Biochemical Pharmacology, Vol. 44, No. 8, pp. 1523–1528, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

REGULATION BY GROWTH HORMONE AND GLUCOCORTICOID OF TESTOSTERONE METABOLISM IN LONG-TERM CULTURES OF HEPATOCYTES FROM MALE AND FEMALE RATS

CONSTANCE VIND,* JOHN DICH† and NIELS GRUNNET†

Department of Biological Sciences, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen; and †PharmaBiotec Research Center, Department of Biochemistry A, Panum Institute, University of Copenhagen, 3 Blegdamsvej, DK-2200 Copenhagen, Denmark

(*Received* 20 *January* 1992; *accepted* 23 *July* 1992)

Abstract—The activities of 2-, 6β-, 7α- and 16α-testosterone hydroxylase and 5α-testosterone reductase were measured in intact hepatocytes from male and female rats cultured for 8 days in a modified Waymouth medium supplemented with 0.1 or 1.0 μM dexamethasone with or without addition of 1 μg/mL growth hormone. During culture of hepatocytes from female rats the activity of the male-specific 16α-testosterone hydroxylase increased. This increase was significantly inhibited at day 8 by 1 μM dexamethasone as well as by growth hormone. Furthermore, in cultures of hepatocytes from male rats, the activity of the constitutive 16α-testosterone hydroxylase was decreased by 1 μM dexamethasone as well as by growth hormone. The induction of 6β-testosterone hydroxylase by dexamethasone was suppressed by growth hormone in hepatocytes from both male and female rats, while the 7α-testosterone hydroxylase activity was unaffected by culture time, hormone additions and gender. The decrease in female-specific 5α-reductase activity with culture time in hepatocytes from female rats was significantly attenuated by growth hormone at 0.1 μM dexamethasone. The effects of growth hormone on testosterone hydroxylase activities in hepatocyte cultures from male and female rats are in accordance with the concept of growth hormone as a "feminization signal". The results suggest that the glucocorticoid-dependent expression of the male constitutive 16α-hydroxylase requires periods of low levels of growth hormone.

The growth hormone secretory profile is different in male and female rats. In male rats the secretory profile is characterized by high peaks every 3–4 hr separated by periods with low to undetectable levels, and in females by a more steady concentration with higher levels of hormone [1, 2]. Growth hormone affects the expression of a variety of hepatic proteins including members of the cytochrome P450 family [3–5]. Particularly, growth hormone is an important regulator of the expression of several sex-specific P450 forms belonging to the P450IIC subfamily [5–7]. The sex-specific differences in P450 forms are only observed in liver microsomes of adults [7].

Sex differences are particularly pronounced in the case of steroid metabolism [8]. Hydroxylation of testosterone in the 2α and 16α positions is catalysed by the main male-specific P450 form, P450IIC11 (h), and hydroxylation in the 2β and 6β positions is catalysed by the male-dominating P450IIIA1 and P450IIIA2 [9]. The 7α-testosterone hydroxylase activity, P450IIA1, is found in both genders, and the reduction of testosterone in the 5α position catalysed by the 5α-reductase is found predominantly in microsomes in the liver of females [10, 11].

In a recent study [12] we have shown that during long-term culture of rat hepatocytes the adult male-dominating 2α-, 6β- and 16α-hydroxylase activities decreased in cultures of hepatocytes prepared from male rats and increased in cultures of hepatocytes prepared from female rats. This change toward an immature state might reflect a deficiency of hormones other than dexamethasone, insulin and glucagon, which were added to the medium to ensure maintenance of hepatocyte-specific parameters for longer periods [13].

In the present study we have investigated the effects of growth hormone on the activities of the sex-specific testosterone hydroxylase and 5α-reductase in cultures of hepatocytes from male and female rats.

MATERIALS AND METHODS

Materials. Human recombinant growth hormone was kindly provided by Dr J. Høiriis Nielsen, Novo Nordisk (Copenhagen, Denmark), and the 5α-reductase inhibitor, *N,N*-diethyl-3-oxo-4*aza*-5α-androstane-17β-carboxamide, was kindly provided by Dr G. H. Rasmusson, Merck, Sharp and Dohme (Rahway, NJ, U.S.A.). [^{3}H]Testosterone (1,2,6,7-[^{3}H]testosterone) was from Amersham International (Amersham, U.K.). All other materials were obtained as indicated previously [12].

Cell isolation and cultures. Hepatocytes were isolated from 16 hr starved adult male or adult female Wistar rats (age 10 weeks), plated in 60-mm collagen-coated Petri dishes and cultured, as described previously [14]. Four hours after plating, the medium was changed to a modified Waymouth medium [15] containing dexamethasone (0.1 or 1.0 μM), insulin (10 nM) and glucagon (0.1 nM). Growth hormone (1.0 μg/mL) was added at each

* Corresponding author.

medium change from 4 hr after plating as indicated. The concentration of growth hormone decreased by less than 20% between medium changes (data not shown). All experiments were started at day 0 and the culture medium was changed on days 1, 3 and 6. The content of DNA and cytochrome P450 and the activities of the 5α-reductase and the testosterone hydroxylases were determined at days 1 and 8.

Analyses. The content of P450 was determined as described earlier [16] except that 2% Lubrol was exchanged with 2% Triton-X100. The concentration of P450 was determined by the method of Omura and Sato [17]. Cells from parallel dishes were harvested by ultra-sonication in 0.1 M Tris pH 8.0 and used for determination of DNA [18]. 5α-Reduction and hydroxylation of testosterone were measured in intact cell monolayers from parallel cell cultures. The testosterone hydroxylase activities were estimated as described previously [12] except for addition of radioactively labelled testosterone. After termination and addition of 17α-methyl testosterone as internal standard the mixture was treated with β-glucuronidase–sulfatase for conjugate hydrolyses, extracted and analysed by HPLC [12].

For further characterization of the metabolites eluted in the different HPLC peaks, some samples were analysed by TLC as described [19]. Samples were dried, suspended in ethylacetate and spotted onto silica gel plates containing a fluorescent indicator (E. Merck, F-254). Plates were first developed in methylene chloride–acetone 4:1 (v/v) and, after air drying, in the same dimension with the second solvent chloroform–ethylacetate–ethanol 4:1:0.7 (by vol.). Radiolabelled metabolites were identified by their co-migration with unlabelled steroid standards detected by UV light. The radioactive zones were then cut out (together with the aluminium backing) for direct quantitation by liquid scintillation counting.

2α- and 2β-hydroxytestosterone are poorly resolved in the present HPLC system. TLC analysis indicated that 2β-hydroxytestosterone was formed during the incubation, although the 2β-hydroxytestosterone fraction in this system might have been contaminated by 16β-hydroxytestosterone [19]. Consequently, the 2α- plus 2β-hydroxytestosterone peak determined by HPLC is reported as 2-hydroxytestosterone. For 6β-, 7α- and 16α-hydroxytestosterone, the results obtained by TLC were in agreement with those obtained by HPLC. The 5α-reductase activity was calculated as the difference between total testosterone metabolized and the accumulation of 254 nm light-absorbing testosterone derivatives, since dihydrotestosterone has no absorbance at this wavelength. Total testosterone metabolism was determined by HPLC analysis of testosterone at the start and at the end of incubation. Parallel cultures with 10 μM 5α-reductase inhibitor added 5 min before testosterone served as blanks. Blank values were close to zero indicating that pathways of testosterone metabolism other than that catalysed by 5α-reductase give rise to 254 nm light absorbing products. The 5α-reductase activity estimated by this method in intact monolayers on day 1 of culture is in accordance with the activity reported in microsomes from female rat liver [10], while the sensitivity of the method was too low to measure the 5α-reductase activity in hepatocytes from male rats.

Table 1. Effect of growth hormone and dexamethasone on P450 content in hepatocyte cultures from male and female rats

Dex (μM)	GH (μg/mL)	P450 (nmol/mg DNA) Days of culture 1	8
Female			
0.1	—	8.61 ± 0.33 (6)	2.64 ± 0.26 (7)
0.1	1.0	8.43 ± 0.42 (6)	5.06 ± 0.54 (7)*
1.0	—	10.10 ± 0.86 (7)	3.45 ± 0.51 (7)
1.0	1.0	9.62 ± 0.43 (7)	3.84 ± 0.35 (7)
Male			
0.1	—	11.34 ± 0.81 (6)	3.05 ± 0.50 (6)
0.1	1.0	12.20 ± 1.00 (6)	4.18 ± 0.45 (6)
1.0	—	12.61 ± 1.56 (6)	5.27 ± 0.51 (6)
1.0	1.0	11.60 ± 1.91 (6)	3.86 ± 0.47 (6)

Hepatocytes were cultured in a modified Waymouth medium.

The results are means ± SEM with the number of hepatocyte preparations shown in parentheses.

Dex, dexamethasone; GH, growth hormone.

* Significant difference from cultures without GH.

Calculation. The content of cytochrome P450 and the 5α-reductase activity were related to the amount of DNA. The testosterone hydroxylase activities were related to the total amount of P450. For the purpose of comparison, 1 mg DNA corresponds to 0.4 g liver wet wt or 16 mg of microsomal protein [20, 21]. Statistical significance was calculated by the *t*-test for paired data obtained on the same day of culture. Comparison between the DNA content in male and female hepatocytes in culture was carried out by Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

The DNA content per dish, a measure of cell number, declined slowly regardless of the hormone concentration in cultures of hepatocytes from both male and female rats, but significantly ($P < 0.01$) faster in cultures from male compared with female rats. At day 8 the content of DNA was 61.0 ± 1.5% ($N = 28$) of the day 1 value in cultures of hepatocytes from male rats, but 68.6 ± 1.7% ($N = 28$) in those from female rats.

The content of cytochrome P450 per milligram DNA (Table 1) declined 60–70% during the 8 days of culture of hepatocytes from female as well as male rats. Growth hormone had no effect except in cultures of hepatocytes from female rats with 0.1 μM dexamethasone, where addition of 1 μg/mL significantly delayed the decrease of the cytochrome P450 to 40% during 8 days of culture.

When measured in intact hepatocytes cultured for 1 day in the presence of 0.1 μM dexamethasone, the activities of 2-, 6β-, 7α- and 16α-testosterone

Table 2. Effect of growth hormone and dexamethasone on the activities of testosterone hydroxylase in cultures of hepatocytes from *female* rats

Dex (μM)	GH (μg/mL)	Product [mol/(mol P450 × min)] Days of culture 1	8
2-Hydroxylase			
0.1	—	0.03 ± 0.01 (3)	0.12 ± 0.02 (3)
0.1	1.0	0.02 ± 0.01 (3)	ND
1.0	—	0.07 ± 0.01 (6)	0.83 ± 0.35 (4)
1.0	1.0	0.06 ± 0.02 (6)	0.18 ± 0.08 (5)*
6β-Hydroxylase			
0.1	—	0.24 ± 0.03 (6)	0.48 ± 0.05 (5)
0.1	1.0	0.22 ± 0.03 (6)	0.08 ± 0.01 (5)*
1.0	—	0.21 ± 0.03 (6)	1.00 ± 0.30 (5)
1.0	1.0	0.21 ± 0.03 (6)	0.28 ± 0.01 (4)*
7α-Hydroxylase			
0.1	—	0.39 ± 0.04 (7)	0.55 ± 0.13 (5)
0.1	1.0	0.38 ± 0.04 (7)	0.54 ± 0.02 (4)
1.0	—	0.30 ± 0.04 (7)	0.55 ± 0.06 (5)
1.0	1.0	0.34 ± 0.04 (7)	0.40 ± 0.07 (5)
16α-Hydroxylase			
0.1	—	0.10 ± 0.01 (6)	0.31 ± 0.06 (3)
0.1	1.0	0.12 ± 0.01 (6)	0.08 ± 0.02 (3)*
1.0	—	0.11 ± 0.03 (5)	0.15 ± 0.03 (4)
1.0	1.0	0.09 ± 0.02 (5)	ND

Hepatocytes were cultured in a modified Waymouth medium.
The results are means ± SEM with the number of hepatocyte preparations in parentheses.
Abbreviations as in Table 1.
ND, not detectable.
* Significant difference from cultures without GH.

hydroxylase in cells from female rats (Table 2) and male rats (Table 3) as well as of the 5α-reductase (Table 4) were in agreement with activities obtained from liver microsomes from adult female and male rats [8, 10, 22, 23], except that 5α-reductase has been reported detectable in livers from male rats. At day 1 of culture neither increasing dexamethasone concentration to 1 μM nor adding 1 μg/mL growth hormone had any effect on 2-, 6β-, 7α- or 16α-testosterone hydroxylase, or 5α-reductase activities in hepatocytes from either male or female rats (Tables 2–4). During culture for 8 days of hepatocytes from female rats with 0.1 μM dexamethasone the adult male-dominating 2-, 6β- and 16α-testosterone hydroxylase activities (Table 2) increased. Addition of growth hormone prevented these increases significantly. Increasing the dexamethasone concentration to 1.0 μM abolished the increase in 16α-testosterone hydroxylase activity found with 0.1 μM dexamethasone. This effect was further accentuated by addition of growth hormone. Addition of 1.0 μM dexamethasone caused a rise in the activities of 2- and 6β-testosterone hydroxylase, which was significantly prevented by addition of growth hormone (Table 2).

7α-Testosterone hydroxylase activity was unaffected by both culture time and hormone additions (Table 2). 5α-Reductase activity (Table 4) was significantly increased by addition of growth hormone to cultures with 0.1 μM dexamethasone; however, no significant effect of growth hormone was found in the cultures with 1.0 μM dexamethasone.

In hepatocytes from male rats, 2-, 6β- and 16α-testosterone hydroxylase activities were unchanged during culture for 8 days with 0.1 μM dexamethasone. Addition of 1.0 μg/mL growth hormone as well as 1.0 μM dexamethasone inhibited the activities of 2- and 16α-hydroxylases, but no additive effect of the two hormones was seen. In contrast, the 6β-hydroxylase activity was induced by 1.0 μM dexamethasone independent of addition of growth hormone. Neither dexamethasone nor growth hormone had any effect on 7α-testosterone hydroxylase activity (Table 3). 5α-Reductase activity was undetectable in all cultures of hepatocytes from male rats (data not shown).

DISCUSSION

The results of the present study show that growth hormone and dexamethasone affect the sex-specific testosterone hydroxylase activities in cultured hepatocytes, and that the effects required several days of hormone exposure to develop, since no effects were observed after 1 or 3 (results not shown) days of culture. The late appearance of growth hormone effects may be due to the reported increase with time of the number of growth hormone receptors in the presence of dexamethasone and glucagon [24]. The change with time of testosterone hydroxylase

Table 3. Effect of growth hormone and dexamethasone on the activities of testosterone hydroxylase in cultures of hepatocytes from *male* rats

Dex (μM)	GH (μg/mL)	Product [mol/(mol P450 × min)] Days of culture 1	8
2-Hydroxylase			
0.1	—	0.89 ± 0.04 (6)	1.77 ± 0.62 (6)
0.1	1.0	0.87 ± 0.07 (6)	0.21 ± 0.05 (6)*
1.0	—	0.87 ± 0.10 (6)	0.36 ± 0.06 (6)
1.0	1.0	1.05 ± 0.15 (6)	0.33 ± 0.12 (5)
6β-Hydroxylase			
0.1	—	0.51 ± 0.13 (5)	0.41 ± 0.11 (6)
0.1	1.0	0.54 ± 0.09 (6)	0.28 ± 0.04 (5)*
1.0	—	0.66 ± 0.20 (5)	0.93 ± 0.30 (6)
1.0	1.0	0.79 ± 0.03 (6)	0.75 ± 0.14 (6)
7α-Hydroxylase			
0.1	—	0.12 ± 0.01 (6)	0.23 ± 0.07 (5)
0.1	1.0	0.12 ± 0.01 (6)	0.18 ± 0.05 (6)
1.0	—	0.13 ± 0.01 (6)	0.18 ± 0.11 (4)
1.0	1.0	0.14 ± 0.02 (6)	0.24 ± 0.11 (4)
16α-Hydroxylase			
0.1	—	1.30 ± 0.16 (4)	1.11 ± 0.35 (4)
0.1	1.0	1.36 ± 0.16 (6)	0.17 ± 0.05 (4)*
1.0	—	1.42 ± 0.22 (5)	0.38 ± 0.07 (4)
1.0	1.0	1.72 ± 0.35 (6)	0.30 ± 0.05 (4)

Hepatocytes were cultured in a modified Waymouth medium.
The results are means ± SEM with the number of hepatocyte preparations in parentheses.
Abbreviations as in Table 1.
* Significant difference from cultures without GH.

Table 4. Effect of growth hormone and dexamethasone on 5α-reductase activity in hepatocytes cultures from female rats

Dex (μM)	GH (μg/mL)	Dihydrotestosterone [nmol/(min × mg DNA)] Days of culture 1	8
0.1	—	121 ± 7 (7)	38 ± 5 (5)
0.1	1.0	121 ± 8 (5)	57 ± 6 (5)*
1.0	—	120 ± 11 (7)	34 ± 8 (5)
1.0	1.0	133 ± 5 (7)	43 ± 6 (5)

Hepatocytes were cultured in a modified Waymouth medium.
The results are means ± SEM with the number of hepatocyte preparations shown in parentheses.
Abbreviations as in Table 1.
* Significant difference from cultures without GH.

activities in cells cultured in the presence of 0.1 μM dexamethasone confirms previous results [12]. In hepatocytes from female rats, the male-specific 16α-testosterone hydroxylase increased three times in 8 days, whereas in hepatocytes from male rats this hydroxylase activity remained constant with time. In cells from female rats, addition of growth hormone abolished the increase in male-specific hydroxylase activity. In cells from male rats, addition of growth hormone suppressed the activity of the male-specific hydroxylase.

These effects are in accordance with the concept of a constant exposure to growth hormone as a feminization signal. However, growth hormone did not give rise to appearance of the female specific 5α-reductase activity in hepatocytes from male rats. This may be due to the continuous presence of dexamethasone in the culture medium, since it has been reported that 1 nM corticosterone was able to reduce the induction of a female-specific isoform in hepatocytes from male rats cultured on a basement membrane matrix [25]. Meanwhile, dexamethasone is necessary to maintain liver-specific characteristics in cultured hepatocytes [13].

Growth hormone caused inhibition of the male-specific hydroxylase activity at a low concentration of dexamethasone, suggesting that the male-characteristic pattern of growth hormone secretion, with periods of very low concentrations, allows the effects of glucocorticoids to be expressed. This suggestion is supported by the report of Waxman *et al.* [26], who demonstrated *in vivo* that the male-specific 2α- and 16α-hydroxylase activities were stimulated only if growth hormone was undetectable for a period of at least 2.5 hr.

In hepatocytes from male rats, a high concentration of dexamethasone (1 μM) suppressed the male-specific hydroxylase activity almost as much as did growth hormone, and there was no additive effect

of the two hormones. In hepatocytes from female rats, 1 μM dexamethasone caused a decrease in the 0.1 μM-induced 16α-hydroxylase activity and the decrease was extended in the presence of growth hormone.

In contrast, the activity of 2-hydroxylase in hepatocytes from female rats increased after addition of 1 μM dexamethasone. This might be due to content of 2β-hydroxytestosterone in the 2α-hydroxytestosterone peak (see Materials and Methods). Dexamethasone at 1 μM is known to induce P450p (P450IIIA1 and IIIA2) with 2β- and 6β-testosterone hydroxylase activities in the ratio 1:12 [8].

The increase in 6β-hydroxylase activity caused by dexamethasone was prevented by growth hormone. These results are in agreement with those of Schuetz *et al.* [27], who found a partial suppression by growth hormone of the induction by dexamethasone of P450p in hepatocytes from male rats cultured on a basement membrane, and with the results of Gulati and Skett [28]. However, the latter mentioned authors found generally lower enzyme activities with the combined addition of growth hormone and dexamethasone than with dexamethasone alone, whereas only the male-dominating hydroxylase activities were suppressed in the present study.

In conclusion, the results demonstrate that growth hormone suppresses the dexamethasone-inducible as well as the constitutive male-specific P450 steroid hydroxylase activities in cultures of hepatocytes from female and male rats. Furthermore, it is suggested that the activity of constitutive, male-specific P450 testosterone hydroxylases is regulated by interplay between glucocorticoids and growth hormone, though unphysiologically high concentrations of glucocorticoids inhibit the male-specific hydroxylases. This last point requires further investigation.

Acknowledgements—We thank Ida Tønnesen, Hanne Wennicke and Bettina Jacobsen for expert technical assistance.

REFERENCES

1. Eden S, Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology* **105**: 555–560, 1979.
2. Clark RG, Carlsson LMS and Robinson ICAF, Growth hormone secretory profiles in conscious female rats. *J Endocrinol* **114**: 399–407, 1987.
3. Chatterjee B, Demyan WF and Roy AK, Interacting role of thyroxine and growth hormone in hepatic synthesis of α-gloculin and its messenger RNA. *J Biol Chem* **258**: 688–692, 1983.
4. Roberts CT, Brown AL, Graham DE, Seelig S, Berry S, Gabbay KH and Rechler MM, Growth hormone regulates the abundance of insulin-like growth factor I RNA in adult rat liver. *J Biol Chem* **261**: 10025–10028, 1986.
5. Mode A, Wiersma-Larsson E, Strøm A, Zaphiropoulos PG and Gustafsson J-Å, A dual role of growth hormone as a feminizing and masculinizing factor in the control of sex-specific cytochrome P-450 isozymes in rat liver. *J Endocrinol* **120**: 311–317, 1989.
6. McClellan-Green PD, Linko P, Yeowell HN and Goldstein JA, Hormonal regulation of male-specific rat hepatic cytochrome P-450g (P-450IIC13) by androgens and the pituitary. *J Biol Chem* **264**: 18960–19865, 1989.
7. Kobliakov V, Popova N and Rossi L, Regulation of the expression of the sex-specific isoforms of cytochrome P-450 in rat liver. *Eur J Biochem* **195**: 585–591, 1991.
8. Sonderfan AJ, Arlotto MP, Dutton DR, McMillen SK and Parkinson A, Regulation of testosterone hydroxylation by rat liver microsomale cytochrome P-450. *Arch Biochem Biophys* **255**: 27–47, 1987.
9. Nebert DW, Nelson DR, Coon MJ, Estabrook RW, Feyereisen R, Fujii-Kuriyama Y, Gonzales FJ, Guengerich FP, Gunsalus IC, Johnson EF, Loper JC, Sato R, Waterman MR and Waxman DJ, The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol* **10**: 15–21, 1991.
10. Waxman DJ, Dannan GA and Guengerich FP, Regulation of rat hepatic cytochrome P-450: age-dependent expression hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24**: 4409–4417, 1985.
11. Colby HD, Regulation of hepatic drug and steroid metabolism by androgens and estrogens. *Adv Sex Horm Res* **4**: 27–73, 1980.
12. Vind C, Dich J and Grunnet N, Effects of cytochrome P450-inducing agents on the monooxygenation of testosterone in long-term cultures from male and female rats. *Arch Biochem Biophys* **275**: 140–150, 1989.
13. Dich J, Vind C and Grunnet N, Long-term culture of hepatocytes: effect of hormones on enzyme activities and metabolic capacity. *Hepatology* **8**: 39–45, 1988.
14. Dich J, Bro B, Grunnet N, Jensen F and Kondrup J, Accumulation of triacyglycerol in cultured rat hepatocytes is increased by ethanol and by insulin and dexamethasone. *Biochem J* **212**: 617–624, 1983.
15. Grunnet N, Vind C and Dich J, Maintenance of alcohol dehydrogenase activity in long-term culture of hepatocytes from female rat. *Alcoholism Clin Exp Res* **13**: 25–28, 1989.
16. Vind C, Dich J and Grunnet N, The content and activity of cytochrome P-450 in long-term cultures of hepatocytes from male and female rats. *Biochem Pharmacol* **37**: 1371–1375, 1988.
17. Omura T and Sato R, The carbon monooxide binding pigment of liver microsomes I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
18. Kissane JM and Robins E, The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *J Biol Chem* **233**: 184–188, 1958.
19. Waxman DJ, Ko A and Walsh C, Regioselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J Biol Chem* **258**: 11937–11947, 1983.
20. Fiszer-Szafarz B, Szafarz D and GuevaraDeMurillo A, A general, fast, and sensitive micromethod for DNA determination: application to rat and mouse liver, rat hepatoma, human leukocytes, chicken fibroblasts, yeast cells. *Anal Biochem* **110**: 165–170, 1981.
21. McLean AEM and Day PA, The use of new methods to measure: the effect of diet and inducers of microsomal enzyme P-450 in liver homogenates and on metabolism of dimethyl nitrosamine. *Biochem Pharmacol* **23**: 1173–1180, 1974.
22. Yamazoe Y, Shimada M, Kamataki T and Kato R, Effects of hypophysectomy and growth hormone treatment on sex-specific forms of cytochrome P-450 in relation to drug and steroid metabolismes in rat liver microsomes. *Jpn J Pharmacol* **42**: 371–382, 1986.
23. Mode A, Norstedt G, Simic B, Eneroth P and Gustafsson J-Å, Continuous infusion of growth

hormone feminizes hepatic steroid metabolism in the rat. *Endocrinology* **108**: 2103–2108, 1981.
24. Niimi S, Hayakawa T and Tanaka A, Effect of cell density on induction of growth hormone receptors by dexamethasone in primary cultured rat hepatocytes. *Biochem Biophys Res Commun* **174**: 928–933, 1991.
25. Tollet P, Enberg B and Mode A, Growth hormone (GH) regulation of cytochrome P-450IIC12, insulin-like growth factor-I (IGF-I) and GH receptor messenger RNA expression in primary rat hepatocytes: A hormonal interplay with insulin, IGF-I, and thyroid hormone. *Mol Endocrinol* **4**: 1934–1942, 1990.
26. Waxman DJ, Pampori NA, Ram PA, Agrawal AK and Shapiro BH, Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic cytochrome P450. *Proc Natl Acad Sci USA* **88**: 6868–6872, 1991.
27. Schuetz EG, Schuetz JD, May B and Guzelian PS, Regulation of cytochrome P-450b/e and P-450p gene expression by growth hormone in adult rat hepatocytes cultured on a reconstituted basement membrane. *J Biol Chem* **265**: 1188–1192, 1990.
28. Gulati P and Skett P, Interactions of insulin, thyroxine and dexamethasone with growth hormone in the control of steroid metabolism by isolated rat hepatocytes. *Biochem Pharmacol* **38**: 4061–4067, 1989.