Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 4, pp. 1101–1104, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00210-X

BRIEF COMMUNICATION

Methadone Levels in Plasma, Urine, and Amniotic Fluid of Methadone-Treated Pregnant Rats

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Received 23 February 1994

PIERCE, T. L. AND W. HOPE. *Methadone levels in plasma, urine, and amniotic fluid of methadone-treated pregnant rats.* PHARMACOL BIOCHEM BEHAV **49**(4) 1101–1104, 1994.—Methadone was administered in the form of a slow release emulsion to nonpregnant rats and pregnant animals at early, middle, or late gestation. Following a 48-h treatment period, plasma, urine, and amniotic fluid were analyzed for methadone and its two major metabolites using solid-phase extraction and reversed-phase HPLC. Methadone and its primary metabolite were detected in plasma and urine of all rats with levels of both compounds comparable at all stages of gestation. No significant difference was found for either methadone or its primary metabolite between gestation groups and nonpregnant animals. Amniotic fluid collected in middle and late gestation groups contained methadone and primary metabolite at levels similar to that of maternal plasma. These data show that methadone can be detected in plasma, urine, and amniotic fluid following short-term treatment of pregnant rats with the opiate. Methadone levels did not alter during pregnancy and were, in fact, similar to that of nonpregnant animals. These results indicate that the fetal environment contains methadone levels comparable to that of the maternal circulation.

Methadone Methadone dependent Pregnant Rat

METHADONE maintenance is the recommended treatment for opiate-dependent pregnant women who seek treatment for their dependency. As methadone is both highly lipid soluble and has a low molecular weight, it can be expected to be readily cleared from mother to fetus. In fact, it is well established that methadone freely crosses the placenta and enters the fetal unit in the rat (5), monkey (2), and sheep (8). In human neonates, methadone has been detected in readily measurable amounts 1 h after birth in both plasma and urine of babies born to mothers on methadone-maintenance treatment and was still present in detectable amounts for up to 4 days postpartum (4).

We have recently described a method (6) to measure methadone and its metabolites in plasma of rats using high-performance liquid chromatography (HPLC). In this communication, we present results from a preliminary study designed to determine whether methadone can be measured in maternal and fetal environments following short-term administration of the drug to pregnant rats. Methadone was formulated as a slow release emulsion and injected subcutaneously to provide a depot from which it is released into the circulation. Following opiate administration, maternal plasma, urine, and amniotic fluid were analyzed for levels of methadone and its two major metabolites (2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine; referred to as primary metabolite and 2-ethyl-5-methyl-3, 3-diphenylpyrroline; referred to as secondary metabolite) at either early, middle, or late gestation. These experiments form a prelude to a long-term study in which methadone is to be administered in the drinking water of the pregnant rat.

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METHOD

Animals

Virgin female Hooded Wistar rats (200–250 g) were placed individually with males of the same strain in wire bottom cages until the presence of a vaginal plug on the floor of the cage indicated that mating had occurred. Following mating, females were housed two per cage until required under controlled conditions of lighting (12 L : 12 D cycle) and temperature (20 ± 2°C). Food and water were available ad lib.

Methadone Administration

Emulsion formulation. Methadone HCl (Wellcome) was formulated into an emulsion at a concentration of 6.25 mg/ml designed to act as a depot from which the drug would be released slowly into the animal following SC administration. Light liquid paraffin oil (BDH) and mannide monooleate (Sigma) were stirred rapidly in a small beaker using a magnetic stirrer. Methadone HCl was dissolved in saline and added slowly to the rapidly stirring oil mixture. The ratio of components light liquid paraffin oil : mannide monooleate : saline was 5 : 2 : 8. Rapid stirring was continued to maintain a consistent emulsion. Formulation was then sonicated (5 min) and stored at 4°C until required for use. Formulations were thoroughly stirred immediately prior to administration.

Treatment schedule. Animals were divided into three groups, referred to as early, middle, and late gestation. Following mating (day 0), methadone emulsion was administered by SC injection into the scruff of the neck on days 5 and 6, 12 and 13, or 18 and 19 at 0, 12 (0.25 ml/100 g) and 24 h (0.5 ml/100 g) giving rise to a daily methadone dose of 31.25 mg/kg. Animals were then sacrificed on days 7 (early), 14 (middle), or 20 (late), respectively, 48 h after the first methadone dose. Nonpregnant animals were also administered methadone emulsion. A group of pregnant and nonpregnant animals received blank emulsion to act as controls for treatment weight loss and to provide blank samples to determine extraction efficiency of the assay. All animals were sacrificed 48 h after the intitial methadone or blank emulsion injection.

During methadone treatment, rats were housed individually in wire cylindrical metabolism cages. Food and water were available ad lib. Urine output was monitored over the 48 h period and collected for analysis of methadone and its major metabolites. Weight of the animals was recorded 0, 12, 24, and 48 h following methadone administration.

Analysis of Methadone Content

Sample collection. At 48 h, animals were anesthetized with halothane (ICI) and blood collected by cardiac puncture into 5 ml plastic syringes preloaded with 0.5 ml 3.2% trisodium citrate. Blood : 3.2% trisodium citrate (9 : 1) were transferred to plastic tubes and centrifuged (Chilspin, MSE) at 600 × *g* for 10 min. Plasma was then removed and stored (−4°C) until analysis.

Animals were killed with an overdose of halothane and dissected to expose the uterus. Amniotic fluid was withdrawn from the uterine cavity using a syringe and 21 gauge needle; thus, no peritoneal fluids could contaminate the sample. Amniotic fluid was then centrifuged (Micro Centaeur, MSE) at high speed for 5 min, the supernatant collected and stored (−4°C) until analysis.

Urine samples were transferred to plastic test tubes, centrifuged (Chilspin) at 600 × *g* (10 min), and then stored for analysis (−4°C). In some instances, urine appeared to have evaporated leaving a solid residue on the base of the beaker. These samples were reconstituted in 2 ml HPLC grade water and treated as described. Immediately prior to sample extraction urine was diluted 1 : 100 with HPLC grade water.

Sample preparation. Samples were extracted using reversed-phase C18 Sep-Pak cartridges (Waters) and analysed by HPLC using a method developed in this laboratory (6). Prior to sample extraction, each cartridge was activated with 6 ml methanol, followed by 6 ml HPLC grade water and then 6 ml phosphate buffer (1/15 M sodium dihydrogen orthophosphate, pH adjusted to 7.0 using 10% NaOH) at a flow rate of 2 ml/min maintained with a B Braun-Melsungen AG syringe pump fixed in a vertical position. Following activation of the cartridge, sample (0.9 ml) was mixed with 0.1 ml 10 μg/ml of the internal standard (IS) difenoxin and loaded at a flow rate of 1 ml/min. The cartridge was then washed at a flow rate of 1 ml/min with 4 ml 10 : 90 acetonitrile : 0.08% diethylamine, pH 2.3. Methadone, its metabolites, and IS were then eluted using 4 ml 40 : 60 acetonitrile : 0.08% diethylamine, pH 2.3 at a flow rate of 5 ml/min. Extracted samples were collected and stored no longer than 2 days prior to HPLC analysis.

Sample analysis. The HPLC apparatus consisted of a Rheodyne 7125 injection valve fitted with a 100 μl loop, a C18 guard column, and an Ultracarb ODS 30 μm analytical column (150 × 4.6 mm, Phenomenex) maintained at 28°C in a Waters CHM column oven. A variable wavelength detector (Waters Lambda-Max Model 481 LC Spectro-photometer) was set at 210 nm. Chromatograms were recorded on a BAS RYT chart recorder (2 mm/min). Mobile phase consisted of 25 : 75 acetonitrile : 0.08% diethylamine, pH 2.3 (800 μl diethyl amine in 1000 ml HPLC grade water, pH adjusted to 2.3 with orthophosphoric acid). Flow rate was 1.5 ml/min. Aliquots (100 μl) were injected onto the HPLC.

Statistics

Weight loss was assessed between control and treated rats using a two-way ANOVA. Levels of methadone and its primary metabolite in plasma and urine methadone levels were compared using a one-way analysis of variance (ANOVA). *t*-Tests were used to determine significant differences in methadone and primary metabolite content of amniotic fluid at middle and late gestation.

RESULTS

Treatment Weight Loss

During the 48 h treatment schedule, methadone-treated rats showed a profound weight loss with early, middle, and late gestation animals losing 19.9 ± 1.7 g, 18.4 ± 2.8 g, and 12.3 ± 3.8 g, respectively (mean ± sem; n = 8–9). In comparison, control pregnant and nonpregnant rats continued to gain weight over the treatment period showing weight increases of 16.8 ± 2.3 g and 2.3 ± 0.9 g for pregnant and nonpregnant rats, respectively (mean ± sem; n = 3–4). Statistical analysis showed there to be a significant difference between all methadone-treated and control animals ($p < 0.01$). In addition, pregnant control animals gained significantly more weight than that of nonpregnant control rats ($p < 0.01$). In a representative sample of animals (n = 3–4), food consumption over the 48 h treatment period was 17.4 ± 4.7, 16.7 ± 1.1, 11.2 ± 6.4, and 30.8 ± 2.9 g in early,

TABLE 1

EXTRACTION EFFICIENCIES (%) OBTAINED FOR METHADONE ITS METABOLITES AND INTERNAL STANDARD, FOLLOWING SOLID-PHASE EXTRACTION IN PLASMA, URINE, AND AMNIOTIC FLUID*

	Primary Metabolite	Secondary Metabolite	Methadone	Difenoxin (IS)
Plasma	92.3 ± 11.6	76.7 ± 5.5	87.5 ± 5.2	98.9 ± 2.6
(*n*)	(6)	(6)	(6)	(26)
Urine	78.7 ± 3.9	75.3 ± 5.4	89.4 ± 5.1	99.3 ± 3.8
(*n*)	(6)	(6)	(6)	(24)
Amniotic	61.3 ± 5.8	66 ± 9.9	77.3 ± 6.9	97.4 ± 2.3
(*n*)	(3)	(3)	(3)	(13)

*Blank samples of plasma, urine, and amniotic fluid were spiked with concentrations of 0.75 μg/ml primary and secondary metabolite and 1 μg/ml methadone and internal standard.

middle, and late gestation and nonpregnant animals, respectively. In these same animals, fluid intake over 48 h was 33.5 ± 5.1, 55.3 ± 17.7, 62.1 ± 17.4, and 60.9 ± 8.1 ml in early, middle, and late gestation and nonpregnant animals, respectively.

Analysis of Tissue and Fluid Samples

Analysis of blank fluid samples to determine the presence of any endogenous compounds that could interfere with the assay showed that plasma, urine, and amniotic fluid were devoid of these. Extraction efficiencies for methadone, its two major metabolites, and IS in plasma, urine, and amniotic fluid as determined by adding known concentrations to samples collected from untreated pregnant animals are given in Table 1.

Analysis of Methadone and its Primary Metabolite

All samples of plasma, urine, and amniotic fluid obtained from methadone-treated rats contained methadone and its primary metabolite. The secondary metabolite, however, was only detected in the urine of four of the nine animals in the early gestation treatment group and in two nonpregnant rats. The mean concentration (± SEM) of the secondary metabolite was 71.56 ± 15.17 μg in the four early gestation rats and 229.67 μg in the two nonpregnant animals. In addition, levels of both methadone and its primary metabolite in plasma and urine were found to vary considerably between rats within the same gestation group.

Maternal plasma. Although plasma methadone levels were elevated in the late gestation group, there were no significant differences in methadone levels at any stage of gestation. Furthermore, plasma methadone levels were not significantly different from those found in nonpregnant animals following the same treatment schedule (Fig. 1).

It was noted that in pregnant rats, levels of primary metabolite were up to four times less than that of methadone. In nonpregnant animals, however, methadone and its primary metabolite were detected in similar levels.

Maternal urine. The total quantity of methadone and its primary metabolite excreted over the 48-h treatment period tended to be highest in the urine of the middle gestation group. Statistical analysis, however, of the data obtained from early, middle, late, and nonpregnant animals found no significant difference in the levels of unchanged methadone or primary metabolite in maternal urine (Fig. 2).

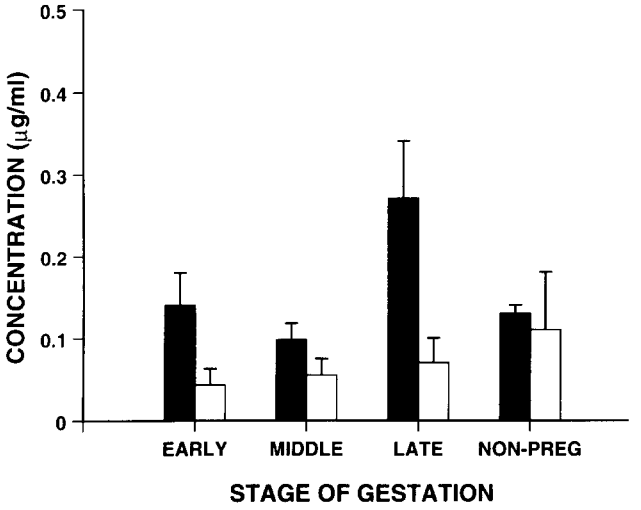

FIG. 1. Concentration (μg/ml; mean ± SEM) of methadone (■) and its primary metabolite (□) in plasma 48 h after administration of methadone emulsion (31.25 mg/kg/day) to nonpregnant rats and animals treated at early, middle, and late gestation (n = 7–9).

Amniotic fluid. At early gestation, placenta, fetal tissue, and amniotic fluid could not be separated; hence, no amniotic fluid sample was collected. At middle and late gestation, however, methadone was readily detected at a level comparable to that found in maternal plasma with the ratio of the two (plasma : amniotic fluid) being 1.3 and 1.2, respectively. Primary metabolite levels were lower than those of methadone in both groups. No significant differences in the levels of either compound was found between the two gestation groups (Fig. 3).

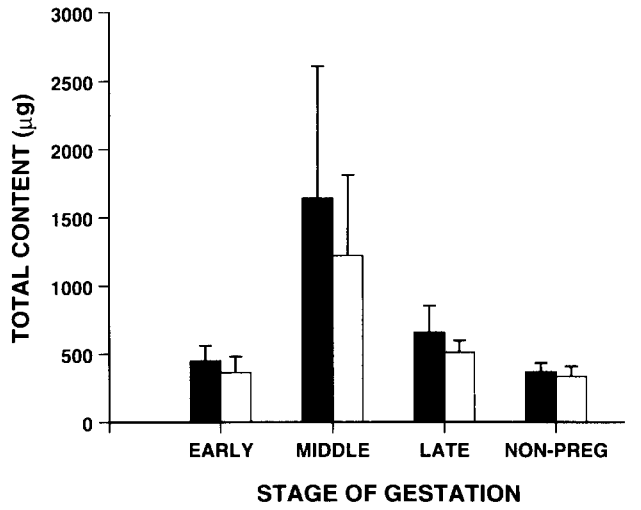

FIG. 2. Total methadone (■) and its primary metabolite (□) excreted (μg; mean ± SEM) in urine collected over the 48-h treatment period following administration of methadone emulsion (31.25 mg/kg/day) to nonpregnant, early, middle, and late gestation animals (n = 8–9).

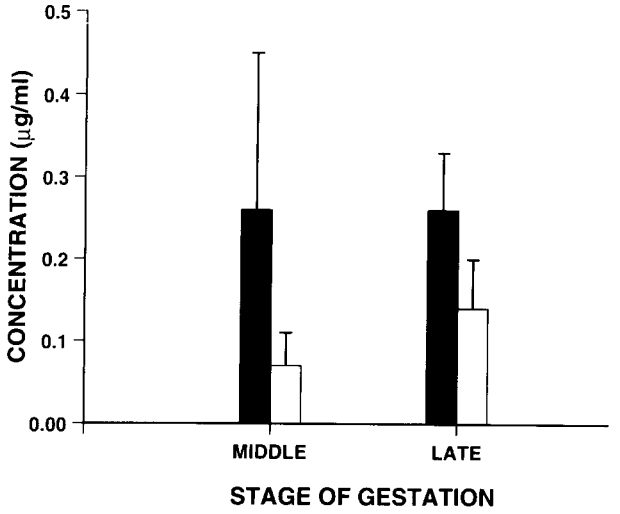

FIG. 3. Concentration (μg/ml; mean ± SEM) of methadone (■) and its primary metabolite (□) in amniotic fluid 48 h after administration of methadone emulsion (31.25 mg/kg/day) to animals treated at middle and late gestation (n = 7–9).

DISCUSSION

The present study has shown, as have others (2,5,8) that methadone readily enters the fetal environment. Although it was not possible to use the same animal at each stage of gestation, plasma levels of methadone and its primary metabolite were similar at all stages of gestation in pregnant and nonpregnant animals following the same treatment schedule, suggesting that maternal handling of methadone is not altered during pregnancy. This, however, may not be typical of all species. It has been reported in methadone-maintained women that plasma methadone levels during pregnancy are lower and clearance more rapid than in the same patients after delivery (7).

Although not noted in this study, others have found that the stage of pregnancy also influences the transfer of methadone across the placenta to the fetus. In the monkey (2), little placental transfer was found early in gestation, whereas maternal and fetal tissues collected during late gestation had equivalent amounts of unchanged methadone. In the present study, a comparison of maternal and fetal environments (plasma and amniotic fluid) at each stage of gestation, showed comparable levels of both methadone and its primary metabolite in the fetal environment. In fact, amniotic fluid contained as much methadone as did maternal plasma in middle and late gestation groups. Two explanations may be presented to explain these observations. Firstly, it is possible that the placenta acts as a reservoir for methadone and steadily releases it, thus giving rise to consistently high levels of methadone into the fetal environment. Secondly, methadone readily crosses the placenta. Whichever explanation is put forward, the fact remains that the fetus is constantly exposed to at least the same opiate level as that of the mother.

There were two obvious signs of methadone intoxication; first, the animals appeared slightly sedated, as evident by decreased motor activity and methadone emulsion administration to pregnant rats was found to result in a marked weight loss over the 48-h treatment period. In comparison, other workers have reported that intraperitoneal (1) or oral (3) methadone administration to the rat during pregnancy is associated with a reduction of maternal weight gain rather than actual weight loss. In addition, in the experiments described, it was found that weight loss over the 48-h period was similar to that of methadone-treated nonpregnant animals of the same strain and weight range. Despite such a drastic weight reduction, animals continued to consume food and water throughout methadone treatment; this, however, was reduced in comparison to animals treated with a blank emulsion. As handling and treatment procedures have been found to have an adverse effect on weight gain during pregnancy (3), the observed weight loss may, in part, be attributable to these factors. Control pregnant animals, however, subject to the same treatment procedure, gained weight as expected. Thus, this would indicate that methadone is a major factor involved in the adverse effect on weight gain and the effect of handling was minimal.

These experiments were a preliminary study to determine the ability to measure methadone in maternal plasma, urine, and amniotic fluid following short-term methadone treatment of pregnant rats. Methadone-maintenance programs, however, involve long-term oral administration of the opioid. A more appropriate animal model for the human situation should, therefore, involve oral drug administration prior to conception and throughout gestation. Because the described analytical technique was successful, further studies are now in progress to investigate the effects of long-term methadone administration on the developing fetus and to monitor concomitant plasma, urinary, and amniotic fluid content of methadone and its metabolites following such treatment.

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