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SHORT COMMUNICATIONS

Morphine-induced effects on hepatic metabolism in the male rat depends on an intact pituitary gland

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Following exposure to morphine a number of endocrine effects have been observed in humans and in animal studies [1–5]. Secretion of gonadal [3] as well as pituitary hormones [4, 5] has been shown to be influenced by morphine administration. Perinatal morphine exposure of rats interferes with development of sexual behaviour [1] and morphine treatment in the neonatal period leads to permanent alterations in the capacity of adult rat liver to perform cytochrome P450 mediated reactions towards several enzyme substrates [6].

Administration of opiates to adult rats has previously been shown to alter the hepatic metabolism of certain drugs and steroids [7, 8]. A number of cytochrome P450 mediated reactions, including N-demethylation of morphine, are sex differentiated in rat liver due to a sexual dimorphism in growth hormone (GH) secretion [9–13].

The present study was designed to investigate whether escalating doses of morphine influence hepatic N-demethylation of morphine and other cytochrome P450 mediated reactions, and to study the role of the pituitary gland in mediating opiate effects on rat liver.

Materials and Methods

Male and female Wistar rats were obtained from Møllegaards Breeding Center, Ejeby, Denmark. Hypophysectomy (hx) of male rats was performed at 39 days of age by an intraauricular method [13]. All rats were kept under standardized conditions of light (lights on 6.00 a.m.–7.00 p.m.) and temperature (21 ± 1°). Body weights were recorded both before any treatment was started and before killing. Rats were starved overnight in order to decrease the hepatic levels of glycogen and were killed by decapitation.

Experiment 1. In experiment 1 two groups of male rats received human GH (hGH; Somatonorm, 2 I.U./mg, KabiVitrum, Stockholm, Sweden) by continuous infusion (6 μg/hr) as previously described [12]. Treatment was started at the age of 46 days and from the next day one group of hGH treated males as well as intact male and female rats received escalating daily doses (between 8.00 and 9.00 a.m.) of morphine hydrochloride (Apoteksbolaget, Gothenburg, Sweden), dissolved in sterile water, by i.p. injections for 2 weeks. Each dose was given for 2 days (20, 30, 60, 80, 100 and 125 mg/kg body wt). Control male and female rats and hGH treated males received saline (0.09%). Rats were killed 24 hr after the last injection.

Experiment 2. Also in experiment 2 intact and hx males received increasing doses of morphine, up to 80 mg/kg, where the escalation was interrupted due to a mortality (1/3) among the hx males receiving morphine, and treatment continued at the same level for the rest of the 2 week period. In addition one group of intact males and one group of hx rats received both morphine and naloxone (1 mg/20 mg morphine).

Methods. Livers were collected at sacrifice and immediately put on ice. Microsomal preparation [14], protein determination [15] and measurement of the microsomal cytochrome P450 content [16] were performed as previously described. Metabolism of 4-androstene-3,17-dione (androstenedione) was measured according to Blanck *et al.* [13] and N-demethylation of morphine according to Rane *et al.* [17]. As several microsomal reactions towards androstenedione provide basically the same information only 7α-hydroxy-androstenedione, a metabolite that does not seem to be GH-regulated, and the 16α-hydroxylated (♂ > ♀) and the 5α-reduced (♀ > ♂) metabolites, representing sex differentiated and GH-regulated pathways, are presented. Arylhydrocarbon hydroxylase activity (AHH) was recorded using the radiometric method described by van Cantfort *et al.* [18]. O-Deethylation of 7-ethoxyresorufin (7-EOR) was monitored continuously at 37° as described by Prough *et al.* [19].

Results and Discussion

Morphine administration led to significant decreases in the total microsomal content of cytochrome P450 in livers from all groups of intact rats. As seen in Table 1 the sex differentiated pathways of androstenedione metabolism, as well as morphine N-demethylation (♂ > ♀) and AHH activities (♂ > ♀), were feminized in morphine treated intact males. Also male rats receiving hGH, with and without morphine, exhibited a similar, feminized, pattern of metabolism. Morphine treatment of female rats led to a decreased capacity for 5α-reduction. No effects of morphine treatment were observed on either 7α-hydroxylation of androstenedione or on the capacity for O-deethylation of 7-EOR. In this experiment morphine treated male rats exhibited a reduction of body growth during the experimental period, compared with saline treated controls (4.9% and 33.4%, respectively). In hGH treated males and in females the morphine induced reduction of body growth was much less pronounced, with a weight gain of approximately 50% of that in saline-treated controls.

In experiment 2 the feminizing effects of morphine to intact male rats on sex differentiated hepatic metabolism were confirmed (Table 2). Concomitant administration of naloxone did not abolish these effects. Morphine treatment of hx rats, with and without administration of naloxone did not alter either 16α-hydroxylation or 5α-reduction of androstenedione although a slight but significant increase with respect to 7α formation was observed in hx rats receiving morphine alone. The combination of morphine and naloxone in experiment 2 significantly attenuated the morphine induced reduction of body growth in intact male rats, with an increase in morphine-treated rats of only 4.9%, compared with 12.3% in rats receiving morphine plus naloxone and 23.4% in saline treated controls. Both morphine alone and morphine plus naloxone significantly decreased the body weight of hx rats.

The morphine induced modifications of sex differentiated pathways of hepatic metabolism in intact but not in hx male rats indicate a role of the pituitary gland as a mediator of the observed effects on rat liver. In a previous report continuous infusion of bovine GH, as well as castration, was shown to feminize N-demethylation of morphine in male rat liver, suggesting a hypothalamo-pituitary regulation of this reaction [9]. Although the decrease in morphine N-demethylation in hGH treated males in the present study

Table 1. Total content of cytochrome P450 and *in vitro* hepatic metabolism in microsomal preparations from male, female and hGH treated male rats receiving escalating doses of morphine (mo) for 2 weeks, compared to controls receiving saline

	Cytochrome P450 (nmol/mg)	7α-OH	Androstenedione (nmol/min × mg) 16α-OH	5α-Reductase	N-Demethylation of morphine (nmol/min × mg)	AHH (nmol/min × mg)	O-Deethylation of 7-EOR (nmol/min × mg)
♂	0.83 ± 0.02	0.23 ± 0.04	2.7 ± 0.6	0.75 ± 0.37	0.15 ± 0.03	0.44 ± 0.04	0.26 ± 0.03
♂ + hGH	0.74 ± 0.05	0.26 ± 0.05	1.2 ± 0.44*	13.8 ± 14.1*	0.08 ± 0.05	0.29 ± 0.05*	0.29 ± 0.04
♀	0.74 ± 0.04	0.26 ± 0.05	0.03 ± 0.01*†	33.3 ± 1.41*†	0.005 ± 0.005*	0.06 ± 0.01*†	0.28 ± 0.05
♂ + mo	0.56 ± 0.04*	0.24 ± 0.06	0.12 ± 0.03*	20.2 ± 2.9*	0.04 ± 0.03*	0.08 ± 0.01*	0.25 ± 0.04
♂ + hGH + mo	0.56 + 0.03†	0.24 ± 0.09	0.07 ± 0.01*†§	23.1 ± 2.2*†‡	0.01 ± 0.01*†	0.08 ± 0.02*†	0.29 ± 0.03
♀ + mo	0.53 ± 0.05‡	0.23 ± 0.03	0.06 ± 0.01§	22.6 ± 1.8‡	0.01 ± 0.004§	0.07 ± 0.02	0.24 ± 0.04

The 7α- and 16α-hydroxylations and 5α-reduction of 4-androstene-3,17-dione (androstenedione) are shown, as well as N-demethylation of morphine, arylhydrocarbon hydroxylase (AHH) activity and O-deethylation of 7-ethoxyresorufin. Each group consisted of four rats. Values are expressed as means ± SD.

* Significantly different from male controls (Wilcoxon rank sum test; $P < 0.05$) [24].

† Significantly different from hGH-treated males.

‡ Significantly different from female controls.

§ Significantly different from morphine-treated males.

was not significant, our data indicate that morphine influences hepatic metabolism via neuroendocrine mechanisms. The increased release of both GH and prolactin (PRL) and the decreased release of luteinizing hormone (LH) and thyreotropin (TSH) following morphine administration reported by Bruni *et al.* [4] support the view that morphine exerts direct effects at the hypothalamic and/or pituitary level. Increasing evidence also indicate that the release of GH and PRL is under tonic control by endogenous opioids [20] and that morphine-induced effects on pituitary hormones are likely to act via hypothalamic transmitter systems, influencing the release of hypothalamic hypophyseotrophic hormones into the pituitary portal vessels [5].

The sex differentiated pattern of GH in male and female rats has been extensively studied [10–12, 21]. In male rats GH is secreted in episodic bursts every 3–4 hr, whereas the basal levels are low. In females the peaks are more irregular and less pronounced and the basal levels are higher than in males. The male pattern of GH secretion is under hypothalamic control, due to the combined action of somatostatin and a GH releasing factor [11] and circulating androgens are needed to maintain this control [21]. A disturbance of the secretory pattern of GH in the male rat by morphine, leading to increased basal levels of GH and feminization of hepatic metabolism, can thus be achieved both at the hypothalamic/pituitary levels and by an influence on gonadotropin secretion and/or peripheral effects on androgen synthesis.

Cicero *et al.* [3] demonstrated a suppressive effect of low doses of morphine on serum testosterone in male rats. The feminizing effect of morphine on male rat liver observed in the present study were, however, much more pronounced than those observed following adult castration of male rats [22]. Consequently, reduced androgen levels might contribute to the observed effects although not as a major determinant of the morphine-induced feminization of male rat liver.

The minor effects of morphine on hepatic metabolism in female rats seen in the present study are somewhat contradictory to the studies by Skett *et al.* [8] who showed that following low doses of morphine to male and female rats a stimulating effect on several pathways of androstenedione metabolism was seen only in the female rat liver. The doses used were at least 80-fold lower than those in the present study and the period for morphine administration shorter. Taken together these findings demonstrate the importance of sex, dose and time for morphine-induced effects on rat liver.

In the present study naloxone attenuated the morphine-induced reduction of weight gain in intact but not in hx rats, whereas no modifying effects of naloxone on hepatic metabolism was seen in morphine treated intact males. These observations suggest that the mechanisms mediating effects of morphine on body growth and hepatic function might be different, possibly due to the fact that multiple opiate receptors are present in the central nervous system and that naloxone might bind more efficiently to some of these receptors than to others [4]. Whereas Bakke *et al.* [23] reported decreased body and thyroid weights, accompanied by decreased levels of pituitary TSH, in morphine treated rats, Bruni *et al.* [4, 20] demonstrated that naloxone counteracted morphine-induced inhibition of TSH-release and that naloxone only partly reversed the effects on the release of GH [4]. These findings support the hypothesis that effects of morphine on TSH-release might be of importance for the observed effects on weight gain. Hx rats were in general more sensitive to morphine administration than intact rats, measured as the mortality rate, and we therefore suggest that the decrease in body weight in hx rats is due to an increased toxicity of morphine in these rats.

The fact that only sex differentiated pathways of hepatic

Table 2. Microsomal metabolism of 4-androstene-3,17-dione (androstenedione) *in vitro* with liver preparations from intact and hypophysectomized (hx) male rats receiving escalating doses of morphine (mo) for 2 weeks, with or without naloxone (nal) treatment

	N	Androstenedione (nmol/min × mg)		
		7α-OH	16α-OH	5α-Reduction
♂	6	0.51 ± 0.09	1.83 ± 0.35	3.9 ± 1.6
♂ + mo	6	0.58 ± 0.12	0.17 ± 0.05*	20.8 ± 9.2*
♂ + mo + nal	6	0.63 ± 0.10	0.26 ± 0.11*	17.3 ± 5.8*
♂ hx	6	0.66 ± 0.07*	0.90 ± 0.11*	1.3 ± 0.32*
♂ hx + mo	4	0.75 ± 0.03†	0.82 ± 0.19	0.83 ± 0.25
♂ hx + mo + nal	6	0.72 ± 0.14	0.87 ± 0.17	0.94 ± 0.19

Controls received saline (0.09%). Values are expressed as means ± SD.
* Significantly different from ♂ controls (Wilcoxon rank sum test; $P < 0.05$) [24].
† Significantly different from ♂ hx.

metabolism were shown to be influenced by morphine in the present study and that this influence seems to be dependent on an intact pituitary gland favours the contention that the hypothalamo–pituitary–liver axis is a mediator of the observed effects. Consequently studies on sex differentiated rat liver functions provides an additional tool by which the complex neuroendocrine mechanisms/effects exerted by endogenous as well as exogenous opiates can be studied.

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Cytosolic phenol and steroid sulphotransferase activities are decreased in a sex-dependent manner in streptozotocin-induced diabetic rats

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Sulphation is an important pathway of deactivation and elimination of potentially toxic compounds from the body, serving both xenobiotics and endogenous compounds [1, 2]. The reactions are performed by a large family of sulphotransferase (ST*) isoenzymes, subdivided according to the class of substrate metabolized, which transfer the sulphate group from the donor molecular 3′-phosphoadenosine 5′-phosphosulphate to the acceptor substrate (see Ref. 2 for review).

The effects of treatment of animals with the diabetogenic antibiotic streptozotocin (STZ) on the activities of many drug metabolizing enzymes have been extensively studied, and such experimental diabetes is known to induce alterations in the levels of activity of many of these enzyme systems, including the cytochrome P450s, the UDP-glucuronosyltransferases, the sulphotransferases and glutathione-*S*-transferases (e.g. Refs 3–12).

Most investigations of the effects of experimental diabetes on conjugation reactions have focused on the metabolism of xenobiotic substrates for the enzymes. However, the STs are also involved in the metabolism of important and potentially toxic endogenous compounds such as steroid hormones, bile salts and catecholamines [12–16], and indeed this may be seen as their primary function. The disruption, in diabetes, of key biochemical processes as a result of alterations in the metabolism of such endogenous molecules may have important consequences for the individual, and this may be particularly true for the sulphation of steroids, since dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S) and oestrogens have been shown to have potent anti-diabetic effects in experimental and genetically diabetic animals [15, 16]. Therefore, in this work we have investigated the effect of experimental diabetes on the sulphation of xenobiotic and endogenous steroid substrates selective for four different ST isoenzymes in STZ-induced diabetic rats. These enzyme activities exhibit well known sexual dimorphisms, so we have examined the effects of diabetogenesis on the activities in both male and female animals.

* Abbreviations: STZ, streptozotocin; ST, sulphotransferase; DHEA, dehydroepiandrosterone, DHEA-S, dehydroepiandrosterone sulphate; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid.

Materials and Methods

1-[1-^{14}C]Naphthol (59 mCi/mmol) and [2,4,6,7-^{3}H]oestrone (107 Ci/mmol) were purchased from Amersham, Aylesbury, U.K. and *p*-[^{3}H(G)hydroxyacetanilide (paracetamol, 1.2 Ci/mmol) and [1,2,6,7-^{3}H]dehydroepiandrosterone (78 Ci/mmol) were from DuPont/NEN (Stevenage, U.K.). BM-Test-Glycemie strips were from Boehringer Mannheim (Lewes, U.K.). Histone 2A, 3′-phosphoadenosine 5′-phosphosulphate, oestrone, paracetamol and dehydroepiandrosterone were purchased from the Sigma Chemical Co. (Poole, U.K.). Glucose-6-phosphate (monosodium salt), 1-naphthol and Scintran Cocktail T (scintillant) were from BDH Ltd (Glasgow, U.K.). All other reagents were of analytical grade and obtained from frequently used local suppliers.

Adult Wistar rats, approximately 12 weeks of age from the colony maintained in the Medical School animal facility, were used throughout, and had access to food and water *ad lib*. Diabetes was induced by a single tail vein injection of streptozotocin (75 mg/kg body wt) in buffered citrate (pH 4.5). Animals were killed 48 hr later, and blood glucose levels determined using BM-Test-Glycemie strips. Control animals received the vehicle only. Liver microsomes and cytosol (from the same livers) were prepared from 25% homogenates in 0.25 M sucrose, 5 mM Hepes, pH 7.4 by differential centrifugation. Briefly, homogenates were centrifuged at 10,000 *g* for 15 min and the resultant supernatant centrifuged at 105,000 *g* for 1 hr. The cytosolic fraction (105,000 *g* supernatant) was harvested avoiding the lipid layer at the surface, and aliquoted and frozen at −70°. The microsomal pellets were resuspended in sucrose/Hepes buffer to a protein concentration of approximately 20 mg/mL, aliquoted and stored frozen at −70°. All samples were used within 2 months of preparation, and microsome samples were frozen and thawed only once before assay in order to maintain the intactness of the microsomal membrane.

Glucose-6-phosphatase activities were assayed and calculated according to the method of Burchell *et al.* [17] following disruption of microsomes by histone 2A, and are expressed as μmol P_i released/min/mg microsomal protein. ST activity was determined with 1-naphthol [18], paracetamol [19], dehydroepiandrosterone [20] and oestrone as substrates. Assay conditions were as follows: 1-naphthol—pH 6.6, 8 μM 1-naphthol, 13 μM PAPS; paracet-