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Suppression of DHEA sulfotransferase (Sult2A1) during the acute-phase response

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Kim, Min Sun, Judy Shigenaga, Art Moser, Carl Grunfeld, and Kenneth R. Feingold. Suppression of DHEA sulfotransferase (Sult2A1) during the acute-phase response. *Am J Physiol Endocrinol Metab* 287: E731–E738, 2004. First published June 15, 2004; 10.1152/ajpendo.00130.2004.—The acute-phase response (APR) induces alterations in lipid metabolism, and our data suggest that this is associated with suppression of type II nuclear hormone receptors that are key regulators of fatty acid, cholesterol, and bile acid metabolism. Recently, the farnesoid X receptor (FXR), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) were found to regulate DHEA sulfotransferase (Sult2A1), which plays an important role in DHEA sulfation and detoxification of bile acids. Because FXR, PXR, and CAR are suppressed during the APR, we hypothesized that Sult2A1 is downregulated during the APR. To induce the APR, mice were treated with LPS, which will then trigger the release of various cytokines, and the mRNA levels of Sult2A1 and the sulfate donor 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (PAPSS2), as well as the enzyme activity of Sult2A1, were determined in the liver. We found that mRNA levels of Sult2A1 decrease in a time- and dose-dependent manner during the LPS-induced APR. Similar changes were observed in the mRNA levels of PAPSS2, the major synthase of PAPS in the liver. Moreover, hepatic Sult2A1 activity and serum levels of DHEA-sulfate (DHEA-S) were significantly decreased in LPS-treated animals. These results suggest that decreased levels or activities of FXR, PXR, and CAR during the APR could contribute to decreases in Sult2A1, resulting in decreased sulfation of DHEA and lower circulating level of DHEA-S. Finally, we found that both TNF and IL-1 caused a significant decrease in the mRNA level of Sult2A1 in Hep3B human hepatoma cells, suggesting that the proinflammatory cytokines TNF and IL-1 mediate the inhibitory effect of LPS on Sult2A1 mRNA level. Our study provides a possible mechanism by which infection and inflammation are associated with altered steroid metabolism and cholestasis.

acute-phase response; lipopolyssacharides; sulfation; pregnane X receptor; dehydroepiandrosterone

SULFOTRANSFERASE (Sult) enzymes catalyze the transfer of a sulfate group from the universal sulfonate (SO_3) donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a wide range of endogenous molecules ranging from neurotransmitters and hormones to xenobiotic compounds (56). Upon the addition of the highly charged sulfonate group, compounds undergo major changes in their physicochemical properties. Sulfonation increases water solubility, converting lipophilic molecules to amphiphiles that remain ionized in solution. As one of the prominent conjugating systems, sulfonation, together with phosphorylation, is essential for normal growth and development, catalyzing an important modification for macromolecules such as proteoglycans and glycosaminoglycans (24, 41), for tyrosine residues of many secretory and membrane proteins and peptides (25, 42), and for sulfoglycolipids such as sphingolipids and galactoglycerolipids (59). Cholesterol and its derivatives (27), bile acids (45), and steroids (12) undergo sulfonation as part of their normal metabolism.

There are two classes of sulfotransferase that are based on their solubility. Membrane-associated insoluble sulfotransferases are located in the trans-Golgi complex, where they are involved in the posttranslational modification of macromolecules (56). Soluble or cytosolic sulfotransferases sulfonate various endogenous compounds, including hormones and neurotransmitters, drugs, and xenobiotics. In mammals, cytosolic sulfotransferases comprise five Sult families with ~40% similarity in amino acid sequences. The Sult1 family transfers sulfonate to catecholamines, estrogenic steroids, thyroid hormones, phenolic drugs, and xenobiotics. The Sult2 family mainly sulfonates neutral steroids and sterols. The Sult3 enzymes catalyze the formation of sulfamates, whereas Sult4 and Sult5 have not yet been characterized.

Dehydroepiandrosterone (DHEA) sulfotransferase (Sult2A1) catalyzes sulfonation of a number of endogenous hydroxysteroids, bile acids, and polycyclic xenobiotics such as certain aromatic carcinogens (30, 44). The substrates of this Sult2A1 family include DHEA, which has been suggested to be a multifunctional hormone with immunoenhancing, anti-cancer, neurotropic, and anti-aging effects (57). Sult2A1 catalyzes conversion of DHEA to DHEA sulfate (DHEA-S), but the physiological significance of this event is not well understood. The serum concentration of DHEA-S is 300–500 times higher than that of DHEA and 20 times higher than that of any other steroid hormone (3). Whereas DHEA-S is bound to albumin and forms a circulating reservoir, DHEA has been reported to be more active at the tissue level (3). Only lipophilic DHEA can be converted intracellularly to androgens and estrogen, and the biosynthesis of all sex steroids in humans proceeds through DHEA. Because only the unconjugated steroid hormone DHEA has growth-promoting activity, changes in the regulation of Sult2A1 activity could be implicated in a variety of physiological states and pathophysiological disorders (3).

Also, sulfation of the toxic bile acid lithocholic acid (LCA) by Sult2A1 increases water solubility, decreases reabsorption, and facilitates the clearance of LCA (46). Therefore, sulfation of LCA protects liver from its toxicity-induced cholestasis (60).

Transcription of Sult2A1 has been shown to be regulated by type II nuclear hormone receptors farnesoid X receptor (FXR)

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(53), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) (55). FXR is involved in the homeostasis of bile acid synthesis from cholesterol and bile acid transport. The primary bile acid chenodeoxycholic acid (CDCA), which is the most potent ligand for FXR, strongly increases the mRNA level of Sult2A1 through an inverted repeat-0, or IR-0, cognate to the FXR/retinoic acid receptor (RXR)α response element (53). PXR has also been shown to regulate Sult2A1. PXR regulates genes involved in the detoxification of xenobiotics, and activation of PXR induces the transcription of target genes that encode oxidative cytochrome *P*-450 enzymes and drug transporters (7–9, 29). Sonoda et al. (55) identified a PXR/RXRα response element in the Sult2A1 gene promoter region and showed that stimulation of PXR with its ligand pregnenolone-16α-carbonitrile (PCN) resulted in marked induction of Sult2A1 and PAPS synthase 2 (PAPSS2) (55). Recently, Maglich et al. (34) and Saini et al. (48) showed that Sult2A1 is also regulated by CAR. Therefore, altered activity of FXR/RXRα, CAR/RXRα, and/or PXR/RXRα under pathological conditions could lead to changes in the mRNA levels of Sult2A1 and PAPSS2, regulating the metabolism of hydroxysteroids and bile acids.

Because activities of PXR (6), CAR (6), and FXR (28), together with their heterodimer partner RXRα, are significantly suppressed during the APR, we hypothesized that mRNA level and enzyme activity of Sult2A1 would be also downregulated. If so, this change could contribute to the decrease in DHEA-S seen during infection and in inflammatory diseases such as rheumatoid arthritis and arteritis (43). To test this hypothesis, we chose C57BL/6 mice as an experimental model because of their high circulating levels of DHEA-S compared with those of other rodents (38) and treated them with very low doses of lipopolysaccharide (LPS), an inducer of APR. Our results demonstrate that the mRNA levels of Sult2A1 and PAPSS2 are dramatically decreased in mouse liver during the LPS-induced APR. Furthermore, we demonstrate that the LPS-induced decrease in Sult2A1 mRNA levels was accompanied by a significant reduction in its activity and in circulating levels of DHEA-S. Our present study provides a possible mechanism for the changes in the sulfation of hydroxysteroids and bile acids observed during the APR.

MATERIALS AND METHODS

Materials. DHEA {[1,2,6,7-^{3}H(N)]-dehydroepiandrosterone} was purchased from PerkinElmer Life Sciences (Boston, MA). DHEA, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), and the PXR agonist PCN were purchased from Sigma.

Animals. All animal experimentation described in the present study was approved by the Animal Studies Subcommittee at the San Francisco VA Medical Center, and the protocol meets current AAALAC standards. Eight-week-old female C57BL/6 mice were purchased from Jackson Laboratory. Only female mice were used in the present study because they do not fight as much as males, and fighting leads to inflammatory responses. The animals were maintained in a normal light-cycle room and were provided with rodent chow and water ad libitum. Their weight was ~20 g at the time of experiment. Anesthesia was induced with halothane. To determine the effect of the APR on Sult2A1 and PAPSS2 mRNA levels, mice were injected intraperitoneally with 100 μg of LPS in saline or with saline alone. For the PCN experiment, mice were injected daily with 40 mg of PCN/kg body wt for 3 days followed by LPS administration for a 16-h period. Food was withdrawn at the time of injection, since LPS induces anorexia in rodents (16). Livers were removed at the time after treatment indicated in the figure legends. The doses of LPS used in this study have significant effects on triglyceride and cholesterol metabolism (13, 14) but are not lethal, because the half-maximal lethal dose (LD_{50}) for LPS in rodents is ~5 mg/100 g body wt.

Preparation of cytosolic extract from mouse liver. Fresh mouse liver was collected into glass tubes and homogenized in buffer containing (in mM) 250 sucrose, 5 HEPES, and 2 2-mercaptoethanol, pH 7.4. Homogenates were centrifuged at 11,000 *g* for 2 min. The resulting supernatants were centrifuged for 30 min at 105,000 *g*, and the cytosolic fractions (supernatants) were stored in small aliquots at −70°C until use.

Estimation of protein. Protein content of hepatic cytosolic extract was estimated using Bio-Rad protein quantitation reagent, with bovine serum albumin as standard.

Measurement of Sult2A1 enzyme activity. The enzyme activity of Sult2A1 was measured on the basis of a technique with isotopically labeled substrate (52). Briefly, the reaction mixture (250 μl) comprised 50 μg of cytosolic protein from mouse liver, 0.1 μCi [^{3}H]DHEA, 0.8 μM unlabeled DHEA, 1 mM MgCl2, 60 μM PAPS, and 60 mM Tris·HCl, pH 7.5. Assays included a control incubation containing no PAPS. After a 20-min reaction at room temperature, 250 μl of 250 mM Tris·HCl, pH 8.7, and 3 ml of chloroform were added, and the tubes were shaken vigorously before centrifugation at 3,000 *g* for 5 min. Two hundred microliters of the supernatant were mixed with 8 ml of scintillation fluid, and radioactivity was quantified by liquid scintillation spectrometry. The enzyme activity was expressed as %control.

RNA isolation and Northern blot analysis. Total mouse RNA was isolated from 300−400 mg of snap-frozen whole liver tissue by use of Tri-Reagent (Sigma). Poly(A)$^{+}$ RNA was subsequently purified using oligo(dT) cellulose. RNA was quantified by measuring absorption at 260 nm. Ten micrograms of poly(A)$^{+}$ were denatured and electrophoresed on a 1% agarose-formaldehyde gel. Total RNA from Hep 3B was isolated from a 100-mm dish by the Tri-Reagent method and resuspended in diethyl pyrocarbonate (DEPC)-water. Thirty micrograms of total RNA were denatured and electrophoresed as described above. The uniformity of RNA loading was checked by UV visualization of 18S and 28S bands on the ethidium bromide-stained gel before electrotransfer to Nytran membrane (Schleicher & Schuell). Prehybridization, hybridization, and washing procedures were performed as described previously (36). Membranes were probed with [α-^{32}P]dCTP-labeled cDNAs with the random priming technique (Amersham Biosciences, Little Chalfont, UK). mRNA levels were detected by exposure of the membrane to X-ray film and were quantified by densitometry. Housekeeping genes such as cylophilin D were used to confirm the equal amount of RNA loading. Sult2A1 and PAPSS2 probes were prepared by use of PCR with the following primers: mouse Sult2A1 5′-GTCAGGAACGAACTGGCTGATT-3′ (upper), 5′-GCTTCAGCTTGGGCTACTGTG-3′ (lower); human Sult2A1 5′-GCACTCAGTGAAAGCGAGAGT-3′ (upper), 5′-TGGGCCACTGTGAAGTGATTT-3′ (lower); Sult2B1 5′-TCTCGGAAATCAGCCAGAAGTT-3′ (upper), 5′-TGCAGCTCCTCGTAGGTGATA-3′ (lower); mouse PAPSS2 5′-GCCCGAAAAATCCACGAATC-3′ (upper), 5′-GACCATTGTGGACAGGATTG-3′ (lower).

Serum DHEA and DHEA-S measurement. Serum concentrations of DHEA and DHEA-S were measured using the ^{125}I RIA kit by following the manufacturer's instruction (ICN).

Statistical analysis. Data are expressed as means ± SE of experiments from 3–5 animals or plates for each groups or time point. The difference between two experimental groups was analyzed using the Student's *t*-test. Differences among multiple groups were analyzed using one-way analysis of variance with the Bonferroni post hoc test. A P value < 0.05 was considered significant.

RESULTS

LPS administration decreases Sult2A1 mRNA levels during the APR. Initially, we determined the effect of LPS administration on the Sult2A1 mRNA level in mouse liver at various times up to 24 h. As shown in Fig. 1*A*, LPS administration caused a significant decrease in the mRNA level of Sult2A1 as early as 4 h after treatment (~50% of the control); this decrease plateaued at 8 h and persisted for at least 24 h. Next, we determined whether Sult2A1 mRNA decreases in a dose-dependent manner in response to LPS administration (Fig 1*B*). Our preliminary studies indicated that the mRNA level of Sult2A1 responded very sensitively with a dramatic reduction, when mice were treated with doses of LPS >0.1 μg. Therefore, we tested a wide range of doses of LPS from 10^{-4} to 10^{2} μg/mouse. As shown in Fig. 1*B*, a statistically significant decrease in Sult2A1 mRNA was caused by a dose of 1 ng/mouse or higher, and the maximal decrease was observed with a dose of ~1 μg/mouse. Figure 1*C* shows that 100 μg of LPS did not affect GAPDH mRNA level in mouse liver, indicating that equal amounts of RNA were loaded and that the effect of LPS on Sult2A1 is specific. These data indicate that the LPS-induced decrease in Sult2A1 mRNA is highly sensitive and dose dependent in mouse liver, with the half-maximal effect occurring at a dose of <1 ng LPS/mouse.

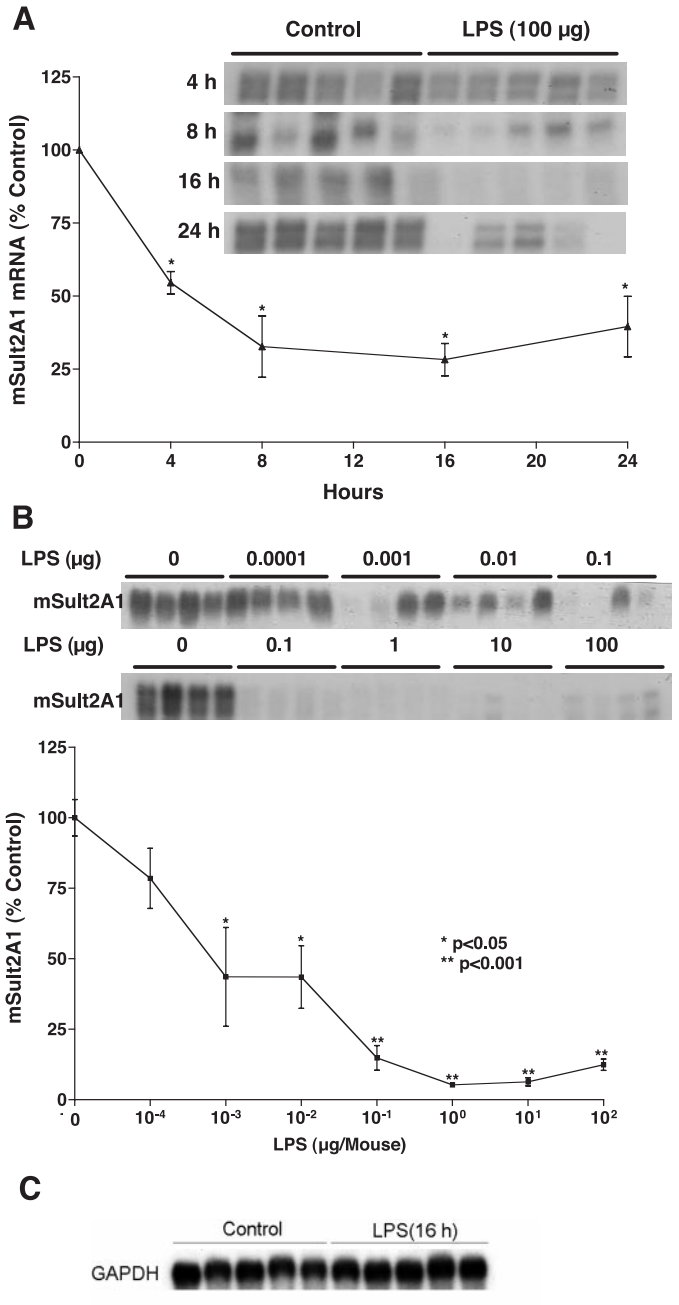


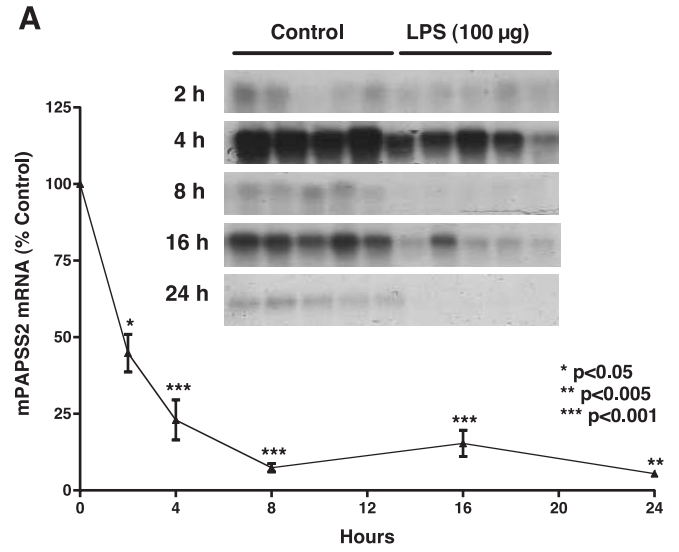
Fig. 1. LPS significantly decreases dehydroepiandrosterone (DHEA) sulfotransferase (Sult2A1) mRNA levels in mouse liver. *A*: time course of C57BL/6 mice injected ip with either saline or LPS (100 μg of LPS per mouse), with animals killed at time indicated after LPS administration. *B*: dose response of C57BL/6 mice injected ip with LPS at various concentrations, as indicated, with animals killed 16 h after LPS administration. *C*: GAPDH mRNA poly(A)$^+$ RNA was prepared from liver, and Northern blot analysis was carried out as described in METHODS. Data (means ± SE; n = 4~5) are expressed as % of control. Variation in Northern blotting of control mice at different times after LPS administration is because hybridizations were performed at different times. *P < 0.05 vs. control; **P < 0.001 vs. control.

It should be noted that the effect of LPS on the mRNA level of Sult2A1 is specific, because LPS did not affect the mRNA level of cholesterol sulfotransferase (Sult2B1) (data not shown).

LPS administration decreases mRNA level of PAPSS2 in mouse liver. Because we observed that the mRNA level of Sult2A1 is regulated during the APR, we next determined whether LPS administration also decreases mRNA level of PAPSS2. This enzyme is primarily responsible for producing the sulfonate group donor PAPS in the liver and has been shown to be activated by PXR (55). As shown in Fig. 2*A*, as early as 2 h after administration, LPS caused a >50% decrease in the mRNA level of PAPSS2. At 4 h after LPS administration, there was a >75% decrease (P < 0.001), with further decreases at 8 h that persisted for a 24-h period. These data indicate that the PAPSS2 mRNA level rapidly decreased after LPS administration and that the decrease in PAPSS2 mRNA after LPS administration was dose dependent. As shown in Fig. 2*B*, LPS administration decreased PAPSS2 mRNA level, with the half-maximal effect occurring at ~0.5 ng/mouse. All doses but the lowest tested in the experiment caused a significant reduction (P < 0.005) in PAPSS2 mRNA levels in mouse liver.

LPS administration significantly decreases Sult2A1 activity in mouse liver homogenate. Having observed a significant reduction in mRNA levels of both Sult2A1 and PAPSS2 in mouse liver, we hypothesized that a reduced mRNA level of these genes would ultimately result in a decrease in Sult2A1 enzyme activity. As shown in Fig. 3*A*, 16 h after LPS administration, Sult2A1 activities in liver were significantly decreased by ~60% (P < 0.01) compared with those of the control mouse. These data clearly demonstrate that an LPS-induced decrease in Sult2A1 mRNA level is associated with a reduced level of hepatic Sult2A1 activity.

LPS administration significantly decreases the level of serum DHEA-S. In mammals, DHEA is the primary substrate for sulfation reaction by Sult2A1, and altered sulfation status of DHEA could affect functionality of DHEA steroid hormones. Because Sult2A1 enzyme activity is downregulated in LPS-treated mouse liver, as shown in Fig. 3*A*, we next determined whether these changes in Sult2A1 activity led to reduced levels of serum DHEA-S. As shown in Fig. 3*B*, 24 h after LPS



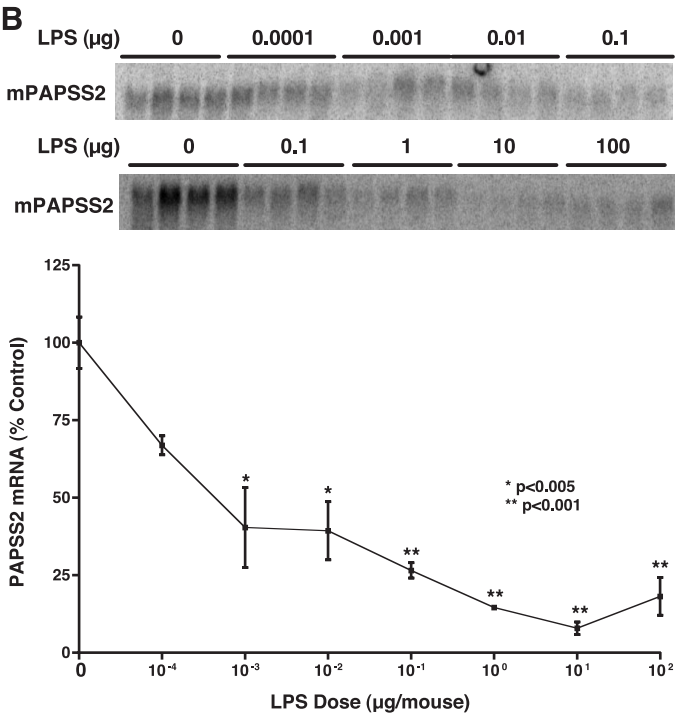


Fig. 2. LPS decreases 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (PAPSS2) expression in mouse liver. *A*: time course of C57BL/6 mice injected ip with either saline or LPS (100 μg of LPS per mouse), with animals killed at time indicated after LPS administration. *B*: dose response of C57BL/6 mice injected ip with LPS at various concentrations, as indicated, with animals killed 16 h after LPS administration. Poly(A)$^+$ RNA was prepared from liver, and Northern blot analysis was carried out as described in METHODS. Data (means ± SE, $n = 4\sim5$) are expressed as % of control. Variation in Northern blotting of control mice at different times after LPS administration is because hybridizations were performed at different times. *$P < 0.05$ vs. control; **$P < 0.001$ vs. control.

administration, serum DHEA-S levels were significantly decreased by 50% compared with control mice. Thus decreased mRNA level of Sult2A1 is associated with reduction in enzyme activity and the circulating levels of DHEA-S during LPS-induced APR.

mRNA level of Sult2A1 is increased by PCN administration and inhibited by LPS administration. Because Sult2A1 has been reported to be regulated by PXR (55), we next determined whether administration of a PXR ligand increased the mRNA level of Sult2A1 and whether LPS still exerted its inhibitory effect on Sult2A1 mRNA level after PXR activation. For this experiment, the PXR ligand PCN (40 mg PCN/kg body wt) was administered to mice for three consecutive days. As shown in Fig. 4*A*, PCN administration caused a more than threefold increase in the mRNA levels of Sult2A1. However, administration of LPS after the 3 days of PCN treatment decreased Sult2A1 mRNA level to 50% of the basal (Control) level. These data confirm that Sult2A1 is activated by PXR and demonstrate that LPS strongly decreases mRNA level of Sult2A1 even in the presence of a PXR ligand. Next, we determined whether the changes of Sult2A1 mRNA level with PXR stimulation were reflected in the circulating levels of DHEA-S. PCN administration caused an almost twofold increase in the serum DHEA-S levels in mice (Fig. 4*B*). How-

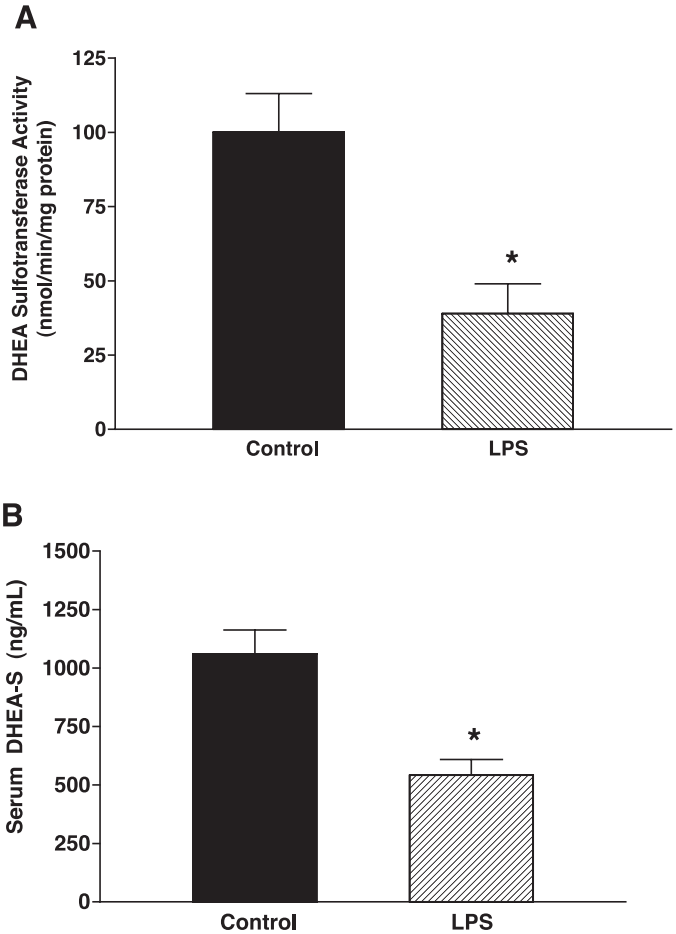


Fig. 3. LPS administration decreases activity of Sult2A1 in mouse liver. *A*: effect of LPS on Sult2A1 activity. C57BL/6 mice were injected ip with either saline or LPS (100 μg of LPS per mouse), and animals were killed at 16 h after LPS administration. Cytosolic extract was prepared and used for the enzyme assay, as described in METHODS. *B*: effect of LPS on serum DHEA sulfate (DHEA-S). C57BL/6 mice were injected ip with either saline or LPS (100 μg of LPS per mouse). Animals were killed 24 h after LPS administration, and serum was collected for the DHEA-S assay using the ^{125}I RIA kit (ICN), as described in METHODS. Data (means ± SE, $n = 5$) are expressed as % of control. *$P < 0.05$ vs. control.

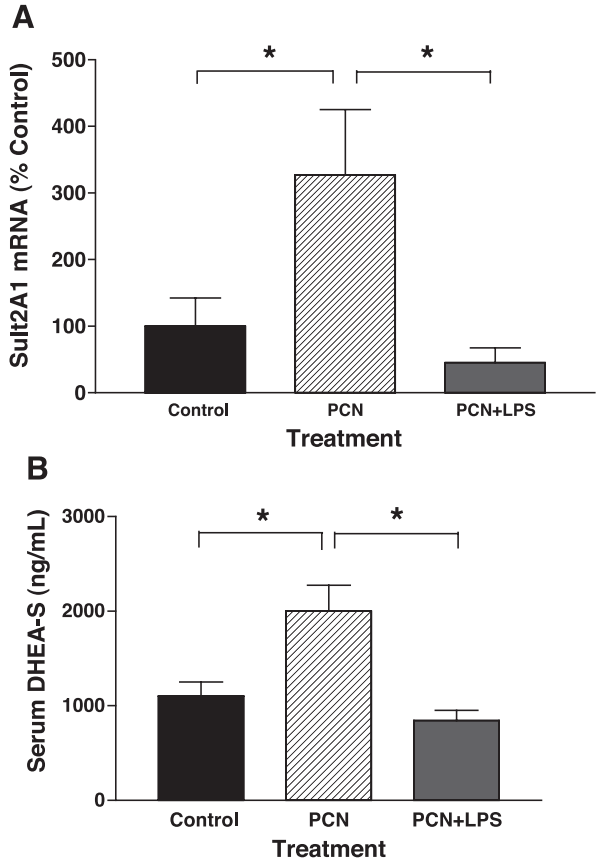


Fig. 4. LPS-induced inhibition of pregnanolone-16α-carbonitrile (PCN)-activated Sult2A1. *A*: effect on Sult2A1 expression. C57BL/6 mice were injected with PCN (0.8 μg/mouse each time) daily for 3 days followed by LPS administration (100 μg of LPS per mouse). After 16 h, animals were killed, and poly(A)$^+$ RNA was prepared from liver. To determine mRNA level of Sult2A1, Northern blot analysis was carried out, as described in METHODS, and the mRNA level of cyclophilin D was used for normalization. *B*: effect on serum DHEA-S. Serum samples were obtained from experiment described in *A*, and level of DHEA-S was measured using the ^{125}I RIA kit (ICN) as described in METHODS. Data (means ± SE, $n = 4\sim5$) are expressed as % of control. *$P < 0.05$ vs. control.

ever, LPS administration in conjunction with PCN decreased serum DHEA-S concentrations to below control levels. It should be noted that the serum concentration of DHEA was not affected by any of these treatments (data not shown). These data strongly support the finding that Sult2A1 is regulated by PXR and demonstrate that the level of serum DHEA-S correlates with the mRNA level of Sult2A1.

TNF and IL-1, but not IL-6, decrease Sult2A1 mRNA in Hep 3B cells. It is well established that the physiological effects of LPS are mediated by proinflammatory cytokines, such as TNF, IL-1, and IL-6. To determine whether these cytokines also decrease Sult2A1 mRNA in vitro, the human hepatoma cell line Hep 3B cells were treated with cytokines at 10 ng/ml for 24 h, and RNA was isolated to determine the level of Sult2A1 mRNA with Northern blot analysis. As shown in Fig. 5, TNF treatment decreased Sult2A1 mRNA by almost 90%, and IL-1 decreased Sult2A1 by almost 80% compared with control levels. However, IL-6 did not significantly affect Sult2A1 mRNA level in Hep 3B cells (data not shown). These results suggest that the effect of LPS on Sult2A1 mRNA level is mediated by TNF and IL-1, but not by IL-6.

DISCUSSION

Infection, inflammation, and trauma induce a wide range of metabolic changes in the liver as part of the APR and result in alterations in the concentrations of plasma proteins that are collectively called acute-phase proteins (4, 32). During the APR, the serum levels of proteins such as C-reactive protein and serum amyloid A (SAA) increase (positive acute-phase proteins), whereas the serum levels of proteins such as albumin and transferrin decrease (negative acute-phase proteins) (4, 32, 39). LPS induces the APR by stimulating the release of cytokines such as TNF, IL-1, and IL-6 from macrophages and other cells (1, 2), which then mediate the biological action of LPS (17, 19, 28). Low doses of LPS induce release of TNF and IL-1 (1), and a dose of as little as 1 ng/mouse can cause the APR, as indicated by the induction of SAA (Kim MS, Moser A, Grunfeld C, and Feingold K, unpublished data).

In the present study, we determined whether the mRNA level and activity of Sult2A1 are altered during LPS-induced APR in a rodent model. Here we demonstrate that LPS, an inducer of the APR, dramatically decreases the mRNA levels of both Sult2A1 and PAPSS2, an enzyme that synthesizes the sulfate donor PAPS in mouse liver. The inhibitory effect of LPS on the mRNA levels of these two enzymes occurred at very low doses; the mRNA levels of both Sult2A1 and PAPSS2 are significantly decreased at a dose of 1 ng of LPS, making these changes among the most sensitive of host responses to LPS. The mRNA levels of the two genes demonstrate a similar time course, but the mRNA level of PAPSS2 decreased earlier and remained at a lower level than that of Sult2A1 during the 24-h period of study. We also show that these changes were accompanied by a decrease in the enzyme activity of Sult2A1. Furthermore, we show that the decrease in

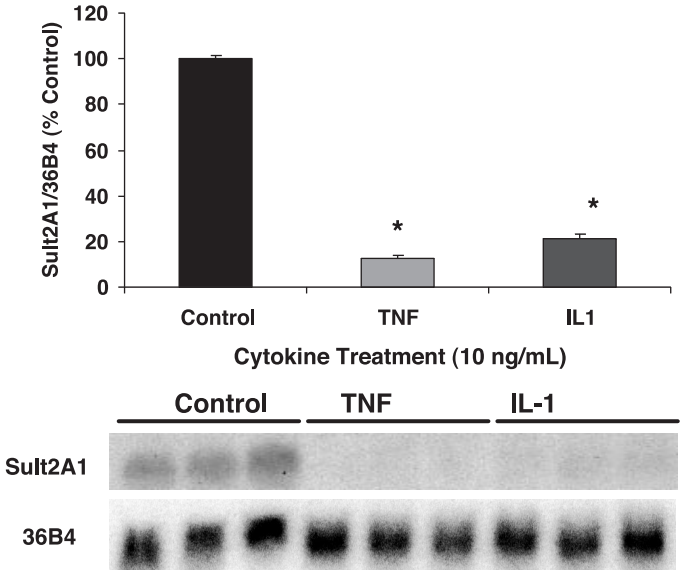


Fig. 5. TNF and IL-1 decrease expression of Sult2A1 in Hep 3B human hepatoma cells. About 7×10^5 Hep 3B cells were plated in 100-mm petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were washed twice with PBS, and medium was replaced with fresh MEM (without serum) plus 0.1% bovine albumin and the appropriate cytokine at 10 ng/ml. After 24-h incubation, total RNA was isolated, and Northern blot analysis was performed as described in METHODS with human Sult2A1 cDNA. Ribosomal protein 36B4 was used as a normalizing control. Data (means ± SE, $n = 3$) are expressed as % of control. *$P < 0.05$ vs. control.

enzyme activity resulted in a significant reduction in the circulating level of DHEA-S, suggesting that there may be significant alterations in the sulfation of hydroxysteroids, drugs, and bile acids during infection and inflammation. Finally, the proinflammatory cytokines TNF and IL-1 caused a significant decrease in the mRNA levels of human Sult2A1 in Hep 3B human hepatoma cells, consistent with our previous findings of an inhibitory effect of TNF and IL-1 on the steady-state level of mRNA along with protein of nuclear hormone receptors, including RXRs, PPARs (peroxisome proliferator-activated receptors), LXR, FXR, CAR and PXR, with minimal effect of IL-6 (28). Therefore, our data suggest that LPS-induced decrease in the mRNA level of Sult2A1 could be mediated by TNF and/or IL-1.

There are several possible mechanisms by which the mRNA level of Sult2A1 is downregulated during the LPS-induced APR. First, LPS-induced repression of PXR/RXRα, CAR/RXRα, and/or FXR/RXRα activity may cause the decreased transcription of Sult2A1. We previously showed that mRNA levels and DNA-binding activity of RXRα and FXR are significantly decreased in the liver of rodents administered LPS (5, 28). Also, decreases in FXR were accompanied by decreased mRNA levels of its target genes, including apolipoprotein CII and small heterodimer partner. Similarly, LPS administration almost abolished hepatic expression of PXR and CAR by 4 h in mouse liver (6). When we consider the regulatory role of PXR, CAR, and FXR on the expression of Sult2A1, it is possible that repression of these nuclear hormone receptors may have caused a reduction in the transcription and, therefore, the mRNA level of Sult2A1. However, the sensitive response of Sult2A1 to LPS-induced APR indicates that repression of FXR/RXRα, CAR/RXRα, and PXR/RXRα may play a limited role in decreasing the mRNA levels of Sult2A1. The half-maximal dose for LPS-induced decrease in the heterodimer partner RXRα and in FXR mRNA was ~0.5 μg/mouse. The half-maximal effect for PXR was observed at ~1 μg of LPS/mouse (Moser A, Grunfeld C, and Feingold K, unpublished data). On the other hand, the half-maximal effect for Sult2A1 was observed at <1 ng of LPS/mouse, a dose which does induce the classic APR, because mRNA levels of SAA are increased (Kim MS, Grunfeld C, and Feingold K, unpublished data). The dose response for Sult2A1 is almost 1,000-fold more sensitive than the dose of LPS that decreases FXR/RXRα or PXR/RXRα. Taken together, repression of RXRα and its heterodimer partners FXR, PXR, and CAR during the APR may coordinately play a role in decreasing mRNA levels of Sult2A1 and PAPSS2, but other factors may contribute.

Second, besides nuclear hormone receptors, other transcription factors may be involved in the regulation of Sult2A1 transcription. Song et al. (54) demonstrated that there are two hepatocyte nuclear factor (HNF)-1 and three CCAAT/enhancer-binding protein (C/EBP) response elements in the rat Sult2A1 promoter, suggesting that they may play a role in the liver-selective expression of the Sult2A1 gene (54). Also, these authors found evidence for the presence of transcription factors other than FXR and RXR at the −169/−193 element, which includes the orphan HNF-4 and possibly Fushi-Tarazu factor-1 or liver receptor homolog-1 (LRH-1). Because mRNA levels of HNF-1, HNF-4, and LRH-1 are significantly decreased during the APR (28, 37), it is possible that decreases in multiple transcription factors may account for the dramatic decreases in the mRNA levels of Sult2A1 during the LPS-induced APR.

Third, altered expression of coactivators and corepressors may be involved in the altered level of Sult2A1. Coactivators do not bind to DNA but play an important role in the transcriptional regulation of nuclear hormone receptors′ target genes (23, 35, 47). Although there is little known about the changes in the mRNA level of corepressors during the APR, hepatic mRNA levels of certain coactivators, including steroid receptor coactivator-1 and PGC-1, are significantly decreased (Kim MS, Grunfeld C, and Feingold K, unpublished data). Because their role in the transcriptional activity of PXR and/or FXR is not clearly understood, further studies are needed to clarify the role of altered mRNA levels of coactivators in the transcriptional regulation of Sult2A1.

Changes in hepatic Sult2A1 can result in alterations in the hormonal activity and responsiveness. It has been shown that androgen sensitivity of the rat liver is reciprocally correlated with the hepatic expression of Sult2A1 (10). Increased Sult2A1 can attenuate androgen receptor function, as reflected by the loss of androgen induction of its target genes (10), whereas decreased Sult2A1 activity may ensure a maximal androgenic responsiveness in selected target tissues. Sult2A1-mediated sulfation of steroids is related to the metabolic inactivation of steroids; therefore, altered expression of Sult2A1 may cause changes in the steroid action in target tissues. The present study demonstrates that the circulating levels of DHEA-S are decreased during the LPS-induced APR, consistent with other reports that serum DHEA-S levels significantly decrease in many chronic inflammatory diseases (18, 20, 22, 43, 49). However, our study found that the circulating levels of DHEA are not affected during LPS-induced APR or by the PCN-induced PXR activation (data not shown). Thus the APR or PXR activation specifically regulates the serum levels of DHEA-S via Sult2A1 but does not affect the synthesis of DHEA. The importance of the circulating levels of DHEA and DHEA-S has not been well elucidated, but they are thought to be markers or risk factors for neoplasia and cardiovascular diseases. The implication of decreased levels of DHEA-S remains to be determined.

Altered sulfation activity caused by decreased Sult2A1 expression could also affect drug metabolism during the APR. PXRs coordinately regulate drug-metabolizing enzymes in the activation (*phase I*) and conjugation/inactivation (*phase II*) processes that include CYP3A and Sult2A1. It has been shown that the common drug acetaminophen is metabolized through PXR-mediated glucuronidation and sulfation, and that both of these processes are enhanced in rodent liver by PCN or dexamethasone treatment (51). In the present study, we show that PCN increases Sult2A1 mRNA and activity and that simultaneous administration of LPS abolishes the induction caused by PCN. Similarly, we have previously shown that another PXR ligand, RU-486, which is a glucocorticoid antagonist, significantly increased mRNA level of the PXR target gene Cyp3A and that this induction was abolished by LPS treatment (6). Therefore, suppression of Sult2A1 together with Cyp3A could contribute to the altered drug metabolism during the APR.

Results from the present study may also help to explain the LPS-induced impairment of bile flow and cholestasis (11).

LCA is a hydrophobic secondary bile acid that is primarily formed in the intestine by the bacterial dehydroxylation of chenodeoxycholic acid, and it has been related to intrahepatic cholestasis (15, 26). Sulfation by Sult2A1 is important for detoxification of LCA, as evidenced by a study in which nonsulfated LCA produced a marked cholestasis when introduced to animals, whereas sulfated LCA did not (33), and by the demonstration of the protective effect of PCN against cholestasis (50). Decreased Sult2A1 activity during the LPS-induced APR, as shown in the present study, could result in decreased sulfation of LCA, contributing to the development of cholestasis. Together with the rapid downregulation of bile acid transporter proteins during the APR (21, 40), the potent inhibitory effect of LPS on the mRNA level and activity of Sult2A1 may explain the mechanisms by which the APR is associated with hepatotoxicity and cholestasis.

The significant downregulation of PAPSS2 mRNA level during the APR could inhibit a broad range of biological processes that require a sulfonate group. PAPSS2 is the primary hepatic synthase of PAPS, the universal sulfate donor used in all known posttranslational sulfation reactions. PAPSS2 is a bifunctional enzyme with ATP sulfurylase/APS kinase activities and catalyzes the metabolic activation of inorganic sulfate to PAPS. Potent inhibition of PAPSS2 mRNA level during the APR found in the current study could be problematic for the sulfation of many biologically important molecules. For example, reduced activities of both ATP sulfurylase and APS kinase, with decreased synthesis of chondroitin sulfate, have been demonstrated in the inherited brachymorphic mouse, whose cartilage is characterized by small, diffuse proteoglycan granules and reduced staining for sulfated glycosaminoglycans (31, 58). Further studies are needed to elucidate the role that downregulation of PAPSS2 plays in the pathophysiology of inflammatory diseases.

In summary, we find that the mRNA level of Sult2A1 is significantly decreased during LPS-mediated APR in rodent liver. Also, the mRNA level of PAPSS2 was similarly downregulated, suggesting that the synthesis of sulfate donor molecule PAPS could be decreased. These changes were accompanied by decreases in the hepatic activity of Sult2A1 and in the circulating levels of DHEA-S, implying that the LPS-induced changes in Sult2A1 expression could alter the sulfation of steroids, drugs, and bile acids. Furthermore, PXR ligand PCN dramatically increased the mRNA level of Sult2A1, and this induction was also abolished by LPS administration, indicating that LPS exerts its inhibitory effect on Sult2A1 expression even in the presence of the PXR ligand PCN. These results help to explain the mechanisms by which various infection and inflammatory conditions are associated with low levels of DHEA-S, altered drug metabolism, and the development of cholestasis.

GRANTS

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