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## DISPOSITION TOLERANCE TO METHADONE

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**Summary**

*Evidence is presented in this paper to indicate that tolerance to methadone, a widely used synthetic narcotic, is dispositional in nature–i.e., metabolic inactivation of this narcotic does increase, with a resulting tolerance to its toxic properties. This is in contrast to tolerance to most narcotics such as morphine, because morphine tolerance seems to be adaptive or cellular tolerance.*

Many investigators have sought to explain narcotic tolerance as a result of increased metabolic inactivation of the drug. No evidence for disposition tolerance has yet been reported, however[1–6]. On the contrary tolerance to most narcotic effects seems to be adaptive or cellular tolerance, that is, a decrease in pharmacological effects even when enough drug is in contact with target cells in the brain to produce marked effects in naive animals[7]. We wish to report however that metabolic inactivation of methadone, a widely used synthetic narcotic does increase with a resulting tolerance to its toxic properties.

Subcutaneous or oral administration of methadone has been shown to prevent one sign of narcotic withdrawal in mice tolerant to and dependent on morphine after implantation of a morphine pellet for 3 days[8]. After 6 d of oral methadone treatment (100 mg $kg^{-1}$ of the hydrochloride salt), these mice display tolerance to the lethal effects of methadone but not to those of morphine (Table I). This observation suggests the existence of disposition tolerance to methadone as an increased $LD_{50}$ for morphine as well as methadone would have been expected had cellular tolerance developed in these animals.

The liver seems to be the chief site of metabolism for methadone[9], which is a substrate for the rat and rabbit hepatic microsomal *N*-demethylase enzymes[10]. We therefore examined the activity of *N*-demethylase in the livers of mice maintained on the

same regimen as that described above. The activity of *N*-demethylase was assayed in the hepatic 12,000 g supernatant fraction[11] 6 d after removal of the morphine pellet. Ten micromoles of D,L-methadone were used as substrate. The 6-d oral administration of methadone brought about a nearly twofold increase in the activity of the enzyme. This increase in enzyme activity may account for the elevation of the methadone $LD_{50}$ which was determined in identically treated animals. Since *N*-demethylation plays only a minor role in the inactivation of morphine[7], the fact that the $LD_{50}$ for morphine was unchanged is not surprising.

To demonstrate that the elevation in the methadone $LD_{50}$ was the result of increased *N*-demethylase activity follwing methadone administration and not residual cellular tolerance brought about by the morphine pellet treatment we gave naive mice phenobarbital intraperitoneally (50 mg $kg^{-1}d^{-1}$), which increases the activity of a variety of microsomal enzymes[12]. After 3 d of treatment, a nearly sixfold increase in *N*-demethylase activity occurred (Table II). There was a concomitant fourfold increase in the oral $LD_{50}$ for methadone. A less dramatic but significant incease of 55% in the intraperitoneal methadone $LD_{50}$ was also noted. In contrast the intraperitoneal morphine $LD_{50}$ was not significantly affected by the barbiturate treatment. Since these mice had never been exposed to narcotics it seems that a change in disposition of methadone did occur.

The increased activity of *N*-demethylase observed as a result of methadone treatment is not a consequence of the morphine pellet treatment since morphine treatment has been shown to lower *N*-demethylase activity significantly[2]. To demonstrate the ability of methadone to increase *N*-demethylase activity, naive mice were given oral doses of methadone (50 mg $kg^{-1}$) daily. After 24 h, a 50% increase in the activity of *N*-demethylase was noted. By day 6, the activity was increased nearly twofold (compare $0.770 \pm 0.124$ with $1.419 \pm 0.124$ μ mol of formaldehyde per 30 min per liver.

Table III demonstrates the similarities between the effects of methadone and phenobarbital on liver protein content and liver weights. Both treatments caused a significant elevation in the liver weights and in the protein of the 12,000 g supernatant fraction and of the microsomal fraction of the liver. The increases in microsomal protein and *N*-demethylase activity after methadone seem to be typical of the phenobarbital type of induction of microsomal enzymes[12].

The principal metabolites of metadone are 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene ($M_1$) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline ($M_2$) [13,14]. Both are formed by *N*-demethylation of methadone and non-enzymatic cyclisation and, in the case of $M_2$, further *N*-demethylation[13]. These metabolites have been isolated from rat bile[14,16] and urine[16], as well as from human plasma[17,18], bile[14,17] and urine[13,15,17,19]. They have no demonstrated analgesic activity[14] and, therefore, probably play no role in the prevention of the narcotic abstinence syndrome. Thus, the increase in the activity of *N*-demethylase following repeated administration of methadone should increase the rate of inactivation of the narcotic and contribute to the development of tolerance to some methadone effects.

There is circumstantial evidence for the role of metabolism in the termination of the pharmacological actions of methadone. Basic metabolites of methadone are significantly elevated in the faeces of rats after repeated methadone administration[20]. These compounds are probably $M_1$ and $M_2$ since both have a higher $pK_a$ than methadone[21] and represent the greatest portion of methadone metablites[14,15]. More recently, preteatment with methadone for 15 d was shown to increase the biliary excretion of $M_1$ by 50% and advance its peak excretion time[16]. Since biliary excretion accounts for the majority of these compounds in the faeces[22], these two observations support the possibility that increased activity of microsomal enzymes is the consequence of the repeated administration of methadone.

Alvares and Kappas[23] showed that phenobarbital pretreatment of rats results in an enhancement of demethylation of methadone *in vitro* by liver microsomal preparations and that the analgesic effect of methadone decreases concomitantly. No increased *N*-demethylase activity was demonstrated following chronic administration of methadone (20 mg $kg^{-1}$, intraperitoneally), however. Peters[24] was also unable to demonstrate enhanced demethylation after daily intraperitoneal injections of methadone (5 mg $kg^{-1}$) in rats. In contrast Misra *et al.*[25] using an oral rather than a parenteral route observed that the biological half life of methadone in the plasma and brain of rats was reduced more than fourfold after repeated administration of the drug (59 mg $kg^{-1}$). Subcutaneous administration of methadone brought about a much smaller decrease in this half life.

These observations may have clinical implications for methadone maintenance in which doses are often greater than one-half the minimal lethal dose in non-tolerant subjects. Rapid inhibition of microsomal metabolism can follow after exposure to a number of drugs and environmental agents[26,27]. Morphine and heroin for example are abused by subjects on methadone maintenance and are effective microsomal enzyme depletors[2,28]. Furthermore the occurrence of microsomal enzyme inhibition is compounded when methadone is taken orally, a route which is associated with a slow onset and prolonged duration of drug action. Thus toxic sequellae may not develop until the subject has left the methadone maintenance for the day.

**Table I**

**Effects of repeated methadone treatment on methadone and morphine lethality and activity of liver N-demethylase in mice rendered tolerant by implantation of a morphine pellet.**

Mice were implanted with 75 mg morphine base pellets for 3 d (ref 8). Treated animals were given 100 mg $kg^{-1}$ of D, L-methadone HCl in water (5mg $ml^{-1}$) orally once a day for 6 d after removal of the pellet. Controls were given equivalent volume of water. All determinations were made 24 h after the last dose. $LD_{50}$s were calculated according to the method of Miller and Tainter[29]. *N*-demethylase activity was assayed using approximately 5 mg of microsomal protein per assay from the 12,000 g supernatant fraction of mouse livers. The assay procedure of Fouts[11], using 10 μmol (2 mM) of D,L-methadone HCl as substrate, was used. Numbers in parentheses indicate number of animals in each group.

| Treatment | Methadone $LD_{50} \pm$ s.e. (mg $kg^{-1}$, p.o.) | Morphine $LD_{50} \pm$ s.e. (mg $kg^{-1}$, i.p.) | *N*-demethylase activity (μmol formaldehyde/ 30 min/liver ± s.e.) |
|---|---|---|---|
| Water | 120 ± 4 (24) | 404 ± 27 (24) | 0.770 ± 0.124 (11) |
| Methadone | 198 ± 21[a](24) | 407 ± 34 (24) | 1.42 ± 0.12[a] (11) |

[a] Significantly different from controls, $P < 0.05$.
i.p. Intraperitoneally; p.o., orally.

**Table II**

**Effect of phenobarbital treatment on methadone and morphine lethality and activity of liver N-demethylase in naive mice.**

Mice were injected intraperitoneally with 50 mg $kg^{-1}$ phenobarbital in saline (5 mg $ml^{-1}$) once a day for 3 d. Controls were injected with saline. All determinations were made 24 h after the last injection using the same procedures as in Table I. Numbers in parentheses indicate number of animals in each group.

| Treatment | Methadone $LD_{50} \pm$ s.e. (mg $kg^{-1}$) | Morphine $LD_{50} \pm$ s.e. (mg $kg^{-1}$) | *N*-demethylase activity (μmol formaldehyde/ 30 min/liver ± s.e.) |
|---|---|---|---|
| Saline | 84.0 ± 4.6 (24), p.o.<br>40.0 ± 2.8 (24), i.p. | 480 ± 44 (24) | 0.598 ± 0.0226 (5) |
| Phenobarbital | 304 ± 42[a](24), p.o.<br>62.0 ± 2.6[a](24), i.p. | 547 ± 23 (24) | 3.58 ± 0.23[a](5) |

[a] Significantly different from controls, $P < 0.05$.

**Table III**

**Effects of phenobarbital and methadone on liver weights and protein in liver fractions**

Naive mice were injected with phenobarbital or saline according to the procedure in Table 2. Mice implanted with 75 mg morphine base pellets were given either D, L-methadone HCl or water orally for 6 d according to the procedure in Table 1. All determinations were made 24 h after the last dose. The microsomal fraction was obtained by centrifuging 1-ml aliquots of the 12,000g supernatant fraction at 105,000g for 1 h. Proteins were determined by the method of Lowry *et al.*[30] No significant differences were noted in the body weight data. Numbers in parentheses indicate number of animals in each group.

| Treatment | Liver weight ± s.e. (g) | Protein in 12,000g supernant fraction of liver ± s.e. (mg) | Protein in microsomal fraction of liver ± s.e. (mg) |
|---|---|---|---|
| Naive mice | | | |
| Saline (5) | 1.42 ± 0·09 | 159 ± 7 | 62.6 ± 2.8 |
| Phenobarbital (5) | 1.68 ± 0.08[a] | 203 ± 11[a] | 85.9 ± 4.3[a] |
| Mice implanted with morphine | | | |
| Water (10) | 1.37 ± 0.06 | 109 ± 8 | 50.4 ± 3.6 |
| Methadone (14) | 1.69 ± 0.06[a] | 127 ± 6[a] | 60.4 ± 2.7[a] |

[a] Significantly different from controls, $P < 0.05$.

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**บทคัดย่อ**

รายงานอันนี้ได้กล่าวถึงการศึกษาที่ได้เหตุผลต่าง ๆ เพื่อที่แสดงว่าการดื้อต่อยาเมทธาโดน (methadone) ซึ่งเป็นยาแก้ปวดชนิดเสพติดนั้น เกิดสืบเนื่องมาจากการที่ตัวยานี้ไปเหนี่ยวนำให้ตับสร้างเอ็นไซม์ขึ้นมาทำลายตัวมันเองได้มากขึ้น จึงทำให้ฤทธิ์ของมันลดน้อยลง การดื้อต่อยาเสพติดด้วยวิธีกาวนี้ยังไม่มีผู้ใดค้นพบมาก่อน เพราะการดื้อยาประเภทนี้ส่วนมากแล้วเกิดจากการที่เซลล์ในสมองเปลี่ยนสภาพไปทั้ง ๆ ที่ระดับยาไม่ได้เปลี่ยนแปลงไปเลย