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Sex differences and endocrine regulation of morphine oxidation in rat liver

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Sex differentiation of rat liver metabolism of steroids is heavily dependent on neonatal imprinting by testicular androgens. This imprinting process has been indicated as a prerequisite for a hypothalamo-pituitary regulation of rat liver metabolism. Castration of male neonates interferes with the hypothalamic imprinting by testicular androgens [1]. The neuroendocrine effects on rat liver are mediated by the sex differentiated secretory pattern of growth hormone (GH) [2, 3] which is regulated by the hypothalamus through the combined action of somatostatin and a GH-releasing factor [4, 5]. The sexual dimorphism in GH-secretion occurs at puberty, in parallel with the development of gonadal function and maintenance of a male secretory pattern in the adult rat is dependent on circulating androgens [2, 6].

Sex differentiated rat liver metabolism has been described for a variety of drugs and steroids [7, 8]. A number of endocrine manipulations modifies hepatic metabolism [1, 6–9]. Castration of male rats at neonatal or at adult age, as well as continuous infusion of GH to male rats tends to feminize hepatic metabolism [1, 3, 4, 6, 9] and hypophysectomy of male and female rats markedly attenuates sex differences in liver metabolism [7, 9].

Perinantal exposure of rats to morphine is known to interfere with the development of sexual behaviour [10, 11] and to interact with certain endocrine parameters [10]. We recently reported on the effects of perinatal exposure to morphine on hepatic metabolism in adult rats of both sexes [12]. The microsomal capacity to perform several cytochrome P450 mediated reactions was significantly different in the morphine treated rats compared to controls and the effects were similar in males and females.

The present study was designed to investigate whether N-demethylation of morphine is regulated via the hypothalamo-pituitary-liver axis in a way similar to that previously described for metabolism of 4-androstene-3,17-dione (androstenedione) [3, 4, 6–9].

Materials and Methods

In experiment 1 male and female Sprague–Dawley rats (Møllegaard Breeding Center, Ejeby, Denmark; were killed at either 21 or at 56 days of age. Hypophysectomy was performed at Møllegaards at the age of 42 days by an intraauricular method under short anaesthesia with metohexital sodium (Brietal). These rats were killed at 56 days of age and were compared to their untreated controls.

In experiment 2 male Sprague–Dawley rats (ALAB, Stockholm, Sweden) were castrated either neonatally, within 36 hr of birth, or at 42 days of age. All rats in the experiment were killed at the age of 61 days.

Adult male rats in experiment 3 were treated with continuous infusion of bGH (6 μg/hr) in Alzet osmotic minipumps, model 2001 (Palo Alta, CA). bGH (USDA-bGH-B-1) was kindly supplied by the NIIDK (MD, U.S.A.). Treatment was started at 49 days of age and the rats, including untreated male and female controls, were killed at 56 days of age.

Methods. The animals were starved overnight before decapitation in order to reduce hepatic levels of glycogen. Liver microsomes were prepared according to Ernster *et al.* [13] and protein was measured as described by Lowry *et al.* [14]. Metabolism of androstenedione was measured as previously described [8] and N-demethylation of morphine according to Rane *et al.* [15]. 4-[4-^{14}C]Androstene-3,17-dione (59 mCi/mmol) was obtained from the Radiochemical Center (Amersham, U.K.) and unlabelled androstenedione from the Upjohn Company (Kalamazoo, NI). Morphine chloride was purchased from Apoteksbolaget (Gothenburg, Sweden).

Statistical analysis. Statistical analysis was performed using the Wilcoxon rank sum test [16] and the level of significance was set at $P < 0.05$. All values were expressed as means ± SD.

Results and Discussion

A pronounced sex difference in the microsomal rate of N-demethylation of morphine was observed. The average activity in liver from adult male controls was 15 to 22 times higher than in livers from the corresponding female rats.

At the age of 21 days, no sex differences in the rate of morphine N-demethylation had developed (Fig. 1a). Hypophysectomy was shown to eradicate the sex difference in adult rats completely as was observed in animals killed at the age of 56 days. The male morphine *N*-demethylase decreased to the level of female rats and of prepubertal rats of both sexes.

Hepatic metabolism of androstenedione was, as expected, sex differentiated in adult but not in prepubertal rats. In accordance with previous findings the sex differences in 16α-hydroxylation (♂ > ♀) and 5α-reduction (♀ > ♂) were markedly attenuated following hypophysectomy [8]. Our data indicate the importance of pituitary maturation and of an intact pituitary gland to maintain the high capacity of male rat liver to perform morphine N-demethylation.

The fact that neonatal castration of male rats had a repressing effect on the morphine N-demethylation in adult male rats (Fig. 2) further strengthens this view. At 61 days of age the enzyme activity in liver from these rats was only 6–7% of the control values. Male rats that were sham operated neonatally exhibited 77% of the activity in intact rats. Castration at adult age decreased the morphine N-demethylation rate only by half. Adult male controls had an activity that was similar to that in male rats sham operated in adult life. As expected, castration feminized 16α-hydroxylation and 5α-reduction of androstenedione. In general the feminizing effect of neonatal castration on rat liver was more pronounced that that of adult castration which is consistent with previous findings [9]. Taken together these data clearly show that morphine N-demethylation is dependent both on a normal imprinting process and on circulating androgens at adult age.

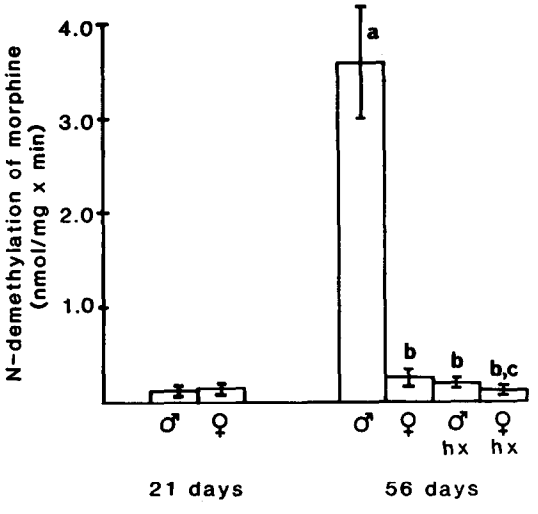

Fig. 1. N-Demethylation of morphine with liver microsomes from prepubertal (21-day-old) and adult (56-day-old) rats of both sexes and from hypophysectomized (hx; 56-day-old) male and female rats. Each group consisted of five rats. Standard deviations are indicated by vertical bars. [a]Significantly different from 21-day-old male and female rats (Wilcoxon rank sum test; $P < 0.05$); [b]Significantly different from adult male controls; [c]Significantly different from adult female controls.

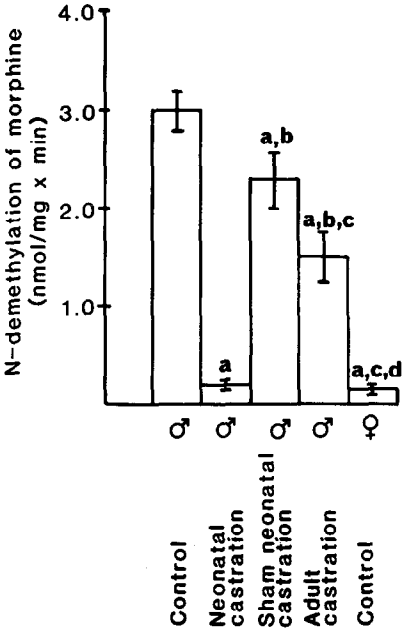

Fig. 2. N-Demethylation of morphine with liver microsomes from male rats castrated at neonatal (within 36 hr of birth) or at adult age (42 days). One group of rats was sham castrated at neonatal age. Livers from untreated males and males sham operated at adult age did not differ with respect to their capacity for N-demethylation and are presented together ("control"; 6 rats). Untreated female rats are also included. All rats were killed at the age of 61 days. In all groups, except the male controls, measurements were performed with liver microsomes from four animals. Standard deviations are indicated by vertical bars. [a]Significantly different from male controls (Wilcoxon rank sum test; $P < 0.05$); [b]Significantly different from male rats castrated at neonatal age; [c]Significantly different from male rats sham castrated at neonatal age; [d]Significantly different from male rats castrated at adult age.

Continuous infusion of bGH to adult male rats caused a significant decrease in the N-demethylation of morphine (Fig. 3) and feminized the metabolism pattern of androstenedione. The features of the hepatic cytochrome P450 responsible for N-demethylation of morphine have previously been shown to be different from many other P450s involved in drug metabolism [15]. Whereas this reaction does not seem to be inducible by phenobarbitone it is down-regulated upon escalating doses of morphine (Blanck *et al.*, unpublished results). Similar observations, following exposure to enzyme inducers/drugs, have been made for hepatic metabolism of several steroid substrates [17]. It is conceivable that the cytochrome P450 species that catalyses N-demethylation of morphine represents only a minor form since e.g. neonatal castration does not significantly perturb the total concentration of hepatic cytochrome P450 [9].

In conclusion the cytochrome P450 responsible for N-demethylation of morphine has so far not been identified. The present study provides strong evidence in favor of a hypothalamo-pituitary regulation of N-demethylation as it seems to be dependent on a normal imprinting process in the neonate male rat as well as on circulating androgens in the adult male. It is also dependent on an intact pituitary function at adult age and is modified following bGH treat-

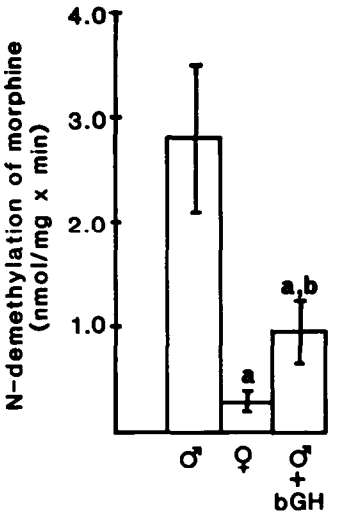

Fig. 3. N-Demethylation of morphine with liver microsomes from adult male and female rats and from male rats receiving bovine growth hormone (bGH; 6 μg/hr for 1 week). Each group consisted of five rats. Standard deviations are indicated by vertical bars. [a]Significantly different from male controls (Wilcoxon rank sum test; $P < 0.05$); [b]Significantly different from female controls.

ment. Further studies are in progress in order to investigate the endocrine effects of exposure to morphine and to identify the cytochrome P450 species involved in hepatic N-demethylation of morphine.

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