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BRIEF COMMUNICATION

Fate of Tritium Derived from Prenatally Administered Tritiated Methadone in Dams and Neonatal Rats[1]

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LEVITT, M., D. E. HUTCHINGS AND S. R. BODNARENKO. *Fate of tritium derived from prenatally administered tritiated methadone in dams and neonatal rats.* PHARMACOL BIOCHEM BEHAV 19(6) 1051–1053, 1983.—Tritiated methadone (^{3}HME) was administered to gravid rats on the last week of gestation, fostered neonates periodically sacrificed, and brain and liver tritium determined by combustion. Concentrations of tritium were highest in brain on the day of birth and declined rapidly so that after 5 days, 20% remained, and after 25 days, 2% remained; similar values were found in liver. In maternal brain, concentrations on the day of birth were essentially the same as offspring brain. The brain concentrations of methadone are discussed in relation to neurobehavioral effects.

Methadone Opiates Persistence Neurobehavioral effects

NEWBORNS passively addicted to methadone show an abstinence syndrome that includes irritability, restlessness and myoclonic twitching. These symptoms gradually subside and are followed by subacute withdrawal consisting of hyperactivity, hypertonicity, disturbed sleep and feeding problems persisting for 3–4 months (for review see Hutchings, [2]). Rats exposed prenatally to methadone show disturbed sleep patterns and hyperactivity for three weeks after birth [3,5]. We have investigated whether the persistence of methadone in neonatal tissue may be related to behavioral effects.

We reported [6] that following the administration of ^{3}HME to gravid rats, methadone persisted in offspring brain and liver up to 30 days after birth. In that study, ^{3}HME was extracted from neonatal tissues and separated by high pressure liquid chromatography. Our procedure distinguished authentic methadone from apparent metabolites but because of low recoveries, we were unable to estimate tissue concentrations. The present study uses tissue oxidation rather than extraction to measure methadone. While this procedure is nonspecific, it has the advantage of high recovery and great sensitivity and permits the estimation of tissue concentrations. We studied tritium concentrations of brain and liver in neonatal tissues from birth to 25 days of age and, in addition, maternal brain after parturition.

METHOD

Individual nulliparous Wistar females (200–224 g, Hilltop Lab Animals, Inc.) were paired with males of the same strain in cages with raised grid floors. The cage floor was examined daily for sperm plugs. The date of finding the plug was designated Day 1 of gestation and the females were then housed 2–3 to a cage on wood chips. Purina Lab Chow and water were provided ad lib. Tritiated l-methadone hydrobromide (Meth-1-^{3}H, 128 mCi/m mol, 98% chemically pure by chromatograph, New England Nuclear) was added to an aqueous solution of methadone hydrochloride (Eli Lilly and Co.) to give a specific activity of 5000 Dpm/μg. All animals received a single daily dose of 5 mg/kg of ^{3}HME by gastric intubation between 1000 and 1300 hr on Days 16 through 22 of gestation. We have not found methadone to alter gestation length, therefore the dams received their last dose approximately 24 hr prior to parturition.

Two days before parturition, animals were separated and housed in individual cages. Within 6–8 hr after birth, intact litters were fostered to surrogate dams of the same strain that had delivered approximately 24 hr earlier. Four treated dams were sacrificed and brains removed. Groups of pups were randomly selected from a total of 14 litters, sacrificed by

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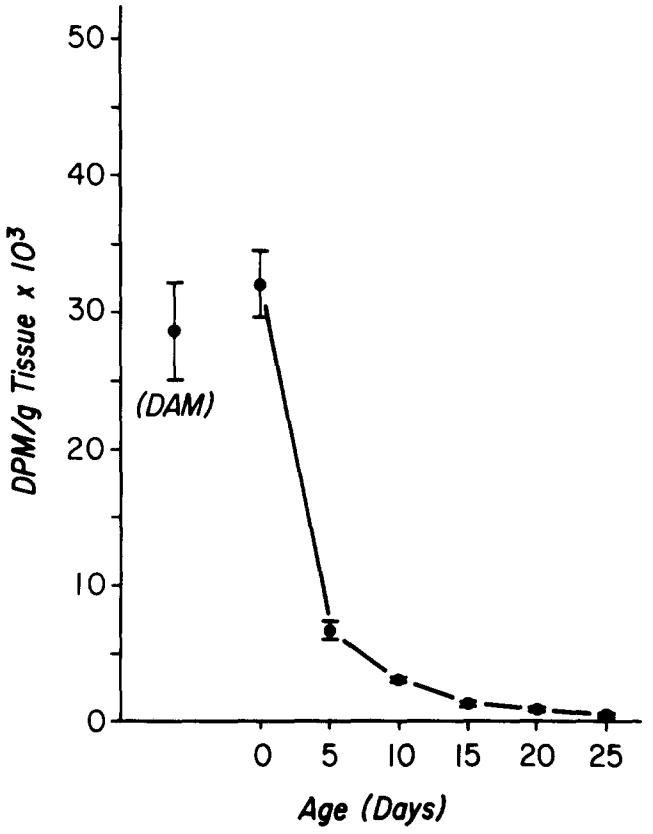

FIG. 1. Changes in brain tritium concentration over time. Valves were obtained by combusting brain as described under the Method section. The values are the mean±SD of 5 samples on day 0 and 5, 4 samples on day 10, 6 samples on day 15, 8 samples on day 20, and 10 samples on day 25.

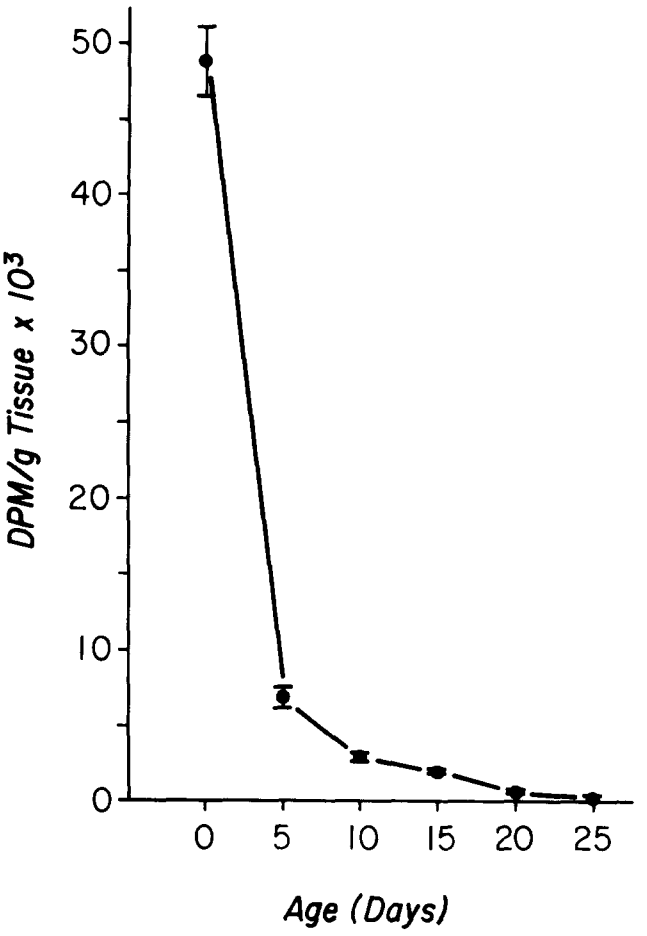

FIG. 2. Changes in liver tritium concentration over time. Values were obtained by combusting liver as described under the Method section. The values are the mean±S.D. of 4 livers on day 0 and 10, 6 livers on day 5 and 15, 8 livers on day 20, and 10 livers on day 25.

decapitation at 5-day intervals from birth (Day 0) to Day 25 and brains and livers removed. All tissue was immediately weighed, wrapped and frozen. Prior to assay, samples were allowed to thaw and partially dessicate and Cumbustaide (Packard) added to each sample.

Tissue tritium concentrations were assayed by combusting the tissues under pressure in an oxygen atmosphere using an automated Packard Tissue Oxidizer. Samples were counted in a Packard Tricarb liquid scintillation spectrometer. The addition of known quanties of ^{3}HME to tissues from untreated animals prior to combustion gave recoveries in excess of 90%. Counting efficiences were calculated for each sample by adding a known amount of tritiated water to each vial and recounting. The values were corrected for efficiency (about 28%) and presented as DPM/g tissue.

RESULTS

In this study we measured the tissue tritium by combustion to tritiated water which we report as apparent methadone.

The mean levels of apparent methadone in maternal brain on the day of birth and those in offspring brain on Days 0 through 25 are shown in Fig. 1.

On the day of birth, the apparent methadone concentration in brain of the offspring is essentially the same as that found in maternal brain. The concentration of apparent methadone in offspring brain is 6.4 μg/g.

At subsequent ages, tritium concentrations in offspring brain rapidly declined so that after 25 days only trace amounts remain. The rate of decrease of apparent methadone in liver is shown in Fig. 2. The mean value for liver is higher than brain on Day 0, declines rapidly and is similar to brain.

The specific activity of the administered ^{3}HME permits the estimation of tissue concentrations of apparent methadone and metabolites from tissue tritium concentrations. The values at Day 0 were obtained approximately 24 hr after the last dose of methadone and have probably decreased from their peak values at birth.

The values for brain continue to decrease so that about 20% remains at 5 days and 1.5% after 25 days. In liver the concentration at day 1 is 9.7 μg/g which declines to 13.5% at day 5 and 0.5% at day 25.

DISCUSSION

Methadone readily crosses the placenta and accumulates

in the tissues of several species including human [13], monkey [1], and rat [4,11]. Misra *et al.* [10] showed that methadone persists in brain and other tissues of the adult rat for several weeks even though it disappears from plasma within hours. This study describes tissue concentrations of methadone in maternal brain at the time of birth and in offspring as a function of postnatal age. The combustion procedure yielded results for offspring that confirm and extend our previous findings [6] that the concentration of tritium in tissue rapidly declines from the initial values so that after 5 days approximately 20% remain and by 25 days only trace amounts are found. Further, concentrations of methadone in neonatal and maternal brain are equivalent at birth (and probably throughout gestation) and may be associated with both short- and long-term neurochemical and behavioral effects [2]. For example, Rech and co-workers [10] reported a significant decrease in dopamine metabolism in limbic regions after 21 to 90 days and differences in behavior 90 days after methadone treatment. Similarly, McGinty and Ford [7] reported that prenatal methadone in rats may modify the growth of catecholinergic axons from the hindbrain to the forebrain. These workers also described marked effects on the uptake mechanisms for norepinephrine and dopamine and on the forebrain amine concentrations of prenatally treated rats.

Short-term dose-related behavioral effects in rat pups prenatally treated with methadone have been described by Hutchings *et al.* [5]. After 10 mg/kg, neonates were hypoactive and appeared sedated at birth while after 5 mg/kg they showed withdrawal-like hyperactivity at 5 days. Possibly the hypoactivity observed soon after birth was mediated by a neonatal brain concentration of methadone to which tolerance had not completely developed in utero. The subsequent hyperactivity may have been produced by the rapid decrease in brain tissue concentrations of methadone that occur during the first five days after birth. These workers also reported sleep disturbance and hyperactivity in the third and fourth postnatal weeks [3].

However, in a preliminary study in our laboratory (unpublished data) we were not able to precipitate abstinence effects (reduced body temperature, weight loss and increased activity) at 2 weeks of age by the administration of naloxone (3 mg/kg). This suggests that methadone is not pharmacologically active at this time.

The complex mechanism(s) by which methadone produces these developmental effects remains poorly understood. Methadone may act as a functional teratogen, altering a developmental pathway to produce relatively permanent neurobehavioral sequelae in the offspring. The abstinence-like effects that appear soon after birth may be mediated by these effects or as a result of the pharmacological activity of the compound and its rapid elimination.

REFERENCES

1. Davis, C. M. and D. C. Fenimore. Placental transfer of methadone in monkeys. *J Pharmacol Exp Ther* **205:** 577–586, 1978.
2. Hutchings, D. E. Methadone and heroin during pregnancy: A review of behavioral effects in human and animal offspring. *Neurobehav Toxicol Teratol* **4:** 429–434, 1982.
3. Hutchings, D. E., E. Feraru, H. S. Gorinson and R. R. Golden. Effects of prenatal methadone on the rest-activity cycle of the preweanling rat. *Neurobehav Toxicol* **1:** 33–40, 1979.
4. Hutchings, D. E., H. F. Hunt, J. P. Towey, T. S. Rosen and H. S. Gorinson. Methadone during pregnancy in the rat: Dose level effects on maternal and perinatal mortality and growth in the offspring. *J Pharmacol Exp Ther* **197:** 171–179, 1976.
5. Hutchings, D. E., J. P. Towey and S. R. Bodnarenko. Effects of prenatal methadone on the activity level in the pre-weanling rat. *Neurobehav Toxciol* **2:** 331–335, 1980.
6. Hutchings, D. E., J. P. Towey, H. S. Gorinson and H. F. Hunt. Methadone during pregnancy: Assessment of behavioral effects in the rat offspring. *J Pharmacol Exp Ther* **197:** 171–179, 1976.
7. Levitt, M., D. E. Hutchings, S. R. Bodnarenko and L. L. Leicach. Postnatal persistence of methadone following prenatal exposure in the rat. *Neurobehav Toxicol Teratol* **4:** 383–385, 1982.
8. McGinty, J. F. and D. H. Ford. Effects of prenatal methadone on rat brain catecholamines. *Dev Neurosci* **3:** 224–234, 1980.
9. Middaugh, L. D. and L. W. Simpson. Prenatal maternal methadone effects on pregnant C57BL/6 mice and their offspring. *Neurobehav Toxciol* **2:** 307–313, 1980.
10. Misra, A. L., S. J. Mule, R. Block and N. L. Vadlamani. Physiological disposition and metabolism of *levo*-methadone-1-^{3}H in nontolerant and tolerant rats. *J Pharmacol Exp Ther* **185:** 287–299, 1973.
11. Peters, M. A., M. Turnbow and D. Buchenauer. Distribution of methadone in the non-pregnant, pregnant and fetal rat after acute methadone treatment. *J Pharmacol Exp Ther* **181:** 273–278, 1972.
12. Rech, R. H., G. Lomuscio and S. Algeri. Methadone exposure in utero: Effects on brain biogenic amines and behavior. *Neurobehav Toxciol* **2:** 75–78, 1980.
13. Rosen, T. S. and C. E. Pippenger. Disposition of methadone and its relationship to severity of withdrawal in the newborn. *Int J Addict Dis* **2:** 169–178, 1975.