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ANALYSIS AND DIFFERENTIATION OF MECHANISM OF TOLERANCE TO METHADONE IN METHADONE-FED RATS*

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Abstract—Rats fed methadone in drinking water (0.5 mg/ml) for 1 or 2 weeks developed tolerance to the analgesic effect of methadone as measured by the hot-plate method. This decreased analgesia was accompanied by an increased methadone *N*-demethylase activity measured *in vitro* and an increased methadone metabolism *in vivo* as judged by increases in the percentages of total ^{14}C as ^{14}C-water-soluble metabolites in the liver and urine 3 hr after administration of [^{14}C]methadone. Two-day oral methadone administration and 4-day phenobarbital pretreatment also increased methadone metabolism both *in vitro* and *in vivo* and decreased the analgesic effect of methadone but not of morphine, indicating that increased methadone metabolism contributed to the development of tolerance to methadone analgesia (dispositional tolerance). Methadone-fed rats also developed cross-tolerance to morphine-induced analgesia. Two-week feeding caused tolerance to the analgesic effects of methadone and morphine to a greater degree. However, there was no significant difference between the increases in methadone metabolism produced by 1- and 2-week feedings. These results indicate that dispositional tolerance did not account for all of the tolerance seen after chronic methadone feeding. This suggests that chronic methadone feeding also caused cellular adaptive tolerance. This suggestion is further supported by the evidence that 4-day phenobarbital pretreatment, enhanced methadone metabolism more than 2-week methadone feeding, but the decrease in methadone analgesia caused by phenobarbital pretreatment was not as great as that caused by 2-week methadone feeding. It is concluded that tolerance to methadone analgesia as a result of chronic methadone feeding arises due to both increased metabolic inactivation of methadone and cellular adaptation to the drug in the brain.

The essential mechanisms underlying tolerance to narcotic analgesics are not known but most of the tolerance seen with these agents has been attributed to some form of cellular adaptation of the nervous system to the effects of the drugs [1, 2]. It has been reported recently that increased metabolic inactivation of methadone as a result of chronic administration of phenobarbital or pentobarbital could be responsible for decreased methadone analgesia and lethality [3, 4]. Many investigators have reported that chronic oral methadone pretreatment enhanced methadone metabolism [5–10] and it has been suggested that this enhanced metabolic inactivation led to tolerance to methadone lethality in mice [7].

Recently, we demonstrated that rats which ingested methadone in drinking water for 2 weeks developed tolerance to methadone analgesia and enhanced biotransformation of methadone [11]. The present studies were designed to investigate, in more detail, the development of tolerance to methadone analgesia. The results indicate that both increased metabolic inactivation of methadone and cellular adaptation to the drug in the brain contribute to the development of tolerance to methadone analgesia in chronically methadone-fed rats.

MATERIALS AND METHODS

Animals and chemicals. Male Sprague–Dawley rats (Spartan Research, Haslett, MI) weighing 150–200 g were used for the acute studies; those weighing 110–140 g at the beginning were used for chronic studies. Rats were fed Purina Laboratory Chow and housed under a light cycle of 8:00 a.m. to 8:00 p.m. in an air-conditioned room.

Morphine sulfate and *dl*-methadone hydrochloride (Mallinkrodt Chemical Co., St. Louis, MO) and *dl*-methadone (heptanone-2-^{14}C) hydrochloride (California Bionuclear Corp., Sun Valley, CA) were used without further purification. The radiochemical purity of [^{14}C]methadone was greater than 98 per cent as judged by thin-layer chromatography.

For injection, the ^{14}C-labeled and nonlabeled methadone were mixed to yield a specific activity of 3 μCi/mg. All the drug solutions were prepared in saline and injected at 2.0 ml/kg of body weight. The solutions of methadone and morphine were administered through the s.c. route while sodium phenobarbital (PB) was given i.p. When methadone was given orally by gastric tube, it was prepared in tap water and administered in a volume of 20 ml/kg. All the doses of drugs referred to, hereafter, will be in terms of their salts.

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Pretreatment of animals. For study of the effect of acute administration of methadone, rats were given two doses of methadone (15 mg/kg) orally by gastric tube at a 24-hr interval. Control rats were given 20 ml/kg of tap water.

To study the effect of chronic methadone ingestion, rats housed in groups of eight were fed methadone in tap water at a concentration of 0.5 mg/ml as the only drinking water for 1 or 2 weeks. The methadone-feeding solution was prepared freshly every other day. The total body weight of rats in the same group was determined and the volume of methadone solution ingested was recorded daily throughout the feeding period. The intake of methadone per group of rats was 40–100 mg/kg/day. Control rats ingested tap water *ad lib.*

Another group of rats was pretreated with PB (90 mg/kg, i.p.) once daily for 4 days. Control rats for this experiment were injected with saline (2.0 ml/kg, i.p.).

All the pretreated rats were used for experiments 1 day after they had received their last injection of PB or ingestion of methadone. Control and pretreated animals were deprived of food, but had free access to water for about 16 hr before being used for experiments.

Effect of pretreatment with PB or methadone on the analgesic responses of methadone or morphine. Analgesia was measured by the hot-plate method of Eddy and Leimbach [12] as modified by Liu and Wang [13]. The surface temperature of the plate (TLI-Thermajust Analgesia Meter, Technilab Instrument, Inc., Pequannock, NJ) was maintained at 58 ± 0.5° throughout the experiment. No reaction in 30 sec was considered a maximal effect. A standard 30-sec cut-off time, as used by other investigators [12, 14], was used for studying the time–response curves of methadone analgesia in order to compare the intensity and duration of methadone analgesia in drug-pretreated rats with those in controls. No tissue damage was ever observed even in the animals that were exposed to the hot plate several times under the experimental conditions used. The reaction time determined 30 min before administration of saline or a challenging dose of methadone (5 mg/kg, s.c.) or morphine (15 mg/kg, s.c.) was used as the predrug reaction time for each rat. Rats which received only saline showed no variability in reaction times during the 2.5-hr hot-plate testing period. The reaction times of these control rats remained within the range of presaline control time.

The effects of pretreatment with PB or methadone on the intensity and duration of the analgesic response to methadone or morphine were examined by plotting the reaction times on the hot plate against time in min after administration of methadone or morphine.

In addition to plotting the analgesic response curve for each group of animals, the total analgesic effect, the analgesic area (see Statistical Analysis of Data), was also used as an indication of the degree of analgesia and for examining the effects of pretreatment with PB or methadone on the analgesic responses of methadone and morphine.

In vitro *metabolism of methadone.* Methadone metabolism *in vitro* was determined by measuring the rate of *N*-demethylation of methadone using liver microsomes prepared from both control and PB- or methadone-pretreated rats. Liver microsomes were prepared as described previously [15]. The assay mixture and assay conditions for *N*-demethylation of methadone were similar to those described in a previous paper [13]. The rate of methadone *N*-demethylation was measured by the formation of formaldehyde according to the method of Nash [16]. Microsomal protein concentration was determined by the method of Lowry *et al.* [17] with crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, MO) used as a standard.

Tissue distribution of [^{14}C]*methadone and its excretion in urine.* Control rats and those pretreated with PB or methadone were injected with the same dose of [^{14}C]methadone (5 mg/kg, 15 μCi/kg, s.c.) and killed by decapitation 3 hr later for tissue distribution study. Before killing, the rat was anesthetized with ether and its penis was clamped with an arterial clamp to prevent urination. Urine was collected from individual cages and urinary bladders 3 hr after administration of [^{14}C]methadone as described previously [11, 18]. Blood samples were collected in heparin-treated tubes, and whole brains and livers were removed quickly. Blood samples were centrifuged within 1 hr after collection for separation of plasma. Samples of plasma, urine, brain and liver were kept frozen until further analysis for concentration of [^{14}C]methadone and its ^{14}C-metabolites.

Analysis of [^{14}C]*methadone and its* ^{14}C-*water-soluble metabolites* (^{14}C-*WSM*). Concentrations of total ^{14}C in brain and liver were determined by mixing 0.5 ml of 20% tissue homogenate, 1.0 ml of NCS tissue solubilizer and 15 ml of Aquasol scintillation solution (New England Nuclear Co., Boston, MA) as described previously [13]. Total ^{14}C concentrations in plasma and urine were determined by directly counting 0.3 ml plasma or 0.05 ml urine with 15 ml of Aquasol scintillation solution.

Determinations of ^{14}C-labeled unchanged methadone and ^{14}C-WSM in the liver were performed by a modification of the solvent extraction method of Inturrisi and Verebely [19], described previously in detail [11].

The relative percentages of [^{14}C]methadone and its major metabolites in the urine were determined by spotting the urine sample directly on a thin-layer chromatography sheet and developing in a solvent system consisting of ethyl acetate–butanol–ethanol–ammonium hydroxide (50:30:15:0.5) as described by Roerig *et al.* [20]. The detailed procedures for quantitation of relative percentages of methadone and its metabolites have been described previously [11, 18].

Statistical analysis of data. The total analgesic effect (area) in terms of min-sec was calculated for each animal according to the method of "Numerical integration using Simpson's one-third rule" [21]. These data were analyzed in the same manner as those for the analgesic reaction time on the hot plate. Student's '*t*' test was used to determine the significance of the difference between the PB- or methadone-pretreated and appropriate control groups. Data were analyzed by means of one-way analysis of variance when more than one pretreated group was

compared to the same control group. Duncan's multiple range test [22] was then applied to examine individual differences between means. In all cases, P values lower than 0.05 were considered significant. All calculations were performed by a Hewlett–Packard 9100A computer (Hewlett–Packard Co., Cupertino, CA) using programs designed by the same company.

RESULTS

Effect of 2-day methadone administration on the time–response curves of methadone and morphine analgesia. Oral administration of methadone for 2 days decreased the intensity and shortened the duration of methadone analgesia as shown in the top panel of Fig. 1. Sixty min after administration of a test dose of methadone (5 mg/kg, s.c.) all the control animals still reached the maximum analgesic effect (30 sec on the hot plate). However, the reaction times in all of the methadone-pretreated rats had dropped sharply from their maximum analgesic effect. In fact, two out of seven methadone-pretreated rats did not reach the peak analgesic effect 30 min after administration of the challenge dose of methadone and returned to the pre-methadone reaction time (0 time on Fig. 1) at the 60-min interval. The analgesic area in methadone-pretreated rats (29.2 ± 1.46 min-sec, mean ± S. E.) was significantly lower than in control rats (45.8 ± 3.43 $P < 0.01$).

Unlike the effect on methadone analgesia, 2-day methadone pretreatment did not produce significant cross-tolerance to morphine-induced analgesia, although the analgesic response with a challenging dose of morphine tended to be decreased in methadone-pretreated rats compared to control rats (lower panel of Fig. 1). The analgesic area in methadone-pretreated rats (35.4 ± 3.64) was not significantly different from controls (42.7 ± 4.26).

Effects of chronic methadone feeding and PB pretreatment on the time–response curves of methadone analgesia. Figure 2 shows the effects of chronic methadone feeding in drinking water on the time–response curves of methadone analgesia. After 1 or 2 weeks of methadone feeding, rats developed tolerance to methadone analgesia, as evidenced by the marked reduction in reaction times after administration of a challenging dose of methadone 24 hr after cessation of methadone feeding. In all methadone-fed rats, the duration of methadone analgesia was much shorter than in controls. The effect of methadone ingestion was time-dependent since the group ingesting methadone for 2 weeks developed more tolerance than the group ingesting for 1 week. There was a significant difference between the reaction times of the two methadone-fed groups at the 30-min interval.

Similarly to chronic methadone feeding, PB pretreatment decreased the intensity and shortened the duration of methadone analgesia, as shown in Fig. 3. However, the degree of decrease in methadone analgesia produced by chronic PB pretreatment was not as great as the decrease resulting from 2-week methadone feeding. A test dose of methadone (5 mg/kg, s.c.), which caused almost no analgesia in methadone-fed rats, produced partial analgesia in PB-pretreated rats up to the 90-min interval. There was no significant difference between the saline-pretreated and control rats at any time interval measured; therefore, the data from these rats were pooled together and used as controls in Fig. 3.

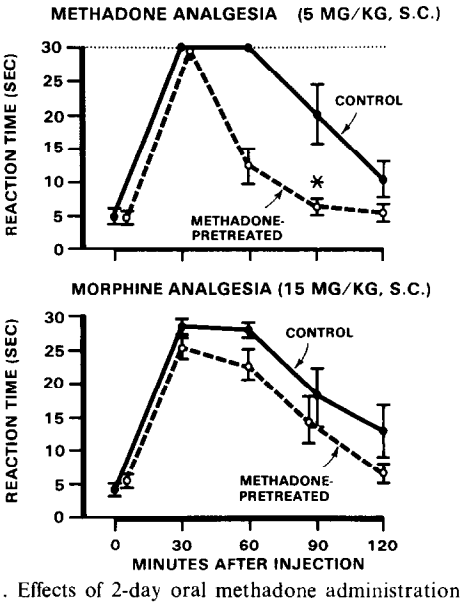

Fig. 1. Effects of 2-day oral methadone administration on analgesia by methadone and morphine. Rats were given methadone (15 mg/kg) orally by gastric tube once a day for 2 days. Control rats received water (20 ml/kg). Twenty-four hr after the second pretreatment, one half of the rats from each group were challenged with methadone and the remaining rats were challenged with morphine for measurement of analgesia. Each point and vertical bar represents a mean ± S. E. from seven rats. An asterisk indicates significant difference from the control at $P < 0.05$.

Comparison of the effects of chronic methadone feeding and PB pretreatment on morphine analgesia. To

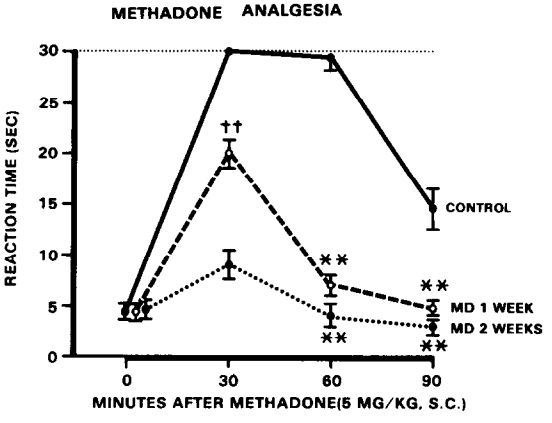

Fig. 2. Comparison of the effect of 1- and 2-week methadone (MD) feedings on methadone analgesia. Rats were fed methadone in drinking water (0.5 mg/ml) for 1 or 2 weeks. Twenty-four hr after withdrawal of methadone, the rats were challenged with methadone for measurement of analgesia. Each point and vertical bar represents the mean ± S. E. from six rats. A double asterisk indicates significant difference ($P < 0.01$) at the indicated interval as compared to the control. A double dagger indicates significant difference ($P < 0.01$) between the 1- and 2-week methadone-fed groups.

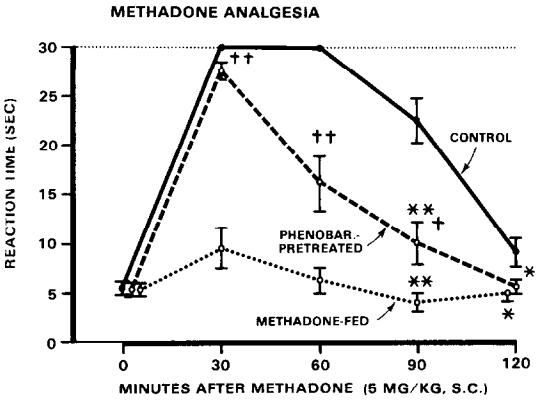

Fig. 3. Effects of chronic methadone feeding and phenobarbital pretreatment on methadone analgesia. Rats were fed methadone in drinking water (0.5 mg/ml) for 2 weeks or pretreated with phenobarbital (90 mg/kg, i.p.) for 4 days. Twenty-four hr after withdrawal of methadone or the last dose of phenobarbital, all pretreated and control rats were challenged with methadone for measurement of analgesia. Each point and vertical bar are the mean ± S. E. from six rats. An asterisk and a double asterisk indicate significant difference from the control group at the indicated interval at $P < 0.05$ and < 0.01 respectively. A dagger and a double dagger indicate significant difference from the methadone-fed group at $P < 0.05$ and < 0.01 respectively.

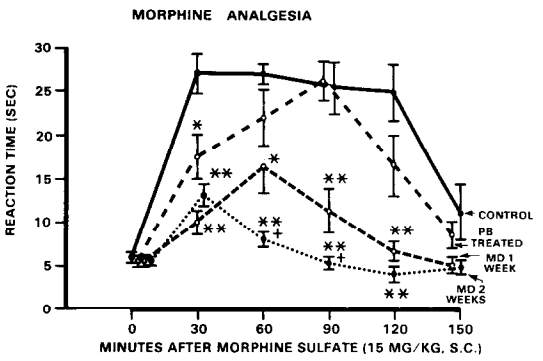

Fig. 4. Effects of chronic methadone feeding and phenobarbital pretreatment on morphine analgesia. Rats were fed methadone in drinking water (0.5 mg/ml) for 1 or 2 weeks or pretreated with phenobarbital (90 mg/kg, i.p.) for 4 days. Twenty-four hr after withdrawal of methadone or the last dose of phenobarbital, all pretreated and control rats were challenged with morphine for measurement of analgesia. Each point and vertical bar represents the mean ± S. E. from seven rats. An asterisk and a double asterisk indicate significant difference from the control group at the indicated interval at $P < 0.05$ and < 0.01 respectively. A dagger indicates significant difference between the 1- and 2-week methadone-fed groups at $P < 0.05$.

assess any cross-tolerance between methadone-induced and morphine-induced analgesia, groups of rats fed methadone for 1 or 2 weeks or pretreated with PB for 4 days were challenged with a test dose of morphine for measurement of morphine analgesia. As shown in Fig. 4, rats which ingested methadone for 1 and 2 weeks developed cross-tolerance to morphine analgesia as indicated by decreases in both the intensity and duration of morphine analgesia compared to control rats. Rats ingesting methadone for 2 weeks developed a greater degree of cross-tolerance to morphine analgesia than those for 1 week since the reaction times in 2-week methadone-fed rats were significantly lower than those in 1-week methadone-fed rats at the 60- and 90-min intervals.

In contrast to methadone feeding, PB pretreatment did not decrease the duration of morphine analgesia; however, it did slightly decrease the analgesic reaction time 30 min after morphine injection. Analysis of the analgesic area of morphine up to 120 min yielded no significant difference between PB-pretreated and control rats.

Effect of 2-day oral methadone administration on methadone metabolism in vitro *and* in vivo. The effect of 2-day oral methadone administration on methadone metabolism was studied, since 2-day methadone pretreatment decreased the analgesic effect of only methadone but not that of morphine, suggesting that methadone pretreatment might enhance its own metabolism. As shown in Table 1, 24 hr after two doses of methadone, the activity of liver microsomal enzymes responsible for methadone *N*-demethylation was increased 38 per cent over the control value. The concentration of total ^{14}C (mostly as unchanged methadone) in the brain of methadone-pretreated rats 3 hr after administration of [^{14}C]methadone was significantly lower than that of controls. The concentration of ^{14}C-WSM in the liver of methadone-

Table 1. Effect of 2-day methadone administration on methadone metabolism*

Pretreatment	*In vitro* *N*-demethylase activity (nmoles HCHO/mg protein/min)	*In vivo* methadone metabolism: Brain total ^{14}C (nmoles methadone Equiv./g)	Liver ^{14}C-WSM (as % of total ^{14}C in liver)	Urine ^{14}C-WSM (as % of total ^{14}C in urine)
Control	2.60 ± 0.153	0.928 ± 0.098	29.5 ± 3.75	14.4 ± 1.38
Methadone (2 days)	3.59 ± 0.284† (+38%)§	0.678 ± 0.037‡ (−25%)	49.0 ± 1.84† (+66%)	23.2 ± 3.86‡ (+61%)

*Twelve rats were given methadone (15 mg/20 ml/kg) orally by gastric tube once a day for 2 days. Twelve controls received water (20 ml/kg). Twenty-four hr after the second pretreatment, six rats from each group were killed and liver microsomes prepared. The remaining rats were given [^{14}C]methadone (5 mg/kg, s.c.) and killed 3 hr later. Urine was collected from cage and bladder. Data are mean ± S. E. from six rats.

†Significantly different from control at $P < 0.01$.

‡Significantly different from control at $P < 0.05$.

§Numbers in parentheses are percentage changes from control value.

Table 2. Comparison of the effects of 1 and 2 weeks of methadone feedings on methadone metabolism*

Pretreatment	*In vitro* *N*-demethylase activity (nmoles HCHO/mg protein/min)	*In vivo* methadone metabolism: Brain total ^{14}C (nmoles methadone Equiv./g)	Liver ^{14}C-WSM (as % of total ^{14}C in liver)	Urine ^{14}C-WSM (as % of total ^{14}C in urine)
Control	2.82 ± 0.238	1.02 ± 0.140	30.2 ± 2.28	15.6 ± 1.43
Methadone (1 week)	4.52 ± 0.571† (+60%)§	0.641 ± 0.082† (−37%)	49.1 ± 1.76‡ (+62%)	24.8 ± 2.04‡ (+60%)
Methadone (2 weeks)	5.19 ± 0.717† (+84%)	0.682 ± 0.039† (−33%)	46.5 ± 1.55‡ (+54%)	27.2 ± 2.25‡ (+74%)

*Rats were fed methadone in drinking water (0.5 mg/ml) for 1 or 2 weeks. Control rats received water *ad lib.* Twenty-four hr after withdrawal of methadone feeding, one half of the rats from each group were killed and liver microsomes prepared. The remaining rats were injected with [^{14}C]methadone (5 mg/kg, s.c.) and killed 3 hr later. Urine was collected from cage and bladder. Data are mean ± S. E. from six rats.

†Significantly different from control at $P < 0.05$.

‡Significantly different from control at $P < 0.01$.

§Numbers in parentheses are percentage changes from control value.

pretreated rats, expressed as a per cent of total ^{14}C in the liver, was significantly higher than that of controls. A similar increase in the concentration of ^{14}C-WSM in the urine as a per cent of total ^{14}C in the urine was found in methadone-pretreated as compared to controls.

Comparison of the effects of chronic methadone feeding and PB pretreatment on methadone metabolism in vitro *and* in vivo. Table 2 shows the effects of 1- and 2-week methadone feedings on methadone metabolism. Liver microsomal *N*-demethylase activity toward methadone was increased by both 1- and 2-week feedings. These increases were significantly different from control value but there was no significant difference between the effects of 1- and 2-week feedings. Three hr after administration of a test dose of [^{14}C]methadone to chronic methadone-fed rats, the brain concentration of total ^{14}C in all the methadone-fed rats was significantly lower than in control rats given an equal dose of [^{14}C]methadone. Chronic methadone ingestion produced an increase in liver concentration of ^{14}C-WSM expressed as a per cent of total ^{14}C in the liver, suggesting an enhancement of methadone metabolism *in vivo*. There was a parallel increase in per cent of total ^{14}C in urine as ^{14}C-WSM in the urine of all methadone-fed rats as compared to control rats. However, it should be noted that the effects of 1- and 2-week methadone feedings were indistinguishable in terms of their ability to decrease the brain concentration of total ^{14}C and increase the concentrations of ^{14}C-WSM in the liver and urine.

Since chronic PB pretreatment is known to stimulate the metabolism of methadone *in vitro*, its effect on methadone metabolism *in vivo* was measured using the indexes we chose as criteria of changes of methadone metabolism *in vivo*, and the results were compared to those obtained from chronic methadone feeding. As shown in Table 3, PB pretreatment increased markedly the methadone *N*-demethylase activity. The increase (102 per cent over the control) in this enzyme activity was significantly greater than the increase (65.3 per cent over the control) produced by 2-week methadone feeding. Table 3 also shows

Table 3. Effects of chronic methadone feeding and phenobarbital pretreatment on methadone metabolism*

Pretreatment	*In vitro* *N*-demethylase activity (nmoles HCHO/mg protein/min)	*In vivo* methadone metabolism: Brain total ^{14}C (nmoles methadone equivalent/g)	Liver ^{14}C-WSM (as % of total ^{14}C in liver)	Urine ^{14}C-WSM (as % of total ^{14}C in urine)
Control	3.05 ± 0.123	1.01 ± 0.086	29.7 ± 2.05	17.3 ± 0.92
Methadone (2 weeks)	5.04 ± 0.311† (+65.3%)§	0.723 ± 0.039‡ (−28%)	44.8 ± 3.26† (+50.8%)	30.5 ± 1.01† (+76%)
Phenobarbital	6.15 ± 0.156†‖ (+102%)	0.542 ± 0.066†¶ (−47%)	64.1 ± 6.91†¶ (+116%)	36.1 ± 2.97†‖ (+109%)

*Rats were fed methadone (0.5 mg/ml) in drinking water for 2 weeks or injected with phenobarbital (90 mg/kg, i.p.) once a day for 4 days. Control rats received no pretreatment. Twenty-four hr after withdrawal of methadone feeding or the last dose of phenobarbital, one half of the rats from each group were killed and liver microsomes prepared. The remaining rats were given [^{14}C]methadone (5 mg/kg, s.c.) and killed 3 hr later. Urine was collected from cage and bladder. Data are mean ± S. E. from six rats.

†Significantly different from control at $P < 0.01$.

‡Significantly different from control at $P < 0.05$.

§Numbers in parentheses are percentage changes from control value.

‖Significantly different from methadone-fed group at $P < 0.05$.

¶Significantly different from methadone-fed group at $P < 0.01$.

that PB pretreatment markedly decreased the brain concentration of total ^{14}C and markedly increased the concentrations of ^{14}C-WSM in the liver and urine expressed as a per cent of total ^{14}C in the liver or urine. In terms of the increase in methadone metabolism *in vivo*, PB pretreatment was more effective than 2-week methadone feeding.

DISCUSSION

The present studies were designed to investigate the effects of chronic methadone ingestion in the rat on the analgesic effect of methadone and the possible mechanism(s) involved. They demonstrate that chronic ingestion of methadone via drinking water results in a time-dependent tolerance to the analgesic effect of methadone and cross-tolerance to that of morphine. Our finding with respect to methadone tolerance is similar to those of Sung *et al.* [23] and Brown and Garrett [24] showing that rats could be made tolerant to methadone-induced analgesia by increasing daily subcutaneous injections of methadone for 14–80 days. However, no conclusive mechanism as to the cause of methadone tolerance was given by Sung *et al.* [23]. The study reported by Brown and Garrett [24] was not intended to elucidate the mechanism involved in the development of tolerance to methadone analgesia.

It is known that, in rats, methadone is metabolized mainly in the liver by *N*-demethylation, cyclization and further *N*-demethylation to form 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) [25, 26]. EMDP is further hydroxylated and glucuronidated to form water-soluble metabolite(s) (WSM) [27, 28]. We have previously reported that WSM is excreted mostly in rat urine [18]. Our previous work [29] showed that the proportion of ^{14}C-WSM, expressed as a percentage of total ^{14}C in the liver or urine, increased progressively with time after administration of [^{14}C]methadone. In other studies, we showed that changes in the percentages of total ^{14}C as ^{14}C-WSM in liver and urine 3 hr after administration of [^{14}C]-methadone could be used as indexes of changes in methadone metabolism *in vivo* [11]. The fact that rats pretreated orally with methadone and rats which ingested methadone in drinking water had significantly higher percentages of total ^{14}C as ^{14}C-WSM in the liver and urine 3 hr after administration of a challenging dose of [^{14}C]methadone clearly indicates an enhanced biotransformation of methadone as a result of methadone ingestion. Apparently, enhancement of methadone biotransformation resulted in a lower brain concentration of unchanged methadone. The lower concentration of unchanged methadone in the brain of methadone-pretreated rats would in turn decrease the duration of methadone analgesia since previous studies [13, 30] suggested that brain concentration of unchanged methadone is one of the primary factors responsible for the duration of methadone analgesia. Thus, the evidence presented in the present studies suggests that tolerance to methadone analgesia after oral methadone administration and chronic methadone feeding can be attributed at least partly to dispositional tolerance as a result of enhancement of its own biotransformation.

Dispositional tolerance could be demonstrated as early as 48 hr after the initial oral administration and after a 1- or 2-week methadone feeding in drinking water. However, there was no significant difference between the effects of 1- and 2-week methadone feedings on the metabolism of methadone. In addition, the effect of a 1- or 2-week methadone feeding on methadone metabolism was not much different from that of a 2-day oral methadone pretreatment. These results indicate that methadone ingestion rapidly results in an enhancement of its own biotransformation (e.g. dispositional tolerance) and reaches its maximal effect but a longer period of ingestion exerts no further enhancement of its own metabolism. Our findings in the rat parallel those of Masten *et al.* [6] that *in vitro* methadone *N*-demethylase activity in chronic oral methadone-pretreated mice measured after 6 and 30 days of pretreatment was approximately the same as it was after 2 days of pretreatment. These same investigators [31], however, reported recently that chronic oral administration of methadone to rats at a very high dose (50 mg/kg compared to 15 mg/kg used in our 2-day experiment) produced a decrease in hepatic methadone *N*-demethylase activity. Nevertheless, neither methadone analgesia nor methadone metabolism *in vivo* was studied by these investigators. The feeding regimen of methadone used in the present studies was similar to that of Spaulding *et al.* [8, 9], who found that rats fed methadone in drinking water for 5–10 days increased *in vitro* methadone *N*-demethylase activity. Misra *et al.* [5] found increased rates of disappearance of methadone from the brain and plasma in rats chronically pretreated with methadone via the oral route. The reports of Anggard *et al.* [32] and Verebely *et al.* [33] indicated an enhanced *N*-demethylation of methadone in man after chronic administration of methadone.

We found that a test dose of methadone (5 mg/kg, s.c.), which caused no obvious analgesia in chronic methadone-fed rats, produced some analgesia in PB-pretreated rats. However, the magnitude of increase in the *in vivo* methadone metabolism induced by chronic methadone feeding was not as great as that induced by PB pretreatment (Table 3). These results indicate that dispositional tolerance did not account for all of the tolerance seen after chronic methadone feeding. This suggests that chronic methadone feeding also caused cellular adaptive tolerance. Our results indicate that the tolerance to methadone analgesia seen in 2-week methadone-fed rats can be attributed more to cellular adaptive tolerance to methadone than can that seen in 1-week methadone-fed rats. This suggestion is supported by the experiments in which analgesia was measured by morphine instead of methadone. All chronic methadone-fed rats developed cross-tolerance to morphine-induced analgesia with rats ingesting methadone for 2 weeks developing cross-tolerance to a greater degree than in rats ingesting methadone for 1 week. Rats pretreated with methadone orally for 2 days and rats pretreated with PB for 4 days did not develop cross-tolerance to morphine analgesia, suggesting that cellular adaptive tolerance to methadone analgesia did not occur at the early stage of methadone ingestion. Very recently, Moreton *et al.* [34] also found that rats made

tolerant by chronic i.v. infusion of methadone developed tolerance to morphine using EEG and behavioral correlates.

Based on the results of metabolic studies and analgesic measurements with methadone and morphine, it is concluded that tolerance to methadone analgesia in chronic methadone-fed rats arises from both increased metabolic inactivation of the drug (dispositional tolerance) and cellular adaptation to the drug in the brain.

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