Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 323 (2004) 235–241
BBRC
www.elsevier.com/locate/ybbrc

Stress regulation of sulfotransferases in male rat liver☆,☆☆

Smarajit Maiti, Shannon Grant, Sharon M. Baker, Subramanya Karanth, Carey N. Pope, Guangping Chen*

Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA

Received 10 August 2004
Available online 27 August 2004

Abstract

Sulfotransferase (SULT) catalyzed sulfation is responsible for hormone regulation and xenobiotic detoxification. Induction of SULTs by various hormones has been reported. Stress regulation of SULTs has not been reported, however. Here we report that rat liver SULTs can be regulated by physical stress (forced running, EX) and chemical stress (the organophosphorus pesticide parathion, PS). Both EX and PS increased rat liver phenol-sulfating SULT1A1 and hydroxysteroid-sulfating SULT2A1 activities. The increase in SULT1A1 activity did not correlate with protein (Western blot) or mRNA (RT-PCR) results but correlated well with increased non-protein soluble thiols. This suggests a possible Cys modification mechanism for stress regulation of SULT1A1. In vitro studies on GSH/GSSG effects on SULT1A1 activity support this conclusion. In contrast, SULT2A1 activity following physical or chemical stress treatments correlated well with protein and mRNA levels. This suggests a stress regulation mechanism of SULT2A1 at the gene transcription level, possibly occurring via hormones.

Keywords: Sulfotransferase; (rat)SULT1A1; (rat)SULT2A1; Chemical stress; Physical stress; Parathion; Forced exercise; Regulation; Non-protein soluble thiol

Sulfotransferases (SULTs) are a family of phase II drug metabolizing enzymes which catalyze sulfuryl group transfer from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to most hydroxyl containing compounds. Substrates for SULTs include endogenous hormones and xenobiotics. The biological functions of SULTs include hormone regulation/metabolism and xenobiotic detoxification. SULTs are regulated primarily by different hormones [1–5]. Xenobiotic induction of SULTs is also known [1,6–8]. In vitro oxidative regulation of bacterial expressed rat aryl sulfotransferase IV ((rat)SULT1A1) has been reported [9–12]. Our recent results indicated that this enzyme can be oxidatively regulated by hyperoxia in rat lung (unpublished observation). To the best of our knowledge, the effect of physical stress or chemical stress on SULTs is not known.

Physical stress resulting from daily exercise can influence the intracellular redox status [13–15] as well as the homeostasis of steroid hormone metabolism [16–18]. Extensive and/or moderate exercise has been shown to differentially regulate plasma cortisol, corticosterone, testosterone, estradiol, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS) levels in military personnel, female athletes, and elderly individuals [19–22]. Repeated forced exercise markedly increased plasma corticosterone levels [23]. Rats forced to exercise experience significantly elevated bile excretion of GSH and GSSG indicating the possible alteration in intracellular thiol homeostasis [19–22,24]. Elevation of GSH and the

☆ This work was supported in part by NIH Grants GM59873 (G.C.), and Grant DAMD17-00-1-0070 from the U.S. Army (C.N.P.).
☆☆ *Abbreviations:* SULT, sulfotransferase; (rat)SULT1A1, rat aryl sulfotransferase IV; (rat)SULT2A1, rat hydroxysteroid sulfotransferase a; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; DHEA, dehydroepiandrosterone; NPSH, non-protein soluble thiol; PNPS, *p*-nitro-phenyl sulfate.
* Corresponding author. Fax: +405 744 8263.
E-mail address: cguang@okstate.edu (G. Chen).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.08.074

influence of different antioxidant enzymes have been linked to the increased level of reactive oxygen species (ROS) in exercised humans [25,26]. This has been supported and demonstrated in terms of elevated levels of lipid peroxides following intensive exercise [27,28]. In general, chronic exercise up-regulates different metabolic processes including oxygen-mediated oxidative stress [29], carbohydrate metabolism [30,31], and pituitary–hypothalamic axis-mediated steroid homeostasis [32,33].

The known chemical stressor, parathion (*O*,*O*′-diethyl-*O*-4-nitrophenyl phosphorothioate, PS), is a prototype organophosphorus pesticide that is converted to its active metabolite paraoxon (PO) upon enzymatic oxidation [34,35]. Worldwide, organophosphorus (OP) results in numerous poisonings each year [36]. PS has been reported to be a strong inhibitor of rat microsomal CYP 450s [34,37]. It has also been shown to inhibit the hepatic CYP 450s mediated monooxygenase activities in microsomes of rainbow trout [38]. OPs can also elevate plasma levels of stress hormones [23,39]. The closely related insecticide methyl parathion (*O*,*O*′-dimethyl-*O*-4-nitrophenyl phosphorothioate) also increases plasma corticosterone levels [39–41]. PS and PO have been reported to interact with the cellular antioxidant defense systems by conjugating glutathione (GSH) in the presence of glutathione-*S*-transferase (GST) resulting in altered thiol status in the cell [36,42,43].

In the present investigation, we examined the effects of PS (chemical stress) and EX (physical stress) alone or in combination on the expression and regulation of (rat)SULT1A1 and (rat)SULT2A1 in male rat liver. These findings may contribute to the understanding of hormone regulation, drug metabolism, drug–drug interactions, and human health under physiological and pathological conditions.

Materials and methods

Materials. Parathion was purchased from ChemService (West Chester, PA). 2-Naphthol, [^{14}C]2-naphthol (4.7 mCi/mmol), *p*-nitrophenyl sulfate (PNPS), 3′-phosphoadenosine 5′-phosphosulfate (PAPS), and [1,2,6,7-^{3}H(*N*)]dehydroepiandrosterone ([^{3}H]DHEA, 60 Ci/mmol) were purchased from Sigma–Aldrich (St. Louis, MO). SDS–polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad (Hercules, CA). Western blot chemiluminescence reagent kits (Super Signal West Pico Stable Peroxide and Super Signal West Pico Luminol/Enhancer solutions) were purchased from Pierce Chemical (Rockford, IL). Total RNA extraction kit (RNeasy mini protection kit) was supplied by Qiagen (Valenica, CA). One-step RT-PCR kit was purchased from Promega (Madison, WI). Antibodies against (rat)SULT1A1 and (rat)SULT2A1 [7,44] were generously provided by Dr. Michael W. Duffel (Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA). Protein assay reagent was purchased from Bio-Rad. All other reagents and chemicals were of the highest analytical grade available.

Animals and drug treatment. Young male Sprague–Dawley (SD) rats (5–7 weeks old, Harlan, Indianapolis, IN) were used in this investigation. Rats were housed in a temperature-controlled (23 ± 1 °C) room with a 12 h light/12 h dark cycle and supplied with standard rodent chow and water ad libitum. All rats were acclimated for at least 1 week prior to use. Two sets of experiments were carried out. For each set, rats were divided into 4 groups ($n = 4$ per group). In one set, 4 treatment groups were designated as control, EX, PS (1.8 mg/kg), and EX + PS (1.8 mg/kg). In the other set, groups were designated as control, EX, PS (3.6 mg/kg), and EX + PS (3.6 mg/kg). The rats in the EX and EX + PS groups were forced to exercise (physical stress) by running on a 4-lane treadmill (OmniPacer Model No. LC4/R-MA; AccuScan Instruments, Columbus, OH, USA). Animals were placed at the back of the treadmill lane which delivered a mild electric shock (1 mA) and speed was set to 15 m/min with an incline of 6°. Rats were placed on the treadmill for 90 min each day. The rats in the PS and EX + PS groups were treated orally with PS as a homogeneous mixture in peanut oil at either 1.8 or 3.6 mg/kg/day for 7 days. PS was administered daily just prior to initiation of forced running. The corresponding group of control rats received only the vehicle. The animals were sacrificed 24 h after the final drug treatment. Livers were collected, washed with sterile, ice-cold NaCl (0.9%) solution, and snap-frozen. All samples were stored at −80 °C until use.

Cytosol preparation. Tissues were homogenized in 50 mM Tris buffer containing 0.25 M sucrose, 0.01 mg/ml trypsin inhibitor, and 10 μg/ml phenylmethylsulfonyl fluoride, pH 7.5. All homogenates were centrifuged at 100,000*g* for 1 h at 4 °C [8]. Cytosol aliquots were collected and preserved at −80 °C for enzymatic assay and Western blot.

Enzyme assays. Two different enzyme assay methods were used. For both PNPS and radioactive assay methods, the linearity and steady state of 30 min reaction period were confirmed with different amounts of cytosolic protein and substrate concentration.

PNPS assay. 2-Naphthol sulfation activity from liver cytosols was determined as previously described [45,46]. This assay determines phenol sulfation activities of different isoforms of phenol sulfating SULTs. Briefly, sulfation activity was determined in a reaction mixture containing 50 mM Tris buffer (5 mM PNPS, 20 μM PAPS, and 0.1 mM 2-naphthol, pH 6.2). Rat liver cytosols (50 μg protein) were used as the enzyme source in a total reaction volume of 250 μl. After 30 min incubation at 37 °C in a shaking water bath, the reaction was stopped by adding 250 μl of 0.25 M Tris, pH 8.7. The reaction mixtures were read at 401 nm in a spectrophotometer. Specific activity (SA) was expressed as nmol/min/mg of protein.

Radioactive assay. DHEA sulfation activities in liver cytosols were determined by the radioactive assay method previously described [7,8,47]. The reaction conditions were same as the PNPS assay mentioned above. To determine DHEA sulfation activity in liver cytosols, [^{3}H]DHEA (diluted to 0.4 Ci/mmol; 2 μM final concentration) was used as substrate. For all assays, 20 μM PAPS was used. Liver cytosol protein (50 μg) was used as the enzyme source in a total reaction volume of 250 μl. After 30 min incubation at 37 °C in a shaking water bath, the reaction was stopped by adding 250 μl of 0.25 M Tris, pH 8.7. Extraction was performed twice by addition of 0.5 ml of water-saturated chloroform. After the final extraction, 100 μl of the aqueous phase was used for scintillation counting. PAPS was eliminated from the controls of both assay methods. Assays were run in duplicate and the average of the results was used for enzyme activity calculations. The data are expressed as means ± SEM from 4 rats.

Western blot analysis. Cytosol proteins from liver (10 μg) were separated on a 12% (w/v) polyacrylamide gel in an electrophoresis system (Novex, San Diego, CA) [44]. After running at 200 V, the protein bands were transferred overnight at 40 V onto a nitrocellulose membrane. All membranes were blocked in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, and 0.05% (v/v) Tween 20) containing 5% (w/v) dried milk for 1 h on a shaker at room temperature. Membranes were incubated with either rabbit anti-(rat)SULT1A1 or rabbit anti-(rat)SULT2A1 (1:5000) in TBST containing 5% (w/v) dry milk for 2 h on a shaker at room temperature. After incubation, membranes were washed with TBST for 4 × 15 min and incubated with secondary antibody (horseradish peroxidase-conjugated Immuno-Pure goat anti-rabbit IgG; H + L) at 1:5000 dilutions in the same buffer for 2 h. The

membranes were washed with TBST for 4 × 15 min and then with Tris-buffered saline (TBS) 3 × 5 min. Fluorescent bands were developed with 1 ml of substrate containing the same volume of each Super Signal West Pico Luminol Enhancer solution and Super Signal West Pico Stable Peroxidase solution at room temperature for 5 min. The X-ray films were exposed to the membrane and then developed. Films were scanned and the densitometry analysis was performed with AAB software in a Gel Documentation and Analysis System from Advanced American Biotechnology (Fullerton, CA).

Extraction of total RNA and RT-PCR. Total RNA was extracted from liver tissues (~20 mg) using RNeasy mini protection kit from Qiagen according to the supplier's guidelines [8]. The concentration and purity of the extracted RNA were checked spectrophotometrically by measuring 260/280 absorption ratios. The primer pair for (rat)-SULT1A1 was designed in our laboratory using the Gene Fisher primer designing and Multialignment software. Using the forward primer (FP) 5′-GTGTCCTATGGGTCGTGGTA-3′/reverse primer (RP) 5′-TTCTGGGCTACAGTGAAGGTA-3′ (GenBank Accession No. X52883), the 299 bp (rat)SULT1A1 cDNA was synthesized. The 264 bp (rat)SULT2A1 cDNA was synthesized using the primer pair FP 5′-TCCTCAAAGGATATGTTCCG-3′/RP 5′-CAGTTCCTTCTCC ATGAGAT-3′ (GenBank Accession No. M33329) [48]. The specificity of all primers was tested using the BLAST from the National Center for Biotechnology Information Open Reading Frame software. cDNA synthesis from total 1 μg of liver RNA was performed in a 50 μl reaction mixture. Concentrations of the different ingredients used followed the supplier's guidelines. For internal control, 500 bp cDNA of rat β-actin was synthesized from the same amount of RNA from respective sources. The primer pair FP 5′-GATGTACGTAGCCA TCCA-3′/RP 5′-GTGCCAACCAGACAGCA-3′ for the synthesis of rat β-actin cDNA was designed in our laboratory using the software mentioned above.

Determination of non-protein soluble thiol. Total non-protein soluble thiol (NPSH) in liver cytosol was determined by the standard DTNB (Ellman's reagent, 5,5′-dithiobis-2-nitrobenzoic acid) method [49] with a slight modification. Liver tissues were homogenized with 50 mM Tris-buffer, pH 7.5, containing 250 mM sucrose, and 5 mM EDTA. Homogenates were centrifuged at 100,000*g* for 1 h. Equal volume of 5% (w/v) 5-sulfosalicylic acid was used to precipitate all proteins from the tissue cytosols. After mild vortexing and centrifugation at 4000*g*, 80 μl of transparent sample supernatant was added to 720 μl of 0.1 M potassium phosphate buffer containing 5 μM DTNB. The absorbance was measured after 5 min at 412 nm in a UV-spectrophotometer. The steady state of the reaction kinetics was checked up to 7 min. A standard curve was generated using GSH and individual sample values were determined from this standard curve.

Statistical analysis. Student's *t* test was performed for the statistical significance between control and treated samples. Data presented in the figures denote means ± SEM of the data collected separately from four individual animals.

Results

Cholinergic toxicity

With the PS dosages used (i.e., 1.8 or 3.6 mg/kg/day), no overt signs of cholinergic toxicity (excessive secretions, muscle fasciculations, and tremors) were noted.

Effect of physical and chemical stress on 2-naphthol sulfation activity

2-Naphthol sulfation activity increased in rat liver cytosols following physical (EX) and chemical (PS) stress (Fig. 1). EX increased the activity significantly (~2-fold, $p < 0.05$) in both sets of experiments. PS treatment at 1.8 mg/kg increased activity 50% whereas that at 3.6 mg/kg increased activity ~2-fold ($p < 0.01$). An additive effect was observed with the combined treatment group at 1.8 mg/kg PS. The pattern and fold of increase (~3-fold, $p < 0.001$) was similar in both EX + PS (1.8 mg/kg) and EX + PS (3.6 mg/kg) groups. Though the higher dose of PS treatment had a greater effect on sulfation activity, this dose along with EX showed no significant difference compared with the corresponding combined treatment group with the lower PS dose.

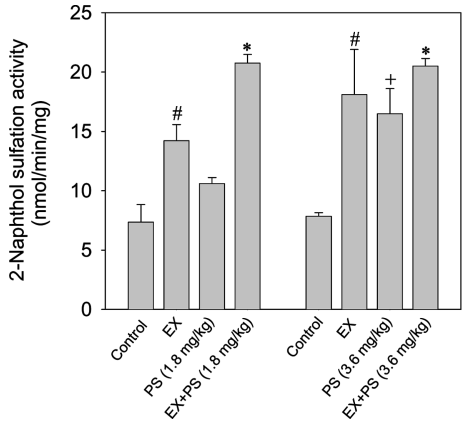

Fig. 1. Effect of forced exercise and parathion on 2-naphthol sulfation activity in rat liver. Enzyme activity was determined by the PNPS assay method using 50 μg of liver cytosolic protein. Values in the figure represent means ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$; and $^{*}p < 0.001$.

Effect of physical and chemical stress on DHEA sulfation activity

EX related physical stress significantly increased (~3-fold, $p < 0.05$) the DHEA sulfation activity in rat liver. PS treatment at 3.6 mg/kg also increased the activity (~2-fold, $p < 0.001$) (Fig. 2). The combined treatment of EX and PS increased the activity by 3- and 4.5-fold ($p < 0.01$) in EX + PS 1.8 mg/kg or EX + PS 3.6 mg/kg group, respectively. Additive effects of either stressor on DHEA sulfation activity were observed.

Western blot analysis of (rat)SULT1A1 and (rat)-SULT2A1 protein following stress treatment

The Western blot results of (rat)SULT1A1 (major protein for 2-naphthol sulfation in rat [50]) and (rat)-SULT2A1 (major protein for DHEA sulfation [51,52]) and their densitometry analysis data are presented in Fig. 3. The densitometry data on (rat)SULT1A1 protein suggest that this protein amount did not change following treatment with either physical or chemical stress alone or in combination (Fig. 3A).

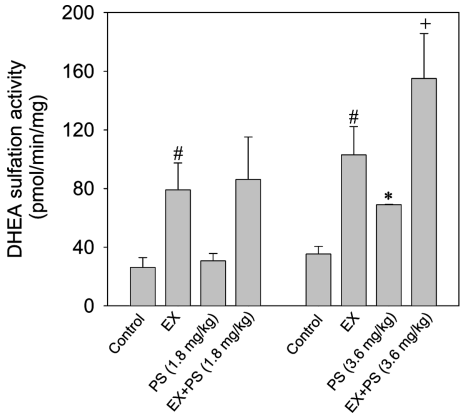

Fig. 2. Effect of forced exercise and parathion on DHEA sulfation activity in rat liver. Enzyme activity was determined by the radioactive assay method using 50 μg of liver cytosolic protein. Values in the figure stand for means ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$; and $^{*}p < 0.001$.

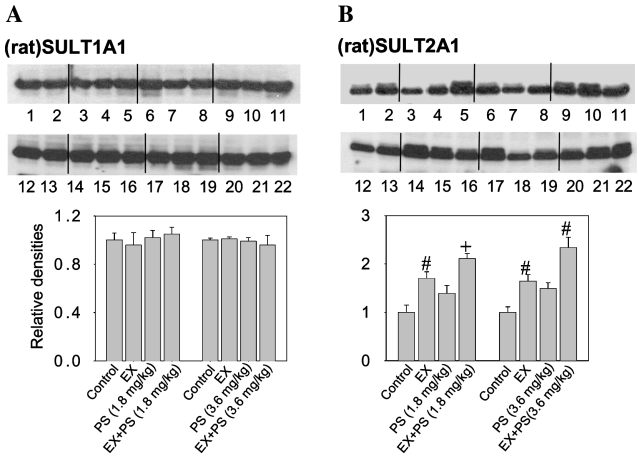

Fig. 3. Representative Western blot and densitometric analysis of (rat)SULT1A1 (A) and (rat)SULT2A1 (B) Ten micrograms of cytosolic total protein was run in 12% bis-acrylamide gel. Two sets of experiments were performed. Samples from 1 to 11 represent the first set and 12 to 22 the second set. Lane description for both (rat)SULT1A1 (A) and (rat)SULT2A1 (B): 1–2 control, 3–5 EX, 6–8 PS: 1.8 mg/kg, 9–11 EX + PS: 1.8 mg/kg. The densitometry data are plotted as the normalized values which denote means ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$.

This was true for all treatment groups. The increase in 2-naphthol sulfation activity, described earlier, does not correlate with the (rat)SULT1A1 Western blot results. Unlike (rat)SULT1A1, the densitometry analysis data of (rat)SULT2A1 protein showed an increase in all treatment groups. The increase in protein level in EX treatment group was found to be significant ($p < 0.05$). The effect was found to be significant and additive in the combined treatment groups (both at EX + PS 1.8 mg/kg ($p < 0.01$) and EX + PS 3.6 mg/kg ($p < 0.05$)). This is consistent with the enzyme activity results mentioned above.

RT-PCR results on (rat)SULT1A1 and (rat)SULT2A1 mRNA expression following stress treatment

RT-PCR results show that (rat)SULT1A1 mRNA expression did not change following treatments either alone or in combination (Fig. 4). This result correlates well with the (rat)SULT1A1 Western blot results but does not agree with the 2-naphthol sulfation activity results. In contrast, (rat)SULT2A1 mRNA results show that either stress treatment increased the expression significantly over the control group and the combined treatment group increased the expression even more than the individual treatment groups. (rat)SULT2A1 RT-PCR results are in basic agreement with the corresponding Western blot and enzyme activity results (Fig. 4).

Non-protein soluble thiol changes following stress treatment

Non-protein soluble thiol (NPSH) increased in the liver tissues following treatment with individual stressors or in combination (Fig. 5). Treatment with EX or PS alone increased the concentration ~2-fold and combined treatment with EX + PS 1.8 mg/kg or EX + PS 3.6 mg/kg group increased the concentration ~3-fold ($p < 0.01$ and $p < 0.001$, respectively).

GSH and GSSG in vitro effect on rat liver cytosol 2-naphthol and DHEA sulfation activity

Rat liver cytosol (in 50 mM Tris, 0.25 M sucrose, pH 7.5) was treated with GSH (0, 1, 2, 4, and 8 mM) or

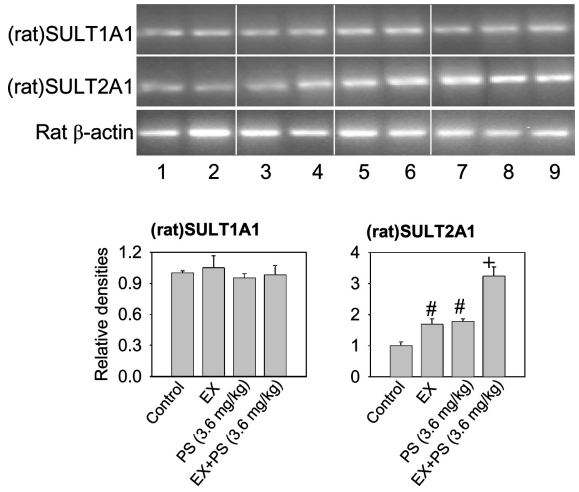

Fig. 4. Representative RT-PCR results of (rat)SULT1A1 and (rat)-SULT2A1 mRNA expression. cDNA was synthesized from 1 μg of total RNA in the presence of a gene-specific primer. The total reaction volume was 50 μl. Ten microliters of product was run in each lane of a 2% agarose gel. Lane descriptions: 1–2, control; 3–4, EX; 5–6, PS:3.6 mg/kg; and 7–9, EX + PS: 3.6 mg/kg. Data are normalized with the corresponding β-actin densities and expressed relative to control. Thirty cycle reactions were performed during this RT-PCR experiment. Values in the figure represent means ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$.

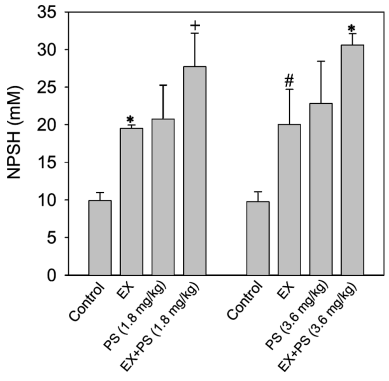

Fig. 5. Effect of forced exercise and parathion on non-protein soluble thiol in rat liver. Total protein in the cytosol fraction was precipitated using sulfosalicylic acid. DTNB was used to react with the free thiol group creating a color reaction read at 412 nm in a UV spectrophotometer. GSH with different concentrations was used to generate a standard curve. Values in the figure represent means ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$; $^{*}p < 0.001$.

GSSG (0, 0.1, 0.2, 0.4, and 0.8 mM) at room temperature for 1 h. The treated cytosols were used for determination of 2-naphthol or DHEA sulfation activities. Results shown in Fig. 6 demonstrate that 2-naphthol sulfation (SULT1A1) activity is increased with added GSH and decreased with added GSSG. DHEA sulfation (SULT2A1) activity was not significantly affected with the addition of GSH or GSSG.

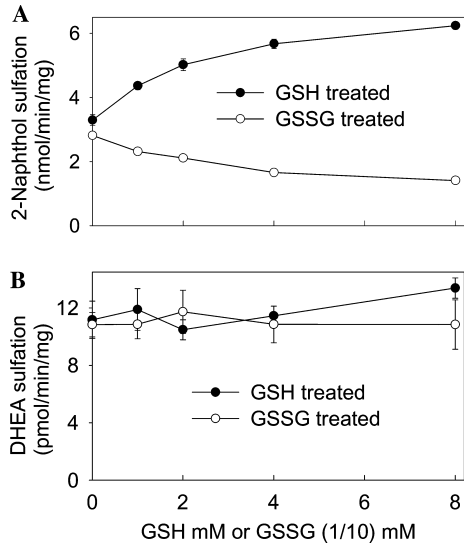

Fig. 6. GSH and GSSG in vitro effect on rat liver cytosol 2-naphthol and DHEA sulfation activities. Rat liver cytosol (in 50 mM Tris, 0.25 M sucrose, pH 7.5) was treated with GSH (0, 1, 2, 4, and 8 mM) or GSSG (0, 0.1, 0.2, 0.4, and 0.8 mM) at room temperature for 1 h. The treated cytosol (50 μg) was used for PNPS assay (0.1 mM 2-naphthol as substrate) or radioactivity assay (2 μM [^{3}H]DHEA as substrate) in triplicate. Mean ± SEM of specific activity was plotted versus GSH or GSSG concentrations.

Discussion

Phase I and phase II drug metabolizing enzymes are responsible for the detoxification of xenobiotics found in food, drugs, and environmental media. SULTs belong to the phase II drug metabolizing enzymes. SULT catalyzed sulfation is an important pathway in endogenous hormone regulation and in the metabolism of exogenous hydroxyl-containing xenobiotics. Regulation of SULTs by different hormones has been well studied. Regulation of SULTs by stressors has not been reported, however. Physical or chemical stress as an exogenous or endogenous source may significantly interfere with physiologic metabolic processes by changing neuroendocrine regulation, hormone regulation, and/or by increasing oxidative stress through alteration of intracellular redox status. Our results demonstrate that both EX and PS can regulate SULT activities in male rat liver.

An increase in (rat)SULT1A1 activity correlated with the increase in cellular NPSH but not with protein or mRNA expression levels. This clearly indicates that the effect of physical and chemical stress on (rat)SULT1A1 occurs through protein modification rather than at the level of gene regulation. Our recent studies (unpublished observation) on in vivo hyperoxic effects in rat lung SULT1A1 suggested that this enzyme can be oxidatively regulated through Cys chemical modification. Our data on stress regulation of this enzyme agree with the Cys modification mechanism. Our results on NPSH changes corresponding to physical and chemical stress agree with literature reports [26,53].

In contrast to (rat)SULT1A1, the increase in (rat)-SULT2A1 activity correlated with the increase in protein and mRNA expression as demonstrated by the Western blot and RT-PCR results. This indicates that stress regulation of (rat)SULT2A1 is at the gene transcription level. It is well known that physical and chemical stressors regulate hormone levels [17,23,26,53,54]. SULTs, which catalyze the sulfation of many hormones, are well known to be regulated through different hormones [2,55,56]. Physical and chemical stress-induced regulation of rat hydroxysteroid SULT2A1 gene expression may therefore be mediated through hormone level changes.

Our in vitro experiments on GSH or GSSG effects on rat liver cytosol 2-naphthol and DHEA sulfation activities further confirmed the above conclusions. GSH/GSSG affected SULT1A1 activity but not SULT2A1 activity (Fig. 6). This demonstrates that Cys residue chemical modification near the active site can significantly alter SULT1A1 activity but not SULT2A1 activity. Computer modeling structures (constructed using Swiss-Model program, data not shown) of (rat)SULT1A1 and (rat)SULT2A1 agree with this conclusion. The three Cys residues (Cys26, Cys54, and Cys198) in SULT2A1 are distant (>13 Å) from the active site (PAPS or substrate) and buried in the internal structure.

They are unlikely to be chemically modified by GSSG. The chemical modification of these Cys residues is also unlikely to inactivate the enzyme. For (rat)SULT1A1, there are five Cys residues in the structure. Cys66, Cys232, Cys283, and Cys289 are located more than 11 Å from PAPS or substrate in the active site. On the other hand, Cys82 is in direct contact with substrate (3 Å). It is exposed on the substrate binding site surface. Cys82 should be easily chemically modified by GSSG (to form a disulfide bond). This modification would prevent the binding of substrate or releasing of product therefore inactivating the enzyme.

Exposure to different forms of stress is a common occurrence in daily life. Stress can regulate the activity of SULTs and thus modulate the metabolism of endogenous and exogenous compounds. Stress regulation of SULTs has not been previously reported. These studies are significant in understanding stress effects on hormone sulfation metabolism, drug and toxicant detoxification, and drug–drug interactions.

Acknowledgment

The authors deeply appreciate the generous gift of aryl SULT IV ((rat)SULT1A1) and hydroxysteroid SULT a ((rat)SULT2A1) antibody from Dr. Michael W. Duffel of University of Iowa.

References

[1] M. Runge-Morris, Regulation of sulfotransferase gene expression by glucocorticoid hormones and xenobiotics in primary rat hepatocyte culture, Chem. Biol. Interact. 109 (1–3) (1998) 315–327.
[2] C.D. Klaassen, L. Liu, R.T. Dunn 2nd, Regulation of sulfotransferase mRNA expression in male and female rats of various ages, Chem. Biol. Interact. 109 (1–3) (1998) 299–313.
[3] Z. Duanmu, T.A. Kocarek, M. Runge-Morris, Transcriptional regulation of rat hepatic aryl sulfotransferase (SULT1A1) gene expression by glucocorticoids, Drug Metab. Dispos. 29 (8) (2001) 1130–1135.
[4] R.T. Dunn 2nd, B.A. Gleason, D.P. Hartley, C.D. Klaassen, Postnatal ontogeny and hormonal regulation of sulfotransferase SULT1B1 in male and female rats, J. Pharmacol. Exp. Ther. 290 (1) (1999) 319–324.
[5] G.L. Rubin, A.J. Harrold, J.A. Mills, C.N. Falany, M.W. Coughtrie, Regulation of sulphotransferase expression in the endometrium during the menstrual cycle, by oral contraceptives and during early pregnancy, Mol. Hum. Reprod. 5 (11) (1999) 995–1002.
[6] M. Runge-Morris, K. Rose, C.N. Falany, T.A. Kocarek, Differential regulation of individual sulfotransferase isoforms by phenobarbital in male rat liver, Drug Metab. Dispos. 26 (8) (1998) 795–801.
[7] S. Maiti, G. Chen, Tamoxifen induction of aryl sulfotransferase and hydroxysteroid sulfotransferase in male and female rat liver and intestine, Drug Metab. Dispos. 31 (5) (2003) 637–644.
[8] S. Maiti, G. Chen, Methotrexate is a novel inducer of rat liver and intestinal sulfotransferases, Arch. Biochem. Biophys. 418 (2) (2003) 161–168.
[9] A.D. Marshall, P. McPhie, W.B. Jakoby, Redox control of aryl sulfotransferase specificity, Arch. Biochem. Biophys. 382 (1) (2000) 95–104.
[10] T.M. Su, Y.S. Yang, Mechanism of posttranslational regulation of phenol sulfotransferase: expression of two enzyme forms through redox modification and nucleotide binding, Biochemistry 42 (22) (2003) 6863–6870.
[11] A.D. Marshall, J.F. Darbyshire, P. McPhie, W.B. Jakoby, A review of the effects of manipulation of the cysteine residues of rat aryl sulfotransferase IV, Chem. Biol. Interact. 109 (1–3) (1998) 107–116.
[12] A.D. Marshall, J.F. Darbyshire, A.P. Hunter, P. McPhie, W.B. Jakoby, Control of activity through oxidative modification at the conserved residue Cys66 of aryl sulfotransferase IV, J. Biol. Chem. 272 (14) (1997) 9153–9160.
[13] A. Agacdiken, I. Basyigit, M. Ozden, F. Yildiz, D. Ural, H. Maral, H. Boyaci, A. Ilgazli, B. Komsuoglu, The effects of antioxidants on exercise-induced lipid peroxidation in patients with COPD, Respirology 9 (1) (2004) 38–42.
[14] B. Akova, E. Surmen-Gur, H. Gur, M. Dirican, E. Sarandol, S. Kucukoglu, Exercise-induced oxidative stress and muscle performance in healthy women: role of vitamin E supplementation and endogenous oestradiol, Eur. J. Appl. Physiol. 84 (1–2) (2001) 141–147.
[15] J. Bejma, P. Ramires, L.L. Ji, Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and liver, Acta Physiol. Scand. 169 (4) (2000) 343–351.
[16] K.T. Borer, L.L. Bestervelt, M. Mannheim, M.B. Brosamer, M. Thompson, U. Swamy, W.N. Piper, Stimulation by voluntary exercise of adrenal glucocorticoid secretion in mature female hamsters, Physiol. Behav. 51 (4) (1992) 713–718.
[17] M.A. Coleman, T. Garland, Jr., C.A. Marler, S.S. Newton, J.G. Swallow, P.A. Carter, Glucocorticoid response to forced exercise in laboratory house mice (*Mus domesticus*), Physiol. Behav. 63 (2) (1998) 279–285.
[18] I. Girard, T. Garland Jr., Plasma corticosterone response to acute and chronic voluntary exercise in female house mice, J. Appl. Physiol. 92 (4) (2002) 1553–1561.
[19] D. Gomez-Merino, M. Chennaoui, P. Burnat, C. Drogou, C.Y. Guezennec, Immune and hormonal changes following intense military training, Mil. Med. 168 (12) (2003) 1034–1038.
[20] J.L. Copeland, L.A. Consitt, M.S. Tremblay, Hormonal responses to endurance and resistance exercise in females aged 19–69 years, J. Gerontol. A Biol. Sci. Med. Sci. 57 (4) (2002) B158–165.
[21] G. Ravaglia, P. Forti, F. Maioli, L. Pratelli, C. Vettori, L. Bastagli, E. Mariani, A. Facchini, D. Cucinotta, Regular moderate intensity physical activity and blood concentrations of endogenous anabolic hormones and thyroid hormones in aging men, Mech. Ageing Dev. 122 (2) (2001) 191–203.
[22] O. Tissandier, G. Peres, J. Fiet, F. Piette, Testosterone, dehydroepiandrosterone, insulin-like growth factor 1, and insulin in sedentary and physically trained aged men, Eur. J. Appl. Physiol. 85 (1–2) (2001) 177–184.
[23] J. Shaikh, S. Karanth, D. Chakraborty, S. Pruett, C.N. Pope, Effects of daily stress or repeated paraoxon exposures on subacute pyridostigmine toxicity in rats, Arch. Toxicol. 77 (10) (2003) 576–583.
[24] J.B. Watkins 3rd, S.T. Crawford, R.A. Sanders, Chronic voluntary exercise may alter hepatobiliary clearance of endogenous and exogenous chemicals in rats, Drug Metab. Dispos. 22 (4) (1994) 537–543.
[25] H.M. Alessio, A.E. Hagerman, B.K. Fulkerson, J. Ambrose, R.E. Rice, R.L. Wiley, Generation of reactive oxygen species after exhaustive aerobic and isometric exercise, Med. Sci. Sports Exerc. 32 (9) (2000) 1576–1581.

[26] A.K. Banerjee, A. Mandal, D. Chanda, S. Chakraborti, Oxidant, antioxidant and physical exercise, Mol. Cell. Biochem. 253 (1-2) (2003) 307–312.
[27] H.M. Alessio, Exercise-induced oxidative stress, Med. Sci. Sports Exerc. 25 (2) (1993) 218–224.
[28] H.M. Alessio, A.H. Goldfarb, R.G. Cutler, MDA content increases in fast- and slow-twitch skeletal muscle with intensity of exercise in a rat, Am. J. Physiol. 255 (6 Pt 1) (1988) C874–877.
[29] R.A. Fielding, M. Meydani, Exercise, free radical generation, and aging, Aging (Milano) 9 (1) (1997) 12–18.
[30] E.M. Gorostiaga, S.M. Czerwinski, R.C. Hickson, Acute glucocorticoid effects on glycogen utilization, O2 uptake, and endurance, J. Appl. Physiol. 64 (3) (1988) 1098–1106.
[31] S.R. McAnulty, L.S. McAnulty, D.C. Nieman, J.D. Morrow, A.C. Utter, D.A. Henson, C.L. Dumke, D.M. Vinci, Influence of carbohydrate ingestion on oxidative stress and plasma antioxidant potential following a 3 h run, Free Radic. Res. 37 (8) (2003) 835–840.
[32] M. Harkonen, H. Naveri, K. Kuoppasalmi, I. Huhtaniemi, Pituitary and gonadal function during physical exercise in the male rat, J. Steroid Biochem. 35 (1) (1990) 127–132.
[33] J.S. Petrides, G.P. Mueller, K.T. Kalogeras, G.P. Chrousos, P.W. Gold, P.A. Deuster, Exercise-induced activation of the hypothalamic–pituitary–adrenal axis: marked differences in the sensitivity to glucocorticoid suppression, J. Clin. Endocrinol. Metab. 79 (2) (1994) 377–383.
[34] A.M. Butler, M. Murray, Inhibition and inactivation of constitutive cytochromes P450 in rat liver by parathion, Mol. Pharmacol. 43 (6) (1993) 902–908.
[35] M. Murray, A.M. Butler, Hepatic biotransformation of parathion: role of cytochrome P450 in NADPH- and NADH-mediated microsomal oxidation in vitro, Chem. Res. Toxicol. 7 (6) (1994) 792–799.
[36] E.L. Abel, T.K. Bammler, D.L. Eaton, Biotransformation of methyl parathion by glutathione s-transferases, Toxicol. Sci. 79 (2) (2004) 224–232.
[37] M. Murray, A.M. Butler, Identification of a reversible component in the in vitro inhibition of rat hepatic cytochrome P450 2B1 by parathion, J. Pharmacol. Exp. Ther. 272 (2) (1995) 639–644.
[38] C.L. Miranda, M.C. Henderson, D.R. Buhler, Evaluation of chemicals as inhibitors of trout cytochrome P450s, Toxicol. Appl. Pharmacol. 148 (2) (1998) 237–244.
[39] B.A. Rattner, J.C. Franson, Methyl parathion and fenvalerate toxicity in American kestrels: acute physiological responses and effects of cold, Can. J. Physiol. Pharmacol. 62 (7) (1984) 787–792.
[40] A.W. Abu-Qare, M.B. Abou-Donia, Inhibition and recovery of maternal and fetal cholinesterase enzyme activity following a single cutaneous dose of methyl parathion and diazinon, alone and in combination, in pregnant rats, J. Appl. Toxicol. 21 (4) (2001) 307–316.
[41] A.W. Abu-Qare, A. Abdel-Rahman, C. Brownie, A.M. Kishk, M.B. Abou-Donia, Inhibition of cholinesterase enzymes following a single dermal dose of chlorpyrifos and methyl parathion, alone and in combination, in pregnant rats, J. Toxicol. Environ. Health A 63 (3) (2001) 173–189.
[42] Y.S. Huang, L.G. Sultatos, Glutathione-dependent biotransformation of methyl parathion by mouse liver in vitro, Toxicol. Lett. 68 (3) (1993) 275–284.
[43] H.X. Zhang, L.G. Sultatos, Biotransformation of the organophosphorus insecticides parathion and methyl parathion in male and female rat livers perfused in situ, Drug Metab. Dispos. 19 (2) (1991) 473–477.
[44] G. Chen, J. Baron, M.W. Duffel, Enzyme- and sex-specific differences in the intralobular localizations and distributions of aryl sulfotransferase IV (tyrosine-ester sulfotransferase) and alcohol (hydroxysteroid) sulfotransferase a in rat liver, Drug Metab. Dispos. 23 (12) (1995) 1346–1353.
[45] G. Chen, E. Battaglia, C. Senay, C.N. Falany, A. Radominska-Pandya, Photoaffinity labeling probe for the substrate binding site of human phenol sulfotransferase (SULT1A1): 7-azido-4-methylcoumarin, Protein Sci. 8 (10) (1999) 2151–2157.
[46] G. Chen, P.A. Rabjohn, J.L. York, C. Wooldridge, D. Zhang, C.N. Falany, A. Radominska-Pandya, Carboxyl residues in the active site of human phenol sulfotransferase (SULT1A1), Biochemistry 39 (51) (2000) 16000–16007.
[47] G. Chen, D. Zhang, N. Jing, S. Yin, C.N. Falany, A. Radominska-Pandya, Human gastrointestinal sulfotransferases: identification and distribution, Toxicol. Appl. Pharmacol. 187 (3) (2003) 186–197.
[48] G.M. Pacifici, M. Franchi, C. Bencini, F. Repetti, N. Di Lascio, G.B. Muraro, Tissue distribution of drug-metabolizing enzymes in humans, Xenobiotica 18 (7) (1988) 849–856.
[49] I. Rahman, B. Mulier, P.S. Gilmour, T. Watchorn, K. Donaldson, P.K. Jeffery, W. MacNee, Oxidant-mediated lung epithelial cell tolerance: the role of intracellular glutathione and nuclear factor-kappaB, Biochem. Pharmacol. 62 (6) (2001) 787–794.
[50] M.H. Parker, D.J. McCann, J.B. Mangold, Sulfation of di- and tricyclic phenols by rat liver aryl sulfotransferase isozymes, Arch. Biochem. Biophys. 310 (2) (1994) 325–331.
[51] T. Watabe, K. Ogura, M. Satsukawa, H. Okuda, A. Hiratsuka, Molecular cloning and functions of rat liver hydroxysteroid sulfotransferases catalysing covalent binding of carcinogenic polycyclic arylmethanols to DNA, Chem. Biol. Interact. 92 (1–3) (1994) 87–105.
[52] K. Ogura, M. Satsukawa, H. Okuda, A. Hiratsuka, T. Watabe, Major hydroxysteroid sulfotransferase STa in rat liver cytosol may consist of two microheterogeneous subunits, Chem. Biol. Interact. 92 (1–3) (1994) 129–144.
[53] R. Della Morte, G.R. Villani, E. Di Martino, C. Squillacioti, L. De Marco, P. Vuotto, M.A. Belisario, N. Staiano, Glutathione depletion induced in rat liver fractions by seven pesticides, Boll. Soc. Ital. Biol. Sper. 70 (8–9) (1994) 185–192.
[54] D.R. Baldwin, Z.C. Wilcox, G. Zheng, The effects of voluntary exercise and immobilization on humoral immunity and endocrine responses in rats, Physiol. Behav. 61 (3) (1997) 447–453.
[55] M. Runge-Morris, W. Wu, T.A. Kocarek, Regulation of rat hepatic hydroxysteroid sulfotransferase (SULT2-40/41) gene expression by glucocorticoids: evidence for a dual mechanism of transcriptional control, Mol. Pharmacol. 56 (6) (1999) 1198–1206.
[56] H.L. Fang, S. Shenoy, Z. Duanmu, T.A. Kocarek, M. Runge-Morris, Transactivation of glucocorticoid-inducible rat aryl sulfotransferase (SULT1A1) gene transcription, Drug Metab. Dispos. 31 (11) (2003) 1378–1381.