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Ontogeny and Hormonal Basis of Male-Dominant Rat Hepatic Sulfotransferases

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SUMMARY

The developmental and hormonal regulation of three male-dominant rat hepatic sulfotransferases (STs) was studied in male and female rats. ST1A1 (phenol ST) mRNA levels increased gradually in both male and female rats after birth until puberty and then declined to a greater extent in female than in male rats. In adult rats, hepatic ST1A1 mRNA levels were ~2-3-fold higher in males than in females. However, ST1C1 and ST1E2 mRNAs (corresponding to *N*-hydroxy-2-acetylaminofluorene ST and estrogen ST, respectively) increased dramatically at puberty in male rats but remained low in female rats. ST1C1 and ST1E2 expression is >10-fold higher in adult male than in adult female rats. Estradiol, progesterone, and testosterone administration to hypophysectomized rats did not have marked effects on hepatic ST expression. Hypophysectomy decreased ST1A1 gene expression in rat liver, but neither intermittent growth hormone (GH) injection (male pattern) nor continuous GH infusion (female pattern) restored ST1A1 mRNA levels. ST1C1 gene expression was abolished by hypophysectomy and reversed by GH injection. Hypophysectomy did not dramatically decrease hepatic ST1E2 mRNA in male rats but markedly increased ST1E2 expression in female rats. GH infusion (female pattern) in hypophysectomized male and female rats decreased ST1E2 mRNA levels. Prolactin increased hepatic ST1C1 mRNA levels, which is similar to the effect of GH. It is concluded that the three male-dominant rat hepatic STs are regulated differently because the developmental pattern of ST1A1 is markedly different from that for ST1C1 and ST1E2. The high expression of ST1C1 in adult males is determined by male GH secretory pattern, whereas male dominance of ST1E2 is due to the suppressive effect of female GH secretory pattern in adult female rats.

STs are a family of enzymes that catalyze the sulfation of xenobiotics and a variety of endogenous molecules. The STs play an important role in xenobiotic detoxication, activation, and hormone homeostasis (1). Originally, STs were named according to their substrate specificities. For example, phenol ST (or aryl ST), *N*-hydroxyarylamine ST, and estrogen ST were STs that catalyze naphthol or nitrophenol, *N*-hydroxy-2-acetylaminofluorene (*N*-OH-2AAF), and estrone or estradiol sulfation, respectively. However, several reports indicate that one aryl ST, termed ASTIV, sulfates all of the substrates (2–4). In addition, at least one substrate, estradiol, can be sulfated by both phenol ST and hydroxysteroid ST (5). These results indicate that STs, as in the case of other drug-metabolizing enzymes, have broad and overlapping substrate specificities. Recently, Yamazoe *et al.* (6) proposed a new nomenclature in which STs are divided into families and subfamilies based on deduced amino acid sequences from cloned ST cDNAs. Alphanumeric names were designated. Among them, ST1A1, ST1C1, and ST1E2 correspond to phenol ST, *N*-hydroxyarylamine or *N*-OH-2AAF ST, and estrogen ST, respectively.

Gender differences in ST activities are observed in rats. For example, ST activity toward *p*-nitrophenol is higher in adult male than in adult female rats (7). Male rats are more susceptible to *N*-OH-2AAF carcinogenesis than were female rats (8) because male rats have considerably higher *N*-OH-2AAF ST activity. Estrone ST activity is also higher in male than in female rats (9). The mRNA levels of these three STs (ST1A1, ST1C1, and ST1E2) exhibit the same male dominance (10, 11, 12). However, it is not clear when this sex divergence emerges and whether the three male-dominant STs follow the same developmental pattern.

Sex hormones are thought to be responsible for the sex differences in the STs. It has been shown that *N*-OH-2AAF ST activity is "feminized" (i.e., decreased) by neonatal castration and restored by administration of testosterone to castrated male rats (13). In addition, uterine estrogen ST enzyme activity in young female pigs was induced by PR (14). However, estrogens and androgens are also known to affect pituitary hormone secretion (15). Whether the effect of sex hormones on STs is direct or mediated through the pituitary has not been examined.

ABBREVIATIONS: ST, sulfotransferase; EB, estradiol benzoate; PR, progesterone; TP, testosterone propionate; GH, growth hormone; PRL, prolactin; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; AR, androgen receptor; CYP, cytochrome P-450.

Gender-dependent expression of CYP is evident and has been studied extensively. The sex differences in CYPs are due to GH secretory patterns, and the effects of sex hormones are of secondary importance to that of GH (16). The GH effect is also observed for one of the male-dominant STs. *N*-OH-2AAF ST activity is feminized by continuous infusion of bovine GH (female GH secretory pattern) (17). Hypophysectomy decreases *N*-OH-2AAF ST activity, and intermittent injection of human GH (male GH secretory pattern) enhances its activity (13). Phenol ST and estrogen ST are also male-dominant STs. Whether GH hormone plays a similar role in phenol ST and estrogen ST regulation is not known.

The current study was therefore undertaken to better understand the regulation of the three male-dominant hepatic STs by examining the ontogenic profiles and hormonal regulation of their gene expression.

Materials and Methods

Animals. Sprague-Dawley rats were obtained from Sasco (Omaha, NE). Rats were bred in an American Association for Accreditation of Laboratory Animal Care-accredited facility for the ontogeny study. Rats for hypophysectomy were purchased at 22 days of age and hypophysectomized 3 days later by IACUC-approved procedures. Hypophysectomized rats were administered 5% glucose for 3 days after surgery. Two weeks of recovery were allowed before starting the various treatments. Body weight gain was monitored after hypophysectomy. None of the hypophysectomized rats gained >7 g/week. All rats were maintained on tap water and standard laboratory rodent chow (Purina Laboratory Chow 5001) *ad libitum* with a 12-hr dark/light cycle.

Hormone administration. EB (200 μg/kg), PR (25 mg/kg), combined EB and PR (same doses as when given alone), and TP (10 mg/kg) were injected subcutaneously daily to hypophysectomized rats. Corn oil (2 ml/kg) was given as the vehicle for the steroid hormones. Rat GH (1.8 IU/mg, AFP-87401; National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) was administered to hypophysectomized male and female rats by either twice-daily subcutaneous injections (600 μg/kg) or continuous infusion through Alzet miniosmotic pumps (model 2001; Alza, Palo Alto, CA) at a rate of 5 μg/hr. Rat PRL (25 IU/mg, AFP-6452B; National Institute of Diabetes and Digestive and Kidney Diseases) was infused into hypophysectomized rats at a rate of 7 μg/hr. The vehicle for GH and PRL was a solution containing 0.15 M NaCl and 0.01 M $NaHCO_3$. No difference was observed between vehicle injection and infusion. All treatments were of 5-day duration.

RNA isolation. Livers were flash-frozen in liquid N_2 and total liver RNA was isolated with the use of RNAzol-B (Tel-Test, Friendswood, TX) according to the manufacturer's directions. Briefly, livers were homogenized in RNAzol-B (2 ml/100 mg of tissue). Chloroform [0.1 chloroform/1 homogenate (v/v)] was added, and the mixture was shaken vigorously, followed by centrifugation. RNA in the aqueous phase was precipitated with the same volume of isopropanol and washed with 75% ethanol. RNA pellets were dissolved in 0.25% SDS. RNA yield, purity, and integrity were determined by absorbance at 260 nm, A_{260}/A_{280} ratio, and electrophoresis in an agarose minigel.

Northern blot and dot blot analyses of RNA. Twenty micrograms of total liver RNA from individual animals was denatured and subjected to 1.2% agarose gel electrophoresis. After electrophoresis, RNA was transferred onto a ζ probe GT membrane (BioRad, Richmond, CA) in 10× SSC buffer (1× = 0.15 M sodium chloride and 0.015 M sodium citrate). Total RNA isolated from livers of rats at specified ages was also analyzed by dot blot analysis. Twenty micrograms of RNA was denatured and directly applied onto the GT membrane via a BioRad dot blot vacuum apparatus (18).

Probes and labeling for Northern blot and dot blot analyses. DNA oligonucleotide probes (20 mer) were designed for each ST. The probe for ST1A1 (5′-CTTCACATGCACTAGCGGTG-3′) was complementary to the corresponding regions (82–101) to the published cDNA sequence of phenol ST (19). The probe for ST1C1 (5′-CACCTAGTGTGGAAGGTCTG-3′) was complementary to nucleotides 1050–1069 of *N*-OH-2AAF ST cDNA (11). The probe for ST1E2 (5′-GCACTCCAGGTCAGGTATTC-3′) corresponds to the complement of nucleotides 364–383 of the cDNA sequence for estrogen ST (12). The probe for 28S rRNA corresponds to the oligonucleotide sequence described by Barbu and Dautry (20). The probes were tailed with [α-^{32}P]dATP (6000 Ci/mmol; Amersham, Arlington Heights, IL). The reaction mixture contained 100 units of terminal deoxynucleotidyltransferase (Boehringer-Mannheim Biochemicals, Indianapolis, IN), 2.5 mM $CoCl_2$, and 3–4.5 pmol of probe in a 1× reaction buffer. The labeled probes were purified on DNA G-25 quick-spin columns (Boehringer-Mannheim Biochemicals).

Hybridization. Prehybridization and hybridization solutions were purchased from Sigma Chemical (St. Louis, MO). SDS, formamide (20%), and tRNA (Boehringer-Mannheim Biochemicals) were added. For ST1A1, prehybridization (20 mM sodium phosphate, pH 7.0, 7% SDS, 5× Denhardt's solution, 100 μg/ml salmon sperm DNA, and 125 μg/ml yeast tRNA) was performed at 52° for ≥4 hr. The hybridization incubation was similar to the prehybridization incubation except that the solution contained 2× 10^6 cpm/ml ^{32}P-labeled probe, and the hybridization time was 16–18 hr. The membranes were then washed in 2× SSC and 2% SDS at 46° for 10 min, twice with 1× SSC and 2% SDS at 46° for 20 min, and then for an additional 20 min at 52°. For ST1C1 and ST1E2, prehybridization was similar to that for ST1A1, except the solution contained 20% formamide and the temperature was 46°. Hybridization for ST1C1 and ST1E2 was carried out overnight with a similar prehybridization solution except that it contained 1× Denhardt's solution and appropriate radiolabeled oligonucleotide probe. Posthybridization washing for ST1C1 and ST1E2 was the same: 2× SSC and 2% SDS for 30 min at 46°, 1× SSC and 2% SDS for 20 min at 46° and 50°, and 0.5× SSC and 2% SDS for 20 min at 50°. Autoradiography was performed by exposing X-ray film (X-Omat, Kodak, Rochester, NY) for an appropriate time (1–7 days). After hybridization for all STs, the blots were stripped and rehybridized with oligonucleotide probe for 28S rRNA. The hybridization methods for 28S rRNA were the same as described for ST1C1 and ST1E2. The autoradiograms were scanned with a densitometer (model CS9000U; Shimadzu, Kyoto, Japan).

Statistical analysis. The data were analyzed by Student's *t* test for comparing two mean values. When more than two groups were compared, analysis of variance followed by Duncan's multiple-range test was applied. The accepted level of significance was set at $p < 0.05$.

Results

Ontogeny of hepatic ST1A1, ST1C1 and ST1E2 gene expression. The ontogenic development of ST1A1 mRNA levels in male and female rats is shown in Fig. 1. In male rats, ST1A1 was detected at birth, increased gradually until puberty, and then declined slowly. In female rats, ST1A1 developed in parallel to male rats, but it decreased after 30 days of age to a greater extent than in male rats. At 60–90 days of age (mature adult rats), ST1A1 mRNA in male rats was ~2-3-fold that in female rats.

In contrast to ST1A1, ST1C1 mRNA was very low in both male and female rats until male rats were 30 days of age (Fig. 2). At that age, the level in male rats was ~30% of that in adult males, which was reached by 45 days of age. In females, the ST1C1 mRNA levels remained low. In adult rats, ST1C1 was 10-fold higher in male than in female rats.

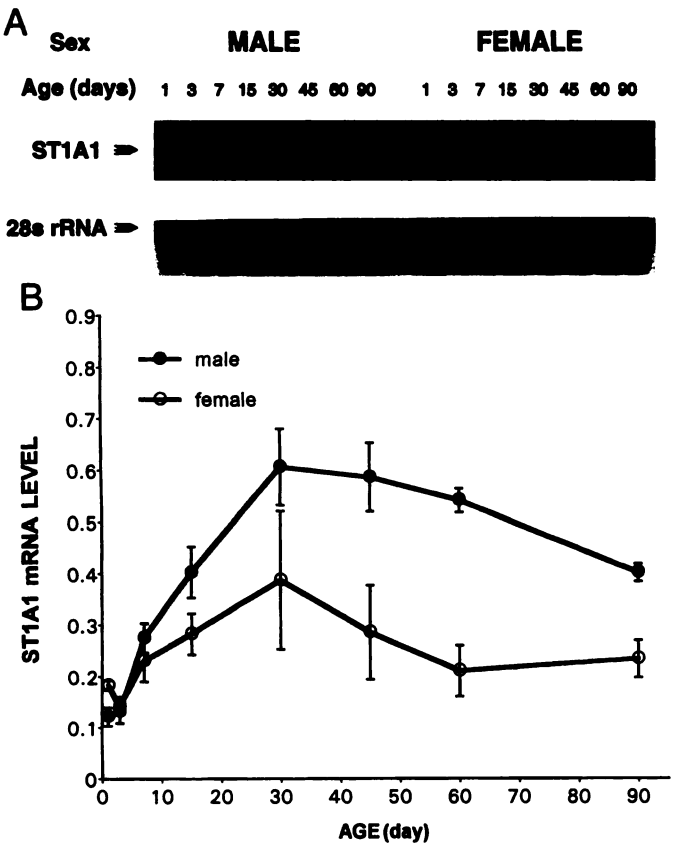

Fig. 1. Hepatic ST1A1 mRNA levels in male and female rats of various ages. A, Representative autoradiogram of Northern blot analysis. Each lane (20 μg of RNA/lane) contains RNA from one animal from a group of three or four rats. B, Graphic presentation of ST1A1 mRNA levels by dot blot analyses. The autoradiograms were scanned with a densitometer. The values are expressed as ratio of densitometric units for ST hybridization signal to the respective 28S rRNA hybridization signal. The results are presented as mean ± standard error for three or four rats.

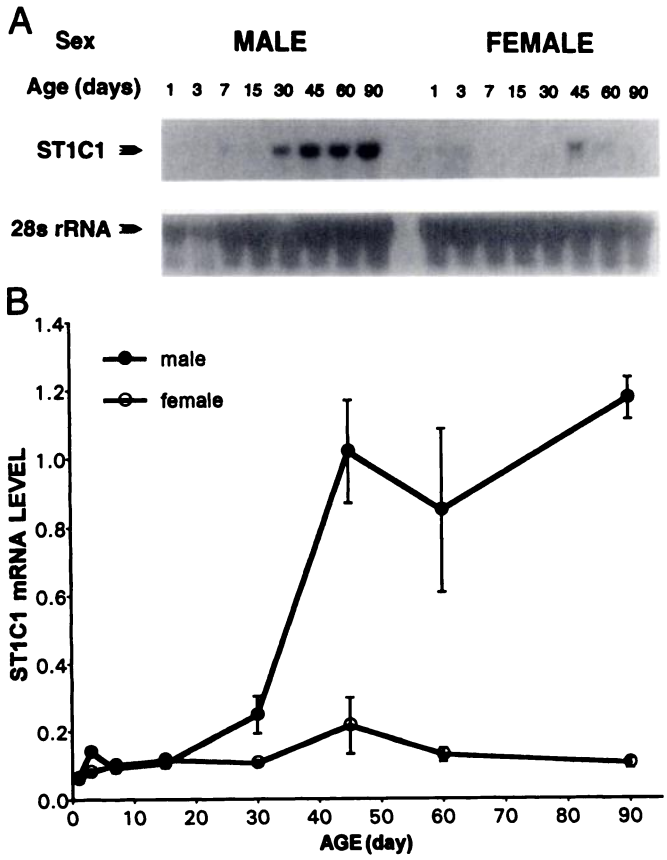

Fig. 2. Hepatic ST1C1 mRNA levels in male and female rats of various ages. A, Representative autoradiogram of Northern blot analysis. Each lane (20 μg RNA/lane) contains RNA from one animal from a group of three or four rats. B, Graphic presentation of ST1C1 mRNA level by dot blot analysis. Values were obtained as described in the legend to Fig. 1.

The developmental pattern of ST1E2 was similar to that for ST1C1 (Fig. 3). The ST1E2 mRNA levels were very low in both male and female rats during the first 15 days of age. A significant increase in ST1E2 mRNA was observed in male rats by 30 days and reached adult levels by 45 days of age. Female rats expressed very low constitutive levels of ST1E2 at all ages. A >10-fold difference in ST1E2 mRNA levels were observed between male and female rats at 60–90 days of age.

Effect of hypophysectomy on hepatic ST1A1, ST1C1, and ST1E2 expression in male and female rats. As shown in Fig. 4, hypophysectomy resulted in a 68% and 74% reduction in ST1A1 mRNA levels in male and female rats, respectively (Fig. 4, *top*). Hypophysectomy, however, abolished the expression of ST1C1 mRNA (Fig. 4, *middle*). For ST1E2, male and female rats responded differently to hypophysectomy (Fig. 4, *bottom*). Large individual variations were observed in hypophysectomized male rats. Compared with intact control rats, ST1E2 mRNA levels increased in some (one of five) hypophysectomized male rats and decreased (two of five) or maintained the same (two of five) in other hypophysectomized rats. On average, ST1E1 mRNA level in male rats tended to be decreased by hypophysectomy, but the decrease was not statistically significant. In contrast, ST1E2 mRNA levels in female rats were markedly enhanced by hypophysectomy.

Effect of steroid hormones on hepatic ST1A1, ST1C1, and ST1E2 expression in hypophysectomized rats. Steroid hormones were administered to hypophysectomized rats, and their effects on STs are shown in Fig. 5. Steroid hormones did not have marked effects on ST1A1 expression in either male or female rats (Fig. 5, *top*). ST1C1 mRNA levels were very low in hypophysectomized rats, and none of the steroid hormones were able to restore ST1C1 expression (Fig. 5, *middle*). ST1E2 expression was increased by EB, PR, and TP in hypophysectomized male rats by 185%, 200%, and 78%, respectively (Fig. 5, *bottom*). Estradiol tended to enhance ST1E2 expression in hypophysectomized female rats.

Effect of GH secretory patterns on hepatic ST1A1, ST1C1, and ST1E2 expression in hypophysectomized rats. Although the developmental pattern of ST1A1 was different from ST1C1 and ST1E2, the sex difference in all three STs became apparent around puberty (30–45 days of age) (Figs. 1–3). Sex differences in the GH secretory pattern in rats also become evident at puberty. Therefore, the effects of GH on the three ST mRNA levels were examined. As indicated in Fig. 6 (*top*), GH injection, which mimics male GH secretory pattern, tended to increase ST1A1 mRNA levels, whereas GH infusion, which mimics female GH secretory pattern, tended to suppress it in both hypophysectomized male and female rats. However, neither pattern of GH administration produced statistically significant changes.

Replacement of GH by injection (male pattern) restored

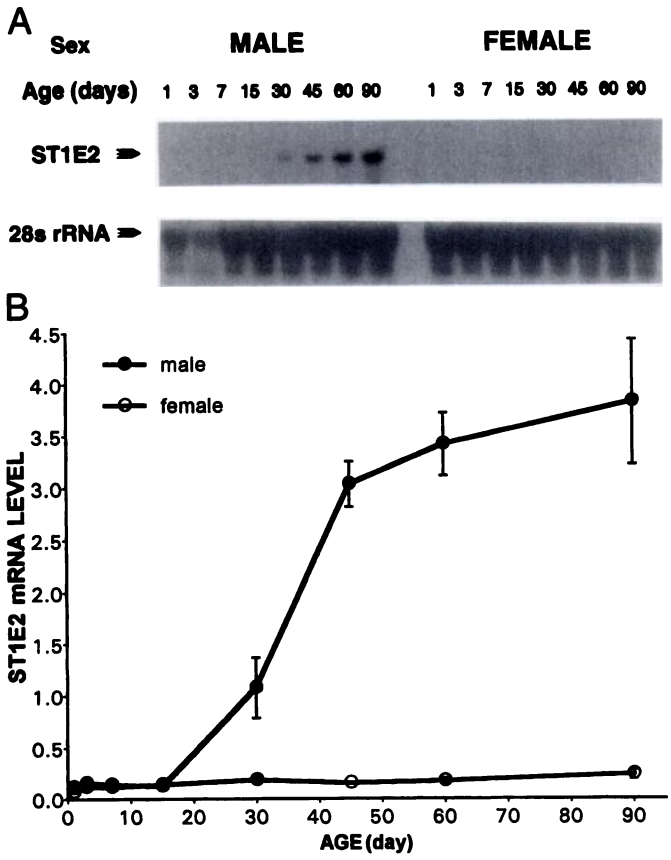

Fig. 3. Hepatic ST1E2 mRNA levels in male and female rats of various ages. A, Representative autoradiogram of Northern blot analysis. Each lane (20 μg of RNA/lane) contains RNA from one animal from group of three or four rats. B, Graphic presentation of ST1E2 mRNA level by dot blot analysis; see legend to Fig. 1.

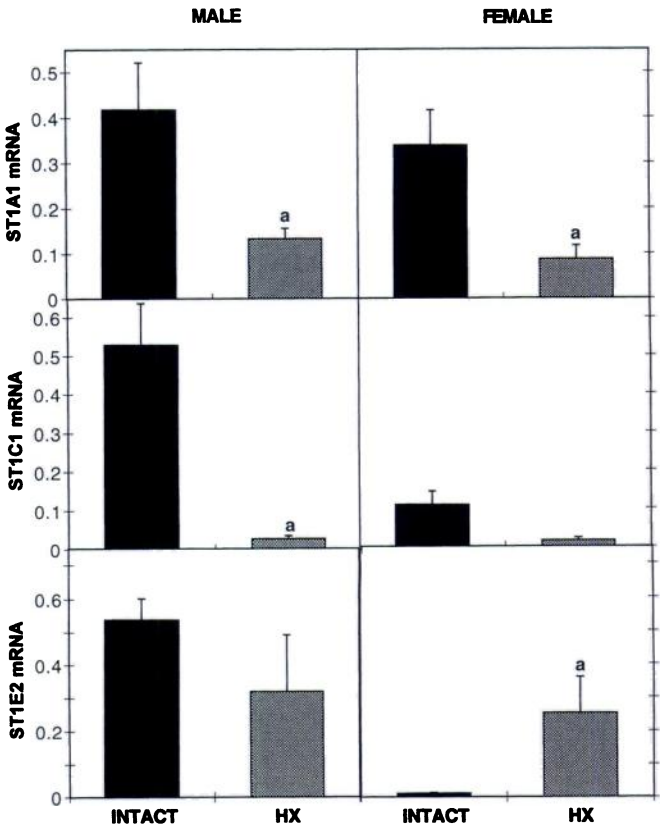

Fig. 4. Effect of hypophysectomy on hepatic ST1A1, ST1C1, and ST1E2 mRNA levels in male and female rats. Total liver RNAs were extracted from intact and hypophysectomized (*HX*) rats, and Northern blot analysis was performed as described in Materials and Methods. The values are the ratios of densitometric units of ST hybridization to 28S rRNA hybridization. Results are mean ± standard error for four or five rats. *a*, Significant difference from intact control rats.

ST1C1 mRNA levels in hypophysectomized male rats and enhanced it in hypophysectomized female rats to the levels observed in intact male rats (Fig. 6, *middle*). GH infusion (female pattern), however, did not affect ST1C1 levels in either male or female rats.

GH injection (male pattern) had minimal effects on ST1E2 mRNA levels in hypophysectomized male rats but seemed to decrease it in female rats. GH infusion suppressed ST1E2 mRNA levels in both hypophysectomized male and female rats to intact female levels (Fig. 6, *bottom*).

Effect of PRL on hepatic ST1A1, ST1C1, and ST1E2 expression in hypophysectomized rats. The effects of rat PRL on the expression of the three male-dominant hepatic ST mRNA levels are shown in Fig 7. Infusion of PRL for 5 days tended to increase ST1A1 expression in male and female rats, but the increases were not statistically significant. However, PRL resulted in ~2- and 6-fold increases in ST1C1 expression in hypophysectomized males and females, respectively. PRL had no appreciable effect on ST1E2 mRNA levels but tended to decrease it.

Discussion

All three of the three hepatic STs examined in this study had higher levels of expression in male than in female rats, as previously reported (10–12). Furthermore, in the current study, the three ST mRNA levels were examined in the same group of male and female rats, and thus the magnitude of the sex differences can be compared. With ST1A1, the mRNA levels in male rats were ~2-3-fold that in female rats, whereas for ST1C1 and ST1E2, the mRNA levels were >10-fold higher in adult male rats than in adult female rats. Expression of ST1C1 and ST1E2 in adult female rats was almost undetectable. Thus, ST1A1 can be considered a male-predominant ST, whereas ST1C1 and ST1E2 were male-specific STs.

The developmental patterns of the three male-dominant hepatic STs are not identical. The ontogenic patterns of male-specific ST1C1 and ST1E2 are similar, but they are different from the developmental pattern of the male-predominant ST1A1. ST1C1 and ST1E2 mRNA levels increased dramatically in male rats around puberty, whereas they remained at low levels in female rats. In contrast, ST1A1 increased gradually in both male and female rats during the first month of life. At puberty (30–45 days of age), a decline in the ST1A1 mRNAs occurred in both sexes, but the decrease was more dramatic in female than in male rats. As a result, adult male rats have higher ST1A1 mRNA levels than adult female rats.

The developmental pattern of ST1A1 gene expression is similar to the pattern of phenol ST activity with *p*-nitrophenol as the substrate in rat liver (7). The activity of *p*-nitrophenol ST increased after birth in both sexes until 30 days of age, which is similar to what was observed with ST1A1

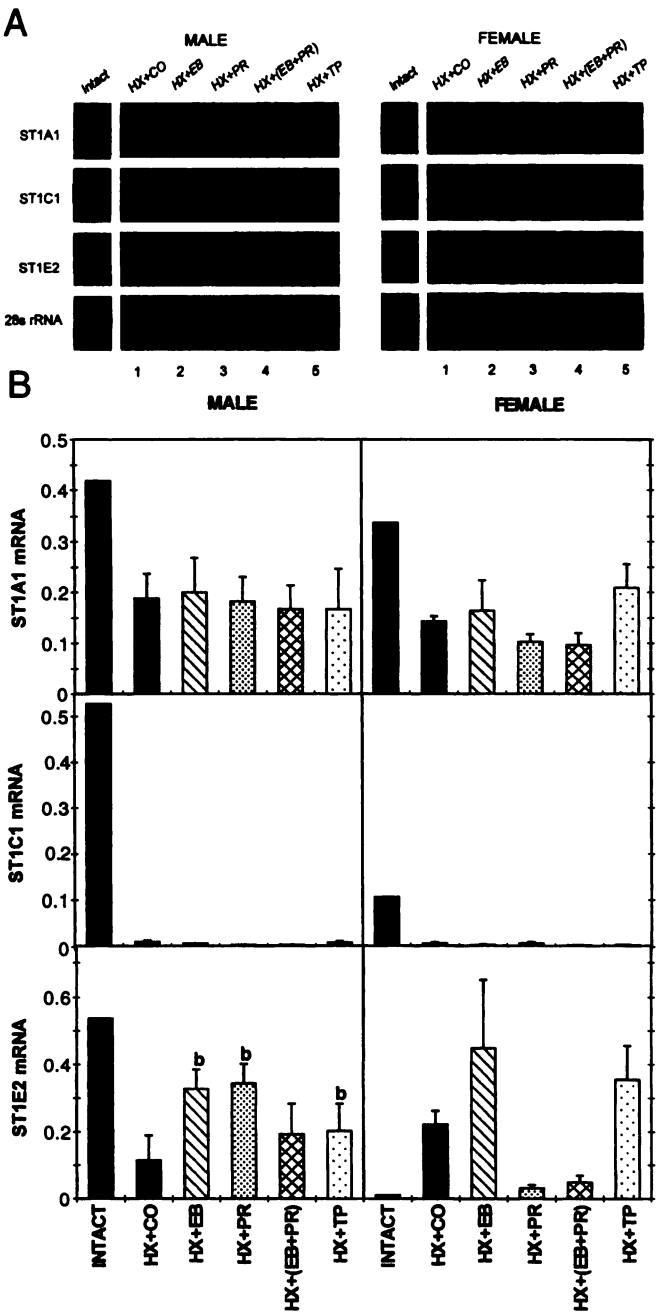

Fig. 5. Effects of EB, PR, and TP on hepatic ST1A1, ST1C1, and ST1E2 mRNA levels in hypophysectomized (*HX*) rats. Male and female hypophysectomized rats were injected subcutaneously with 200 μg/kg EB, 25 mg/kg PR, mixed EB and PR (same dose as given alone), and 10 mg/kg TP for 5 days. Hypophysectomized control rats were given corn oil (*CO*) alone. The intact rats used were the same rats as used for Fig. 4. Total liver RNAs were extracted and analyzed by Northern blot analysis. A, Representative autoradiograms of Northern blot analysis. B, Graphs showing the relative amount of the mRNA levels. The values are the ratio of densitometric units of ST hybridization to 28S rRNA hybridization. Results are mean ± standard error for four or five rats. *b*, Significant difference from corn oil control rats.

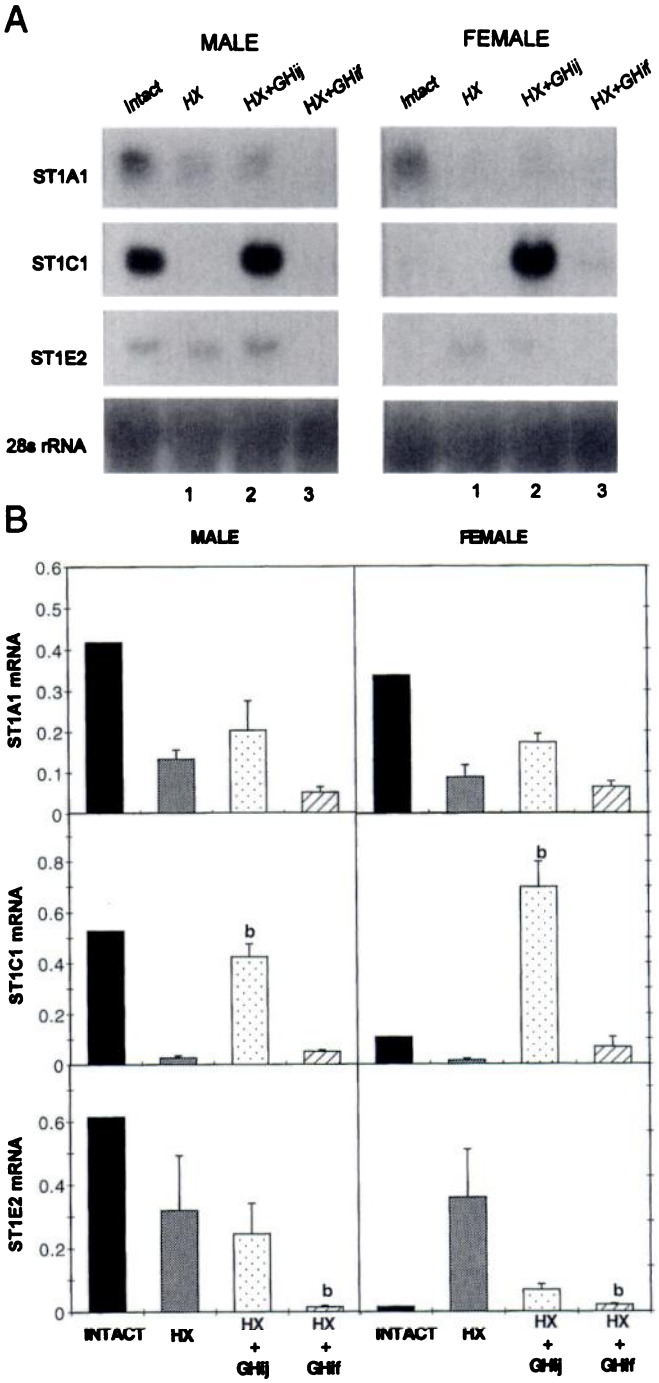

Fig. 6. Effect of intermittent GH injection and constant GH infusion on hepatic ST1A1, ST1C1, and ST1E2 mRNA levels in hypophysectomized (*HX*) rats. Rat GH was injected subcutaneously twice daily at 600 μg/kg (*HX+GHij*) (mimicking male GH secretory pattern) or infused with a miniosmotic pump at 5 μg/hr (*HX+GHif*) (mimicking female GH secretory pattern) for 5-day −40-day-old hypophysectomized male and female rats. The intact and hypophysectomized rats used were the same rats as used for Fig. 4. See legend to Fig. 5. The results are mean ± standard error for four or five rats. *b*, Significant difference from hypophysectomized control rats.

mRNA levels. The ST activity then increased in male rats, reached adult values at ~40 days of age, and declined after 60 days of age. Female rats did not show the increase in *p*-nitrophenol ST activity between 30 and 40 days of age, but activity did decline after 60 days of age. ST1A1 cDNA encodes a ST that sulfates *p*-nitrophenol (10). The data in this study indicate that the development of phenol ST activity might be due to changes in mRNA levels of ST1A1.

Sex steroid hormones may be responsible for the gender-dependent expression of STs. Yamazoe *et al.* (13) reported that administration of androgens to gonadectomized male

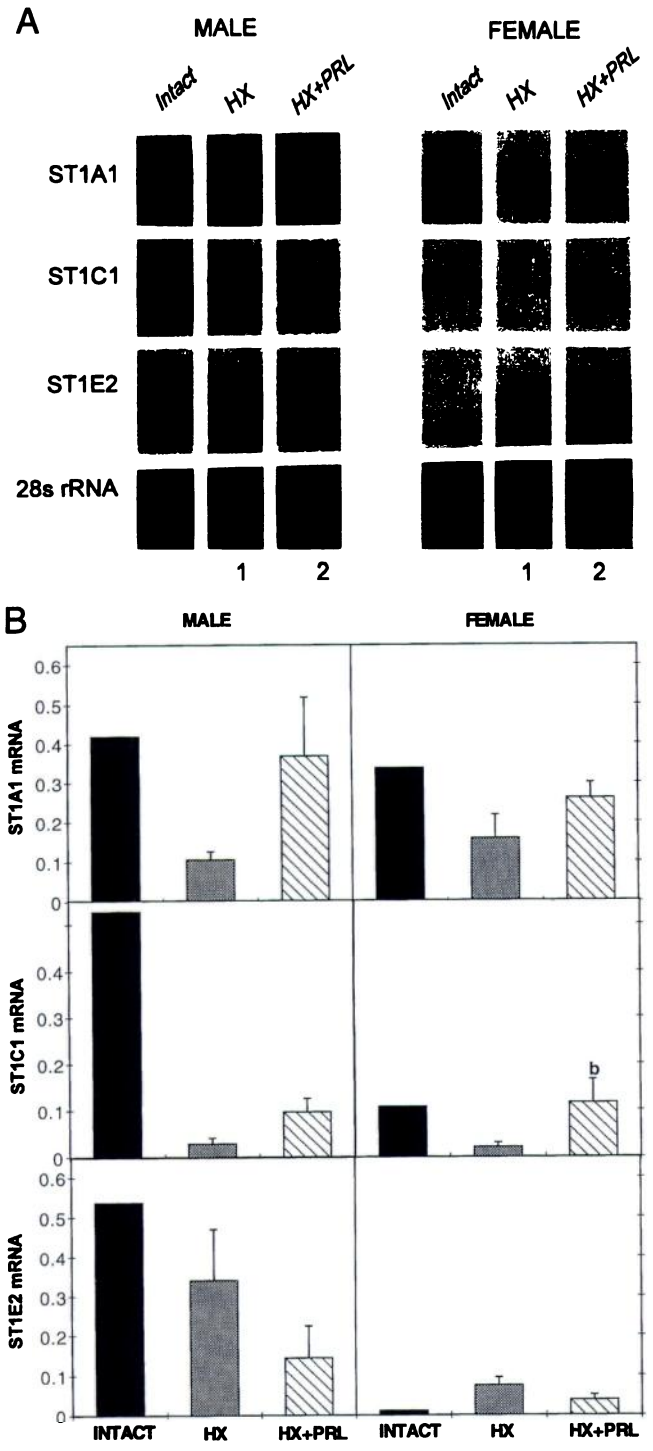

Fig. 7. Effect of PRL on hepatic ST1A1, ST1C1, and ST1E2 mRNA levels in hypophysectomized (*HX*) rats. Two weeks after hypophysectomy, male and female rats were administered rat PRL at a rate of 7 μg/hr for 5 days. The controls were given vehicle (0.15 M NaCl, 0.01 M $NaHCO_3$) alone. The intact and hypophysectomized rats used were the same rats as used for Fig. 4. A, Representative autoradiogram of Northern blot analysis. B, Relative amount of mRNA level; see legend to Fig. 5. The results are mean ± standard error for four or five rats. *b*, Significant difference from hypophysectomized control rats.

and female rats increased *N*-OH-2AAF ST enzyme activity. Similarly, daily treatment of ovariectomized female rats with androgens enhanced estrogen ST expression (12). Both of these studies were performed in gonadectomized rats with intact pituitaries. In the current study, male and female sex hormones did not have appreciable effects on ST1A1 and ST1C1 mRNA levels in hypophysectomized rats. Although androgen administration increased ST1E2 expression in hypophysectomized male and female rats, as was seen in ovariectomized female rats, the increase was less than a doubling. Estrogens were more effective than androgens in enhancing ST1E2 expression in the current study. However, the effects of estrogen on ST1E2 gene expression cannot account for the sex- and age-specific ST1E2 expression. Progesterone induces estrogen ST in the uterus (14), but the current study indicates that PR has minimal effects on estrogen ST expression in liver. Overall, the results with hypophysectomized animals indicate that sex hormones alone are unlikely to be the primary determinants of the sex difference in ST expression. Sex hormones are known to affect the secretion of hypothalamic somatostatin and GH-releasing factor, which regulates GH secretion (15). Thus the effect of gonadal hormones in nonhypophysectomized animals might be mediated by altering GH secretion.

GH secretory patterns seem to be a major regulator for the expression of some hepatic ST genes. In male rats, plasma GH levels exhibit regular peaks every 3–4 hr, exhibiting high amplitude pulsation with an undetectable interpulse baseline. In female rats, the pulsation is more frequent with a low amplitude and higher trough levels (21). These dimorphic GH secretory patterns are thought to be one of the reasons for sex-dependent hepatic enzyme activity. In the current study, hypophysectomy abolished hepatic ST1C1 mRNA expression in male rats. A decrease in ST1C1-encoded ST activity and corresponding protein (*N*-hydroxyarylamine ST) content was also observed in hypophysectomized male rats (13, 22). Intermittent injection of GH, mimicking male GH secretory pattern, increases ST1C1 mRNA, *N*-hydroxyarylamine ST protein, and enzyme activity in both male and female rats to the levels observed in intact males, although the continuous infusion of GH, mimicking female GH secretory pattern, had no effect. The results of the current study indicate that GH secretory patterns regulate *N*-hydroxyarylamine ST at the pretranslational level.

The stimulating effect of the male GH secretory pattern on hepatic ST1C1 expression does not serve as a general mechanism for regulating the two other male-dominant hepatic STs. Hypophysectomy produced a ~70% reduction in ST1A1 mRNA in both male and female rats; however, replacement of GH with either a male or female GH secretory pattern did not restore ST1A1 mRNA levels. ST1A1 mRNA expression and the ST activity are detected at birth, and they gradually increase in both male and female rats before puberty (7), which also supports the conclusion that GH secretory pattern does not play a major role in ST1A1 regulation. ST1E2 is also a male-specific ST; however, ST1E2 mRNA levels in rat liver were not significantly decreased by hypophysectomy in male rats. In female rats, hypophysectomy increased ST1E2 mRNA levels. Infusion of GH (female pattern) suppressed ST1E2 expression in hypophysectomized female rats to the intact female levels, and it also decreased ST1E2 expression in hypophysectomized male rats. Therefore, during puberty, establishment of the female GH secretory pattern inhibits hepatic ST1E2 expression in female rats, whereas in male rats, due to the lack of continuous GH secretion, hepatic ST1E2 expression increases.

GH effects on STs might be secondary to the changes in the AR expression. The AR expression in rat liver shows sexual dimorphism, with higher expression in adult males than that in adult females. The expression of AR in liver is also regulated in an age-dependent manner (23). Sex- and age-dependent expression of AR is very similar to that of male-specific STs. In intact rats, androgens increase AR expression and up-regulate hepatic synthesis of estrogen and other aryl STs (24). Overexpression of AR in transgenic mice suppresses the expression of androgen STs (25). AR expression seems to be programmed by sex hormones (26). However, GH (male pattern) also increased AR expression in rat prostate (27, 28). Direct effects of GH independent of AR on liver protein were also observed (29).

The ontogenic pattern of male-dominant STs and the differential effects of GH secretory pattern on STs are similar to that of some male-dominant CYPs such as CYP3A2, CYP2C11, and CYP2A2. Similar to ST1A1, CYP3A2 is expressed in immature rats and is suppressed after puberty in female rats but not in male rats (30). However, different from ST1A1, CYP3A2 is increased instead of decreased by hypophysectomy. Replacement of GH by intermittent injection (male pattern) decreases the levels of CYP3A2 in both hypophysectomized male and female rats, and GH infusion completely suppresses CYP3A2 in intact adult male rats (31). Similar to ST1C1, CYP2C11 increases dramatically at puberty in male but not in female rats (30). As with ST1C1, CYP2C11 expression is reduced by hypophysectomy, and this effect is reversed by GH injection (32, 33). Male GH pattern also increases CYP2C11 mRNA levels in hypophysectomized female rats (33), as observed for ST1C1. Similar to ST1E2, CYP2A2 increases markedly at puberty in male rats but with undetectable levels in female and immature rats (31, 34). CYP2A2 is also suppressed by female GH secretory pattern. Therefore, CYP2A2 is elevated on hypophysectomy in females, as it is for ST1E2. However, in contrast to ST1E2, which is not dramatically affected by hypophysectomy of male rats, CYP2A2 expression is increased by hypophysectomy in male rats as well. Intermittent GH injection reverses the induction of CYP2A2 in both sexes (31). These similarities and differences suggest that cytosolic ST and some microsomal CYPs may be under similar, but not identical, regulatory mechanisms.

In summary, male-dominant hepatic ST1A1, ST1C1, and ST1E2 can be further divided into male-predominant (ST1A1) and male-specific isoforms (ST1C1 and ST1E2), based on mRNA levels in adult rats. The developmental pattern for the male-predominant ST is different from the male-specific STs, but sex-dependent expression of all occurs around puberty. The sex and age differences of ST1A1 do not seem to be due to any of the hormones examined. However, male-specific ST1C1 expression in postpubertal male rats is determined by male GH secretory pattern, and male-specific ST1E2 expression in mature male rats is due to the lack of inhibitory female GH secretory pattern.

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