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Species differences in the effects of chronic, oral administration of methadone on oxidative drug metabolism

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While nearly all vertebrate species have in common a similar system for the metabolism of drugs, variations in the patterns of drug biotransformation have been observed. The rate of metabolism of a drug through a given pathway may differ from species to species, or the pathway itself may be quite different. For example, hydroxylation is the main route of biotransformation of amphetamine in the rat, but deamination is a more important route, quantitatively, in the mouse and in man [1].

Numerous drugs and environmental chemicals can inhibit or enhance the actions of drugs by altering the activity of the drug-metabolizing enzymes located in liver microsomes. Again, species variations can occur with regard to the capability of a drug to potentiate the activity of the microsomal system. For example, dichlorodiphenyltrichloroethane (DDT) stimulates the metabolism by liver microsomes of various drugs in rats but is without effect in mice [2].

In this communication, we report that the manner in which the microsomal drug-metabolizing system in different species responds to the chronic administration of methadone is unusual if not quite unique. The drug has the peculiar property of acting as an inducer of drug-metabolizing activity and exerting an anabolic effect on the liver in one species (mouse) while bringing about precisely the opposite effects in other species (rat and guinea pig).

Methods

Animals. ICR (male) and Swiss–Webster (male and female) mice, 25–30 g, were obtained from Simonsen Laboratories, Gilroy, Calif. Horton Laboratories, Oakland, Calif., supplied male ICR mice, 25–30 g, for some experiments. Rats (Sprague–Dawley males, 150–175 g) and guinea pigs (random bred, Hartley strain, males, 150–275 g) were also purchased from Simonsen Laboratories. All animals had free access to food and water, and their cages were cleaned twice a week to avoid accumulation of urine and feces which is known to depress drug-metabolizing activity [3].

Chemicals. d,l-Methadone·HCl was obtained from the Mallinckrodt Chemicals Works, St. Louis, Mo. All other chemicals used in the study were purchased from Sigma Chemical Co., St. Louis, Mo.

Administration and dosage. The methadone was administered once a day, orally, by means of intubation. The solution used for dosing contained 5 mg *d,l*-methadone·HCl/ml (aqueous). All control animals received equivalent volumes of water orally, also by intubation.

Tissue preparations. Rat livers were quickly perfused *in situ* with 50 ml of cold KCl (1.15%) solution and homogenized in 3 vol. of 0.1 M Tris–HCl, pH 7.4, 24 hr after the last dose of methadone or water, as described previously [4]. Microsomes were prepared according to Mazel [5] by centrifuging aliquots of the 12,000 *g* supernatant fractions from the above homogenates at 105,000 *g*. The final microsomal pellet was stored under 1 ml of the Tris–HCl buffer.

Enzyme assays. N-demethylase activity was assayed using the procedure of Fouts [6]. Ten μmoles *d,l*-methadone·HCl (2 mM) served as the substrate [4]. Aniline hydroxylase was assayed after method 2 described by Mazel [5], except that 0.1 M Tris–HCl was used as the buffer. Approximately 3–5 mg of protein from the 12,000 *g* supernatant fraction of the homogenized livers was used in the assays for methadone *N*-demethylase and aniline hydroxylase activities. The reactions were found to be linear with time for at least 30 min. Activities are expressed as μmoles of product formed/30 min/liver in order to convey information concerning the capability of the animal, rather than just a portion of its liver, to demethylate methadone.

Protein. Protein concentrations were determined by the procedure of Lowry *et al.* [7].

Sleeping times. Twenty-four hr after the last dose of methadone, pentobarbital sleeping times in rats and mice were determined after administration of sodium pentobarbital (50 mg/kg) intraperitoneally. The time elapsed from the loss of righting reflex to its return was defined as the sleeping time. The elapsed time between the injection and loss of righting reflex was also noted, but no significant differences were found among the tested group of animals with regard to this parameter.

Results and discussion

Effects of methadone in the mouse. Chronic, oral administration of methadone to mice resulted in 2-fold increases in the activities *in vitro* of the enzymes that *N*-demethylate methadone and hydroxylate aniline (Table 1). The findings *in vivo* were consistent with these results in that pentobarbital sleeping time was decreased, suggesting that the rate of barbiturate metabolism by a microsomal alkyl hydroxylase was also increased. These findings confirm previous studies in this laboratory which showed that daily oral administration of methadone (50 mg/kg) to mice will effect, within 24 hr, increases in the activity of methadone *N*-demethylase [4]. There was low mortality (5 per cent) and minimal overt evidence of toxicity associated with this dose of methadone.

Table 1 further shows that animal weights and liver weights were not significantly changed compared to those of controls given equivalent volumes of water over the first 6 days. Longer periods (30 days) of administration, however, resulted in significant decreases in both body and liver weights in the treated animals. Methadone seemed to exert anabolic effects on the liver at 6 days as judged by increases in microsomal protein, but by day 30, the increases in microsomal protein were no longer seen. At both test days, pentobarbital sleeping time was markedly shortened in the methadone-treated group.

Effects of methadone in the rat. Rats, in marked contrast to the mice, exhibited, during a similar regimen of methadone administration (50 mg/kg), a depression in the activities of methadone *N*-demethylase and aniline hydroxylase and slightly prolonged pentobarbital sleeping time. Table 2 indicates that, for the first 6 days, methadone was without effect on any of the parameters examined in rats. (Since a dose of 25 mg/kg also proved to be without effect, this lower dose was discontinued.) It can be seen that, later

Table 1. Effect of chronic administration of methadone in the mouse*

Treatment	Days of treatment	Animal wt (g)	Liver wt (g)	Methadone N-demethylase activity†	Aniline hydroxylase activity‡	Microsomal protein (mg/g liver)	Pentobarbital sleeping time (min)
H_2O	6	29.0 ± 0.9	1.51 ± 0.04	0.925 ± 0.124	1.20 ± 0.14	36.7 ± 3.5	58.1 ± 8.5
Methadone	6	29.4 ± 0.5	1.64 ± 0.05	1.80 ± 0.08§	2.47 ± 0.18§	47.0 ± 2.5§	19.5 ± 0.8§
% of Control		101	109	195	206	128	34
H_2O	30	37.6 ± 1.2	2.16 ± 0.09	0.761 ± 0.094		27.2 ± 0.9	64.3 ± 8.3
Methadone	30	31.8 ± 1.2§	1.56 ± 0.07§	2.01 ± 0.18§		27.9 ± 2.3	20.3 ± 1.4§
% of Control		85	72	263		103	32

* Male ICR mice (Simonsen), initially weighing 25–30 g, were dosed orally on a daily basis with *d,l*-methadone HCl (50 mg/kg). Controls received equivalent volumes of water. Each value is the mean ± S. E. M. for four to six animals.

† Expressed as μmoles HCHO formed/liver/30 min.

‡ Expressed as μmoles *p*-aminophenol formed/liver/30 min.

§ Significantly different ($P < 0.05$) from controls.

into the regimen (by day 30), the various parameters relating to the function of the hepatic drug-metabolizing system were markedly depressed by methadone, and pentobarbital sleeping time was prolonged. Treated animals failed to gain weight as rapidly as water-treated controls, and as a result body and liver weights were significantly lower in the narcotic-treated animals, even at day 6. By day 60, body and liver weights were only approximately 75 per cent of controls.

The rats were found to be more sensitive to methadone than mice, and a number of signs of toxicity were noted. Mortality was higher (10/46; 22 per cent) over the 60-day treatment period, although about $\frac{1}{2}$ of the deaths occurred after the first or second dose, some within minutes after administration. Five rats (11 per cent) exhibited alopecia on portions of their bodies. In six animals (13 per cent), small wounds on the tails and paws failed to heal and sometimes became quite extensive. None of these signs were seen in any water-treated controls.

Effects of methadone on guinea pigs. A lower dose of methadone (25 mg/kg) was used on the guinea pigs because these animals proved to be extremely sensitive to the depressant effects of the narcotic. Although none of the four guinea pigs given methadone over a 6-day period died, this dose resulted in a decrease in body and liver weights (Table 3).

The inhibitory effect of the drug on the capability of the liver to metabolize drugs was evident by day 6: both *N*-demethylase and aniline hydroxylase were markedly decreased.

Effects of methadone in different mouse strains. The antithetical results obtained in the different species caused

Table 2. Effect of chronic administration of methadone in the rat*

Treatment	Days of treatment	Animal wt (g)	Liver wt (g)	Methadone N-demethylase activity†	Aniline hydroxylase activity‡	Microsomal protein (mg/g liver)	Pentobarbital sleeping time (min)
H_2O	6	178 ± 6	7.57 ± 0.45	8.90 ± 1.06	3.40 ± 0.20	37.7 ± 2.1	
Methadone	6	159 ± 7	6.41 ± 0.39	8.70 ± 0.84	3.47 ± 0.35	40.5 ± 3.4	
% of Controls		89	85	98	102	107	
H_2O	30	366 ± 13	16.3 ± 2.5	16.6 ± 0.7	4.25 ± 0.43	21.4 ± 0.9	54.8 ± 1.5
Methadone	30	290 ± 12§	14.0 ± 1.3	5.90 ± 2.61§	1.92 ± 0.48§	14.1 ± 1.9§	67.0 ± 3.8§
% of Controls		79	86	35	45	66	122
H_2O	60	418 ± 10	17.1 ± 1.1	12.8 ± 2.2	7.76 ± 0.94	21.3 ± 1.2	
Methadone	60	302 ± 22§	13.8 ± 1.0§	2.39 ± 0.43§	2.34 ± 0.25§	20.6 ± 1.2	
% of Controls		72	78	19	29	97	

* Male Sprague–Dawley rats, initially weighing 150–175 g, were dosed orally on a daily basis with *d,l*-methadone HCl, 50 mg/kg. Each value is the mean ± S. E. M. for five or six animals.

† Expressed as μmoles HCHO formed/liver/30 min.

‡ Expressed as μmoles *p*-aminophenol formed/liver/30 min.

§ Significantly different from water controls ($P < 0.05$).

Table 3. Effects of chronic administration of methadone in the guinea pig*

Treatment	Days	Animal wt (g)	Liver wt (g)	Methadone N-demethylase activity†	Aniline hydroxylase activity‡	Microsomal protein (mg/g liver)
H_2O	6	324 ± 7	15.9 ± 0.7	22.9 ± 1.0	5.94 ± 1.01	33.7 ± 1.5
Methadone	6	216 ± 9§	7.45 ± 0.67§	10.2 ± 1.2§	3.42 ± 0.34§	40.3 ± 2.9
% of Controls		67	47	44	58	120

* Male Hartley strain guinea pigs, initially weighing 250–275 g, were dosed orally on a daily basis with *d,l*-methadone HCl (25 mg/kg). Each value is the mean ± S. E. M. for three or four individual animals.

† Expressed as μmoles HCHO formed/liver/30 min.

‡ Expressed as μmoles *p*-aminophenol formed/liver/30 min.

§ Significantly different ($P < 0.05$) from water controls.

Table 4. Effects of 6-day administration of methadone in different strains and both sexes in mice*

Strain and sex	Treatment	Body wt (g)	Liver wt (g)	Methadone *N*-demethylase activity†	Aniline hydroxylase activity‡	Microsomal protein (mg)/g liver	Pentobarbital sleeping time (min)
ICR (Horton)	H_2O	32.5 ± 0.8	2.11 ± 0.13	0.680 ± 0.083	0.82 ± 0.09	45.0 ± 0.8	22.2 ± 2.4
male	Methadone	30.7 ± 0.8	2.04 ± 0.09	1.33 ± 0.17§	1.12 ± 0.17	45.1 ± 1.8	13.4 ± 1.0§
% of Control		94	97	196	137	100	60
Swiss Webster	H_2O	26.9 ± 1.4	1.41 ± 0.11	0.316 ± 0.063	1.35 ± 0.15	49.3 ± 2.3	27.0 ± 4.3
male	Methadone	24.7 ± 1.6	1.45 ± 0.10	0.694 ± 0.078§	1.63 ± 0.26	60.2 ± 3.6§	22.1 ± 2.5
% of Control		92	103	220	121	122	82
Swiss Webster	H_2O	31.4 ± 1.3	1.62 ± 0.11	0.644 ± 0.067	1.05 ± 0.13	43.1 ± 2.0	22.6 ± 3.5
female	Methadone	27.6 ± 1.1§	1.56 ± 0.08	1.27 ± 0.20§	1.74 ± 0.27§	55.1 ± 3.7§	10.1 ± 1.7§
% of Control		88	96	197	166	128	45

* Mice, initially weighing 25–30 g, were dosed orally on a daily basis with *d,l*-methadone HCl (50 mg/kg). Each value is the mean ± S. E. M. for five to seven animals.

† Expressed as μmoles HCHO formed/liver/30 min.

‡ Expressed as μmoles *p*-aminophenol formed/liver/30 min.

§ Significantly different from water controls ($P < 0.05$).

us to consider the possibility that the effects of methadone, which seemed to be acting as a typical inducer of drug-metabolizing activity, were peculiar to the one mouse strain (Simonsen ICR). Therefore, two other strains of mice, Horton ICR and Simonsen Swiss–Webster, were given 50 mg/kg of methadone for 6 days. Both sexes of the latter strain were used. The results (Table 4) made it quite evident that the induction phenomenon was not confined to the one mouse strain. In all cases, the activity of *N*-demethylase approximately doubled after methadone despite disparate endogenous activities in the various strains and sexes. The activity of aniline hydroxylase, on the other hand, was not significantly increased in Horton ICR and Swiss–Webster males. In contrast, Simonsen ICR (Table 1) and Swiss–Webster females (Table 4) showed significantly increased aniline hydroxylase activity. Pentobarbital sleeping time was reduced by the methadone treatment in all strains, but there were quantitative variations from strain to strain. In male Swiss–Webster mice, for example, sleeping time was not quite lowered by the methadone treatment to a level of significance (Table 4), but in the other three groups, the treated animals manifested a loss of righting reflex that lasted only 30–60 per cent of controls (Tables 1 and 4).

In summary, the daily oral administration of *d,l*-methadone to mice of various strains and either sex brought about increases in the metabolism *in vitro* of methadone (*N*-demethylation) and aniline (hydroxylation). In addition, pentobarbital sleeping time was reduced in the treated mice, and microsomal protein concentrations were increased. In contrast, the administration of methadone to rats or guinea pigs resulted in changes in the above parameters that were in the opposite direction to that of the mice. These studies indicate that, while methadone stimulates the hepatic mixed function oxidase system in mice, it depresses this microsomal drug-metabolizing system in rats and guinea pigs.

Various hypotheses and related experiments are being considered to delineate the mechanism(s) underlying these diametrically different effects of methadone. It might be pointed out, however, that, when the enzyme activities and microsomal protein were expressed as concentrations per g of liver for the guinea pig (Table 3), the values were unaffected by the methadone treatment, indicating, perhaps, a non-specific catabolic effect on the liver. This was not true for the rat where the enzyme activities were depressed regardless of how they were expressed (Table 2).

Spaulding *et al.* [8], administering methadone via drinking water (0.5 mg/ml) to rats, reported an increment in the activity of the enzyme responsible for the *N*-demethylation of methadone. It is possible, therefore, that methadone depresses the activity of the mixed function oxidase system only at high doses and not when it is administered gradually in relatively low doses.

The effects of methadone on the metabolism of drugs by the liver raises the possibility that some aspects of the tolerance seen to methadone, at least in mice and any other species in which the drug acts as an inducer of the mixed function oxidases, may be a function of its biotransformation (disposition tolerance). The metabolites (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline) that result from *N*-demethylation are analgesically inactive [9] and, therefore, an increment in the activity of *N*-demethylase will increase the rate of inactivation of the narcotic. In addition to these inactive, oxidized metabolites, an analgesically active, reduced metabolite of *d*-methadone, *l*-methadol, has been described. However, this reductive pathway is thought to be a relatively minor one [10].

Studies to date indicate that, in man, chronic administration of methadone results in increased demethylation, and dispositional tolerance may be involved in the development of tolerance to methadone [10, 11].

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Department of Pharmacology, University of California, San Francisco, Calif. 94143, *U.S.A.*

GEORGE R. PETERSON*
LAWRENCE W. MASTEN†
ALAN BURKHALTER
E. LEONG WAY

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*Department of Biological Chemistry, Wright State University, School of Medicine, Dayton, Ohio.

† Department of Pharmacology, University of Mississippi, School of Pharmacy, Oxford, Miss.

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