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PITUITARY INVOLVEMENT IN THE SEXUAL DIFFERENTIATION AND 3-METHYLCHOLANTHRENE INDUCTION OF RAT LIVER MICROSOMAL MONOOXYGENASES

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Abstract—Liver microsomal monooxygenase activities with several drugs and model substrates were determined for male rats, that had been given a pituitary implant or which had been hypophysectomized and injected with 3-methylcholanthrene. The effect of implanting a male or female rat pituitary into an immature male rat was to cause a change to a more typically female pattern of monooxygenase activities, i.e. ethylmorphine and aminopyrine *N*-demethylases and ethoxycoumarin *O*-deethylase, which are less active in adult female than male rats, were suppressed and ethoxyresorufin *O*-deethylase, which is more active in females, was induced. The effects of pituitary implantation on the biphenyl and benzo(a)pyrene hydroxylases were apparently unrelated to sex-differences in the activities of these enzymes. The inducibility by 3-methylcholanthrene of cytochrome P448-catalysed reactions was decreased 2–3-fold by prior hypophysectomy of adult male rats.

Sex differences in rat liver microsomal drug- and steroid-metabolizing reactions become apparent at puberty [1–5]. Since similar types of cytochrome P450-mediated monooxygenase enzymes are probably involved in the hepatic metabolism of both drugs and steroids, the question follows of whether similar mechanisms and controls of sex-differences operate for the metabolism of both drugs and steroids [3, 6–8]. Current ideas of the physiological control of sex-differences are clearer for steroid metabolism than for drug metabolism, whereas identification of the sex-differentiated steps in the monooxygenase reactions is more complete for drug metabolism than for steroid metabolism [9–19].

Most of the drugs investigated for sex-differences in hepatic metabolism, for example ethylmorphine, aminopyrine and hexobarbital, are more actively metabolised in male than in female rats [1, 2, 4, 10, 13, 17, 20–22]. The magnitudes of the type I binding of drugs and steroids to cytochrome P450 are also higher in males [10, 17, 18]. These drugs also stimulate the possible rate-limiting enzyme in their metabolism, NADPH-cytochrome P450 reductase, to a larger extent in males than in females [19]. However, the hepatic microsomal concentrations of cytochrome P450 and its reductase are not significantly different in male and female rats. Sex differences in drug metabolism have been found with rats, mice and tree-shrews, but they are apparently not present in several other species [20, 25]. Definitive experiments have yet to be reported for humans.

Most liver steroid hydroxylation and reduction reactions, including those of progesterone, testosterone and 5α-androstane-3α,17β-diol, are also more active in adult male than in female rats [3, 5, 18, 24].

The distinctive masculine patterns of rat liver steroid and drug-metabolism are controlled by exposure to physiological androgen [3, 9, 10, 11, 13, 18, 24, 26, 27]. Androgen control is mediated through the pituitary [14, 16]. In the female rat the post-pubertal levels of steroid-metabolising reactions are apparently controlled entirely through the hypophyseal secretion of a so-called feminizing factor [15, 16, 28]. However, the effects of hypophysectomy on ethylmorphine demethylation suggest a different control for this activity in female rats [12].

The male rat pituitary *in situ* is inhibited by the hypothalamus from secreting feminizing factor [15]. Either male or female autonomous pituitaries, implanted under the renal capsule and therefore free of direct hypothalamic influence, feminize the pattern of steroid metabolism in adult male rat liver [14].

We have investigated the role of the pituitary gland in mediating both the sex-differences and the 3-methylcholanthrene-induction of rat liver microsomal xenobiotic metabolism.

MATERIALS AND METHODS

Animal experiments. Sprague–Dawley rats were used. 18-day old males were given implants, under the renal capsule, of pituitaries from adult (56-day) male or female rats. After 7 days, during which the rats were allowed food (a commercial pellet diet, "R3", manufactured by Astra-Ewos, Södertälje, Sweden) and water *ad libitum*, they were sacrificed, their livers were removed and their microsomal activities were measured. A further group of 56-day old male rats were hypophysectomized and kept for 11 days on the pellet diet with a physiological saline–5.5 per cent glucose solution to drink. On the 7th to 10th days after hypophysectomy one group was injected with 3-methylcholanthrene (i.p. 20 mg per kg body wt, in corn oil, final volume 0.5 ml) and a

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Table 1. Age- and sex-dependence of rat liver microsomal biphenyl- and ethoxyresorufin metabolism

Age, days	Sex	Biphenyl 2-Hydroxylation	Biphenyl 4-Hydroxylation	Ethoxyresorufin O-Deethylation
1	M	0.014 ± 0.007	0.23 ± 0.03	0.012 ± 0.005
	F	0.018 ± 0.005	0.21 ± 0.04	0.013 ± 0.003
20	M	0.130 ± 0.010	1.61 ± 0.08	0.089 ± 0.006
	F	0.122 ± 0.011	1.50 ± 0.02	0.099 ± 0.004
56	M	0.046 ± 0.009	1.20 ± 0.04*	0.020 ± 0.003*
	F	0.050 ± 0.007	0.98 ± 0.06	0.052 ± 0.004
90	M	0.031 ± 0.008	1.09 ± 0.03*	0.016 ± 0.002*
	F	0.033 ± 0.005	0.95 ± 0.01	0.045 ± 0.005

Results (nmoles/min/mg protein) are means ± S.D. for four rats of each age and sex, all killed on the same day. The activities were measured as described in Methods.
* Male activity significantly different from female ($P < 0.01$).

control hypophysectomized group was injected with corn oil (i.p. 0.5 ml). Two additional groups of sham-operated 56-day old rats were at the same time injected with either 3-methylcholanthrene or corn oil. These four groups of rats were killed on the 11th day after hypophysectomy for determination of liver enzyme activities.

Incubations with liver microsomes. The rats were killed by cervical dislocation and liver microsomes prepared as previously described [29]. Freshly prepared liver microsomes were incubated with the following six substrates and the monooxygenase activities determined as previously described: biphenyl [29], benzo(a)pyrene [30], ethylmorphine and aminopyrine [31], ethoxycoumarin [21] and ethoxyresorufin [32]. Results were statistically analyzed using Student's '*t*' test.

RESULTS

Sex-differences in monooxygenase activities. Hepatic microsomal aminopyrine and ethylmorphine *N*-demethylases [4], ethoxycoumarin *O*-deethylase [22] and benzo(a)pyrene hydroxylase [21] are three to five times more active in the adult male than female rat. In contrast, biphenyl 4-hydroxylase was almost as active in female as in both young and adult male rats, similarly there was no significant sex-difference in the activity of hepatic biphenyl 2-hydroxylase ([25] Table 1). Ethoxyresorufin *O*-deethylation was more active in adult female than male rats (Table 1). The activities for biphenyl 2-hydroxylation and ethoxyresorufin *O*-deethylation were very low in these normal rats, because the reactions are catalyzed by an abnormal, aromatic hydrocarbon-inducible form of cytochrome P450, cytochrome P448 ([33] Table 3). Table 1 also shows that peak biphenyl 4-hydroxylase and ethoxyresorufin deethylase activities occurred in both sexes at around puberty but that the sex difference in ethoxyresorufin metabolism became manifest only at a later age.

Effects of pituitary implants on monooxygenase activities. Sex-differences in rat liver steroid metabolism due to hypophyseal secretions occur only after puberty [3]. For this reason we picked prepubertal male rats with which to investigate the effects of pituitary implants. The recipient rats were not hypophysectomized since their pituitaries were sexually undifferentiated and therefore would not be expected to interfere with the feminizing effect of the implanted autonomous pituitary. Intact male rats, 18-days old, were given an implant, under the renal capsule, of an autonomous pituitary, taken from adult (56-day) male or female rats. A control group of male rats was sham-operated. Seven days after these operations (i.e. at 25 days) the rats were killed and various of their liver microsomal xenobiotic metabolizing activities were determined. The effects of pituitary implant on monooxygenase acti-

Table 2. Effect of pituitary implants in immature male rats on liver microsomal xenobiotic metabolism

Reaction	Sham-operation	Pituitary implant: Female adult Pituitary	Pituitary implant: Male adult Pituitary
Biphenyl 4-hydroxylation	1.48 ± 0.12	2.46 ± 0.02*	1.96 ± 0.10*
Biphenyl 2-hydroxylation	0.15 ± 0.01	0.13 ± 0.03	0.04 ± 0.01*
Benzo(a)pyrene hydroxylation	0.44 ± 0.01	0.64 ± 0.03*	0.59 ± 0.01*
Ethylmorphine *N*-demethylation	1.19 ± 0.05	0.95 ± 0.04*	0.82 ± 0.05*
Aminopyrine *N*-demethylation	1.33 ± 0.10	0.99 ± 0.07*	1.01 ± 0.08*
Ethoxycoumarin *O*-deethylation	0.52 ± 0.08	0.40 ± 0.04†	0.35 ± 0.02*
Ethoxyresorufin *O*-deethylation	0.06 ± 0.01	0.11 ± 0.01*	0.16 ± 0.02*

Results (nmoles/min/mg protein) are means ± S.D. for four rats in each group, all operated on and later killed on the same days. Male rats 18-days old were either sham-operated or given an implant under their renal capsule of a pituitary from adult (56-day) male or female rats. They were killed 7 days later and their liver microsomal xenobiotic-metabolizing activities measured as described in Methods. Reaction rates significantly different from sham-operated are: * $P < 0.01$; † $P < 0.05$.

vities are presented in Table 2. With two exceptions there were essentially no differences in effect between male or female implanted autonomous pituitaries, in that differences due to donor sex were of small magnitude compared to the effects of implantation *per se*. The exceptions were biphenyl 2-hydroxylation, which was decreased by a "male" but not affected by a "female" pituitary, and biphenyl 4-hydroxylation, which was increased much more by a "female" than by a "male" pituitary. It is difficult to suggest an explanation for these sex-differences and why they occurred only with biphenyl hydroxylation. An implanted pituitary stimulated benzo(a)pyrene hydroxylation and ethoxyresorufin *O*-deethylation, but suppressed ethylmorphine and aminopyrine *N*-demethylations and ethoxycoumarin *O*-deethylation. Although the effects of the pituitary implants were statistically significant, they were of only moderate magnitude: the largest degree of suppression was a little more than 3-fold and the greatest enhancement was almost 2.5-fold.

Effects of hypophysectomy on induction of monooxygenases. In view of the observation that the steroid induction of liver microsomal steroid metabolism in male rats is apparently mediated through the pituitary, we investigated the effects of hypophysectomy on 3-methylcholanthrene induction of xenobiotic metabolism ([14, 16] Table 3). Three of the reactions measured were chosen because they are specifically or preferentially catalyzed by the 3-methylcholanthrene-inducible form of cytochrome P450, cytochrome P448, and biphenyl 4-hydroxylation was included for a comparison, since it is catalyzed by both cytochromes P450 and P448 [33]. There were no decreases in basal activity due to hypophysectomy, but the extents of 3-methylcholanthrene induction of all the reactions were diminished by prior hypophysectomy of the rats. The degrees of induction of biphenyl 2- and 4-hydroxylation were halved by prior hypophysectomy, although the ratio of induced 2- to 4-hydroxylation activities was not affected, while the extent of induction of ethoxyresorufin *O*-deethylation in hypophysectomized rats was only 40 per cent of that in intact rats. Hypophysectomy had a smaller effect on the 3-methylcholanthrene induction of benzo(a)pyrene hydroxylation, the extent of which was decreased 1.5-fold compared to intact rats.

DISCUSSION

The results of this investigation suggest that the pituitary plays an important role in regulating the hepatic microsomal drug metabolizing monooxygenase enzymes of rats.

Gustafsson and co-workers have postulated that a pituitary not under hypothalamic control secretes a so-called feminizing factor, which induces a feminine pattern of hepatic steroid metabolism, either when *in situ* in female rats or when implanted in male rats [14, 16].

In this present study, rat pituitaries renally implanted in male rats stimulated biphenyl 4-hydroxylase, benzo(a)pyrene hydroxylase and ethoxyresorufin *O*-deethylase activities and suppressed biphenyl 2-hydroxylase, ethylmorphine- and aminopyrine *N*-demethylase and ethoxycoumarin *O*-deethylase activities. Thus, a considerable feminization of the liver microsomal monooxygenase pattern was caused by the autonomous pituitary, in that all but one of the activities (benzo(a)pyrene hydroxylase) that were less active in the adult female than in the male rat were suppressed and ethoxyresorufin deethylation, which was more active in the female, was stimulated. Feminization by an autonomous pituitary of the pattern of androstenedione metabolism also involves the suppression of some reactions and the enhancement of others [14]. The identity of the feminizing factor, which presumably caused these changes, is still unknown. It is probably not prolactin, LH or FSH [14, 34].

The effects of pituitary implantation on the biphenyl or benzo(a)pyrene hydroxylases did not however relate simply to the pattern of sex-differences in the reactions.

Pituitary glands *in situ* mediate the steroid-induction of hepatic steroid hydroxylases and ethylmorphine demethylase [12, 14]. Our observations with hypophysectomized rats indicate that the hypophysis also mediates the 3-methylcholanthrene-induction of cytochrome P448-type monooxygenases. 3-Methylcholanthrene-induction was at least twice as effective in intact than in hypophysectomized rats. It is probably relevant in this context that there are geometrical similarities between the

Table 3. Effect of hypophysectomy of adult male rats on induction by 3-methylcholanthrene of liver microsomal xenobiotic metabolism

Physiological state	Pre-treatment	Biphenyl 2-Hydroxylation	Biphenyl 4-Hydroxylation	Benzo(a)-pyrene hydroxylation	Ethoxy-resorufin *O*-deethylation
Sham-operated	Control	0.06 ± 0.01	1.00 ± 0.04	0.25 ± 0.01	0.04 ± 0.01
	3-MC	1.43 ± 0.09	3.18 ± 0.10	1.15 ± 0.06	16.35 ± 0.82
Hypox	Control	0.07 ± 0.02	1.18 ± 0.07	0.24 ± 0.01	0.04 ± 0.01
	3-MC	0.89 ± 0.07*	2.20 ± 0.09*	0.66 ± 0.04*	6.50 ± 0.33

Results (nmoles/min/mg protein) are means ± S.D. for four, 56-day-old male rats in each group, all operated on and later killed on the same days. Rats were either sham-operated or hypophysectomized (hypox); on days 7, 8 and 9 after operation they were injected once i.p. with either corn oil (control; 0.5 ml) or 3-methylcholanthrene (3-MC; 20 mg/kg body wt, in corn oil); the rats were killed and the activities were measured on day 10 after the operations, as described in Methods.

* Activity in hypox significantly different from activity in sham-operated ($P < 0.01$).

3-methylcholanthrene and steroid molecules, and it is possible that a common, pituitary-mediated factor is involved in their inducing actions.

We have reported here for a selection of drug- and general xenobiotic-metabolizing enzymes that their basal and 3-methylcholanthrene-inducible activities show similarities to steroid hydroxylases in respect to their control by the hypophysis. There are, however, major dissimilarities for certain xenobiotic substrates, notable biphenyl and benzo(a)pyrene. If the physiological control of xenobiotic-metabolizing enzymes is similar to that of hepatic steroid metabolism, then alteration of gonadal and hypothalamic-pituitary functions in either neonatal or adult humans might conceivably modify the ability of the adult to detoxify hazardous compounds.

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