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Morphine in Fetuses after Maternal Injection: Increasing Concentration with Advancing Gestational Age (40666)

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The opioids are used clinically and abusively during human pregnancy. It is important to know the drug concentrations in fetuses of varying gestational age after administration of the drug to the mother.

Several previous studies have shown the amount of opiates crossing the placenta in rats during late gestation (19 and 21 days). Considerable quantities of dihydromorphine cross the placenta at 19 and 21 days of gestation (1, 2). Sixteen hours after injection, fetal brain still contains measurable amounts of dihydromorphine while maternal brain contains only trace amounts (1). When dihydromorphine is injected into "tolerant" rats on the 21st day of gestation the peak concentration found in the fetuses is earlier than in nontolerant fetuses (3).

Recently, Davis and Fenimore (4) have found that very little methadone crosses the placenta during early gestation in monkeys, but equivalent concentrations are found in maternal and fetal tissues during late gestation.

The present study was undertaken to determine the amount of morphine in rat fetuses on various days of gestation after subcutaneous injection to the dam, the time intervals after injection that morphine can be measured in the fetuses, and to compare morphine levels in 15-day fetuses of dams which were naive and those chronically exposed to morphine.

Materials and methods. Pregnant Wistar rats (weighing approximately 200 g) were obtained from Charles River Laboratories on the 5th day after conception. The rats were housed singly in wire-bottomed cages in a 12-hr light–dark cycle and a temperature- and humidity-controlled environment. At 10:00 AM on gestation Days 11, 13, 15, 17, and 19, five rats for each day were injected s.c. with 1 ml of physiological saline containing 180 μCi of n-[1-^{3}H]morphine 28 Ci/mmole (Amersham) with an appropriate amount of nonradioactive morphine sulfate (Mallinckrodt) to result in a final morphine dose of 4.8 mg/kg (expressed as free base) adjusted for the weight of each animal. This dose has been shown to be an analgetic dose of morphine in the rat (5) and affects fetal activity *in utero* (6). The purity of the labeled morphine had been checked previously by thin-layer chromatography (absolute ethanol:acetic acid: water, 6:3:1) which showed that 93% of the radioactivity was associated with morphine.

At 30 min, 1, 2, 6, and 12 hr after injection the rats were anesthetized with ether and the fetuses were delivered by Caesarean section, frozen in liquid nitrogen, and stored in liquid nitorgen until the tissues could be analyzed. Eight individual whole fetuses were analyzed at each time on Day 11 of gestation. Five individual fetuses from each time on Days 13, 15, 17, and 19 were divided into head and trunk pieces which were assayed separately. Five placentas for each time and three pieces of maternal liver (always taken from the same lobe) and part of the maternal brain, divided into lower brainstem and diencephalon/caudate pieces were also assayed. One additional pregnant rat from each day was not injected, and the same tissues were analyzed to obtain blank values.

One group of five pregnant rats was chronically injected with morphine prior to the experiment. Beginning on Day 7 of gestation these rats were injected with 1 ml of nonradioactive morphine in normal saline s.c. in the shoulder region, every 12 hr (10:00 AM and 10:00 PM). The first two injections were 5 mg/kg (expressed as free base) and all subsequent injections were 10 mg/kg until Day 15. On Day 15 of gestation these rats were injected with tritiated morphine at 10:00 AM and processed as described above.

The frozen tissues were weighed and homogenized in 1 vol of absolute ethanol in glass homogenizers. The resulting homogenate was spun in a clinical centrifuge and 50

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μl of the supernatant was added to 10 μl of cold morphine carrier (1.5 mg/ml). Fifty microliters of this solution was spotted onto Gelman silica gel ITLC sheets with fluorescent indicator, and developed with absolute ethanol:acetic acid:water (6:3:1). The R_f for morphine in this sysgem is 27 and has been reported to be different from the Rfs of the major metabolites (7). The plates were dried and the morphine spots visualized under a fluorescent lamp. The spots were cut out and placed in scintillation vials with 5 ml of scintillation cocktail and counted in a Beckman Model LS-230 liquid scintillation counter. Recovery of labeled morphine from the homogenates was 90%.

Various dilutions of the injection solution were made with absolute ethanol or water and taken through the analytical procedure. Known amounts were taken from the aqueous dilutions and added to liver homogenates and analyzed. In all cases a linear relationship was observed when net dpm values were plotted against the volume of the labeled injection solution. Taking the recovery of the morphine and various dilutions during the assay procedure into consideration, it was calculated that 1 ng of labeled morphine corresponded to 178.15 ± 4.54 dpm (SEM). The results (ng/wet wt of tissue) refer to the total amount of free morphine only.

The data was analyzed by an analysis of variance for each tissue on individual days. The concentration of morphine in the head and body were found to be the same by a Student's t test and were pooled and compared by an analysis of variance for each time after injection.

Results. There was no difference in the amount of morphine in the placenta (per gram of tissue) at the same time after injection, regardless of the day of gestation and these values were pooled (Table I). The peak concentration of morphine in the placenta was at 1 hr after injection. This dropped sharply by 6 hr and was about 100 ng/g by 12 hr after injection.

The concentration of morphine in Day 11 fetuses was highest (65 ng/g) at 1 and 2 hr, and dropped to negligible values by 12 hr after injection (Table I). The peak morphine concentration in the fetuses was progressively higher on Days 13, 15, 17, and 19 ($P < 0.001$). These concentrations represented only a fraction of 1% of the dose administered. The highest morphine concentration in the fetuses was observed at Day 17, 1 hr after drug administration to the mother. At this time, morphine concentration in the fetuses represented 65% of that in the placenta. The peak concentration on Day 19 represented only 72% of the peak concentration at Day 17. On all of the days measured, the concentrations in the fetuses dropped markedly by 6 hr and were quite low by 12 hr after injection although morphine was still measurable.

The peak concentrations in maternal tissues were at 1 hr (Table I). These include maternal liver, brainstem, and caudate–diencephalon pieces. The peak concentration

TABLE I. MORPHINE CONCENTRATION (ng/g wet wt) IN FETAL AND MATERNAL TISSUES AT VARIOUS TIMES AFTER SUBCUTANEOUS INJECTIONS OF MORPHINE TO THE MOTHER

	Hours after injection				
	½	1	2	6	12
Fetal tissue					
Day of Gestation					
11	9.9 ± 1.9[a]	63.5 ± 8.5	54.1 ± 9.8	43.4 ± 8.5	2.2 ± 1.5
13	192.5 ± 29.1	179.8 ± 21.3	168.7 ± 19.6	15.0 ± 5.0	11.0 ± 4.5
15	243.9 ± 17.1	405.9 ± 10.3	396.0 ± 13.0	85.4 ± 5.6	64.7 ± 3.6
17	176.2 ± 9.1	714.2 ± 31.8	461.2 ± 15.4	76.7 ± 3.7	54.3 ± 8.1
19	356.4 ± 2.0	519.6 ± 17.9	367.5 ± 21.4	183.7 ± 9.8	76.5 ± 6.4
Placenta	746.4 ± 41.4	1092.2 ± 100.9	721.7 ± 73.3	182.4 ± 17.5	100.5 ± 6.4
Maternal tissues					
Liver	904.3 ± 55.0	1442.7 ± 171.0	713.0 ± 91.6	626.9 ± 53.1	329.2 ± 51.9
Brainstem	144.6 ± 12.1	262.3 ± 29.9	83.2 ± 16.6	12.5 ± 3.8	37.3 ± 3.9
Diencephalon	87.6 ± 7.6	170.0 ± 47.2	72.3 ± 6.7	14.7 ± 2.9	5.6 ± 3.0

[a] Variation is expressed as SEM.

TABLE II. MORPHINE CONCENTRATION (ng/g wet wt) IN FETUSES AND PLACENTAS ON DAY 15 OF GESTATION AFTER CHRONIC EXPOSURE TO MORPHINE

	½	1	2	6	12
Fetuses	561.0 ± 31.1[a]	456.8 ± 35.6	319.9 ± 19.3	72.1 ± 6.7	47.2 ± 5.7
Percentage of acute	230	113	80	84	73
Placentas	1372.4 ± 53.1	1167.6 ± 47.1	583.0 ± 27.8	166.7 ± 20.0	63.8 ± 3.8
Percentage of acute	181	107	81	91	63

[a]Variation is expressed as SEM.

in liver was 5.5 times higher than the peak brainstem concentration. Twelve hours after injection the maternal brain tissues contained only trace amounts of morphine while the liver concentration was still higher than any of the other tissues analyzed.

Chronically injected animals measured on Day 15 of gestation showed a peak concentration at ½ hr after injection (Table II). The concentration was 230% higher than in naive animals ½ hr after injection. By 1 hr after injection the morphine in chronically injected animals was 113% that of naive animals. At 2, 6, and 12 hr the concentration in chronically exposed fetuses and placentas was less than in naive fetuses.

Discussion. The major finding of this paper, that morphine passes the placenta with decreasing difficulty as gestation progresses, is consistent with the morphological development of the placenta. In the rat the placenta is hemochorial. The villi are bathed in maternal blood. This arrangement provides two separate compartments, i.e., the fetus and the mother, with a barrier in between. The morphological barrier separating maternal from fetal blood consists of (i) synctiotrophoblast, (ii) cytotrophoblast, (iii) basal lamina of the cytotrophoblast cells, (iv) mesenchyme, (v) basal lamina, and (vi) endothelium of fetal capillaries. Early in pregnancy this barrier is relatively thick and becomes much thinner as pregnancy progresses. The cytotrophoblast layer disappears altogether leaving the synctiotrophoblast and basal lamina (8). In addition, during pregnancy there is a progressive decrease in the diameter of the terminal villi with a corresponding increase in their number. This results in a larger functional surface for fetal–maternal exchange (9).

Very few vital materials cross the placenta by simple diffusion. However, drugs are thought to cross mainly by diffusion (10). Since the placental membrane is lipoprotein in character, the rate of diffusion of a drug is dependent on its lipid/water partition ratio. Thus drugs with a high lipid solubility are transferred most rapidly. In this respect the placental barrier resembles the blood brain barrier and the gastrointestinal barrier (11). Drugs with a molecular weight up to 600, M_r are thought to cross readily, but this is dependent on several factors, including pH (affecting dissociation), rate of administration, concentration in maternal plasma, and rate of metabolism of the drug by the mother, placenta, and fetus (11).

Several studies have shown that different opioids pass readily from maternal blood into the fetus at 21 days of gestation (1–3). As shown in the present study, this is not the case during earlier stages of gestation. There is also some difference in the times of peak concentrations of different opioids. Blane and Dobbs (2) found a peak of etorphine in the fetus at 1 hr after injection while the peak for dihydromorphine was at 2 hr after injection. Etorphine reaches its peak concentration in maternal blood and brain more rapidly than dihydromorphine. Etorphine has a higher lipid solubility than dihydromorphine (2).

Very low levels of the enzymes catalyzing glucuronidation are detectable in the livers from fetal and newborn rats (12, 13). However, morphine glucuronyl transferase activity of 3-day-old rats reaches the adult level (14). Yeh and Woods (3) found a significant amount of conjugated [^{3}H]dihydromorphine in 21-day fetal rats and speculated that this probably originated from synthesis in both the placenta and fetus. In the present study there was a statistically significant drop in free morphine in the fetus between Days 17 and 19 of gestation which may be accounted for by an increase in morphine conjugation to morphine glucuronide.

The peak concentration of morphine was at 30 min after injection in 15-day fetuses chronically exposed to morphine. In fetuses exposed acutely to morphine the peak con-

centration was achieved at 1 hr after injection. This result is consistent with that of Yeh and Woods (3) who found earlier peak concentrations of dihydromorphine (1 hr in tolerant, 2 hr in nontolerant) of 21-day fetuses. Yeh and Woods (3) measured the disappearance of [^{3}H]dihydromorphine from the injection site and found that disappearance is more rapid in tolerant than in nontolerant animals. This might be caused by either an increase in vascularity or blood flow through the area or both.

Summary. Pregnant rats were injected s.c. with [^{3}H]morphine on Days 11, 13, 15, 17, and 19 of gestation. At ½, 1, 2, 6, and 12 hr after injection the fetuses were delivered by Caesarean section and were analyzed for [^{3}H]morphine along with placentas and various maternal tissues. Very little morphine was found in the fetuses on Day 11. On each subsequent day until Day 17, the amount of morphine increased in the fetuses. On Day 19 the morphine in the fetuses was lower than on Day 17, possibly because of morphine metabolism by the placenta and fetus. Peak concentrations of morphine occurred in the fetuses at 1 to 2 hr after injection up to Day 15, while peaks occurred on Days 17 and 19 at 1 hr after injection. In addition, one group of pregnant animals was injected chronically with 10 mg/kg of morphine twice daily between Days 7 and 15 of gestation. On Day 15, these animals were injected with [^{3}H]morphine and the fetuses analyzed at the times stated above. These fetuses showed a peak concentration at ½ hr after injection which was higher than the 1- to 2-hr peak in naive fetuses on the same day. In conclusion, these results show that morphine passes through the placenta more easily as gestation progresses and that the concentration of morphine in the fetuses can be altered by prior morphine exposure.

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