Thyroid hormone and estradiol have overlapping effects on kidney glutathione S-transferase-α gene expression

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Faustino LC, Almeida NA, Pereira GF, Ramos RG, Soares RM, Morales MM, Pazos-Moura CC, Ortiga-Carvalho TM. Thyroid hormone and estradiol have overlapping effects on kidney glutathione S-transferase-α gene expression. Am J Physiol Endocrinol Metab 303: E787–E797, 2012—First published July 24, 2012; doi:10.1152/ajpendo.00223.2012.—Class GST (Gsta) represents an essential component of cellular antioxidant defense mechanisms in both the liver and the kidney. Estrogens and thyroid hormones (TH) play central roles in animal development, physiology, and behavior. Evidence of the overlapping functions of thyroid hormones and estrogens has been shown, although the molecular mechanisms are not always clear. We evaluated an interaction between TH and estradiol in regulating kidney Gsta expression and function. First, we observed that female mice expressed greater amounts of Gsta compared with males and showed an opposite pattern of expression in TRβ knock-in mice. To further investigate these sex differences, hypothyroidism was induced by a 5-propyl-2-thiouracil diet, and hyperthyroidism was induced by daily T3 injections. Hypothyroidism increased kidney Gsta expression in male but not in female mice, indicating that sex hormones could be influencing the regulation of Gsta by thyroid hormones. To analyze this hypothesis, ovarioctomized females were subjected to hypo- and hyperthyroidism, which led to a male profile of Gsta expression. When hypo- or hyperthyroid ovarioctomized mice were treated with 17β-estradiol benzoate, we were able to confirm that estradiol was interfering with TH modulation; Gsta expression was increased by T3 when estradiol is present and decreased by T3 when estradiol is absent. Using proximal tubule cells, we also showed that estradiol and T3 worked together to modulate Gsta expression in an overlapping fashion. In summary, 1) the sex difference in the basal expression of Gsta impacts the detoxification process, 2) kidney Gsta expression is regulated by TH in males and females but in opposite directions, and 3) T3 and estradiol interact directly in renal proximal cells to regulate Gsta expression in females.

GLUTATHIONE S-TRANSFERASES (GSTs) are a superfamily of ubiquitous dimeric detoxification isoenzymes that conjugate many substrates to reduced glutathione (GSH), including several xenobiotic and endogenous electrophiles (26). Mammalian cytosolic GSTs represent the largest family of such transferases and have been divided into seven different classes (α, μ, π, σ, θ, ω, and ξ) (2, 27, 28). The α-class of GST (Gsta) is found in several organs, such as the liver, kidney, lung, stomach, and gonads, some of which exhibit sexual dimorphism (35). In the liver, the important role played by GST in cellular detoxification and in many other known functions has already been well described (26). However, in other tissues, the role of GST is still not well characterized.

It has been established that GSTα is a biomarker of renal toxicity that aids in the detoxification of endogenous and exogenous compounds and in drug metabolism (2). Renal GST isoforms are differentially expressed along nephron segments; expression also depends on species. In the human kidney, GSTα is found predominantly in the proximal convoluted tubule, and low levels of GSTα have been detected in the loop of Henle (24). In rats, immunohistochemical analysis has revealed that GSTα is expressed in the proximal convoluted tubules and in the straight portion of the proximal tubules (54). GSTα is highly expressed in the proximal tubule cells and comprises ~2% of all of the soluble proteins present in this nephron segment (54).

Bass et al. (5) were the first to report that higher levels of urinary GST occur in rats following a nephrotoxic injury, a phenomenon that can be related to either increased leakage from tubular cells into the lumen or epithelial proximal tubule necrosis. According to several authors, urinary GSTα is a good noninvasive biomarker of cytoplasmic leakage from early tubular damage before regeneration occurs (23, 44, 55).

GSTα isoenzymes are also implicated in tumorogenesis. They are involved in the kidney activation of cytotoxic compounds (46) because the circulating glutathione S conjugates are bioactivated in the kidney by γ-glutamyltransferase and β-lyase, which create these nephrotoxins (14). It has been known for some time that, in the liver, GST gene expression and activity are regulated by several exogenous factors, such as dexamethasone and phenobarbital (45), and by a variety of antioxidant compounds (18, 28, 47). Less is known about GST regulation by hormones. Adrenocorticotropic hormone (39), glucocorticoids (19, 33), growth hormones (54), thyroid hormones (TH) (6, 11, 31), and sex steroids (7, 25, 30) are able to modulate GST.

Thyroid hormones, including thyroxine (T4) and triiodothyronine (T3), are essential during embryonic development and play critical roles in several physiological processes in adult organisms (36). It is agreed that most of the TH effects are mediated by nuclear action via a superfamily of ligand-modulated transcription factors that modify target gene transcription (36). Regarding GSTα regulation by thyroid hormones, TH suppress both the activity and gene expression of GSTα in the liver (1, 11, 20, 43, 48, 57). Recent data from our group have shown that liver GSTα is inhibited by T3 in a hypothyroid state but not when mice are euthyroid (EU). This finding was

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dependent on the cooperative actions of the two different TH receptor isoforms, TRα1 and TRβ1 (20). The main estrogen, 17β-estradiol (E2), plays important regulatory functions in a wide variety of biological processes, including reproduction, cell differentiation, cell proliferation, apoptosis, inflammation, cellular metabolism, and brain function (60). The most common mechanism of E2 action is mediated by two main subtypes of nuclear estrogen receptors, ERα and ERβ, which are members of a superfamily of nuclear receptors (40).

Estradiol modulates GST in different tissues. In the ovaries of immature rats treated with E2, the total GST activity was elevated (7). Igarashi et al. (30) detected reduced immunoreactivity and activity of total GST in the liver after E2 administration but not in the expression of the α-class. Coecke et al. (11) observed that the treatment of hepatocytes with E2 increased the content of the α-class subunits A1 and A2 of GST.

In past years, several lines of evidence have shown overlapping functions of TH and estrogens. Studies have shown that these two endocrine systems can interact, with cross-regulation between TH and estradiol receptors that modulates genes involved in reproduction and sexual behavior (16, 58). Additionally, manipulations of the levels of TH and estradiol in vitro potentiate or mutually inhibit effects on gene expression (3). Similarly to TH (13), sex steroids (32) also demonstrate nongenomic action (12). Presently, there is a growing body of evidence showing cross-talk between TH and estradiol activity, both genomic and nongenomic, in breast tumor, thyroid, and neural cell lines (13).

In the present study, we evaluated possible cross-talk between TH and estradiol modulating GST expression in the kidney.

**EXPERIMENTAL PROCEDURES**

**Ethical approval.** All of the procedures were performed in accordance with the care and use guidelines established by the Fund for the Replacement of Animals in Medical Experiments Guide and were approved by the Universidade Federal do Rio de Janeiro Institutional Committee on Animal Care and Use under the protocol IBCCF1002.

**Animals.** Female and male wild-type mice, aged 8–10 wk, were used in this study. A total of 106 females and 28 males were used.

One transgenic mouse line expressing mutant TRβ, TRβKP377T/KO377T, was used for one experiment (50). The genotypes of the animals were determined as described previously (50). Littermate wild-type (TRβwt/wt) controls were used in the experiment. A total of 13 males and 13 females were used.

All of the mice were from the same mixed genetic background (C57BL/129Svj) and were maintained under 12:12-h light-dark cycles (beginning at 7 AM and 7 PM); the room temperature was held at 22°C. Standard rodent chow and tap water were provided ad libitum.

In all of the experiments, the mice were euthanized by CO2 inhalation, and the kidneys were excised, immediately frozen in liquid nitrogen, and stored at −70°C before the extraction of total RNA or protein. Serum was obtained from trunk blood and kept frozen at −20°C for measurements of total T3, T4, and TSH, and E2.

**Kidney tubular dissection.** The nephron segments, proximal tubule, and collecting ducts were isolated from cortical and medullary slices and incubated at 37°C for 30 min in digestion solution with continuous shaking. Proximal and collecting tubule dissections were performed under a dissecting microscope. The tubules were homogenized immediately upon dissection. RNA was isolated as described below. We dissected each kidney into 80 to 100 2-mm segments.

**Ovariectomy and E2 supplementation.** Female mice were anesthetized with a combination of ketamine hydrochloride (Dopalen, Sao Paolo, Brazil) and xylazine hydrochloride (Amasedan, Sao Paolo, Brazil) (50 and 5 mg/kg, respectively, diluted in saline 0.9% vol/vol) via intraperitoneal (ip) injection. After anesthesia was established, the animals were submitted to bilateral ovariectomy (OVX), and the sham-operated group was submitted to surgical stress. Vaginal cytology was performed on the sham-operated group to determine the phase of the estrous cycle. The uteri were weighed and used as a control for surgical efficiency. After 2 wk of recovery, the OVX mice were injected subcutaneously (sc) with 10 (EB10) or 100 (EB100) μg/kg body wt of E2 benzoate (Sigma) every 4 days for 15 days. Control OVX females received sc sesame oil every 4 days for 15 days.

**Thyroid status manipulation and estradiol supplementation.** OVX mice (Fig. 3C) were induced to become hyperthyroid (HYPER) by daily sc injections of T3 (50 μg/100 g body wt) for 15 days. Hypothyroidism (HYPO) was induced by feeding OVX animals a diet containing 0.15% 5-propyl-2-thiouracil (PTU; Sigma) for 4 wk, as described previously (20). After the induction of hypo- and hyperthyroidism, the OVX mice were injected sc with 100 μg/kg E2 benzoate (Sigma) every 4 days for an additional 15 days throughout T3 and PTU treatments to ensure that, at the last time point, the OVX animals were hypo- or hyperthyroid plus E2 benzoate supplemented (Fig. 5C).

**Serum T3, T4, TSH, and E2 measurements.** The serum T3 and T4 concentrations were determined using an RIA-coated tube from ICN Pharmaceuticals and according to the manufacturer’s instructions. The minimum detection limit for the assay was 25 ng/ml for T3 and 1 ng/dl for T4, and the intra-assay variations were 1.5 and 1.2% for T3 and T4, respectively. The serum TSH was measured in duplicate, and a specific mouse TSH RIA reagent, acquired from the National Hormone and Peptide Program (Torrance, CA), was used as described previously (42). The minimum detection limit of the assay was 30 ng/ml, and the intra-assay variation was 8%. The serum E2 levels were measured using an RIA-coated tube from Siemens Healthcare Diagnostics and according to the manufacturer’s instructions. The minimum detection limit of the assay was 20 pg/ml, and the intra-assay variation was 6%.

**Cell culture and hormone treatments.** Immortalized rat proximal tubule cells (iRPTCs) were used from passages 28 to 36. Serial cultures were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum (Gibco, Grand Island, NY), 25 mM glucose, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco). The cells were grown at 37°C and 95% humidified air with 5% CO2 (pH 7.4) in a CO2 incubator (Sanyo). The cells were harvested with trypsin ethylene glycol tetra-acetic acid (0.02%, Gibco), seeded on sterile glass coverslips or 12-well plates, and incubated again for 24–48 h in the same medium to become partially confluent.

After 90% confluence was reached, the cells were incubated with DMEM supplemented with 0.5% fetal bovine serum for 6 h and treated with T3, E2 benzoate, or both hormones together (10−6 and 10−8 M) for 24 h. After this procedure, total RNA was extracted.

**RNA analysis.** Total RNA was extracted using a standard method (TRizol Reagent; Invitrogen). RT-PCR analyses were conducted, taking as the template 1 μg of total RNA extracted from the kidney using the Superscript III kit (Invitrogen) according to the manufacturer’s instructions.
Real-time RT-PCR analyses were performed in a fluorescent temperature cycler (Applied Biosystems 7500; Life Technologies). After an initial incubation at 50°C for 2 min and 95°C for 10 min, the reactions were cycled 40 times using the following parameters for all genes studied: 95°C for 15 s, 60°C for 30 s, and 72°C for 45 s. Go Taq (Promega) fluorescence was detected at the end of each cycle. The primers were synthesized by Integrated DNA Technologies. The forward and reverse primers used in this study have been described previously by our own laboratory and by others (9, 38, 43, 48, 50). The sequences for the forward and reverse primers were, respectively, 5’-GCAATGCGCCGGAACG-3’ and 5’-ACCTGATGCCTCAT-TCTGC-3’ for glutathione-S-transferase-α (Gsta), 5’-TTGTTGGCTC-CTAACTGCTC-3’ and 5’-GGTTGATTGTTGTCCTTCT-3’ for estrogen receptor-α (Esr1), 5’-CAGGCTGGGAACAGGCA-GAA-3’ and 5’-TCAGACATTCTACACGTCTC-3’ for thyroid hormone receptor-β (Thrb), and 5’-TGGTTGAACACCGAG-CATT-3’ and 5’-CCAGAGCAACGTGGTG-3’ for 36B4 (Rplp0). All of the primer sets were verified to produce a single peak on a melting curve analysis.

The relative mRNA levels (2^ΔΔCT) were determined by comparing the PCR cycle threshold (Ct) between the groups after correcting for the internal control 36B4 (GenBank MGI: 1927636). The assays were repeated two to three times, and the data were merged after normalization. The results are expressed relative to the values of the control group, which were considered to be equal to 100%.

Western blot analysis. The kidneys were excised and homogenized in an Ultra-Turrax T25 basic (IKA Werke, Staufen, Germany) using lysis buffer (50 mM Tris-HCl, 0.25 mM sucrose, 10 mM KCl, and 5 mM MgCl2, pH 7.5) with the Complete protease inhibitor cocktail (Roche, Indianapolis, IN). Samples containing 20 μg of total protein were resolved by SDS-PAGE on a 15.0% gel, as described previously for the liver (20). The antibodies and dilutions were as follows: goat anti-Gsta (1:3,000 dilution for male samples and 1:30,000 for female samples); rabbit anti-ERα (1:200; Santa Cruz BioMolecules), rabbit anti-β-actin (1:10,000; Amersham Biosciences), and oxidase-labeled anti-rabbit IgG (1:10,000 dilution; Amersham Biosciences) and exposed on autoradiograph film (Eastman Kodak). Densitometry measurements were performed using Kodak ID 3.5 software. The blots were repeated two to three times, and the data were merged after normalization. The results are expressed relative to the values of the control group, which were considered to be equal to 100%.

Treatment with a low dose of microcystin-LR. Microcystins are toxic heptapeptides that act as potent serine/threonine protein phos- phatase inhibitors, which are produced by several cyanobacteria (21). To investigate sex differences in its clearance related to treatment with a low dose of microcystin-LR, animals were exposed to a single sublethal dose of microcystin-LR (leucine and arginine) by ip injection at a 10 μg/kg dose. Control animals received ip saline injections. After 24 h, urine was collected and stored at −20°C for posterior microcystin-LR quantification by mass spectrometry coupled to liquid chromatography (LC/MS-MS) after previous cleanup with solid phase extraction cartridges. The detection occurred through electron spray ionization in positive polarity. Multiple resolution reaction mode was used for microcystin-LR detection. Two transitions were used for quantitation and identification. The product ions used in quantitation transitions were mass-to-charge ratio (m/z) 135 and identification m/z 223. The method was based on Spoo et al. (53) and Dell’Aversano et al. (15).

Statistical analysis. The data are reported as means ± SE. The number of animals is indicated in the figure legends. One-way ANOVA followed by Student-Newman-Keuls multiple comparison tests or t-tests was employed for the assessment of significance when comparisons were made within the same sex. A two-way ANOVA was employed when mice of different treatments were compared (GraphPad Prism version 5.0; GraphPad Software). The differences were considered to be significant at P < 0.05.

RESULTS AND DISCUSSION

Females express increased Gsta in the kidneys compared with males. As shown in Fig. 1A, Gsta mRNA levels, as assessed using real-time RT-PCR, were increased greatly in female whole kidneys compared with those of male wild-type mice (P < 0.001). The protein expression showed a similar magnitude of difference, and for this reason we were not able to show male and female Gsta levels in the same blot.

To further investigate the regulation of kidney Gsta expression by TH in males and females, we examined TRβ337T/337T animals. The total serum T3 levels of male and female TRβ337T/337T mice were similar and five to seven times higher compared with wild-type animals, as reported before (20, 50). The TRβ337T/337T mouse is a model in which T3, even when present in high levels, is unable to bind to TRβ. Gsta expression was opposite in homozygous males and females (Fig. 1, B–E). In agreement with our previous data from the livers of male and female TRβ337T/337T mice (20), the kidneys of male TRβ337T/337T mice exhibited elevated Gsta mRNA and protein levels compared with wild-type mice (Fig. 1, B and D). We and others (1, 20, 38, 48) have shown that T3 downregulates liver Gsta through TRβ and that this regulation is impaired when TRβ is absent or not functional. Surprisingly, in female homozygous mice, the Gsta expression was lower compared with that of TRβ337T/337T. However, the Gsta levels in female TRβ337T/337T mice were still higher compared with those of male mice independent of genotype (data not shown). Taken together, these data suggest that sexual hormones could be responsible for the higher expression of Gsta in females and for the antagonistic response to TH.

Hypothyroidism increased Gsta expression in the kidneys of male but not female mice. To further understand the differences observed in the TRβ337T/337T animals, we were due to T3 levels and sex difference or to the mutation, we induced hypo- and hyperthyroidism in wild-type males and females. After PTU treatment (HYPO), animals showed undetectable levels of T4 (EU: 3.17 μg/dl). The serum T3 concentrations confirmed the hyperthyroid status (EU: 117.4 ± 7.4 ng/dl; HYPO: 50.2 ± 3.7 ng/dl; HYPER: >400 ng/dl). Hypo- and hyperthyroidism status in the kidney were also confirmed by analyzing the expression of Dio1, a highly T3-responsive gene in the kidney. Figure 2A shows Dio1 expression in males and females combined. There is no sex difference in Dio1 expression (data not shown).

We next evaluated kidney Gsta mRNA levels and protein in males and females (Fig. 2). Confirming the data obtained from TRβ337T/337T animals, we observed a sex-specific regulation of Gsta according to thyroid status. When considering males, hypothyroidism caused a significant increase (5 times) in Gsta expression compared with that of euthyroid males, as observed previously in the liver (20). Conversely, as depicted in Fig. 2, hypothyroidism in females caused a 20–50% decrease in Gsta expression compared with that of euthyroid females (Fig. 2). To the best of our knowledge, as of this writing there are no published data showing the regulation of Gsta by TH in the

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kidney. The present knowledge of Gsta regulation by TH is restricted to the enzyme in the livers of rats and mice or heterologous cells. Our group and others have demonstrated that TH are able to inhibit liver Gsta expression in hypothyroid animals (11, 20, 48, 57). In this study, we have shown for the first time that Gsta is regulated by T3 in the kidney. In addition, we showed that not only is basal expression of Gsta higher in the female kidney but that there is also a unique profile of

![Graphs showing expression levels of Gsta in male and female kidneys](image)

**Fig. 1.** The expression of glutathione S-transferase-α (Gsta) mRNA and protein in the kidneys of male and female mice. A: the Gsta mRNA levels in male and female wild-type mice according to real-time RT-PCR analysis. B and C: the Gsta mRNA levels of wild-type (TRβ<sup>wt/wt</sup>) and homozygous (TRβ<sup>337T/337T</sup>) male (B) and female mice (C). The data were normalized for each mRNA level relative to the values for TRβ<sup>wt/wt</sup>. D and E: representative autoradiographs of male (D) and female (E) Gsta (26 kDa) and cyclophilin (Cyclo; 19 kDa) according to Western blotting and a respective analysis by densitometry. The data are shown as means ± SE of at least 3 independent experiments. Five to eight animals were evaluated in each group.
regulation by T3 in each sex, suggesting that sex hormones may be involved in TH regulation.

It is known that several GST class proteins are also subject to modulation by estrogens and that Gsta activity and gene expression can be stimulated by E2 in various tissues (7, 11). However, an interaction between thyroid status and estradiol has never been reported before in the kidney or for Gsta expression in any other tissue.

OVX restored the male profile of Gsta expression in response to hypo- and hyperthyroidism. To investigate the possible role of estradiol in the TH regulation of Gsta, we studied Gsta expression in hypo- and hyperthyroid female mice 2 wk after OVX (Fig. 3). Uterus weight and estradiol concentrations were reduced (data not shown). As observed in males (Fig. 2), Gsta expression was increased in the absence of TH, and T3 treatment was not able to change the Gsta expression in the kidneys of OVX female mice (Fig. 3). The protein and mRNA presented the same expression profile, which was similar to that of male mice, suggesting that the absence of the ovary restored the “male profile” of Gsta regulation. The absence of the ovary takes away several factors and hormones, including progesterone and E2. Because it was known previously that E2 regulates Gsta in other tissues (11), we decided to investigate how estradiol affects the TH regulation of Gsta.

E2 benzoate increases renal Gsta expression in female mice. Kidney Gsta mRNA levels (Fig. 4, A and B) were increased in sham-operated and E2-treated (10 or 100 mg/kg body wt)
females compared with OVX and male mice ($P < 0.05$ or less). Figure 4, C and D, shows the $E_2$ levels and uterus weights, respectively, of manipulated females, confirming the efficacy of OVX and estradiol replacement. OVX was also able to increase the kidney expression of estrogen receptor ($Esr1$) and TH receptor-$\beta$ ($Thrb$) compared with all groups (Fig. 4E), as has already been described in the literature (4, 8).

As with our findings, Kireev et al. (34) showed that OVX in aged rats led to reduced activity of the GST proteins in the liver, which was reversed by $E_2$ treatment (34). However, concerning estrogen regulation of $Gsta$ expression in the kidney, to our knowledge, no data have been published.

Taking into account that the ovary produces several steroids and protein hormones, we then repeated the same experiment described in Fig. 3 but by treating OVX animals with 100 mg/kg body wt $E_2$ benzoate (EB100) to confirm that $E_2$ was interfering with TH regulation. The levels of $Gsta$ mRNA increased significantly in the hyperthyroid animals after OVX and $E_2$ benzoate ($P < 0.01$; Fig. 5, A and B). Comparing Figs. 3 and 5, in OVX females the maximum expression of kidney $Gsta$ is observed in the absence of TH, whereas in the presence of $E_2$ (OVX + EB), higher $Gsta$ levels were observed with an excess of $T_3$.

To better visualize the interaction between thyroid and ovary status, Fig. 6 shows OVX and OVX + EB in eu-, hypo-, and hyperthyroidism. Kidney $Gsta$ expression is increased by $T_3$ when $E_2$ is present and decreased by $T_3$ when $E_2$ is absent.

According to the present data, it appears that there is cross-talk between the thyroid- and $E_2$-signaling pathways that modulates renal $Gsta$ expression. There are several lines of evidence showing the integrated actions of $T_3$ and $E_2$ in models of tumors (13), reproduction, and sexual behavior (17, 58). Because we performed in vivo experiments, it was impossible to be certain that $E_2$ and $T_3$ interact in renal tissue to regulate the expression of this enzyme. To further investigate whether this interaction was direct, we also performed in vitro experiments.

$E_2$ and $T_3$ act together in the proximal tubule cells to regulate $Gsta$ expression. To choose the best cell line to perform our experiments, we evaluated $Gsta$ expression after microdissection of the renal tubules. Figure 7A shows that female mice expressed increased $Gsta$ in isolated proximal tubules compared with male mice. Collecting duct $Gsta$ mRNA expression in males and females did not differ and was lower compared with that of the proximal tubules. These data confirm that in the mouse kidney, as well as in humans (24), $Gsta$ mRNA is expressed mainly in the proximal tubules.

To further investigate whether $E_2$ and $T_3$ act together in the proximal tubule cells to regulate $Gsta$ expression, we performed in vitro experiments using IRPTC cells. First, we confirmed by Western blotting that the IRPTC cells in the

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**Fig. 3.** The expression of $Gsta$ in the kidneys of ovariectomized (OVX) EU, HYPO, and HYPER mice. A: the $Gsta$ mRNA levels of mice according to real-time RT-PCR analysis. B: representative autoradiographs of $Gsta$ (26 kDa) and Cyclo (19 kDa) according to Western blotting and a respective analysis by densitometry. The data are shown as means $\pm$ SE of at least 3 independent experiments. Six to eight animals were evaluated in each group. C: experimental design.
specific passages we were using expressed *Esr1* and *Thrb* receptors. Both receptors were highly expressed (data not shown).

We then treated IRPTC cells with 10^{-6} and 10^{-8} M of T_{3}, EB, or T_{3} + EB. After 24 h of incubation, T_{3} at a physiological dose (10^{-8} M) was able to decrease the expression of *Gsta* by 50%, as described before by several studies using liver cell culture (11, 57). However, when the 10^{-6} M dose was used, T_{3} increased *Gsta* threefold. EB alone or EB + T_{3} at both 10^{-6} M was able to increase the *Gsta* mRNA levels (*P* < 0.01 or less; Fig. 7B). However, this effect was not observed in shorter times (data not shown). Thus, in higher concentrations, both T_{3} and E_{2} were able to increase *Gsta* expression, but the effect was not synergistic. In a way, these results are in conflict with our data in vivo. Because IRPTC cells were developed from male animals (56), it is possible that a different balance of receptor expression between male and female tissue might cause these results.
Functions of Gsta in the kidney. Very little is known about renal Gsta function and its regulation by exogenous factors and hormones. It is believed that the detoxifying actions of this protein are related to the activation of carcinogens, inducing renal tumors and developing resistance to chemotherapeutic agents (14). Kidney GSTα has already been associated with tumorigenesis (52), and the study of the sex-dependent regulation of this gene can help elucidate differences in renal detoxification and the tendency for tumor growth. Bass et al. (5) first reported that higher levels of urinary GST occur in rats following a nephrotoxic injury, which can be related to either an increased leakage from tubular cells into the lumen or an epithelial proximal tubule necrosis. According to recent studies by Ozer et al. (44), Harpur et al. (23), and Swain et al. (55), urinary GSTα is a good noninvasive biomarker of cytoplasmic leakage, detecting early tubular damage before regeneration occurs.

Considering that GSTs detoxify a broad range of compounds by catalyzing the conjugation of reduced GSH with several xenobiotic and endogenous electrophiles (26), we attempted to understand the physiological role of sex-specific Gsta regulation. Therefore, we treated males and females with a single sublethal injection of microcystin-LR, a toxic heptapeptide, to...
we were able to identify possible TREs and EREs next to (29, 51). However, by analyzing the challenge in TH-negative regulation for the past 25 years been described yet. Describing a negative TRE has been a region of have described hormone response elements in the promoter response elements (TREs) of target genes. Several studies (22, 60). In a similar way, TRs also interact with the thyroid DNA of target genes, modulating transcriptional activity and bind to estrogenic response elements (EREs) in the complexes, following which they homo- or heterodimerize been shown previously (35) that basal monal regulation of kidney GSTs is quite intriguing. It has been published; however, here we use a lower dose, regarding the effect of microcystin in the kidneys of females recently (37) and includes an increase in urinary flow. No data microcystin on kidney function in male rats was published lism in the liver involves the GST class (21). The effect of known that microcystin is a hepatotoxin and that its metabo- in male mice. The relevance of this result is unclear; it is obtained results may be interpreted as an indication that more original microcystin-LR was conjugated to GSH in female than in male mice. The relevance of this result is unclear; it is known that microcystin is a hepatotoxin and that its metabolism in the liver involves the GST class (21). The effect of microcystin on kidney function in male rats was published recently (37) and includes an increase in urinary flow. No data regarding the effect of microcystin in the kidneys of females have been published; however, here we use a lower dose, aiming to not affect renal function.

Final considerations. The functional relevance of the hor- monal regulation of kidney GSTs is quite intriguing. It has been shown previously (35) that basal Gsta expression is different between males and females in certain tissues, such as in the kidney and intestines, but the physiological relevance is still unknown.

In this study, we showed an in vivo interaction between T3 and E2 in Gsta regulation in females. It is known that after interaction with E2, ERs are released from inactive complexes, following which they homo- or heterodimerize and bind to estrogenic response elements (EREs) in the DNA of target genes, modulating transcriptional activity (22, 60). In a similar way, TRs also interact with the thyroid response elements (TREs) of target genes. Several studies have described hormone response elements in the promoter region of Gsta (19, 45, 47); however, no TREs or EREs have been described yet. Describing a negative TRE has been a challenge in TH-negative regulation for the past 25 years (29, 51). However, by analyzing the Gsta promoter region, we were able to identify possible TREs and EREs next to each other based on comparisons to uncommon hormone response elements (10, 49, 59). According to the present data, it is possible that T3 and E2 receptors interact in the same DNA region and compete for the same hormone response elements. We propose the following, in a very simplified model, when E2 is absent and T3 is present: T3, bound to its receptor, decreases the rate of Gsta transcription (as described in the liver and other tissues). When E2 is present, the complex ER-E2 displaces TR-T3 from the DNA, increasing Gsta expression directly and indirectly avoiding downregulation by T3.

In summary, we have shown for the first time that 1) the sex difference in the basal expression of Gsta impacts the detoxification process, 2) kidney Gsta expression is regulated by TH in males and females in opposite directions, 3) T3 and E2 interact directly in renal proximal cells, regulating Gsta expression in females, and 4) TH and E2 do not have synergic effects.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


![Fig. 7. The expression of kidney Gsta in vivo and in vitro. A: Gsta mRNA levels in the proximal tubules and collecting ducts after microdissection. B: Gsta mRNA levels in mice according to cell culture treated with $10^{-6}$ and $10^{-8}$ M of EB, T3, and EB + T3 after 24 h of incubation. The results are expressed relative to the values of the group treated with the vehicle, which were considered to be equal to 100%. The data are shown as means ± SE of at least 3 independent experiments. Nine to 12 replicates were evaluated in each group. C: urinary microcystin in males and females. The data are shown as means ± SE of 1 experiment. Five animals were evaluated in each group.](image-url)
prepared the figures; L.C.F. drafted the manuscript; L.C.F., N.A.S.A., G.F.P., R.G.R., R.M.S., M.M.M., C.C.P.-M., and T.M.O.-c. approved the final version of the manuscript; R.M.S., C.C.P.-M., and T.M.O.-c. edited and revised the manuscript.

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