0026-895X/87/040536-06$02.00/0

MOLECULAR PHARMACOLOGY, 32:536-541

Regulation of Hepatic Sulfotransferase Catalyzing the Activation of *N*-Hydroxyarylamide and *N*-Hydroxyarylamine by Growth Hormone

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Received May 4, 1987; Accepted July 3, 1987

SUMMARY

The regulatory mechanism of hepatic sulfation of *N*-hydroxyarylamine and *N*-hydroxyarylamide by endocrine factors has been studied in rats. The cytosolic sulfations of *N*-hydroxy-2-acetylaminofluorene (*N*-hydroxy-AAF) and 2-hydroxyamino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (*N*-hydroxy-Glu-P-1), which were determined by the reductive formations of 2-acetylaminofluorene and Glu-P-1, were seven to nine times and three times higher, respectively, in male than female rats. Hypophysectomy of male rats decreased their activities to 23% and 41% of the levels of the untreated animals, respectively. Intermittent treatment of hypophysectomized male and female rats with human growth hormone (hGH) significantly increased their sulfating activities of both compounds. Infusion of hGH also enhanced the sulfating activity of *N*-hydroxy-AAF but not of *N*-hydroxy-Glu-P-1. The sulfating activity of *N*-hydroxy-AAF was also decreased by castration at neonate and was increased by the administration of testosterone propionate to gonadectomized male and female rats. Testosterone propionate and estradiol benzoate had no effect on hypophysectomized rats, but estradiol benzoate repressed the sulfating activities of *N*-hydroxy-AAF and *N*-hydroxy-Glu-P-1 in hGH-treated hypophysectomized male rats. These results indicate that sex steroids elicit their effects on the sulfations of *N*-hydroxyl-aryl compounds through modulating the action of growth hormone at hypothalamus-pituitary and hepatic levels in rat livers.

Cytosolic sulfotransferase, which exists in the liver and other tissues of mammals, catalyzes sulfation of diverse chemicals including steroids, bile acids, alcohols, phenols, and arylamines (1–3). In rats, appreciable age- and sex-associated differences are observed on their steroid (4–6), alcohol (7), and *N*-hydroxyarylamide sulfations (8–10). For the first two substrates, the higher activities are generally detected in hepatic cytosols prepared from female rather than male rats, whereas the activities for *N*-hydroxy-AAF are known to be higher in the male than the female.

DeBaun *et al.* (8) and Lotlikar (9) reported that hepatic activity of *N*-hydroxy-AAF sulfation was increased by the treatment of female rats with testosterone propionate and was decreased by hypophysectomy or thyroidectomy of male rats. These results suggest the involvement of sex-steroid and pituitary hormone on the expression and maintenance of sulfotransferase catalyzing the sulfation of *N*-hydroxy-AAF. However, the exact mechanism by which the endocrine factor regulates the sulfotransferase activity has not been studied in detail.

In our recent studies on the regulatory mechanism of male-specific forms of microsomal cytochromes P-450, P-450-male (11, 12), and P-450$_{6\beta}$ (PB IIb) (13) in rat livers, growth hormone was demonstrated to act as both a stimulating and suppressing factor on the expression of these isozymes.

On the activation mechanism of arylamines and arylamides, *O*-sulfation as well as *N*,*O*-acetyltransfer of *N*-hydroxyarylamides, which occur after the initial *N*-hydroxylation of arylamides, seems to be necessary for the activation to form the covalent interaction with cellar DNA (14, 15). However, the role of direct *O*-esterification of *N*-hydroxyarylamines in arylamine-induced carcinogenesis is still unclear.

Recently, we (16, 17) and Flammang *et al.* (18) provided evidence for an alternate activating pathway by which *N*-hydroxyarylamines undergo direct *O*-acetylation by cytosolic *N*-hydroxyarylamine acetyltransferase to cause the DNA damage. Other recent studies on arylamine carcinogenesis also suggest the importance of *O*-sulfation on the metabolic activation of *N*-hydroxyarylamines (19, 20).

Portions of this work were supported by grants-in-aid from the Ministry of Education, Science, and Culture and a grant-in-aid for cancer research from the Ministry of Health and Welfare, Japan, and the Nissan Science Foundation.

ABBREVIATIONS: AF, 2-acetylaminofluorene; AF, 2-aminofluorene; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; *N*-hydroxy-AAF, *N*-hydroxy-2-acetylaminofluorene; *N*-hydroxy-Glu-P-1, 2-hydroxyamino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; DTT, dithiothreitol; hGH, human growth hormone; PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

Thus, we have studied the role of pituitary growth hormone on the hepatic level of sulfotransferase catalyzing the activations of carcinogenic *N*-hydroxyarylamide and *N*-hydroxyarylamine in male and female rats. The data obtained indicate that the sulfating activity of *N*-hydroxy-AAF and *N*-hydroxy-Glu-P-1 is regulated mainly by the pulsatile secretion of growth hormone in rat livers and the sex-related difference in the sulfating activity is caused by the difference in the secretory pattern of growth hormone in male and female Sprague-Dawley rats.

Materials and Methods

Chemicals. Glu-P-1 was supplied under the Research Resource Program for Cancer Research of the Ministry of Education, Science, and Culture, Japan. *N*-Hydroxy-AAF and *N*-hydroxy-Glu-P-1 were synthesized as reported previously (21, 22). DTT and ellipticine were obtained from Sigma (St. Louis, MO), and analytical grade of pentachlorophenol was from Wako Pure Chemicals, Osaka, Japan. PAPS and hGH (Somatonorm, Kavi Vitrum, Stockholm, Sweden) were geneous gifts from Dr. K. Iwasaki, Fujisawa Pharmaceutical and Sumitomo Pharmaceutical (Osaka, Japan), respectively.

Animal treatment. Sprague-Dawley rats were obtained from Clea Japan (Tokyo). Castration and ovariectomy were performed, respectively, within 24 hr after postpartum (23) and on the twenty-second day. Some animals were treated with testosterone propionate (dissolved in corn oil) subcutaneously on the second, fourth, and sixth days after birth (20 mg/kg body wt) and/or at 8 wk of age five times every other day (10 mg/kg). Estradiol benzoate (500 μg/kg, dissolved in corn oil) was given to 8-wk-old hypophysectomized rats on every other day for 7 days. Hypophysectomy was performed at 7 wk of age (11). The animals, left to recover for at least 1 week, were given a subcutaneous injection (0.2 IU/100 g body wt, twice a day) or infusion (0.002 or 0.01 IU/hr) of hGH for 7 days. For the infusion, an osmostic minipump (Alzet 2001, Alza, Palo Alto, CA) was implanted on the back of the rats. Anterior pituitary gland of male rats was implanted under the kidney capsule of the hypophysectomized male rats as described previously (24). The livers were homogenized with 1.15% potassium chloride containing 100 mM potassium phosphate (pH 7.4). The cytosol (105,000 × *g* supernatant fraction) was prepared as described (11) and was kept at −80°C after the addition of DTT (final concentration 1 mM). Protein concentration was determined by the method of Lowry *et al.* (25).

Sulfotransferase assay. We have developed an easy method for sulfation of the *N*-hydroxy group by a cytosolic sulfotransferase based on the reductive formation of arylamine and arylamide from the *O*-sulfonyloxy derivatives in the presence of DTT. A possible mechanism of the reductive formation of AAF and Glu-P-1 from their *N*-hydroxy derivatives is shown in Fig. 1. Although the exact mechanism of the reductive formation of AAF and Glu-P-1 from their *O*-sulfonylated derivatives is unknown, sequential one-electron reduction of nitrenium ion by ascorbate is proposed for the reductive formation of AAF (26). A procedure used for the measurement of the direct *O*-acetylation of *N*-hydroxy-Glu-P-1 (27) was applied for the sulfation assay. The typical reaction mixture consisted of 50 mM bicine-KOH (pH 7.8), 0.5 mg/ml hepatic cytosol, 250 μM PAPS, 1 mM DTT, and 20 μM *N*-hydroxy-AAF or *N*-hydroxy-Glu-P-1 in a final volume of 0.2 ml and was incubated for 10 min at 37°C. If necessary, 10 μM pentachlorophenol or ellipticine was also included in the mixture. For the sulfation of *N*-hydroxy-Glu-P-1, the reaction was terminated by addition of methanol (600 μl), and then the fluorescence in the supernatant was measured after centrifugation at 2,500 rpm at 5 min. Emission and exitation wavelengths at 445 and 376 nm, respectively, were used for the determination of Glu-P-1. The reductive formation of AAF was quantified by high performance liquid chromatography. To the reacted mixture, 400 μl of acetonitrile containing 6 nmol diphenylamine (internal standard) were added, and the mixture was centrifuged at 2,500 rpm after vortexing.

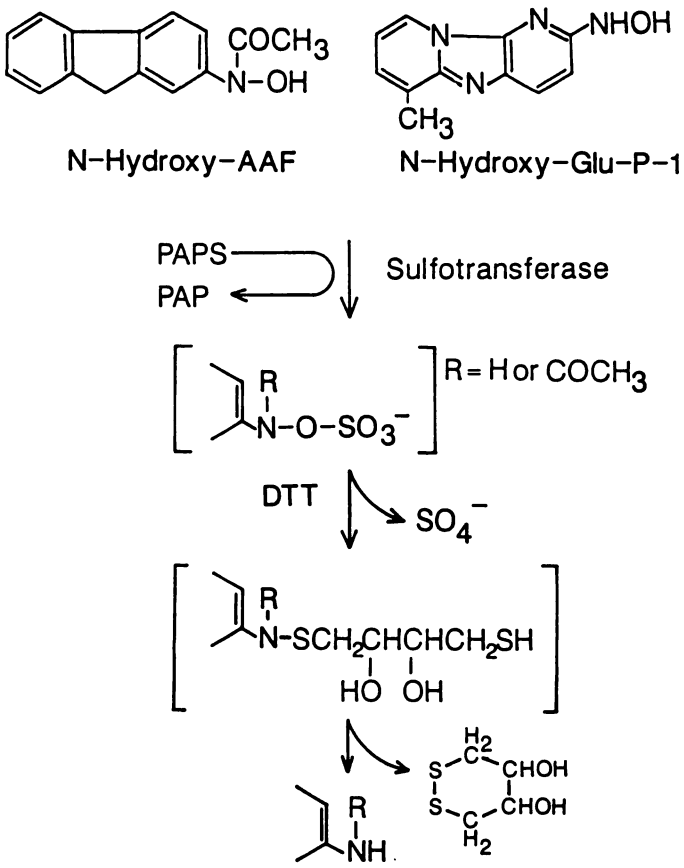

Fig. 1. A possible formation mechanism of arylamine and arylamide from their *N*-hydroxy compounds by sulfotransferase-mediated *O*-sulfonylation and subsequent reductive cleavage in the presence of DTT. PAP, 3′-phosphoadenosine 5′-phosphate.

The metabolites were separated with a nucleosil $_7C_{18}$ column (3.9 × 300 mm) and detected by their absorbances at 280 nm. A mixture of acetonitrile and 20 mM dihydrogen potassium phosphate (60:40) was used as the mobile phase at the flow rate of 1.2 ml/min. AAF, AF, and diphenylamine were eluted at their retention times of 7.0, 8.5, and 12.5 min, respectively, but *N*-hydroxy-AAF was retained in the column and not eluted in this condition. To ascertain the usefulness of the new method, hepatic sulfotransferase activities of *N*-hydroxy-AAF were measured by a different method that is based on the sulfate transfer from 4-nitrophenylsulfate to a substrate in the presence of 3′,5′-adenosine diphosphate (28, 29). The rate of 4-nitrophenol formation (921 ± 59 pmol/mg protein/min) with hepatic cytosols of four male rats was in good agreement with that of the reductive formation of AAF (841 ± 31 pmol/mg protein/min). For the quantification, the amounts formed nonenzymatically (<4 and 2 pmol/mg protein/min for *N*-hydroxy-AAF and *N*-hydroxy-Glu-P-1, respectively) were subtracted from the experimental values. In the sulfation of *N*-hydroxy-AAF using hepatic cytosols, measurable amounts of AAF were formed without the addition of PAPS. On the basis of sex-dependent formation rate (male/female) and inhibition by pentachlorophenol, the activity is considered mainly to be mediated by sulfotransferase that utilizes a residual cofactor in hepatic cytosols. Thus the basal cytosolic activity obtained without the addition of PAPS is not subtracted from the each experimental value. Statistical analyses were performed using Student's *t* test.

Results

Conditions for sulfation. Effects of changes in the concentration of cytosolic protein, cofactor and substrate, and in the

reaction period were examined as shown in Fig. 2. In the presence of 0.25 mM PAPS, the reductive formation of AAF and Glu-P-1 increased with the amounts of hepatic cytosols of male rats between 0 and 0.5 mg/ml. The formations of AAF and Glu-P-1 were low in the absence of PAPS and were stimulated by the increasing concentrations of the cofactor up to 0.2 mM. The rates of their sulfations also depended on the concentration of substrate and linearly increased within the range of 0–20 μM. Both the formations of AAF and Glu-P-1 showed linearity for at least the initial 20 min of the incubation period. Thus the reactions in the following experiments were conducted with the conditions of 0.25 mM PAPS, 20 μM substrate, and 0.5 mg/ml cytosolic protein and were performed for 10 min at 37°C.

In this condition, sulfotransferase-mediated formation of AAF was inhibited by 90% in the presence of 10 μM pentachlorophenol, a sulfotransferase inhibitor (30), but the formation was decreased by only 12% by the addition of 10 μM ellipticine, an acetyltransferase inhibitor[1] (Table 1). In the *N*-hydroxy-AAF sulfating system, AF was also formed, but the amount corresponded only to 1–2% of AAF formed in the reaction with hepatic cytosol of male rats. In addition, formation of AF was inhibited by the addition of 10 μM ellipticine but not of 10 μM pentachlorophenol (data not shown). These results indicate that small amounts of AF are derived from cytosolic *N*,*O*-acetyltransfer of *N*-hydroxy-AAF. A similar inhibitory effect of pentachlorophenol was also observed with the sulfotransferase-mediated formation of Glu-P-1. The formation of Glu-P-1 was reduced to 15% of the control in the presence of 10 μM pentachlorophenol. These results confirmed that sulfotransferase mediated the reductive formation of AAF and Glu-P-1 from their *N*-hydroxy-derivatives.

Effect of gonadectomy of rats on the activity of *N*-hydroxy-AAF sulfation. In accordance with previous reports (8, 9), the hepatic sulfating activity of *N*-hydroxy-AAF was 7.3 times higher in males than females (Table 2). To understand the role of sex steroid on the sex-related difference in the sulfating activity, effects of gonadectomy and administration of testosterone propionate on male and female rats were examined with *N*-hydroxy-AAF sulfation. Although gonadectomy of male rats is reported to cause little change in the *N*-hydroxy-AAF sulfating activity (8), castration at day 1 resulted in significant reduction of the sulfating activity in adult life. Replenishment of testosterone at the neonatal or adult period partially restored the sulfating activity to the 28% and 41% levels of untreated male rats. Castrated rats treated at both neonate and adult periods with testosterone propionate showed activity higher than the animals treated at either neonate or adult periods. Ovariectomy at 22 days after birth of female rats slightly increased the sulfating activity in the adult age. Further enhancement of sulfating activity was observed after treatment of ovariectomized rats with testosterone propionate.

Sulfation of *N*-hydroxy-AAF by hepatic cytosol of hypophysectomized and hormone-supplemented male and female rats. In our previous studies (11–13), the hepatic level of sex-specific forms of cytochrome P-450 in rat livers has been shown as regulated by growth hormone. To verify the possibility that cytosolic sulfotransferase is also under the regulation of serum growth hormone levels, effects of hypophysectomy and administration of sex steroid and growth hormone were examined on the sulfation of *N*-hydroxy-AAF.

As shown in Fig. 3A, continuous infusion of hGH to adult male rats decreased by 50% the hepatic activity of *N*-hydroxy-AAF sulfation. The more profound decrease was observed after hypophysectomy. The activity in hypophysectomized male rats corresponded to 23% of that of untreated male rats. Secretory pattern of growth hormone is sex-related in rats (31): pulsatile secretion with the low or undetectable trough level is observed in the male, whereas the relatively constant secretion is detected in the female. Thus hGH was given to hypophysectomized rats by two different ways to produce the male-mimicked (two intermittent injections per day) and female-mimicked

[1] Y. Yamazoe *et al.*, unpublished data.

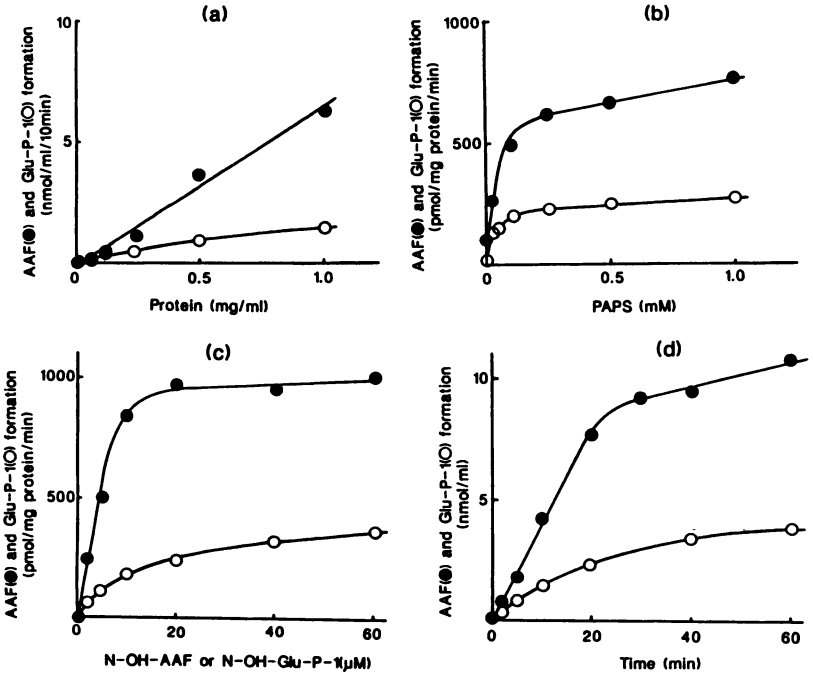

Fig. 2. Sulfating activities of *N*-hydroxy-AAF and *N*-hydroxy-Glu-P-1 in rat liver cytosols and requirements for a cofactor. Unless otherwise indicated, the typical reaction mixture (0.2 ml) contained 0.25 mM PAPS, 20 μM substrate, 0.1 mg cytosolic protein, 1 mM DTT and 50 mM bicine-KOH (pH 7.8) and was incubated for 10 min. The sulfating activities of *N*-hydroxy-AAF and *N*-hydroxy-Glu-P-1 were determined as described in Materials and Methods.

TABLE 1

Effects of pentachlorophenol and ellipticine on the reductive formation of AAF and Glu-P-1 in cytosolic sulfating system

Values are means ± SD with hepatic cytosols of three different untreated male rats. Numbers in parentheses indicate percent relative activity compared with respective control. Methanol (2 μl) was used as a vehicle (1% amounts of total incubation volume). Other experimental details are described in Materials and Methods.

Chemicals	AAF	Glu-P-1
	pmol/mg protein/min	
Control	667.1 ± 65.2 (100)	223.3 ± 9.2 (100)
10 μM Pentachlorophenol	66.5 ± 5.1 (10.0)	34.0 ± 3.5 (15.2)
10 μM Ellipticine	594.3 ± 7.5 (89.1)	200.4 ± 9.4 (89.7)

TABLE 2

Cytosolic sulfating activity of *N*-hydroxy-AAF in the livers of gonadectomized male and female rats

Testosterone propionate (TP) was administrated to male rats castrated within 24 hr after postpartum and the female rats ovariectomized at 22 days of age. Other experimental procedures are described in Materials and Methods.

Treatment	No. of rats	Activity	% Control
		pmol/mg protein/min	
Male			
Control	5	790.4 ± 64.1	100
Castration	3	162.9 ± 24.4[a]	20.6
Castration plus TP at neonate age	4	222.6 ± 17.7[a]	28.2
Castration plus TP at adult age	4	323.1 ± 25.2[a]	40.9
Castration plus TP at both neonate and adult ages	3	508.1 ± 45.9[a]	64.3
Female			
Control	4	109.4 ± 9.2	100
Ovariectomy	5	140.0 ± 15.4[a]	128.0
Ovariectomy plus TP at adult age	4	321.9 ± 105.7[a]	294.2

[a] Differ significantly from the control ($p < 0.05$).

(continuous infusion) patterns. Both intermittent injection and continuous infusion of hGH resulted in increased activities of *N*-hydroxy-AAF sulfation. Extent of enhancement is higher in the hypophysectomized rats given the intermittent injection; the activity was completely restored up to the level of untreated male rats. The levels of the sulfating activities in hGH-infused hypophysectomized rats were largely the same as those in hGH-infused nonoperated male rats, and no significant difference in that activity was observed between the two doses of the infusion given to hypophysectomized rats. Anterior pituitary gland implanted ectopically under the renal capsule is known to maintain low levels of growth hormone (32). Transplantation of anterior pituitary gland to hypophysectomized male rats resulted in an increase in the sulfating activity to a level similar to that of hGH-infused hypophysectomized rats, indicating that the stimulating effect is not specific to hGH (data not shown).

In addition to growth hormone, androgen and estrogen also affected the level of *N*-hydroxy-AAF sulfation in rat livers (Table 2). Thus to understand the relationship between sex steroid and growth hormone, effects of administration of sex steroids to hypophysectomized male rats was examined. Although no significant difference was observed in the activity of hypophysectomized rats before and after treatment with testosterone propionate or estradiol benzoate, the repressive effect of estrogen was observed in hypophysectomized male rats

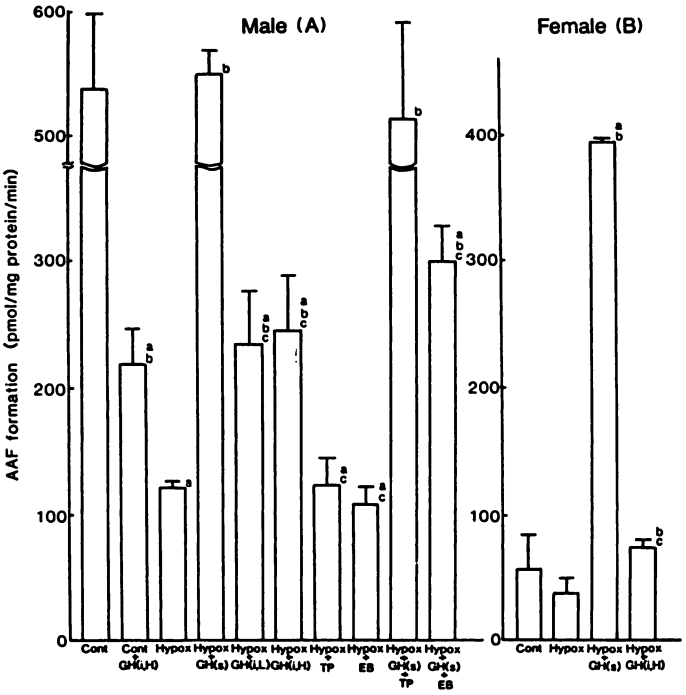

Fig. 3. Effects of hypophysectomy and treatments with growth hormone and/or sex steroid on the sulfation of *N*-hydroxy-AAF by hepatic cytosols of male (A) and female rats (B). hGH was given to adult control (Cont) and hypophysectomized rats (Hypox) with an osmotic minipump at the rate of 0.01 IU/hr (GH(i,H)) and 0.002 IU/hr (GH(i,L)) for 7 days. Testosterone propionate (TP), estradiol benzoate (EB) and/or hGH (GH(s)) were also given to hypophysectomized rats intermittently twice a day for 7 days. Data are means (*column*) ± SD (*bar*) from five different rats; a, b, and c, significantly different from the corresponding control, hypophysectomized, and hypophysectomized rats treated intermittently with hGH, respectively ($p < 0.05$).

treated with hGH. As shown in Fig. 3B, the *N*-hydroxy-AAF sulfating activity of female rats tended to be decreased by hypophysectomy, and the level was one-third that of hypophysectomized male rats. Treatment of hypophysectomized female rats with intermittent injection of hGH enhanced by 10.4-fold the activity of *N*-hydroxy-AAF sulfation. Continuous infusion of hGH restored the activity to the level of untreated female.

Sulfation of *N*-hydroxy-Glu-P-1 by hepatic cytosol of hypophysectomized and hGH-supplemented rats. On the metabolic activation of an amino acid-pyrolysate, Glu-P-1, *N*-hydroxylation by cytochrome P-450 and esterification by cytosolic enzyme of the *N*-hydroxy derivatives are suggested to play major roles in producing DNA damage (17, 33, 34). Although most arylamines ingested in the body are considered to be readily *N*-acetylated to the corresponding arylamides, Glu-P-1 and other pyrolysate heterocyclic arylamines are known to be relatively less susceptible to this reaction (35). In our preliminary experiment, *N*-hydroxy-Glu-P-1 was found to be activated in the cytosol system, including PAPS or acetyl coenzyme A, to the derivatives that bind to DNA. Recent studies (19, 20) also suggest the involvement of hepatic sulfotransferase on the activation of *N*-hydroxyarylamines as well as *N*-hydroxyarylamides. However, knowledge of the characteristics of sulfotransferase catalyzing the activation of *N*-hydroxyarylamine and on the endocrine factor controlling the enzyme is still very limited. Thus, effects of pituitary factors, especially growth hormone, on the *N*-hydroxyarylamine sulfation was also examined with *N*-hydroxy-Glu-P-1 as a substrate.

As shown in Fig. 4, a sex-related difference was observed on the rate of *N*-hydroxy-Glu-P-1 sulfation, although the extent of the sex difference (3.4-fold) was lower than that with *N*-hydroxy-AAF (9.4-fold). The hepatic *N*-hydroxy-Glu-P-1 sulfating activity of male rats was decreased by hypophysectomy to 41% of the untreated level and was restored by the treatment of hypophysectomized male rats with intermittent injection of hGH. Treatment of hypophysectomized male rats with continuous infusion of hGH (female mimicked) had no effect on the sulfation of *N*-hydroxy-Glu-P-1, although the same treatment enhanced the sulfating activity of *N*-hydroxy-AAF. Treatment with testosterone propionate or estradiol benzoate of hypophysectomized male rats did not affect the sulfation of *N*-hydroxy-Glu-P-1. However, estrogen, but not androgen, reduced the activity in hGH-treated hypophysectomized rats. As shown in Fig. 4B, the sulfating activity of *N*-hydroxy-Glu-P-1 was decreased slightly by hypophysectomy of female rats. Intermittent injection of hGH enhanced the activity of hypophysectomized female rats, although the extent of the increase was less in the female (59%) than the male (87%).

Discussion

The present study has demonstrated clearly that hepatic activity of sulfotransferase in catalyzing *O*-sulfation of *N*-hydroxy-AAF is regulated by serum growth hormone levels in rats. As shown in Fig. 3, the *N*-hydroxy-AAF sulfation was decreased by hypophysectomy to 23% of that of untreated male rats and was restored by the intermittent injection of hGH to hypophysectomized rats. Similar enhancement was also observed by the intermittent injection of hGH to hypophysectomized female rats. These results indicate that the pulsatile secretion of growth hormone, which is detected in adult male rats (31), is a main determinant for expressing the male pattern of *N*-hydroxy-AAF sulfating activity. On the activity of sulfotransferase in catalyzing *O*-sulfation of *N*-hydroxy-Glu-P-1, pulsatile secretion of growth hormone was also necessary for maximal expression. These results may indicate that hepatic sulfation of *N*-hydroxy-Glu-P-1 and *N*-hydroxy-AAF are catalyzed at least partially by the same or related sulfotransferase(s), which probably corresponds to the purified enzyme reported previously (10, 36), although the contribution of the growth hormone-dependent enzyme was higher in the sulfation of *N*-hydroxy-AAF than *N*-hydroxy-Glu-P-1.

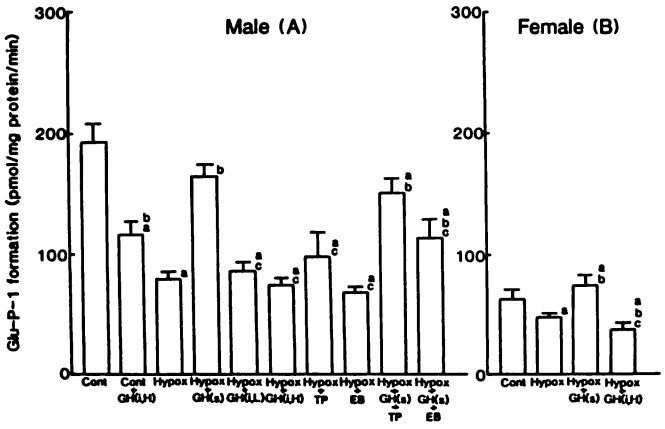

Fig. 4. Effects of hypophysectomy and treatment with growth hormone and/or sex steroid on the sulfation of *N*-hydroxy-Glu-P-1 by hepatic cytosols of male (A) and female rats (B). Data are means (*column*) ± SD (*bar*) of the values from five rats. Abbreviations are the same as in Fig. 3. Other experimental details are described in Materials and Methods.

Treatment of hypophysectomized male rats with the continuous infusion of hGH, which mimicked the female secretory pattern, also increased the sulfating activity of *N*-hydroxy-AAF. The same treatment on female hypophysectomized rats also enhanced the sulfating activity to a level similar to that of untreated female rats. However, the sulfating activity of *N*-hydroxy-Glu-P-1 was not increased by the continuous infusion of hGH. These results strongly suggest that hepatic cytosol contains at least two distinct forms of sulfotransferases catalyzing the *O*-sulfation of *N*-hydroxy-AAF, which are distinguishable from each other by their difference in the dependency on growth hormone-secretory patterns. Continuous infusion of hGH to normal male rats resulted in the decrease in the hepatic sulfation of *N*-hydroxy-AAF to a level similar to that of hypophysectomized rats given the infusion of hGH. The mechanism of the decrease is unclear but might be the result of the disruption of pulsatile pattern that leads to female-type secretion. Similar differences in the dependency to growth hormone-secretory pattern are also observed with hepatic cytochrome P-450 isozymes (11–13, 37).

In the present study, testosterone was shown to increase the activity of *N*-hydroxy-AAF sulfation in castrated rats but not in hypophysectomized male rats. Similarly, estradiol did not affect the level of sulfating activity in hypophysectomized rats, although it repressed the stimulatory action of growth hormone in hGH-supplemented hypophysectomized rats. These results indicate that androgen and estrogen affect the levels of *N*-hydroxyarylamide and *N*-hydroxyarylamine sulfations mainly through altering the action of growth hormone in rats. At the site where the steroids exert their effects, estradiol and testosterone are reported to modulate the serum secretory pattern of growth hormone in normal or gonadectomized rats (31). Thus these steroids are likely to act mainly at hypothalamus and/or pituitary levels. However, the suppressive effect of estradiol in hGH-treated hypophysectomized rats suggests that estradiol also acts at hepatic level to reduce the stimulatory effect of growth hormone.

In the present study, sex-related difference in the sulfating activity of *N*-hydroxy-AAF in Sprague-Dawley rats was caused by the difference in the secretory patterns of growth hormone in male and female rats. However, we also observed differences in the levels of sulfating activities of *N*-hydroxy-AAF and *N*-hydroxy-Glu-P-1 between hypophysectomized male and female rats. These results implicate the participation of an unknown factor, other than pituitary hormones, on the regulation and sex-related difference in hepatic sulfotransferase activities.

In addition, a large strain difference in the case of *N*-hydroxy-AAF sulfation is reported between Sprague-Dawley, Wistar, and Fischer 344 strains of female rats (38). Female rats of Fischer 344 strain show activity six times higher than that of Sprague-Dawley strain, whereas the activities of the male rats of these three strains are similar.

Further studies are necessary to identify the unknown regulatory factor and to assess whether the growth hormone-dependent regulation of sulfotransferase is peculiar to Sprague-Dawley rats.

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