Hormonal and Developmental Regulation of Expression of the Hepatic Microsomal Steroid 16α-Hydroxylase Cytochrome P-450 Apoprotein in the Rat*

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The hormonal regulation of the sexually differentiated cytochrome P-450 isozyme which catalyzes 16α-hydroxylation of testosterone and 4-androstene-3,17-dione in male rat liver (P-45016α) was investigated. Estradiol valerate injection of male rats caused a decrease in P-45016α levels to almost the female level, while methyltrienolone injection had the reverse effect in female animals. Hypophysectomy abolished the sex difference in P-45016α levels. Human growth hormone infusion into male rats, mimicking the female pattern of growth hormone secretion, caused a feminization of P-45016α levels. The same effect was also seen in hypophysectomized rats of both sexes. In contrast, a different administration schedule involving 12 h injections of human growth hormone, mimicking the male pattern of growth hormone secretion, caused a masculinization of P-45016α levels in hypophysectomized rats, at a daily dose which causes feminization when given by infusion. Thus, the level of expression of P-45016α in the liver is dependent on the temporal pattern of blood growth hormone levels. While infusion of rat growth hormone into male rats also feminized the P-45016α levels, infusion of ovine prolactin had no effect. Ontogenic studies showed that the developmental pattern of P-45016α expression in the liver coincided with the known pattern of development of the sexual differentiation of hepatic steroid 16α-hydroxylase activity and of the diurnal pattern of growth hormone secretion.

The steroid 16α-hydroxylase activity of rat liver microsomes is one of a number of sexually differentiated liver functions which are under a unique type of hormonal regulation (1, 2). The differential expression of these functions is controlled at two levels: the sexually dimorphic, diurnal variation in blood growth hormone levels appears to be responsible for the regulation of the specific liver functions, while the presence of androgens in the neonatal period imprints the hypothalamus to generate a male profile of GH secretion, and hence to express male-specific liver functions in the adult phase. This profile is maintained by circulating androgens in adult life.

Two laboratories, including our own, have shown that the male-specific testosterone and 4-androstene-3,17-dione 16α-hydroxylase activities in the rat reside in a specific isozyme of cytochrome P-450 (3, 4). The isozyme is termed RLMα (5), or 2c (3), and probably is also identical to isozymes designated UT-A (6), P-450h (7), and P-450 male (8). For the purpose of clarity, we will refer to the isozyme as P-45016α in this report. The isozyme is also active in 2- and 17-hydroxylation of the above steroids and in oxidation of other substrates (3-8).

We, and others, have shown that androgen imprinting, resulting in male levels of 16α-hydroxylase activity, is achieved by regulation of the level of the specific P-45016α apoprotein in the microsomes (3, 4). Thus, levels of P-45016α are approximately 20-fold higher in mature male rats than in mature female rats or in adult males which were castrated in the neonatal period. In order to investigate further the mechanisms of gonadal and pituitary regulation of this enzyme in the rat, we have studied the role of pituitary hormones on the regulation of P-45016α apoprotein levels in rat liver. In addition, the ontogenesis of P-45016α expression in rats of both sexes was investigated. In doing so, we also wished to test the suitability of P-45016α as a model protein for the study of mechanisms of GH action in the liver.

EXPERIMENTAL PROCEDURES

The preparation of hepatic microsomes, the purification of P-45016α, and preparation of specific, immunoabsorbed rabbit anti-P-45016α IgG have been described previously (4). Specific levels of P-45016α in microsomes were quantitated by a modification of the method of Towner et al. as described in Ref. 4. The proteins, separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and blotted on nitrocellulose filters, were incubated at a primary antibody concentration of 20 μg of IgG/ml. The antigen-antibody complex was detected using 125I-Protein A (2–10 μCi/mg, New England Nuclear) followed by autoradiography. The radioactivity in the bands was then counted. The radioactivity was proportional to the amount of purified or microsomal protein applied on the gel within the range of 50–500 ng and 5–50 μg, respectively.

Continuous infusion of hormones was achieved by administration from Alzet osmotic minipumps (Alza Corp., Palo Alto, CA) implanted subcutaneously on the back of the animals. Minipumps with filling volumes of 225 and 2000 μl, and pumping rates of 1 and 10 μl/h at 37°C, respectively, were used. Control animals were implanted with a piece of silicone tubing of similar size. Unless otherwise stated, hormones were dissolved in physiological saline for administration to the rats. Where necessary, a small amount of NaOH was added to dissolve GH, and the pH of the solution was readjusted to 7.4.

Human GH (Crescormon®), 2 IU/mg, was from Kabi-Vitrum, Sweden, and estradiol valerate (Progynon depot) was from Schering Inc., G.P.R. Methyltrienolone (R1881) was kindly supplied by Dr. J.-P. Raynaud, Roussel-UCLAF, Paris. Rat GH (Bk, 1.8 units/mg)

1 The abbreviations used are: GH, growth hormone; hGH, human GH; rGH, rat GH; P-45016α, isozyme of cytochrome P-450 catalyzing steroid 16α-hydroxylation in male rat liver, otherwise designated RLMα, or 2c; oPRL, ovine prolactin; methyltrienolone, 17β-hydroxy-1α-methyl-4,9,11-estratrien-3-one; IgG, immunoglobulin G; Hx, hypophysectomized.

2 The major phenobarbital-inducible P-450 isozyme also has steroid 16α-hydroxylase activity but is present at extremely low levels in uninduced rats and is not sexually differentiated.

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and ovine prolactin (P-S-14, 31 units/mg) were kindly supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. Sprague-Dawley rats were used throughout this study. Hypophysectomized rats were obtained from Mallegards Avelslaboratorium, Skensved, Denmark.

All results are expressed as the mean ± S.E. for each group of animals. One-way analysis of variance was used to test for significant differences among the means. The level of significance was set at p < 0.05.

RESULTS AND DISCUSSION

The expression of the hepatic 16α-hydroxylase apoprotein in adult male rats is imprinted by androgen in the neonatal period (3, 4), and it is known that the male level of 16α-hydroxylase activity is also maintained by circulating androgen in the adult animal (1, 9). However, when administered to adult rats, exogenous sex steroids can masculinize or feminize the hepatic pattern of steroid metabolism (1, 10, 11). Therefore, we investigated the effects of androgen and estrogen administration on P-450160 levels in the adult rat. As observed in Table I, injection of the potent synthetic androgen methyltrienolone to female rats raised the P-450160 content of hepatic microsomes by approximately 60-fold, to 61% of the male value. Conversely, a single injection of estradiol valerate in male rats caused a 90% decrease in the P-450160 level to a value which was only slightly higher than the female value. These striking effects of sex steroids are similar to those observed for 16α-hydroxylase activity (1, 10), which are only seen in the presence of an intact pituitary (1, 11).

Since evidence is accumulating that the episodic pattern of pituitary growth hormone secretion is the major determining factor in regulation of sexually differentiated liver function (1, 12), we studied the involvement of GH in the regulation of the microsomal P-450160 levels. The episodic pattern of plasma GH levels differs markedly between the sexes. Male rats show regular peaks of high GH levels every 3-4 h, interspersed by troughs with almost null levels (13). In contrast, females have peaks only at irregular intervals, and the trough values are higher than in the male. This sex difference in GH secretory pattern is seen in adult animals only, the sexual differentiation beginning at puberty (13). Thus, by infusing GH continuously by way of osmotic minipumps, we are able to mimic the female pattern of GH levels. Alternatively, we can try to simulate the male pattern of GH levels by injecting GH intermittently. Hypophysectomy of male rats caused a decrease, and of female rats an increase, in P-450160 levels to values intermediate to those of the intact males and females (Fig. 1). Thus, the sex difference in P-450160 was abolished by hypophysectomy (Fig. 1). Continuous infusion of hGH to intact male rats caused a decrease in P-450160 levels to a value which was not significantly different from the female level (Fig. 1). A similar effect was observed in hypophysectomized rats of both sexes, although the magnitude of effect was slightly less in the hypophysectomized females. These effects of hypophysectomy and GH infusion are identical with those observed for 16α-hydroxylase activity (12, 14) and suggest an essential role of GH in regulation of the sexually differentiated P-450160 isozyme.

FIG. 1. Effects of hGH infusion and hypophysectomy on hepatic microsomal levels of P-450160 in the rat.

TABLE I  Effects of androgen and estrogen administration on P-450160 levels in intact adult rats

| Group of animals | P-450160 content of microsomes (ng/µg microsomal protein) |
|------------------|---------------------------------------------------------|
| δ                | 49.8 ± 5.1*                                             |
| δ + estradiol valerate | 1.9 ± 1.2b, 0.8 ± 0.2b, 30.5 ± 4.3b                |
| δ + R1881        | 6.8 ± 0.5b                                             |

* Significantly different from untreated male value.

TABLE II  Effects of intermittent hGH administration on P-450160 levels in hypophysectomized male rats

| Group of animals | P-450160 content of microsomes (ng/µg microsomal protein) |
|------------------|---------------------------------------------------------|
| Hx (4)           | 8.9 ± 1.3                                               |
| Hx + hGH, 10 µg/day (5) | 11.3 ± 0.8                                           |
| Hx + hGH, 50 µg/day (5) | 30.0 ± 5.8*                                           |

* Significantly different from Hx male value.

Effects of intermittent hGH administration on P-450160 levels in hypophysectomized male rats

hGH was injected subcutaneously at 12-h intervals (8 a.m. and 8 p.m.) for 7 days into Hx male rats. The rats were 4 weeks old at the beginning of the experiment, and had been operated on 2 weeks previously. After 7 days’ treatment, microsomes were prepared from the three different groups of animals, and P-450160 was quantified. a, significantly different from intact male value; b, significantly different from intact female value.
Taken together, the results presented in Fig. 1 and Table II strongly support the hypothesis that the up- and down-regulation of P-450<sub>16α</sub>, by GH is dependent on the plasma pattern of GH levels. The slightly lower daily dose of GH used in the injection experiment was not the reason for the difference in effects, since the same low dose administered to Hx males by infusion causes a feminization (not shown). It should be noted that a given dose of GH causes the same rate of growth in rats, irrespective of whether it is administered by infusion or by 12 h injections (15).

hGH can bind to both PRL and GH receptors in rat liver (16). It was therefore essential to determine whether the observed effects of hGH on P-450<sub>16α</sub> levels were due to a somatogenic or lactogenic action. Fig. 2 shows the effects of infusion of rGH, which has no lactogenic activity in the rat, on P-450<sub>16α</sub> levels in intact male rats. In addition, we studied the effect of administration of an identical daily dose of rGH by subcutaneous injection every 12 h. Whereas oPRL infusion had no effect on P-450<sub>16α</sub> levels, infusion of rGH caused a 75% decrease, and hGH a 94% decrease. This favors the idea that the effects of GH are achieved by an action on somatogenic receptors. The lower potency of the rat hormone compared to the human preparation may be explained by a reduced stability of rGH in aqueous solution at physiological pH (17), effectively resulting in a lower dose of active hormone reaching the liver. In contrast to the effect of rGH infusion, 12-h injections of the hormone at the same daily dose had no effect on P-450<sub>16α</sub> levels (Fig. 2), again emphasizing the importance of continuous blood GH levels, i.e., a female-type pattern of pituitary GH secretion, for a feminization of P-450<sub>16α</sub> levels in the liver.

The sex difference in the pattern of plasma GH levels is observed first at puberty, and is developed fully in the sexually mature animal (12, 13). The sex difference in steroid 16α-hydroxylating activity also follows the same developmental pattern, with the male activities increasing at puberty while female values remain low (3, 18). In addition, Maeda et al. (19) have demonstrated a similar ontogenesis of "P-450 male" apoprotein levels. Therefore, we investigated the development of P-450<sub>16α</sub> expression in liver microsomes of male and female rats from the ages of 6 days to 8 months (Fig. 3). P-450<sub>16α</sub> levels are very low in female rats of all ages. The levels are also low in males until the age of puberty. At 35 days, just after the onset of puberty, the levels are about 46% of the levels in the intact male. The male level of expression is fully developed at 54 days and is maintained until the animal is at least 8 months old. Thus, the development of P-450<sub>16α</sub>, expression in rat liver coincides with the development of microsomal steroid 16α-hydroxylase activity and of the male pattern of pituitary GH secretion.

Some variation is observed in the absolute levels of P-450<sub>16α</sub> in microsomes of adult male rats, as measured by the Western blot immunoassay. This variation is puzzling, but, as we have noted previously (4), the Western blot technique may not be suited to the measurement of absolute contents of proteins in microsomes. However, we feel that the assay is satisfactory for the measurement of relative amounts of P-450<sub>16α</sub> in microsomal samples assayed at the same time, based on our observations that the relative amounts of P-450<sub>16α</sub> among samples are constant if the same samples are measured on repeated occasions (results not shown).

In conclusion, our results support the hypothesis that the episodic plasma pattern of GH levels is the ultimate determining factor in the expression of the sexually differentiated P-450<sub>16α</sub>, isozyme and that the hormone acts via somatogenic receptors. In addition, the data shows that the P-450<sub>16α</sub> level in the liver is regulated by steroid hormones and by GH, in precisely the same manner as has been shown for steroid 16α-hydroxylase activity, providing further, strong evidence that P-450<sub>16α</sub> is the enzyme which is responsible for that activity in rat liver microsomes. The mechanism(s) by which the liver can recognize the male and female pattern of GH secretion, and respond with either expression or nonexpression of the P-450<sub>16α</sub>, apoprotein is still unclear.

![Fig. 2. Effect of oPRL, hGH, and rGH infusion and rGH injection of P-450<sub>16α</sub> levels in rat liver.](image-url)
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