treatment of rats causes an enhancement of the rate of biliary secretion of methadone (primarily as products of biotransformation) and DBSP by the isolated perfused liver. This effect cannot be attributed to the weight loss of the pretreated rats, since although the drug doses during liver perfusion were based on the body weights of the donor rats on the day of perfusion, the absolute amounts of ^{3}H-methadone and DBSP administered per g of liver were higher for the pretreated groups (Table 1).

A change in the rate of methadone metabolism by the pretreated livers is not likely as: firstly, the large amounts of unchanged methadone present in the controls and pretreated livers after 1 hr of perfusion are nearly identical (Table 2), suggesting that metabolism is taking place at similar rates in both groups and secondly, the biliary excretion of DBSP, which does not involve biotransformation, is also more rapid in the pretreated livers.

The more rapid appearance of methadone metabolites and DBSP in the bile of the pretreated livers appears to be correlated with the increased bile flow in these livers (Table 1). It is unlikely that the small amount of methadone administered during the 12 days of pretreatment has an osmotic effect on bile flow 24 hr after the last pretreat-

ment dose, since at least 80 per cent of a subcutaneous dose of methadone to rats is eliminated in 24 hr.[1]

It is tempting to compare the effects of methadone pretreatment on bile flow in rats with that of phenobarbital pretreatment, which enhances bile flow apparently by stimulation of the bile salt-independent fraction of canilicular bile production.[19] Phenobarbital pretreatment, however, stimulates microsomal drug metabolism, including that of methadone,[8] and enhances the rate of plasma disappearance of a variety of compounds, including DBSP.[15,20] Methadone pretreatment shares neither the former[8] nor, at least for methadone and DBSP (Tables 2 and 3), the latter property.

A further characteristic of methadone which should be discussed is its ability to restrict bile flow, either *in vivo* or in isolated preparations in which the terminal bile duct is intact, by producing spasm of the sphincter of Oddi.[21] It is likely that in a study such as this, the control rats would be more sensitive to this effect than the methadone pretreated rats, which are partially tolerant to the drug. However, this possibility is precluded by the fact that in preparing the livers for perfusion the terminal bile duct is eliminated from the preparation.

It may be concluded that chronic methadone treatment of rats has a stimulatory effect on bile flow *via* an unknown mechanism, resulting in increased excretion of methadone metabolites in the bile but does not affect the rate of plasma disappearance or pattern of bio-transformation of the drug.

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