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Induction of Hepatic Estrogen Sulfotransferase Expression by Hypophysectomy in Female Rats

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We have examined the effects of hypophysectomy and treatment with thyroxine (T_4) on enzyme activity and expression (as determined by immunoblot analysis) of members of the three principal sulfotransferase (ST) sub-families (phenol STs, PST; estrogen STs, EST; hydroxysteroid STs, HST) in cytosols prepared from female Wistar rat livers. The results demonstrate that in female rat liver cytosol, EST activity was decreased by treatment with T_4, increased following hypophysectomy and that treatment of hypophysectomized animals with T_4 also greatly reduced EST activity. T_4 had no significant effect on PST or HST activity in normal animals, but it decreased HST activity in hypophysectomized rat liver cytosol. Immunoblot analysis of these cytosols with antibodies recognising HST and PST indicated that where changes in enzyme activity occurred they mirrored changes in enzyme protein expression. In normal adult female rat livers, EST protein is not expressed, and the small residual activity results predominantly from the action of HST. Hypophysectomy induced EST activity and the expression of EST enzyme protein in female rat liver cytosol, and T_4 treatment of hypophysectomized animals reduced the activity to below normal levels without reducing the corresponding enzyme protein levels, indicating that T_4 regulation of EST in females is via a post-translational mechanism.

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INTRODUCTION

Sulfation (or more correctly sulfonation) is an important reaction in the metabolism and detoxication of a host of xenobiotics and endobiotics such as steroid hormones, bile acids and monoamine/catecholamine neurotransmitters, and is catalysed by the products of the cytosolic sulfotransferase (ST) gene superfamily [1–4]. The STs catalyse the transfer of the sulfonate group from the active donor molecule 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an appropriate acceptor group on the substrate. Sequence homology analysis indicates that two distinct subfamilies of cytosolic STs exist in mammalians, phenol (or aryl) STs (PSTs) and hydroxysteroid STs (HSTs) [4, 5]. The PST subfamily also includes the estrogen STs (ESTs) [4–6]. In normal young adult rats, members of these different ST sub-families are subject to sex-specific and age-specific regulation of their expression. For example, EST(s) are male-specific, whereas HSTs are predominantly expressed in females [7, 8]. We have previously raised polyclonal antibodies against purified rat liver EST [7], HST [8] and PST [9].

Work from this and other laboratories indicates that in rats, pituitary-derived hormones play a key role in regulating ST expression. For example, hypophysectomy down-regulates the sulfation of an aromatic hydroxylamine and a hydroxamic acid [10] and also cortisol ST activity [11] and HST mRNA levels [12]. We have recently studied the effect on hypophysectomy and/or thyroxine (T_4) administration on the expression EST, PST and HST in adult male rats [13]. Treatment of hypophysectomized rats with T_4 virtually abolished EST activity and this was manifested in decreased levels of the enzyme protein. In contrast this treatment had no effect on HST or PST activity or protein levels [13]. Work from Roy's laboratory suggests that the age-related male-specific

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Abbreviations: ST, sulfotransferase; PST, phenol sulfotransferase; EST, estrogen sulfotransferase; HST, hydroxysteroid sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; DHEA, dehydroepiandrosterone; T_4, thyroxine.

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expression of EST in rats is related to hepatic androgen sensitivity, and EST could be induced in female rats by ovariectomy followed by administration of the androgen 5α-dihydrotestosterone [14]. We have therefore now extended our studies to determine the effects on STs representative of EST, HST and PST of hypophysectomy and/or T_4 administration to adult female rats. Our results indicate that hypophysectomy also induces the activity and expression of hepatic EST in female rats, and that T_4 treatment of hypophysectomized rats abolishes the increase in EST activity by a mechanism other than decreasing EST enzyme protein levels.

MATERIALS AND METHODS

Chemicals

1[1-^{14}C]Naphthol (53 mCi/mmol) was from Amersham U.K., Ltd., Aylesbury, and 1-naphthol was obtained from Merck Ltd., Glasgow, U.K. [2,4,6,7-^{3}H(N)]Estrone (90.5 Ci/mmol), [2,4,6,7-^{3}H(N)]estriol (91 Ci/mmol) and [1,2,6,7-^{3}H(N)]dehydroepiandrosterone (114.7 Ci/mmol) were from Du Pont/NEN, Stevenage, U.K. 3′-Phosphoadenosine 5′-phosphosulfate (PAPS), goat anti-rabbit IgG, nitroblue tetrazolium, 5-bromo-4-chloro-3-indolyl phosphate (*p*-toluidine salt), DL-thyroxine (free acid), dexamethasone and unlabelled steroids were obtained from Sigma Chemical Co. Ltd., Poole, U.K.

Animals and tissue preparation

Adult female (> 12 weeks, 200–250 g) Wistar rats were used throughout—normal rats were from the colony maintained in this institute, and those used for surgical procedures were obtained from Charles River U.K. Ltd, Margate. These suppliers performed all hypophysectomy and sham hypophysectomy procedures, and for hypophysectomy the standard parapharyngeal method was used as described in [15]. All procedures and methods for preparation of hepatic cytosols were performed as described previously [13].

Enzyme assays and protein determination

The sulfation of estrone, estriol, 1-naphthol and DHEA by hepatic cytosolic STs was determined using radioactive substrates as described previously [7]. The assay conditions used have been extensively optimized in the laboratory with respect to substrate and PAPS concentrations, pH, cytosolic protein content and incubation time [7]. Protein content of hepatic cytosols was estimated by the method of Lowry *et al.* [16] with bovine serum albumin as standard.

SDS–PAGE and immunoblot analysis

Immunoblot analysis was performed on cytosolic fractions which were resolved on 11% acrylamide monomer denaturing SDS–polyacrylamide gels and transferred to nitrocellulose as first described by Laemmli [17] and Towbin *et al.* [18], respectively. Immunochemical detection was performed using the alkaline phosphatase method (at room temperature) exactly as described previously [19] with the exception that the pH of the buffer used for all incubation and washing steps was 9.0. Blots were probed with the polyclonal antibodies previously raised against rat liver EST [7], PST [9] and HST [8]. These antibodies, raised in rabbits, have been well characterized, and on Western blot analysis recognise the antigens against which they were raised.

RESULTS

Because of the possibility of an effect of the hypophysectomy operation procedure on levels of enzyme activity and expression, we have included data from sham-operated animals, which shows no significant change in ST activity with all four substrates measured (Table 1). For the sake of simplicity, therefore, all

Table 1. Hepatic cytosolic sulfotransferase activities in normal and hypophysectomized adult female rats

	Sulfotransferase activity (pmol/min/mg)			
Treatment	Estrone	Estriol	DHEA	1-Naphthol
Normal ($n = 7$)	9.3 ± 1.5	4.9 ± 0.9	368 ± 53	1117 ± 200
Normal + T_4 ($n = 3$)	1.1 ± 0.1*	3.2 ± 0.6	320 ± 3	924 ± 54
Sham Hypox ($n = 3$)	6.8 ± 2.4	4.8 ± 2.2***	369 ± 31***	805 ± 133
Hypox ($n = 4$)	24 ± 9	35 ± 9**	218 ± 43	681 ± 87
Hypox + T_4 ($n = 3$)	1.3 ± 0.7††	2.7 ± 1.6†	85 ± 9†	772 ± 74

Data are expressed as mean ± SEM for duplicate determinations on the number of liver samples indicated. statistical analysis was performed using Student's *t*-test (except where indicated) with the 'Instat' software package for Apple Macintosh computers. Hypox, hypophysectomised; T_4, thyroxine.

*$P < 0.01$ Normal vs Normal + T_4. **$P < 0.005$ Normal vs Hypox. ***P0.05 Sham vs Hypox. †$P < 0.05$ Hypox vs Hypox + T_4. ††$P < 0.05$ Hypox vs Hypox + T_4 (Mann-Whitney test).

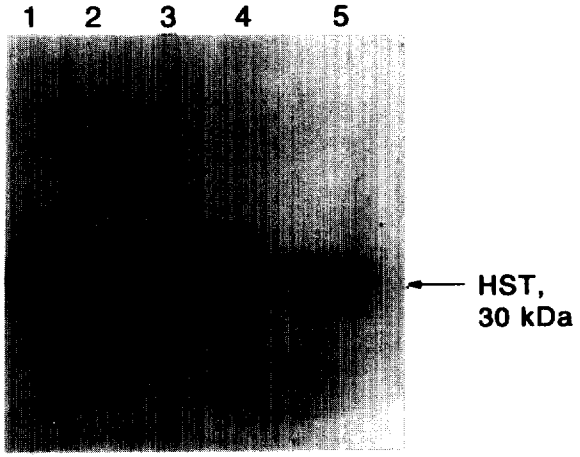

Fig. 1. Immunoblot analysis of HST expression in female rat liver cytosols. Cytosolic proteins from female rat livers (10 μg protein) were resolved on polyacrylamide gels (11% acrylamide monomer) in the presence of 0.1% SDS and electroblotted onto nitrocellulose. Blots were probed with anti-(rat liver HST) at a concentration of 8 μg/ml for 60 min and immunoreactive proteins visualized using an alkaline phosphatase-conjugated secondary antibody. Lane 1, normal; lanes 2 and 3, hypophysectomized; lanes 4 and 5, hypophysectomized + T_4.

statistical analysis was performed against the data from normal adult female rats (Table 1).

Normal adult female rats do not express EST [7, 14], and the small residual acitivty towards substrates such as E_1 and E_3 is most likely due to the action of another ST isoenzyme, and we have previously shown this to be HST by antibody inhibition experiments [8]. Hypophysectomy resulted in a statisticaly significant decrease in ST activity towards DHEA and 1-naphthol (Table 1). In contrast this treatment resulted in an increase in the sulfation of E_1 and E_3 (Table 1). Treatment of hypophysectomized animals with T_4 resulted in no change in the ST activity towards 1-naphthol but decreased the activity towards DHEA, E_1 and E_3 (Table 1). Treatment of normal adult female rats with T_4 resulted in a small but not statistically significant decrease in ST activity towards DHEA, 1-naphthol and E_3 and a significant decrease in ST activity towards E_1 (Table 1).

Immunoblot analysis using our antibodies against rat liver EST [7], HST [8] and PST [9] isoforms has previously been validated as a means of investigating the mechanism of changes in levels of expression of these enzyme proteins and of correlating these changes to enzyme activities [e.g. 13]. Western blots of total liver cytosolic protein probed with anti-(rat liver HST) demonstrated a small decrease in HST enzyme protein levels in hypophysectomized compared to normal animals, but a much larger decrease in hypophysectomized animals which had been treated with T_4 (Fig. 1). Figure 2 shows results of immunoblot analysis of liver cytosols with anti(rat liver PST). We observed a decrease in PST enzyme protein levels in hypophysectomized animals treated with T_4 compared to either

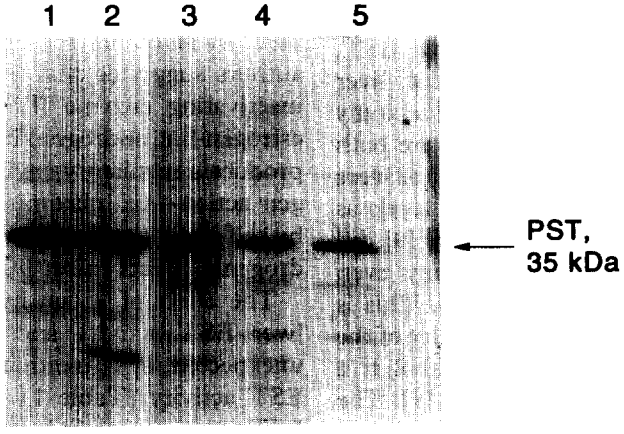

Fig. 2. Immunoblot analysis of PST expression in female rat liver cytosols. Cytosolic proteins from female rat livers (50 μg protein) were resolved on polyacrylamide gels (11% acrylamide monomer) in the presenceof 0.1% SDS and electroblotted onto nitrocellulose. Blots were probed with anti-(rat liver HST) at a concentration of 6.7 μg/ml and immunoreactive proteins visualized by alkaline phosphatase-conjugated secondary antibody. Lane 1, normal; lanes 2 and 3, hypophysectomized; lanes 4 and 5, hypophysectomized + T_4.

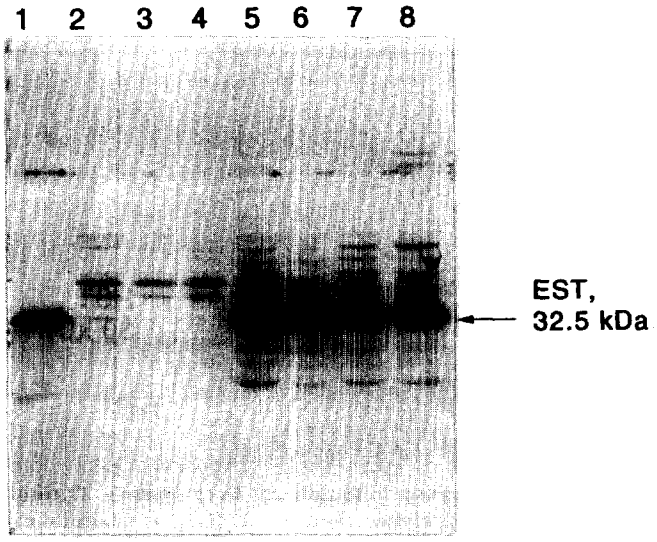

Fig. 3. Immunoblot analysis of EST expression in female rat liver cytosols. Cytosolic proteins from female (50 μg protein) and male (5 μg protein) rat livers were resolved on polyacrylamide gels (11% acrylamide monomer) in the presence of 0.1% SDS and electroblotted onto nitrocellulose. Blots were exposed to anti-(rat liver EST) at a concentration of 4.8 μg/ml and immunoreactive proteins visualized by alkaline phosphatase-conjugated secondary antibody. Lane 1, normal male; lanes 2 and 3, normal female; lane 4, sham-operated female; lanes 5 and 6, hypophysectomized female; lanes 7 and 8, hypophysectomized female + T_4.

hypophysectomized animals or normal animals. Immunoblotting with anti-(rat EST) confirmed the absence of EST protein in both normal and sham-operated adult female rat liver cytosols (Fig. 3). Hypophysectomy resulted in the strong induction of an immunoreactive polypeptide with the same electrophoretic mobility as that seen in adult male liver cytosol, and which we know corresponds to EST [7]. High levels of this protein were still evident following treatment of hypophysectomized animals with T_4 (Fig. 3) despite the fact that this treatment reduced the EST enzyme activity towards both E_1 and E_3 (Table 1).

DISCUSSION

We have examined the effect of hypophysectomy on sulfotransferase enzyme activity and enzyme protein expression in female rats, and determined that EST, which is not expressed in normal female rat liver cytosol, is induced following hypophysectomy (Table 1; Fig. 3). HST and PST activities were both decreased following hypophysectomy (Table 1) as were HST protein levels (Fig. 1). Enzyme protein levels as detected by our anti-(rat liver PST) antibody [9] did not apparently decrease after hypophysectomy even though the sulfation of 1-naphthol decreased. This is likely due to the overlapping substrate specificity of the members of the rat PST family, as 1-naphthol is a substrate for more than one of these isoforms [9]. The antibody was raised against a rat liver PST which had a high activity towards paracetamol [9], but also had significant activity with 1-naphthol as substrate [9]. The exact identity of this paracetamol-sulfating PST has yet to be determined. Our data indicate that there are significant sex differences in the regulation of these enzymes by pituitary hormones, since we have previously shown that in male rats, hepatic EST activity and enzyme protein was not altered by hypophysectomy, whereas HST activity increased and PST decreased [13]. Additionally, the effect of T_4 on hypophysectomized female rat liver HST (decreased activity) was not observed in male animals [13].

These data provide evidence for the involvement of pituitary hormones in the sex-specific regulation EST in rats, since removal of the pituitary gland from female rats resulted in the induction of a protein which is normally only expressed in male animals (Table 1; Fig. 3). Hormonal regulation of EST expression in female rat liver has been previously demonstrated following the treatment of ovariectomized rats with 5α-dihydrotestosterone [14], indicating the requirement of androgen for the expression of EST. These authors suggested that up-regulation of the estrogen-inactivating enzyme (EST) by a physiological anti-estrogen (i.e. androgen) in the liver may be required to produce a regulatory environment conducive to androgen action—i.e. maximising androgen-responsiveness by self-potentiation. Similarly, estrogens have anti-androgenic effects in rat liver [20, 21].

The effect of T_4 on hypophysectomized female rat liver EST activity and enzyme protein levels was unexpected, as T_4 treatment virtually abolished hepatic EST activity (Table 1) without apparently reducing EST enzyme protein levels (Fig. 3). This suggests that T_4 reduces EST activity via a post-translational mechanism. This is in direct contrast to male rats, where T_4 treatment following hypophysectomy results in a decrease in both enzyme activity and enzyme protein

levels [13]. Examples of the inactivation of EST by hormones and other small biomolecules are known; for example androgens, retinoic acid and free fatty acids [22, 23], however the effect of T_4 appears to be selective for EST induced in female rats by hypophysectomy. Whether this apparent discrepancy is the result of the EST whose expression can be stimulated in female rats being a different isoenzyme to that constitutively expressed in adult male animals, or that differential splicing of 5′ upstream regulatory elements occurs in male and female rats remains to be determined.

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