0026-895X/89/050707-06$02.00/0

MOLECULAR PHARMACOLOGY, 35:707-712

Regulation of Hepatic Cortisol Sulfotransferase in Rats by Pituitary Growth Hormone

YASUSHI YAMAZOE, DAWEI GONG, NORIE MURAYAMA, MEDHAT ABU-ZEID, and RYUICHI KATO

Department of Pharmacology, School of Medicine, Keio University, Tokyo 160, Japan

Received December 14, 1988; Accepted February 14, 1989

SUMMARY

Cytosolic sulfating activities of 4-pregnen-11β,17α,21-triol-3,20-dione (cortisol) to the 21-sulfate were 4 to 5 times higher in livers of female than male adult rats. The activity was decreased by administration of testosterone propionate (TP) to ovariectomized, but not to intact, female rats. In male rats, the rate of cortisol sulfation was elevated by neonatal castration and was restored in part by the administration of TP to the castrated rats. In addition, the sulfating activity in adult male rats was increased by the treatment with estradiol benzoate. Hypophysectomy almost completely decreased cytosolic cortisol-sulfating activity in male rats. The activity in hypophysectomized male rats was not increased by the treatment with hydrocortisone, TP, estradiol benzoate, or somatomedin C but was restored by the intermittent injection of human growth hormone (hGH). Further, the continuous infusion of hGH, to mimic the female secretory pattern, increased more efficiently the rate of cortisol sulfation. Hypophysectomy of female rats also decreased, but not completely, the sulfating activity. Treatment of female hypophysectomized rats intermittently with hGH had no appreciable effect, but the continuous infusion increased the activity effectively. The involvement of pituitary growth hormone in the hepatic cortisol sulfation was also supported by the experiment using neonatally glutamate-treated rats and by the observation of developmental changes in the cortisol-sulfating activity. These results indicate that pituitary growth hormone is one of the major factors regulating hepatic levels of cortisol sulfation in rats and that the higher activity in the female than the male is due mainly to the difference in the secretory pattern of growth hormone in the adult animals.

Sulfation is a main metabolic pathway of steroids and xenobiotics that is catalyzed by cytosolic PAPS-dependent sulfotransferases. In mammalian livers, multiple forms of sulfotransferase exist and are divided into several groups depending on their substrate specificities (1, 2). Steroid sulfotransferase, showing high catalytic activities for steroids and alcohols, is contained in high amounts in livers. The activity of steroid sulfotransferase is known to vary depending on the age and sex and to be influenced in pathophysiological conditions (3–8). Singer (9) investigated the regulatory mechanism of this enzyme and showed that hepatic steroid sulfotransferase is under the regulation of sex steroids and adrenal steroids in rats. However, recent studies on the hormonal regulation of liver-specific proteins including albumin (10), α_{2u}-macroglobulin (11), and cytochrome P-450s (12–15) demonstrate the involvement of pituitary hormones in the expression and maintenance of their proteins. In addition, our recent study indicates that the hepatic sulfotransferase catalyzing the sulfation of *N*-hydroxyarylamine and *N*-hydroxyarylamide is regulated by pituitary growth hormone in rats (16). Secretory profiles of pituitary growth hormone are sex-differentiated in adult rats (17); high amplitudes of the pulsatile secretion with low or undetectable levels in trough periods is observed in the adult male, whereas a fairly constant secretion is maintained in female rats. Steroid and bile acid sulfotransferase activities are, in contrast to male-dominant *N*-hydroxyarylamide sulfations, higher in livers of female than male rats (6, 7, 18). These results suggest that steroid sulfotransferase might also be under the regulatory influence of pituitary growth hormone, but in a manner different from the male-dominant sulfotransferase in rat livers. Therefore, the role of growth hormone on hepatic steroid sulfotransferase has been studied using cortisol as a substrate. The present data clearly indicate the involvement of growth hormone on the steroid sulfation in rat livers.

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, and Culture of Japan.

Materials and Methods

Chemicals. Cortisol, cortisol 21-sulfate, and PAPS were obtained from Sigma Chemical Co. (St. Louis Mo). Somatomedin C and hGH (Somatonorm; Kavi Vitrum, Sweden) were generous gifts from Fujisawa and Sumitomo Pharmaceutical, Ltd. (Osaka, Japan), respectively.

Animal treatment. Sprague-Dawley rats obtained from Clea Japan (Tokyo) were used in this study. Castration and ovariectomy were performed within 24 hr after birth and on the 22nd day, respectively

ABBREVIATIONS: PAPS, phosphoadenosylphosphosulfate; hGH, methionylated human growth hormone; HPLC, high performance liquid chromatography; MSG, monosodium glutamate.

(19). Hypophysectomy was performed at 7 weeks of age, and the animals were used for experiments after at least 1 week of recovery (20). Some animals were treated with testosterone propionate (dissolved in corn oil) subcutaneously on the 2nd, 4th, and 6th days after birth (20 mg/kg of body weight) or at 8 weeks of age five times every other day (10 mg/kg). Estradiol benzoate (200 μg/kg, dissolved in corn oil) was given subcutaneously to 8-week-old normal or hypophysectomized rats on every other day for 7 days. Some animals were given a subcutaneous injection (0.2 IU/100 g of body weight, twice a day) or continuous infusion (0.01 IU/hr) of hGH for 7 days as previously described (20). Synthetic somatomedin C was given to hypophysectomized rats by subcutaneous injection (100 μg/kg twice a day for 7 days) or infusion (100 μg/day for 7 days). Hydrocortisone acetate (30 mg/kg) was given intraperitoneally to hypophysectomized male rats once a day for 3 days. For the experiment with MSG, rats were treated intraperitoneally with MSG (4 mg/g of body weight) five times at 1, 3, 5, 7, and 9 days after birth (21). These male and female animals at 9 weeks of age showed significant reduction in respective pituitary weights (4.8 ± 0.7 and 5.8 ± 1.1 mg; mean ± SD) as compared with those of intact animals (12.2 ± 0.7 and 11.9 ± 1.4). The livers were homogenized with 1.15% potassium chloride containing 100 mM potassium phosphate (pH 7.4). Cytosol fractions obtained were kept at −80° after addition of dithiothreitol (final concentration, 1 mM), as previously described (16).

Cortisol sulfation. A typical reaction mixture consisted of 50 mM Tris·HCl (pH 7.4), 20 mM $MgCl_2$, 1 mM dithiothreitol, 1.5 mg/ml cytosol, 50 μM cortisol, and 125 μM PAPS in a final volume of 0.2 ml. The reaction was started by the addition of PAPS and terminated by the addition of 100 μl of acetonitrile containing an internal standard, phenacetin, after 90 min of incubation at 37°. For analysis of the metabolite, the deproteinated supernatant of the reaction mixture was injected onto HPLC. The HPLC system consisted of a Waters M6000 pump, 440 absorbance detector, Jasco autosampler, and a Nucleosil $_7C_{18}$ column (4 × 300 mm) proceeded by the guard column (4 × 50 mm). Metabolites were eluted with a mobile phase of 30% acetonitrile/70% 20 mM potassium phosphate monobasic, at the flow rate of 1.0 ml/min at 37°, and were detected by the absorbance at 254 nm. Peak area of cortisol sulfate increased linearly with increasing amounts added in the range of 20 to 1000 pmol/injection.

Statistical analyses were done using Student's *t* test.

Results

Conditions for cortisol sulfation. To quantitate unequivocally the formation of cortisol 21-sulfate, we developed a HPLC method, as shown in Fig. 1. Cortisol 21-sulfate (7.5 min) was well separated from cortisol (13 min) and other components included in a cytosol reaction mixture (1 to 6 min). In this procedure, extraction of the metabolites with organic solvents is not necessary. Acetonitrile-treated supernatant from the reaction mixture is directly injected to HPLC and detected by the absorbance at 254 nm.

The amount of cortisol 21-sulfate linearly increased during 120 min of incubation (Fig. 2A) and with the increase of hepatic cytosol within the range from 0.25 to 1.50 mg/ml (Fig. 2B). Rates of sulfation attained a maximal level at a cortisol concentration of 50 μM (Fig. 2C). The concentration of PAPS also affected the rate of the reaction. Sulfation increased with the addition of PAPS in the range from 0 to 100 μM, reached a maximum level at around 125 μM, and then slightly declined with further increase in the amount of PAPS (Fig. 2D). Therefore, the reactions in the following experiments were performed under the conditions of 1.5 mg/ml cytosolic protein, 125 μM PAPS, and 50 μM cortisol for 90 min at 37°.

Developmental profile of cortisol sulfation. Activity of hepatic cortisol sulfation was low at 8, 14 and 20 days of age and did not differ between the female and male rats (Fig. 3). The sulfating activity was slightly increased at 26 days of age, and the sex-related difference appeared at 34 days of age. In female rats, cortisol-sulfating activity was abruptly increased

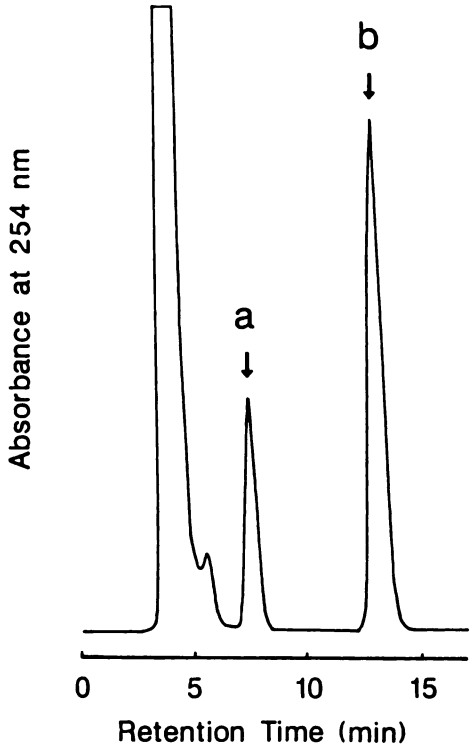

Fig. 1. Elution profile of cortisol and cortisol 21-sulfate. Cortisol 21-sulfate (*a*) and cortisol (*b*) are separated by reversed phase HPLC and are detected by absorbance at 254 nm. Other experimental details are described in Materials and Methods.

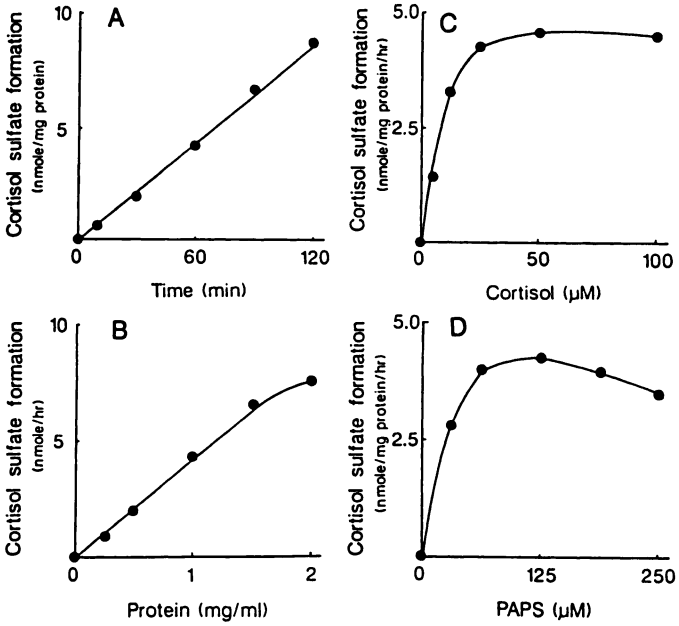

Fig. 2. Effects of incubation period (A) and concentrations of cytosolic protein (B), cortisol (C), and PAPS (D) on enzymatic cortisol sulfation. Unless otherwise indicated, a typical incubation mixture consisted of 50 μM cortisol, 125 μM PAPS, 1-5 mg/ml of cytosolic protein obtained from livers of female rats, 1 mM dithiothreitol, 20 mM $MgCl_2$, and 50 mM Tris·HCl (pH 7.4) and was incubated for 90 min at 37°.

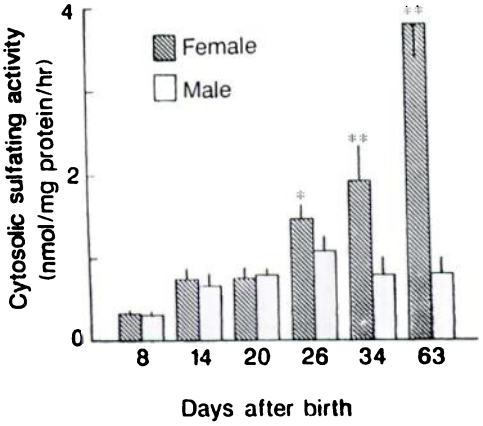

Fig. 3. Developmental profile of cortisol sulfation. Values are expressed as mean (*column*) ± standard deviation (*bar*) of three or four different animals.*, **Significantly different between male and female of same age groups ($p < 0.05$ and $p < 0.01$, respectively).

TABLE 1

Effect of gonadectomy and supplement of steroids on hepatic sulfating activity of cortisol in adult rats

Data represent the mean ± standard deviation. Number of animals is shown in parentheses, except that the average of two different rats is shown in gonadectomized male rats. Testosterone propionate was given to both neonate and 8-week-old rats. Estradiol benzoate was given to 8 week-old rats. Castration was performed at the first day after birth and overiectomy was done at 22 days after birth.

Treatment	Rate of sulfation	
	Female	Male
	nmol/mg of protein/hr	
None	3.78 ± 0.40 (3)	0.61 ± 0.12 (4)
Testosterone	4.21 ± 0.39 (3)	Not determined
Estradiol	Not determined	2.20 ± 0.21 (3)[a]
Gonadectomy	2.08 ± 0.06 (5)[a]	2.74 (3.05, 2.43)[a]
Gonadectomy plus testosterone	1.36 ± 0.46 (4)[a]	1.21 ± 0.26 (4)[a]

[a] Significantly different from the untreated rat ($p < 0.01$).

at puberty and was 4- to 6-fold higher than that in male adult rats.

Effects of sex steroids on cortisol sulfation. To understand the mechanism underlying the sex-related expression of steroid sulfotransferase, effects of androgen and estrogen were examined using gonadectomized rats. As described in Table 1, cortisol-sulfating activity in adult female rats was not decreased by treatment with testosterone propionate at both neonatal and adult periods but was decreased by the pubertal ovariectomy. The sulfating activity was further decreased by the treatment of ovariectomized rats with testosterone propionate at 8 weeks of age. In male rats, the rate of hepatic cortisol sulfation was low but was increased 3- to 4-fold by the administration of estradiol benzoate. Neonatal castration was also effective in enhancing the sulfating activity in adult male rats, and the treatment of neonatally castrated rats with testosterone propionate decreased the sulfating activity to a level similar to that of testosterone-treated ovariectomized rats.

Effect of hypophysectomy and supplement of growth hormone. As shown in Fig. 4, continuous infusion of hGH to normal female rats slightly enhanced the cytosolic activity of cortisol sulfation, although the administration of hGH intermittently to female rats had no appreciable effect on the cytosolic sulfation (data not shown). Depletion of growth hormone by hypophysectomy caused an effective decrease in female rats.

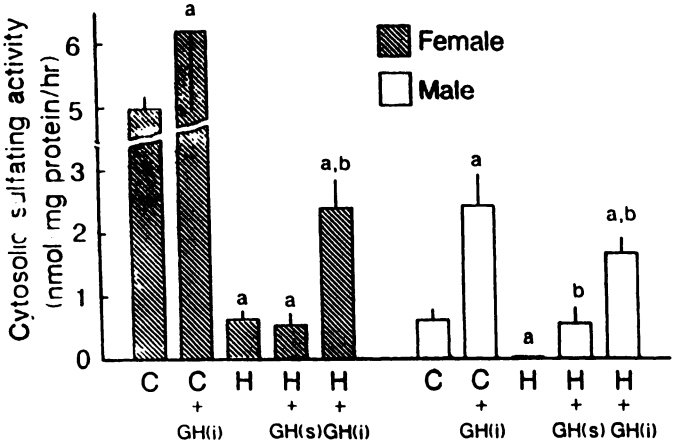

Fig. 4. Effects of hypophysectomy and supplement of growth hormone on cortisol sulfation. hGH was given to adult control (*C*) and hypophysectomized (*H*) rats by a continuous infusion (0.01 IU/hr) [*GH(i)*] or subcutaneous injection (0.2 IU/100 g of body weight twice a day) [*GH(s)*] for 7 days. *Columns* and *bars* indicate the mean ± standard deviation of four to six different animals. *a* and *b*, significantly different from the corresponding control and hypophysectomized rats, respectively ($p < 0.05$).

TABLE 2

Effect of endocrine hormones on cytosolic sulfation of cortisol in hypophysectomized male rats

Data represent the mean ± standard deviation from the number of the animals shown in parentheses. Symbols *s* and *i* indicate the subcutaneous treatment of hormone by the intermittent injection and continuous infusion, respectively.

Treatment		Rate of sulfation	
		pmol/mg of protein/hr	%[a]
None	(4)	88 ± 47	100
Testosterone propionate	(4)	78 ± 30	89
Estradiol benzoate	(4)	40 ± 30	45
hGH(s)	(4)	353 ± 105[b]	401
hGH(s) plus testosterone propionate	(4)	351 ± 105[b]	399
hGH(s) plus estradiol benzoate	(4)	105 ± 61	119
Hydrocortisone	(2)	80 (84, 77)	91
Somatomedin C(i)	(3)	58 ± 23	66
Somatomedin C(s)	(3)	28 ± 15	32

[a] Percents relative to the value of nontreated are shown.
[b] Significantly different from control ($p < 0.01$).

Although the treatment of hypophysectomized female rats intermittently with hGH had no significant effect, continuous infusion of hGH to female hypophysectomized rats, to mimic the secretion pattern of growth hormone in adult female rats, enhanced 4-fold the cortisol-sulfating activity.

In male rats, cortisol-sulfating activity was increased 3- to 4-fold by the continuous infusion of hGH. Hypophysectomy of male rats almost completely abolished the sulfating activity. Intermittent injection of hGH, which mimicked the secretory pattern of growth hormone in adult male rats, restored the sulfating activity to the level of normal male rats. In addition, continuous infusion of hGH to hypophysectomized male rats caused a further increase in the activity.

Although steroid receptor or binding protein is shown to exist in the liver of rats (22), sex steroids are also known to exert their effects through the hypothalamus and pituitary. To understand the relationship between steroid- and growth-hormone-mediated actions on hepatic cortisol sulfation, the effects of steroids on hypophysectomized male rats were examined, as described in Table 2. Treatment of hypophysectomized male rats with estradiol benzoate or testosterone propionate had no

appreciable effect on the rates of hepatic cortisol sulfation. Testosterone was also ineffective in hGH-treated hypophysectomized rats. However, estradiol benzoate slightly decreased the rate of cytosolic cortisol sulfation in hGH-treated hypophysectomized rats, which is in contrast to the treatment in normal male rats (Table 1).

Adrenal steroids are suggested to influence the level of hepatic steroid sulfotransferase (7), but the administration of hydrocortisone to hypophysectomized male rats had no appreciable effect on cortisol sulfation. In addition, somatomedin C, the level of which is also dependent on the level of serum growth hormone, is known to participate in the regulation of skeletal growth (23, 24). However, the treatment of hypophysectomized male rats with somatomedin C had no enhancing effect on cytosolic cortisol sulfation.

Effect of neonatal glutamate treatment. Administration of MSG during neonatal periods is known to cause a reduction of the serum growth hormone level in adult rats without causing severe reduction of other pituitary hormones (25, 26). Therefore, hepatic cortisol-sulfating activity in MSG-treated rats was measured to confirm the effect of growth hormone. As shown in Fig. 5, the rate of cortisol sulfation was decreased to less than 40% in both sexes of MSG-treated rats, supporting the conclusion that cortisol sulfation is under the regulation of pituitary growth hormone in rat livers.

Time-course of induction. In the experiment shown in Fig. 4, infusion of hGH stimulated the hepatic cortisol-sulfating activity in both sexes of hypophysectomized rats. The activities, however, did not reach the level of intact female rats. The protocol used for the administration of hGH is known to be sufficient to yield the optimal response of another female-specific enzyme, P-450-female (12, 20), but it is possible that steroid sulfotransferase is regulated by pituitary growth hormone in a mode different from that of P-450-female in rat livers. Therefore, the time-course of the induction of hepatic cortisol sulfation by growth hormone was studied, using hypophysectomized female rats. As shown in Fig. 6, cortisol-sulfating activity in 1-day-infused rats was not significantly different from that in nontreated hypophysectomized rats, but the activity was clearly increased after 3 days of infusion of hGH. The sulfating activity increased with further continuous infusion of hGH and attained nearly the maximum level after 7 days of infusion to hypophysectomized female rats.

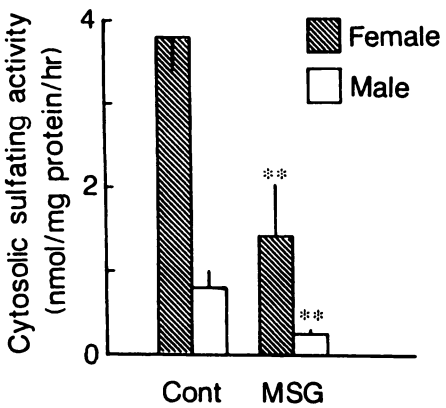

Fig. 5. Effect of neonatally administered MSG on hepatic cortisol sulfation of rats. *Cont*, control, *MSG*, neonatally MSG-treated. Data are shown as a mean (*column*) ± standard deviation (*bar*) of four different animals. **Significantly different from the corresponding control group ($p < 0.01$).

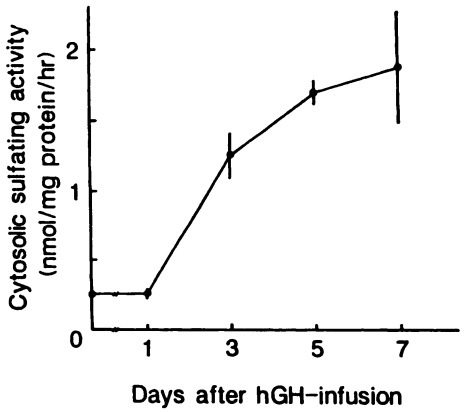

Fig. 6. Time course of the induction of cytosolic cortisol sulfation by hGH infusion in hypophysectomized female rats. Data are expressed as a mean ± standard deviation of three or four different animals.

Discussion

Steroid sulfotransferase has been shown to play a vital role in the metabolism of steroids and alcohols, by converting these chemicals to more hydrophilic sulfates (1, 2). The rates of sulfation of steroids and alcohols are often higher in female than male rats, which is in contrast to a male-dominant sulfation of *N*-hydroxyarylamines and *N*-hydroxyarylamides. As shown in Fig. 3, the level of hepatic cortisol-sulfating activity was low in neonates and was increased in female rats at puberty, similar to the developmental profile of other enzymes including P-450-female (12, 20), steroid 5α-reductase (27), and alcohol dehydrogenase (28) in livers. Singer and Bruns (2, 7) investigated the mechanism of the hormonal regulation of steroid sulfation and proposed that gonadal and adrenal steroids are the main determinant for the hepatic levels of cortisol sulfotransferase. In the present study, pituitary growth hormone has also been shown to be a main determinant in the age- and sex-related difference in hepatic cortisol-sulfating activity. As shown in Fig. 4, hepatic cortisol-sulfating activity was decreased by 87% in hypophysectomized female rats. The continuous infusion of hGH to hypophysectomized female rats, to mimic the secretion pattern of growth hormone in female rats, effectively increased the sulfating activity. In addition, hypophysectomy almost completely abolished the sulfating activity in male rats, and the intermittent injection of hGH to hypophysectomized male rats, to mimic the secretion pattern in male rats, restored the sulfating activity to the normal male level. Infusion of hGH was also effective in male hypophysectomized rats and enhanced the sulfating activity to a level similar to that in hGH-infused hypophysectomized female rats. Although Singer (9) reported that hypophysectomy had no appreciable effect on the cortisol sulfation in male rats, the loss of the sulfating activity by hypophysectomy was consistently observed in three different experiments.

Neonatal treatment with MSG causes a reduced level of serum growth hormone in adult rats (25, 26). As shown in Fig. 5, the hepatic level of cortisol-sulfating activity was lower in MSG-treated than control male and female rats. These results also support the role of growth hormone in the maintenance of steroid sulfotransferase in rat livers. In addition, the results obtained in this study showed a clear contrast in the mode of growth hormone regulation with a male dominant *N*-hydroxyarylamine (amide) sulfotransferase activity (16). Although the

exact mechanism of growth hormone-dependent dual regulation is unclear, alternative expression of sex-related proteins by growth hormone is also observed for cytochrome P-450, P-450-male and P-450-female, in rat livers (12, 20).

In the present study, the role of estrogen and androgen on hepatic cortisol sulfation was also confirmed as previously reported (29). Neonatal castration caused the enhancement of the sulfating activity in adult male rats, and the supplement of androgen to castrated males decreased the activity. In females, hepatic sulfating activity was reduced by ovariectomy. Administration of estrogen to normal male rats also caused an increase in the sulfating activity. However, treatment of hypophysectomized rats with testosterone propionate or estradiol benzoate had no appreciable effect on the sulfating activity, similar to the case with P-450-male and P-450-female (30).

Estradiol is reported to modify the secretory profile of growth hormone and increased the trough levels in adult male rats (31). Castration of male rats is also known to result in the modulation of the growth hormone secretory pattern (31). In addition, coadministration of estradiol benzoate to hGH-treated rats had no stimulatory effect on the sulfating activity, although a significant increase was observed with the treatment of normal male rats. Therefore, androgen and estrogen are likely to exert their effects on hepatic sulfation mainly through the hypothalamus and pituitary, where these hormones affect the secretion of growth hormone.

In the present study, the sex-related difference in cortisol sulfation is diminished by hypophysectomy, but not completely abolished. Further, intermittent injection of hGH increased the cortisol-sulfating activity in hypophysectomized male, but not in hypophysectomized female, rats. The reason that the sex-related difference remains in hypophysectomized rats is unclear, but the hepatic level of growth hormone receptor in hypophysectomized rats is reported to be sex independent (32). Thus, these results might be indicative of the additional involvement of an endogenous factor other than pituitary growth hormone, although treatment with hydrocortisone had no significant effect on hypophysectomized male rats in the present study. Singer and Bruns (7) and Lyon and Jakoby (33) reported that at least three forms of steroid sulfotransferases exist in livers of rats, and these forms are affected noncoordinately by steroidal hormones. Therefore, the sex-related difference in hypophysectomized rats may also be attributed to the difference in the isozyme composition in the two sexes.

In conclusion, the present study clearly indicates the essential role of pituitary hormones, especially growth hormone, on the hormonal regulation of hepatic cortisol sulfation in livers of rats, although the permissive or additional role of some other endogenous factor is also suggested.

References

1. Jakoby, W. B., R. D. Sekura, E. L. Marcus, and J.-L. Wang. Sulfotransferase, in *Enzymatic Basis of Detoxication.* (W. B. Jakoby, ed.), Vol. 2. Academic Press, New York, 199–228 (1980).
2. Singer, S. S. Preparation and characterization of different kinds of sulfotransferase, in *Biochemical Pharmacology and Toxicology. Methodological Aspects of Drug-Metabolizing Enzymes* (D. Zakim and D. A. Veaaey, eds.), Vol. 1. Wiley, Chichester, 95–159 (1985).
3. DeBaun, J. R., E. C. Miller, and J. A. Miller. *N*-Hydroxy-2-acetylaminofluorene sulfotransferase: its probable role in carcinogenesis and in protein (methion-*S*-yl) binding in rat liver. *Cancer Res.* **30**:577–595 (1970).
4. Carlstedt-Duke, J., and J.-A. Gustafsson. Sexual differences in hepatic sulphurylation of deoxycorticosterone in rats. *Eur. J. Biochem.* **36**:172–177 (1973).
5. Iwasaki, K., T. Shiraga, K. Tada, K. Noda, and H. Noguchi. Age- and sex-related changes in amine sulphoconjugations in Sprague-Dawley strain rats: comparison with phenol and alcohol sulphoconjugations. *Xenobiotics* **16**:717–723 (1986).
6. Roy, A. B. Comparative studies on the liver sulphatases. *Biochem. J.* **68**:519–528 (1958).
7. Singer, S. S., and L. Bruns. Enzymatic sulfation of steroids-hepatic cortisol sulfation and glucocorticoid sulfotransferases in old and young male rats. *Exp Gerontol.* **13**:425–429 (1982).
8. Hammerman, K. J., L.-J. Chen, A. Fernandez-Corugedo, and D. L. Earnest. Sex differences in hepatic sulfation of taurolithocholate in the rats. *Gastroenterology* **75**:1021–1025 (1978).
9. Singer, S. S. Glucocorticoid sulphotransferases in rats and other animal species. *Biochem. Soc. Trans.* **12**:35–39 (1984).
10. Keller, G. H., and J. M. Taylor. Effect of hypophysectomy and growth hormone treatment on albumin mRNA levels in the rat liver. *J. Biol. Chem.* **254**:276–278 (1979).
11. Kulkarni, A. B., R. M. Gubits, and P. Feigelson. Developmental and hormonal regulation of α_{2u}-globulin gene transcription. *Proc. Natl. Acad. Sci. USA* **82**:2579–2582 (1985).
12. Kato, R., Y. Yamazoe, M., Shimada, N. Murayama, and T. Kamataki. Effect of growth hormone and ectopic transplantation of pituitary gland on sex-specific forms of cytochrome P-450 and testosterone and drug oxidations in rat liver. *J. Biochem.* **100**:895–902 (1986).
13. Yamazoe, y., M. Shimada, N. Murayama, S. Kawano, and R. Kato. The regulation by growth hormone of microsomal testosterone 6β-hydroxylase in male rat liver. *J. Biochem.* **100**:1095–1097 (1986).
14. Yamazoe, Y., M. Shimada, N. Murayama, and R. Kato. Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. *J. Biol. Chem.* **262**:7423–7428 (1987).
15. Morgan, E. T., C. MacGeoch, and J.-A. Gustafsson. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P-450 apoprotein in the rat. *J. Biol. Chem.* **260**:11895–11898 (1985).
16. Yamazoe, Y., S. Manabe, N. Murayama, and R. Kato. Regulation of hepatic sulfotransferase catalyzing the activation of *N*-hydroxyarylamide and *N*-hydroxyarylamine by growth hormone. *Mol. Pharmacol.* **32**:536–541 (1987).
17. Eden, S. Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology* **105**:555–560 (1979).
18. Kane, R. E. III, L. J., Chen, and M. M. Thaler. Regulation of bile salt sulfotransferase isoenzymes by gonadal hormones. *Hepatology* **4**:1195–1199 (1984).
19. Shimada, M., N. Murayama, Y. Yamazoe, T. Kamataki, and R. Kato. Further studies on the persistence of neonatal androgen imprinting on sex-specific cytochrome P-450, testosterone and drug oxidations. *Jpn. J. Pharmacol.* **45**:467–478 (1987).
20. Yamazoe, Y., M. Shimada, T. Kamataki, and R. Kato. Effects of hypophysectomy and growth hormone treatment on sex-specific forms of cytochrome P-450 in relation to drug and steroid metabolisms in rat liver microsomes. *Jpn. J. Pharmacol.* **42**:371–382 (1986).
21. Yamazoe, Y., M. Shimada, N. Murayama, K. Yamauchi, and R. Kato. Alteration of hepatic drug metabolizing activities and contents of cytochrome P-450 isozymes by neonatal monosodium glutamate treatment. *Biochem. Pharmacol.* **37**:1687–1691 (1988).
22. Lax, E. R., F. Rumstadt, H. Plasczyk, A. Peetz, and H. Schriefers. Antagonistic action of estrogens, flutamide, and human growth hormone on androgen-induced changes in the activities of some enzymes of hepatic steroid metabolism in the rat. *Endocrinology* **113**:1043–1055 (1983).
23. Jansen, M., F. M. A. VanShaik, A. T. Ricker, B. Bullock, D. E. Woods, K. H. Gabbay, A. L. Nussbaum, J. S. Sussenbach, and J. L. Vanden Brande. Sequence of cDNA encoding human insulin-like growth factor I precursor. *Nature (Lond.)* **306**:609–611 (1983).
24. Froesch, E. R., C. Schmid, J. Schwanda, and J. Zapf. Actions of insulin-like growth factors. *Annu. Rev. Physiol.* **47**:443–467 (1985).
25. Nemerroff, C. B., R. J. Konkol, G. Bissette, W. Youngblood, J. B. Martin, P. Brazeau, M. S. Rone, A. J. Prange, Jr., G. R. Breese, and J. S. Kizer. Analysis of the disruption of hypothalamic-pituitary regulation in rats treated neonatally with monosodium L-glutamate (MSG): evidence for the involvement of tuberoinfundibular cholinergic and dopaminergic systems in neuroendocrine regulation. *Endocrinology* **101**:613–622 (1977).
26. Terry, L. C., J. Epelbaum, and J. B. Martin. Monosodium glutamate: acute and chronic effects on rhythmic growth hormone and prolactin secretion, and somatostatin in the undisturbed male rat. *Brain Res.* **217**:129–142 (1981).
27. Einarsson, K., J.-A. Gustafsson, and A. Stenberg. Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. *J. Biol. Chem.* **48**:4987–4997 (1973).
28. Crabb, D. W., W. F. Bosron, and T.-K. Li. Role of the pituitary and neonatal and androgenic imprinting in the hormonal regulation of liver alcohol dehydrogenase activity. *Biochem. Pharmacol.* **35**:1527–1532 (1986).
29. Singer, S. S., and S. Sylvester. Enzymatic sulfation of steroids. II. The control of the hepatic cortisol sulfotransferase activity and the individual hepatic steroid sulfotransferase of rats by gonads and gonatal hormones. *Endocrinology* **99**:1346–1352 (1976).
30. Kamataki, T., M. Shimada, K. Maeda, and R. Kato. Pituitary regulation of

sex-specific forms of cytochrome P-450 in liver microsomes of rats. *Biochem. Biophys. Res. Commun.* **130**:1247–1253 (1985).
31. Jansson, J.-O., S. Eden, and O. Isaksson. Sexual dimorphism in the control of growth hormone secretion. *Endocrine Rev.* **6**:128–150 (1985).
32. Baxter, R. C., and Z. Zaltsman. Induction of hepatic receptors for growth hormone (GH) and prolactin by GH infusion is sex independent. *Endocrinology* **115**:2009–2014 (1984).
33. Lyon, E. S., and W. B. Jakoby. The identity of alcohol sulfotransferases with hydroxysteroid sulfotransferases. *Arch. Biochem. Biophys.* **202**:474–481 (1980).

Send reprint requests to: Yasushi Yamazoe, 35 Shinanomachi, Shinjuku-ku, Department of Pharmacology, School of Medicine, Keio University, Tokyo 160, Japan.