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Depression of Constitutive Murine Cytochromes P450 by Staphylococcal Enterotoxin B

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ABSTRACT. Most *in vivo* studies demonstrating decreased activities of hepatic cytochromes P450 with inflammation have used Gram-negative bacterial lipopolysaccharide (LPS) as the inflammatory stimulant. But products of Gram-positive bacteria, such as staphylococcal enterotoxin B (SEB), also stimulate inflammatory mediators, albeit with a different pattern than LPS. Therefore, effects of SEB on the regulation of murine constitutive P450s were determined in this study and compared with those of LPS. LPS-responsive C3H/HeN and LPS-unresponsive C3H/HeJ mice were injected with either LPS (0.5 mg/kg) or SEB (0.66 to 6.6 mg/kg), and hepatic cytochromes P450 and serum tumor necrosis factor-α, interleukin-6, nitrate/nitrite, and serum amyloid A concentrations were determined up to 24 hr. HeJ mice were generally less responsive than HeN mice to both stimuli, with lower cytokine, nitrate/nitrite, and serum amyloid A responses. However, in both mouse strains SEB caused more prolonged cytokine, higher nitrate/nitrite, and lower serum amyloid A concentrations than LPS. Despite these differences, in HeN mice, after both SEB and LPS administration, total P450 concentrations were equally depressed by 40%. Both SEB and LPS depressed CYP1A1 and 1A2 microsomal protein concentrations by 45 and 30%, respectively; CYP2E1 by 64%; and CYP3A by 70%. There was comparable inhibition of enzymatic activities. In HeJ mice, SEB was only slightly more effective in depressing P450s than LPS, as might be expected. These data showed that the Gram-positive bacterial inflammatory stimulant SEB caused effects on murine hepatic cytochromes P450 similar to those of LPS, even though the pattern of inflammatory mediators induced after SEB exposure was different. BIOCHEM PHARMACOL **59**;10:1295–1303, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. staphylococcal enterotoxin B; lipopolysaccharide; murine; C3H; HeJ mice; cytokines; cytochrome P450; mixed-function oxidases; nitric oxide

The inflammatory acute phase response induced by Gram-negative bacterial endotoxin is associated with down-regulation of hepatic cytochromes P450 [1–4]. The use of endotoxin (LPS§) in most studies reflects the clinical interest in pathophysiological responses to Gram-negative bacterial sepsis. However, Gram-positive organisms and their toxic products are just as important in causing the "systemic inflammatory response syndrome" in humans [5]. Infection with the Gram-positive bacterium *Listeria monocytogenes* has been shown to suppress hepatic P450 activities and mRNA in mice [6, 7], with the secreted hemolysin from this microbe being a necessary factor for these effects [8]. The staphylococcal enterotoxins [9] are a family of proteins secreted by another Gram-positive bacterium that is a common cause of sepsis. All the staphylococcal enterotoxins—A, B, C, D, and E—can induce extensive pathological changes in humans and animals. The mechanisms of their toxicity are not defined completely, but it is known that they are serologically distinct polypeptide chains (22–35 kDa) sharing significant sequence homology [9, 10]. By binding to major histocompatibility complex II proteins, they can stimulate T cells [11–13], macrophages [14, 15], and natural killer cells [16]. Because they stimulate T cell proliferation and cytokine production by binding to the V_β component of the T cell receptor without the need for macrophage processing, they have been called "superantigens."

It has long been known that purified SEB can cause shock [17], and the effects of Gram-positive bacterial products such as SEB have been compared with those of Gram-negative LPS in septic shock models [18–23], with similarities in cytokine cascades [21]. In the D-galactosamine-treated mouse model, SEB causes hepatotoxicity similar to that of LPS [22], although others have shown less sensitization to lethality and less TNF release with *Staphylococcus aureus* infection in this model when compared to *Escherichia coli* infection [23]. Furthermore, the murine cecal ligation and puncture model of sepsis, which includes both Gram-negative and Gram-positive toxic mediators, has demonstrated the existence of endotoxin- and TNF-

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§ *Abbreviations*: EROD, ethoxyresorufin-*O*-deethylase; ERY-N-DM, erythromycin *N*-demethylase; IL-6, interleukin-6; LPS, lipopolysaccharide; PNP-OH, *p*-nitrophenol hydroxylase; SAA, serum amyloid A; SEB, staphyloccocal enterotoxin B; and TNF, tumor necrosis factor-α.

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independent pathways activating widespread apoptosis [24]. It is possible that these alternate pathways are mediated by Gram-positive bacterial toxins. It is not yet clear whether there are major differences in the way the hepatocyte responds to SEB administered *in vivo*, and no one has yet reported the effect of SEB on hepatic P450 regulation.

This study compares the effects of SEB and LPS in both LPS-responsive C3H/HeN mice and LPS-unresponsive C3H/HeJ mice, which have a mutation in the *Tlr4* gene, rendering them less responsive to LPS signaling [25, 26]. The data shows that in both mouse strains, SEB provokes an acute phase response, but with a pattern of inflammatory mediators slightly different from that of LPS. However, despite these differences, SEB depresses total hepatic P450 concentrations and decreases microsomal concentrations of CYPs 1A1, 1A2, 2E1, and 3A proteins and their corresponding enzymatic activities, with effects comparable to those of LPS.

MATERIALS AND METHODS

Chemicals

All reagents used were of the highest purity commercially available and were purchased from the Sigma Chemical Co. or Amersham. Equipment and reagents for SDS–PAGE and western blotting were obtained from Bio-Rad Laboratories and Amersham. Antibodies used were as follows: rabbit anti-mouse CYP1A1 and 1A2 were gifts from Drs. J. Sinclair and P. Sinclair (VA Medical Center); a rabbit anti-rat CYP3A western blot kit was purchased from Amersham; goat anti-rat CYP2E1 antibody was purchased from Gentest. Rabbit anti-mouse SAA polyclonal antibody was prepared by harvesting serum from BALB/c mice injected with 25 μg/kg body weight of *E. coli* 0111:B4 endotoxin (LPS-Difco) for 24 hr, isolating plasma high density lipoproteins, and separating the fractions containing SAA by gel filtration [27]. The pooled and concentrated SAA fraction was sent to LabLogix for polyclonal antibody production in rabbits, and this antibody was used for Western immunoblot analysis as described below. Mouse TNF and IL-6 ELISA kits were purchased from Genzyme.

Animals and Treatments

The Lexington VA Animal Studies Subcommittee of the Research Service approved these studies. Six- to eight-week-old male C3H/HeN and C3H/HeJ mice weighing 25–30 g were obtained from Harlan Sprague Dawley Inc. and Jackson Laboratories, respectively. Animals were housed in plastic cages with cedar bedding in Bioclean units with a 12-hr light/dark cycle and were acclimated to the animal facility for at least 1 week before use. Mice received Mouse Chow Diet-50 (Purina) and water *ad lib*.

LPS at a dose of 0.5 mg/kg or SEB from *S. aureus* (Toxin Technology Inc.) at doses from 0.66 to 6.6 mg/kg was dissolved in pyrogen-free saline and administered i.p. to mice in a final volume of 0.1 to 0.15 mL. Control groups received equivalent volumes of pyrogen-free saline. Similarly treated mice were housed together and marked by ear notching. After treatment, mice were denied food but allowed water *ad lib*. At various periods of time after injection up to 24 hr, mice were anaesthetized with inhaled methoxyflurane. Blood was collected by retroorbital bleeding, the abdominal cavities were opened, and the livers were removed quickly. Then the mice were euthanatized by cervical dislocation.

Analytical Techniques

MICROSOMAL PREPARATION. Livers were weighed and rinsed with cold saline, and a 20% homogenate was prepared at 4° in 0.15 M KCl/0.25 M phosphate buffer, pH 7.4. The microsomal fraction was isolated as described previously [28]. A final 0.15 M KCl wash removed any residual red blood cells. Total cytochrome P450 concentrations, expressed as picomoles per milligram of microsomal protein, were assayed on a Hewlett-Packard 8451A diode array spectrophotometer using CO-reduced minus reduced difference spectra, and proteins were assayed by the Lowry method.

MIXED-FUNCTION OXIDASE ACTIVITIES. EROD for CYP 1A1/1A2 was measured in the microsomal fraction by the method of Burke and Mayer [29]. ERY-N-DM for CYP3A was measured with erythromycin as a substrate by the method of Matsubara *et al.* [30], and the amount of formaldehyde produced was determined colorimetrically with Nash reagent. PNP-OH for CYP2E1 was assayed using a 4-nitrophenol to 4-nitrocatechol conversion measurement [31]. Activities were expressed as picomoles (EROD, ERY-N-DM) or nanomoles (PNP-OH) per milligram of microsomal protein per minute of incubation time.

CYTOKINE ASSAY. After collection into 1-mL borosilicate tubes, blood was allowed to clot at room temperature for 1 hr, and was centrifuged at 2000 *g* for 30 min at 4°; serum was transferred to Eppendorf tubes and stored at −20°. Murine TNF and murine IL-6 were determined in 50 μL of serum with ELISA kits (Genzyme) according to the manufacturer's specifications, and values were expressed as picograms per milliliter of serum.

SERUM NITRITE/NITRATE ANALYSIS. Total NO_2 + NO_3 levels in the serum were measured by the procedure described by Green *et al.* [32] as modified by Vodovotz [33]. Nitrates (NO_3) were reduced to nitrites (NO_2) with metallic cadmium and assayed using Griess reagent. Results were expressed as micromolar concentrations. Serum samples from LPS- and SEB-treated animals were diluted 3-fold prior to the assay.

WESTERN IMMUNOBLOT TECHNIQUES. Concentrations of SAA protein and CYPs 1A1, 1A2, 2E1, and 3A proteins were determined using appropriate antibodies. Sera were

TABLE 1. Dose–response effect of SEB in comparison to LPS on hepatic P450s in C3H/HeN mice

	Controls	LPS (0.5 mg/kg)	SEB (0.66 mg/kg)	SEB (3.3 mg/kg)	SEB (6.6 mg/kg)
SAA	0.14 ± 0.07	2.45 ± 0.12*	0.74 ± 0.11*	1.47 ± 0.12*	2.25 ± 0.23*
(relative units)	**100%**	**1750%**	**528%**	**1050%**	**1607%**
Total P450	0.96 ± 0.08	0.55 ± 0.09*	0.62 ± 0.06*	0.55 ± 0.02*	0.52 ± 0.11*
(nmol/mg protein)	**100%**	**60%**	**68%**	**60%**	**57%**
CYP1A1 protein	0.31 ± 0.06	0.34 ± 0.05	0.25 ± 0.08	0.26 ± 0.05	0.17 ± 0.05*
(relative units)	**100%**	**109%**	**81%**	**84%**	**55%**
CYP1A2 protein	1.44 ± 0.32	1.04 ± 0.34	1.09 ± 0.32	0.92 ± 0.09*	1.00 ± 0.05*
(relative units)	**100%**	**72%**	**76%**	**64%**	**69%**
EROD (1A1/1A2)	82.46 ± 16.1	46.2 ± 6.8*	61.62 ± 10.6*	58.33 ± 8.0*	50.48 ± 7.1*
(pmol/mg/min)	**100%**	**56%**	**75%**	**71%**	**61%**
CYP2E1 protein	0.44 ± 0.16	0.16 ± 0.05*	0.16 ± 0.06*	0.14 ± 0.03*	0.17 ± 0.07*
(relative units)	**100%**	**36%**	**36%**	**32%**	**39%**
PNP-OH (2E1)	1.08 ± 0.2	0.40 ± 0.05*	0.59 ± 0.1*	0.47 ± 0.1*	0.45 ± 0.1*
(nmol/mg/min)	**100%**	**37%**	**55%**	**44%**	**42%**
CYP3A protein	2.48 ± 0.14	0.74 ± 0.04*	0.89 ± 0.19*	0.84 ± 0.27*	0.82 ± 0.10*
(relative units)	**100%**	**30%**	**36%**	**34%**	**33%**
ERY-N-DM (3A)	4.68 ± 0.4	3.35 ± 0.01*	3.64 ± 0.32*	3.28 ± 0.27*	2.18 ± 0.18*
(pmol/mg/min)	**100%**	**72%**	**78%**	**70%**	**47%**

C3H/HeN mice were injected with either LPS or SEB at the doses shown and were euthanatized 24 hr later. Hepatic microsomes were prepared, the activities of EROD, PNP-OH, and ERY-N-DM were determined, and CYPs 1A1, 1A2, 2E1, and 3A protein concentrations were analyzed as described in the text. Values are the means ± SD of 4–9 animals from 2–3 different experiments. Numbers in bold represent percent of control.

*Significantly different from control ($P < 0.05$).

separated on a gradient (5–20%) minigel PAGE, and microsomal proteins on 10% minigel PAGE. Electrophoresis was carried out for 50 min at 200 V and 4°. For CYP1A1/1A2, a modified electrophoresis buffer system of mixed anionic detergent/aliphatic alcohols [34] was used to facilitate separation of the individual isoforms. Separated proteins were electrotransferred onto nitrocellulose membranes and visualized using enhanced chemiluminescence. Detection reagents were horseradish peroxidase bound to a secondary antibody (anti-rabbit or anti-goat) to catalyze oxidation of luminol in the presence of hydrogen peroxide and an enhancer reagent (Amersham). The emitted light was captured on x-ray film (Kodak TMG/RA-1) for 15 sec to 5 min to give a stable hard copy of the results. All visualized bands were scanned on an Ultroscan XL image analyzer (Pharmacia), and data were expressed as relative absorbance units.

Statistics

To determine statistical significance, one-way ANOVA was used. If a significant F-statistic was found at $P = 0.05$, pairwise comparisons were made between experimental groups according to Fisher's protected least significant differences method.

RESULTS

Physical Responses of Mice to SEB and LPS

Two to three hours after SEB or LPS administration, all C3H/HeN mice developed symptoms of lethargy and ruffled fur. Diarrhea and poor water intake were more pronounced after LPS treatment than after SEB treatment. No lethality occurred by 24 hr, but all mice (controls and treated) lost similar amounts of body weight (1–3 g) due to food deprivation. In contrast to the C3H/HeN mice, no visible symptoms of toxicity were noted up to 24 hr in C3H/HeJ mice treated with LPS or SEB.

Dose-Dependent Changes in Inflammatory Mediators Caused by SEB and Compared with LPS in C3H/HeN Mice

Table 1 shows the changes in SAA proteins and hepatic cytochromes P450 for C3H/HeN mice 24 hr after treatment with LPS and increasing doses of SEB. A dose-dependent increase in SAA concentration over control was seen after SEB treatment with doses of 0.66, 3.3, and 6.6 mg/kg, corresponding to increases of 5-, 10-, and 16-fold; the highest dose provoking a response was equivalent to 0.5 mg/kg of LPS (17-fold). Total constitutive microsomal cytochrome P450 concentration demonstrated decreases ranging from 68 to 57% of the control value, but with no statistically significant differences between the 0.66, 3.3, and 6.6 mg/kg doses. These decreases were comparable to those of LPS (60% of control).

Several individual constitutive P450 isoforms in the uninduced mouse liver were then evaluated. We chose to investigate the CYPs 1A, 2E, and 3A subfamilies, since these P450s represent important human isoforms. As shown in Table 1, at 24 hr after LPS or SEB treatment, decreases were noted for CYPs 1A, 2E1, and 3A P450 proteins and their corresponding EROD, PNP-OH, and ERY-N-DM activities. SEB administered at a dose of 6.6 mg/kg decreased the respective P450 proteins to 55, 69, 39, and 33% of control for 1A1, 1A2, 2E1, and 3A, which correlated

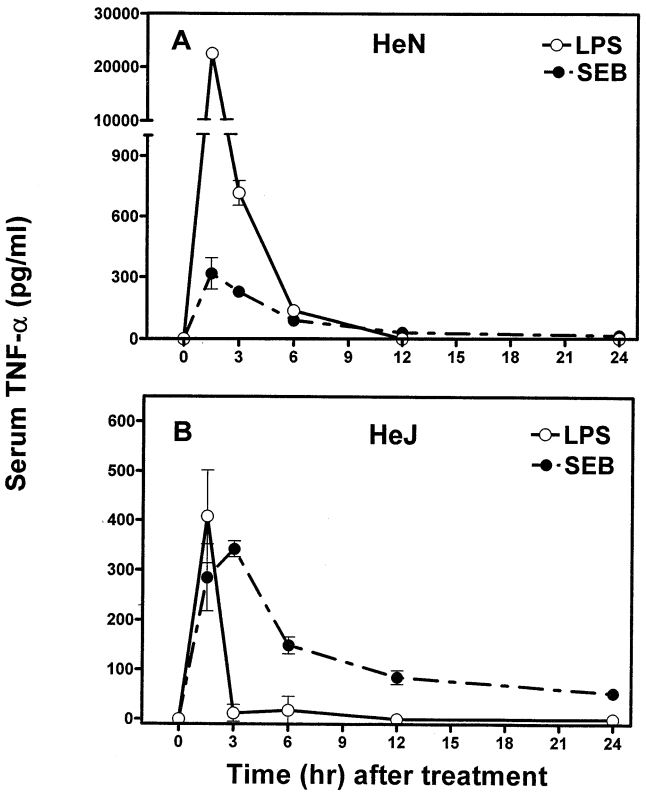

FIG. 1. Serum TNF concentrations at different times after LPS (○, 0.5 mg/kg) and SEB (●, 3.3 mg/kg) administered i.p. to LPS-responsive C3H/HeN (A) and LPS-unresponsive C3H/HeJ (B) mice. Each point represents the mean ± SD of 3 animals in each group.

well with corresponding decreases in activities of 61, 42, and 47% of control for EROD, PNP-OH, and ERY-N-DM. LPS at a dose of 0.5 mg/kg depressed proteins to 72, 36, and 30% of control for CYPs 1A2, 2E1, and 3A, corresponding to 56, 37, and 72% of control for EROD, PNP-OH, and ERY-N-DM. For this LPS dose, decreases in protein levels correlated well with decreased activities. For SEB the decreases were nearly maximal at the lowest dose used (0.6 mg/kg). CYP1A1, which represented approximately 18% of 1A protein, was not decreased except at the SEB dose of 6.6 mg/kg. Because SEB at a dose of 3.3 mg/kg seemed to cause changes equal to those of LPS at a dose of 0.5 mg/kg, we carried out the remainder of our experiments with this dose of SEB.

Time Course of Changes in Inflammatory Mediators after SEB Injections Compared with LPS in Both Mouse Strains

The changes in P450s in the C3H/HeN mice most likely were due to effects of inflammatory cytokines produced after SEB and LPS administration. We next examined both C3H/HeN and C3H/HeJ mice at various time points after SEB and LPS treatment for elaboration of the serum cytokines TNF and IL-6 as well as for serum nitrate/nitrite concentrations. As shown in Fig. 1, LPS caused a transient rise in serum TNF, maximal at 1.5 hr. As would be expected, TNF was much higher in the LPS-responsive HeN mice than in the LPS-unresponsive HeJ mice (22,560 vs 408 pg/mL, a 55-fold difference). SEB also caused peaks in serum TNF, the values being much lower than after LPS in the HeN mice, but of the same magnitude as after LPS in the HeJ mice. After SEB, TNF concentrations remained elevated for much longer (Fig. 1). The HeJ mice still had detectable serum TNF levels 24 hr after SEB. The *Tlr4* mutation in the HeJ mice, which inhibits their response to LPS [25, 26], did not depress the TNF response to SEB, and TNF concentrations at all time points after 1.5 hr were higher than in the HeN mice.

Figure 2 demonstrates serum IL-6 responses. LPS again provoked a much higher peak concentration of 6146 pg/mL at 3 hr in the HeN mice than the peak of 260 pg/mL at 1.5 hr in the HeJ mice. Unlike the TNF response, however, after SEB administration, IL-6 concentrations in the sera of HeN mice were much higher (2158 and 2695 pg/mL at 3 and 6 hr), than in the sera of the HeJ mice (580 and 560 pg/mL at 3 and 6 hr). Despite an approximately 5-fold difference in magnitude, the time-course profile of the IL-6 response after SEB was similar in the two mouse strains, and again showed a more prolonged presence in the serum than after LPS. The time courses of changes in nitrite/nitrate concentrations (stable end products of NO oxidation) in

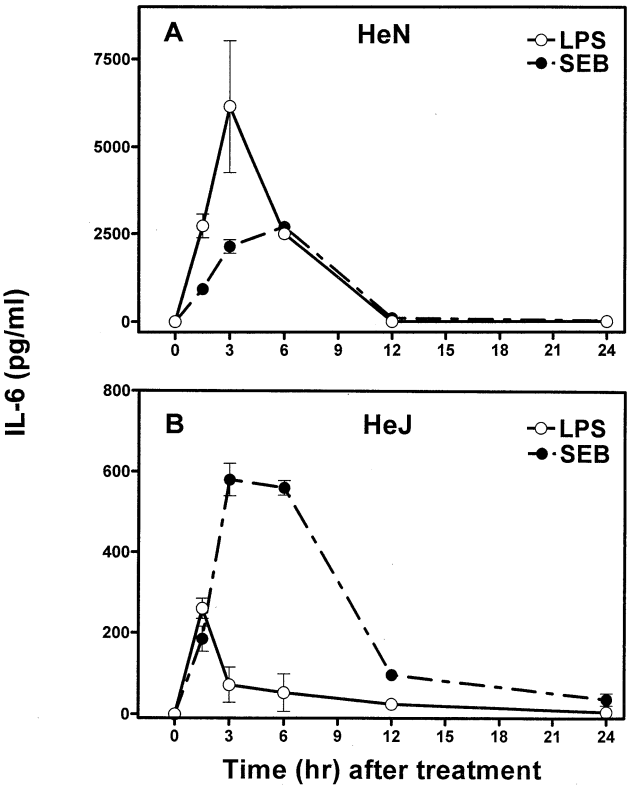

FIG. 2. Serum IL-6 concentrations at different times after LPS (○, 0.5 mg/kg) or SEB (●, 3.3 mg/kg) administered i.p. to LPS-responsive C3H/HeN (A) and LPS-unresponsive C3H/HeJ (B) mice. Each point represents the mean ± SD of 3 animals in each group.

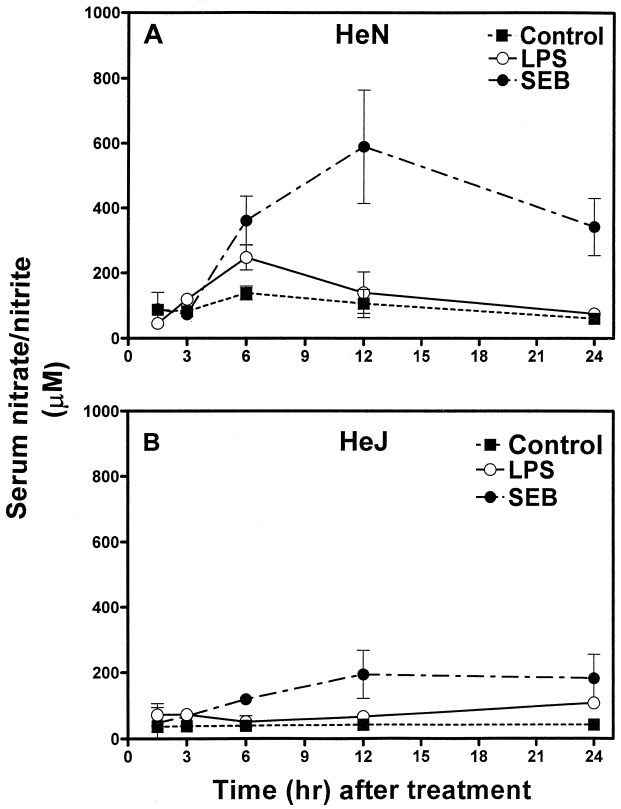

FIG. 3. Time-course changes of serum nitrate/nitrite levels in C3H/HeN (A) and C3H/HeJ (B) mice after a single injection of saline vehicle (■), LPS at a dose of 0.5 mg/kg (○), and SEB at a dose of 3.3 mg/kg (●). The micromolar concentration levels are expressed as means ± SD of 3 animals in each group.

mouse serum were studied in both mouse strains after LPS and SEB administration (Fig. 3). For both LPS and SEB, the nitrite/nitrate response was much more pronounced in the C3H/HeN mice. Peak levels were detected between 6 and 12 hr after both treatments and corresponded to 247 ± 38 and 589 ± 176 μM for LPS and SEB, respectively, in C3H/HeN mice, and 107 ± 6 and 194 ± 73 μM for LPS and SEB, respectively, in C3H/HeJ mice. These results were intriguing because they showed a greater NO response to SEB treatment than to LPS treatment in both mouse strains.

Time-course data were also obtained for SAA responses after exposure to SEB and LPS. As shown in Fig. 4, LPS caused a more rapid acute phase response in the HeN mice, with 10-fold elevations by 3–6 hr as opposed to 2- to 4-fold elevations in the HeJ mice. By 24 hr, however, SAA levels in both mouse strains were not statistically different, with 14- to 20-fold elevations over control. After SEB, the SAA response was again more rapid in the HeN mice, but not as high as with LPS at 24 hr (very similar to the data in Table 1), and the HeJ mice clearly had lower responses than the HeN mice.

Time Course of Changes in Cytochromes P450 after SEB Treatment in C3H/HeN Mice

Changes in the concentrations of total cytochromes P450 as well as in the three subfamilies (CYPs 1A, 2E1, and 3A) after 3.3 mg/kg of SEB at 6, 12, and 24 hr and after 0.5 mg/kg of LPS at 24 hr in C3H/HeN mice are shown in Table 2. Although different mice were used for these experiments than for the experiments presented in Table 1, decreases at 24 hr after LPS and SEB were similar. Most decreases did not become significant until 12 hr after treatment (except for EROD activity and CYP3A protein activity) and were maximal at 24 hr. Time points beyond this were not assessed.

Comparison of the Effects of SEB to Those of LPS in C3H/HeJ Mice

Effects of both SEB (3.3 mg/kg) at 6, 12, and 24 hr and LPS (0.5 mg/kg) at 24 hr after treatment of C3H/HeJ mice are shown in Table 3. By 24 hr, total P450 concentrations were decreased to 69% of control after SEB, and after LPS treatment, the drop was to 81% of control. There were associated decreases in activities of EROD, PNP-OH, and ERY-N-DM and their corresponding concentrations of P450 proteins. SEB generally was more potent at depressing activities, to 66, 56, and 51% of the control values, respectively, than LPS treatment, which reduced them to 78, 89, and 58% of control.

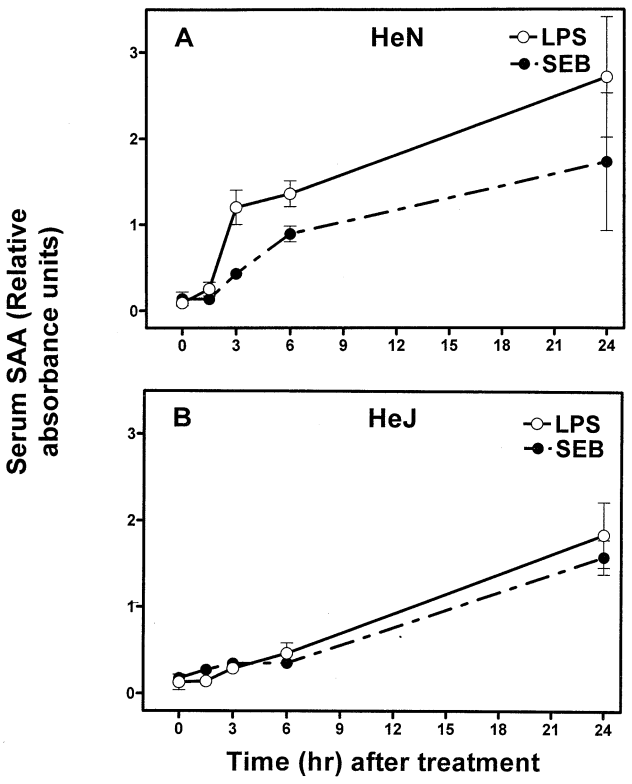

FIG. 4. SAA concentrations at different time points after LPS (○, 0.5 mg/kg) or SEB (●, 3.3 mg/kg) administration to LPS-responsive C3H/HeN (A) and LPS-unresponsive C3H/HeJ (B) mice. Results from immunoblots (see Materials and Methods) are expressed in relative absorbance units, and each point is the mean ± SD from 3 animals (for 1.5 hr and 3 hr) or 5 animals (for control, 6 hr, and 24 hr) in each group.

TABLE 2. Time–course effect of SEB in comparison to LPS on hepatic P450s in C3H/HeN mice

	Control	LPS (24 hr)	SEB (6 hr)	SEB (12 hr)	SEB (24 hr)
Total P450	0.87 ± 0.13	0.59 ± 0.08*	0.74 ± 0.05	0.61 ± 0.07*	0.56 ± 0.03*
(nmol/mg protein)	**100%**	**67%**	**85%**	**70%**	**64%**
CYP1A1 protein	0.45 ± 0.04	0.42 ± 0.10	0.32 ± 0.04	0.39 ± 0.08	0.33 ± 0.06
(relative units)	**100%**	**93%**	**71%**	**86%**	**73%**
CYP1A2 protein	1.73 ± 0.13	1.35 ± 0.13	1.67 ± 0.19	1.29 ± 0.07*	1.05 ± 0.16*
(relative units)	**100%**	**78%**	**96%**	**75%**	**61%**
EROD (1A1/1A2)	70.54 ± 8.7	59.92 ± 4.6*	58.35 ± 14.7*	58.13 ± 8.8*	45.05 ± 9.0*
(pmol/mg/min)	**100%**	**85%**	**82%**	**82%**	**64%**
CYP2E1 protein	0.87 ± 0.11	0.57 ± 0.09	0.84 ± 0.03	0.55 ± 0.04*	0.50 ± 0.01*
(relative units)	**100%**	**65%**	**96%**	**63%**	**57%**
PNP-OH (2E1)	1.59 ± 0.1	1.16 ± 0.1*	1.57 ± 0.3	1.15 ± 0.2*	0.94 ± 0.1*
(nmol/mg/min)	**100%**	**73%**	**98%**	**72%**	**59%**
CYP3A protein	1.82 ± 0.12	0.33 ± 0.07*	1.42 ± 0.07*	1.01 ± 0.10*	0.51 ± 0.05*
(relative units)	**100%**	**18%**	**78%**	**55%**	**28%**
ERY-N-DM (3A)	4.72 ± 0.4	2.27 ± 0.3*	2.02 ± 0.3*	1.97 ± 0.2*	1.74 ± 0.1*
(pmol/mg/min)	**100%**	**48%**	**42%**	**42%**	**36%**

C3H/HeN mice were injected with either LPS (0.5 mg/kg) or SEB (3.3 mg/kg) and were euthanatized 24 hr later. Hepatic microsomes were prepared, the activities of EROD, PNP-OH, and ERY-N-DM were determined, and CYPs 1A1, 1A2, 2E1, and 3A protein concentrations were analyzed as described in the text. Values are the means ± SD of 4–14 animals from 2–3 different experiments. Numbers in bold represent percent of control.

*Significantly different from control ($P < 0.05$).

The immunoblots performed for CYP1A1/1A2, CYP2E1, and CYP3A showed statistically significant changes only in CYP3A protein concentration at 12 hr (55% of control) and 24 hr (51% of control) after SEB administration in these HeJ mice. Despite the semiquantitative nature of immunoblots, the relative units of CYP3A 24 hr after SEB (0.32 ± 0.03) were significantly lower than after LPS (0.52 ± 0.10). The CYP2E1 protein amount decreased to 73% of control, and CYP1A1/1A2 proteins (which could not be separated into distinctly measurable bands) to 75% of control; these changes did not reach statistical significance. There were no significant decreases 24 hr after LPS in any of the specific proteins. The immunoblot data from the HeJ mice compared with that of the HeN mice (Tables 1 and 2) might suggest that control constitutive concentrations of CYP2E1 proteins were much higher and CYP3A proteins much lower than in the HeN mice. However, because the microsomal proteins from the HeJ and HeN mice were not analyzed in the same minigels, and values could only be expressed as relative units, it is not possible to draw such a conclusion. In fact, since P450 activities are so consistent between the HeN and HeJ mice and since it is likely that the specific activities of the P450 isoforms are the same, the differences in relative units of the proteins between Tables 1, 2, and 3 are probably due to differences in the immunoblot technique. However, the immunoblot data in Table 3 do show that in the HeJ mice, SEB was more potent than LPS at depressing CYP3A protein.

TABLE 3. Effects of SEB and LPS on hepatic P450s in C3H/HeJ mice

	Controls	LPS (24 hr)	SEB (6 hr)	SEB (12 hr)	SEB (24 hr)
Total P450	0.88 ± 0.09	0.65 ± 0.03*	0.98 ± 0.13	0.60 ± 0.04*	0.61 ± 0.11*
(nmol/mg)	**100%**	**81%**	**111%**	**68%**	**69%**
CYP1A1/1A2 protein	1.36 ± 0.23	0.99 ± 0.33	1.45 ± 0.40	1.11 ± 0.44	1.03 ± 0.31
(relative units)	**100%**	**73%**	**106%**	**81%**	**75%**
EROD (1A1/1A2)	96.1 ± 4.4	75.9 ± 0.13*	91.1 ± 6.17	63.2 ± 5.60*	63.2 ± 7.8*
(pmol/mg/min)	**100%**	**78%**	**94%**	**66%**	**66%**
CYP2E1 protein	1.74 ± 0.44	1.89 ± 0.51	1.23 ± 0.22	1.29 ± 0.16	1.27 ± 0.23
(relative units)	**100%**	**108%**	**70%**	**74%**	**73%**
PNP-OH (2E1)	1.31 ± 0.06	1.17 ± 0.18	1.13 ± 0.17	0.77 ± 0.07*	0.74 ± 0.05*
(nmol/mg/min)	**100%**	**89%**	**86%**	**59%**	**56%**
CYP3A protein	0.63 ± 0.17	0.52 ± 0.10	0.64 ± 0.16	0.35 ± 0.13	0.32 ± 0.03*
(relative units)	**100%**	**83%**	**101%**	**56%**	**51%**
ERY-N-DM (3A)	5.61 ± 1.06	3.27 ± 0.22*	4.34 ± 0.61	3.09 ± 0.86*	2.85 ± 0.30*
(pmol/mg/min)	**100%**	**58%**	**76%**	**55%**	**51%**

C3H/HeJ mice were injected with either LPS (0.5 mg/kg) or SEB (3.3 mg/kg) and were euthanatized 24 hr later. Hepatic microsomes were prepared, the activities of EROD, PNP-OH, and ERY-N-DM were determined, and CYPs 1A1, 1A2, 2E1, and 3A protein concentrations were analyzed as described in the text. Results are expressed as means ± SD of 3–5 animals in each group. Numbers in bold represent percent of control.

*Significantly different from control ($P < 0.05$).

DISCUSSION

It is well known that the inflammatory response elicited by LPS includes depressed hepatic cytochrome P450-dependent drug metabolism in mice, rats, and humans [1–4]. However, since Gram-positive bacterial sepsis is as common as Gram-negative sepsis [5], it is also important to examine effects of Gram-positive inflammatory stimulants on hepatic drug metabolism. Renton and coworkers [6–8] have shown that the Gram-positive *L. monocytogenes* hemolysin can depress hepatic drug metabolism. It was the purpose of the current study to extend these data by looking at a toxic secreted product from *S. aureus*, which is a more common cause of Gram-positive sepsis. Because the inflammatory response seen after SEB has many of the same features as that induced by LPS [18–23], it was decided to directly compare the effects of both inflammatory mediators on the regulation of hepatic P450s in an *in vivo* murine model.

In the current study, SEB or LPS was administered to both C3H/HeN LPS-responsive and C3H/HeJ LPS-nonresponsive mice (with a *Tlr4* mutation that causes defective LPS signaling [25, 26]) to see if the effects of SEB on P450s might be mediated by the same pathways as the effects of LPS. By comparing cytokine and NO responses with changes in P450s in both strains given SEB or LPS, it was hoped that a more complete picture could be derived as to how SEB might affect P450 regulation. Because the dose of the inflammatory stimulant and the subsequent intensity of the acute phase response are probably critical factors in altering P450s, initial dose-finding experiments were performed in the HeN mice (Table 1). These data determined that SEB at 3.3 mg/kg, i.p., depressed total microsomal P450 concentrations to a level that corresponded to an LPS dose of 0.5 mg/kg. At these doses, both inflammatory stimulants markedly induced the hepatic production of the major murine acute phase protein, SAA, after 24 hr, but with LPS slightly more potent (17.5-fold) than SEB (10.5-fold).

Because much evidence suggests the importance of proinflammatory cytokines in the regulation of hepatic P450s [28, 35–38], serum TNF and IL-6 were monitored after LPS and SEB administration. These cytokines also up-regulate inducible nitric oxide synthase (iNOS), which produces NO, a mediator that not only plays an important role in the pathogenesis of septic shock but also has been implicated in regulating P450s during inflammation [39–41], although this point has been controversial [42–44]. Others [45–47] have reported previously the differences in cytokine and NO responses between HeN and HeJ mice after LPS, but in those studies 10-fold higher doses of LPS were used [46] or live *E. coli* bacteria were administered [45, 47]. The data from the current study are in general agreement with these previous studies showing attenuated cytokine responses in HeJ mice, but may conflict with regard to the NO response (see below).

The current study shows that in the LPS-responsive HeN mice, LPS caused much higher concentrations of TNF and IL-6 than SEB, but with SEB the cytokines remained in the serum slightly longer. In the LPS-unresponsive HeJ mice, there were still easily measurable cytokine responses after LPS, but they were attenuated, as would be expected. With SEB, the TNF response was not attenuated in the HeJ mice compared with the HeN mice, but the IL-6 response clearly was attenuated (Figs. 1 and 2). In addition, after both LPS and SEB administration the HeJ mice did not develop as many physical signs of distress as did the HeN mice. A muted reactivity to both SEB and LPS in the HeJ mice was also seen in the SAA responses (Fig. 4) and the NO responses (Fig. 3). With regard to the latter, although the current results do not seem to agree with data reported by Evans *et al.* [45], they do agree with recent data from Nowicki *et al.* [47]. Despite this possible discrepancy in assessing the NO response, all of the above findings suggest that the defect in the HeJ mouse, which is a mutation in the Tlr4 receptor important for cellular LPS signaling [25, 26], must also attenuate responses to other inflammatory stimuli such as SEB.

In SEB-treated mice (both strains), serum NO_2/NO_3 concentrations were higher and more sustained than in LPS-treated mice (Fig. 3). The above findings support the proposed protective role of NO in SEB-induced shock, as has been suggested [48]. But a higher serum NO_2/NO_3 concentration in response to SEB was associated with more lethality in IL-10 knockout mice [49]. Despite this difference, these data confirm that SEB potently induces iNOS. In fact, the current study suggests that SEB induces iNOS to a greater extent than LPS, at least at the doses of SEB and LPS used, which depress hepatic P450s equally. However, an explanation for this difference is not apparent from the current data, and a higher dose of LPS might generate a maximal NO response that surpasses that of SEB.

With regard to the role of NO in depressing hepatic P450s during inflammation, initial studies [39, 40] suggested that hepatic NO production by increased iNOS in hepatocytes might explain the depression seen in P450s in rat livers after LPS. Unfortunately Sewer and Morgan [42, 43] were unable to confirm these findings *in vivo* or in primary rat hepatocytes, and Sewer *et al.* [44], using an iNOS knockout mouse, have now shown convincingly that NO is not necessary for the suppression of CYPs 2C-like, 2E1, and 3A-like mRNAs and proteins in mice 24 hr after LPS. The data from the current study also cast doubt on a role for NO in suppressing activities of hepatic P450s, since SEB stimulated more serum NO_2/NO_3 than LPS in both strains of mice, but was no more effective in suppressing P450s.

Because SEB elicited different cytokine (TNF and IL-6) and NO responses than LPS, it was hypothesized that there would be major differences in the regulation of P450s after each of these inflammatory stimuli. However, despite there being differences in the patterns of cytokine responses and NO production after LPS and SEB in both strains of mice, these differences were mainly quantitative rather than

qualitative. Probably because of the broad overlap and redundancies of the inflammatory response, the decreases seen in total P450 concentrations and in concentrations and activities of individual P450 isoforms after SEB were not significantly different from what was found after LPS. In addition, the differences between the responses of HeN and HeJ mice again were more quantitative than qualitative, in that the HeJ mouse was generally less responsive to both inflammatory stimulants. Since it is still not yet known by what mechanisms P450s are altered during inflammation, it is possible that the changes seen after Gram-negative (LPS) and Gram-positive (SEB) stimulants are mediated via different pathways, which may or may not converge in a common mechanism of repression and which may affect individual P450s differentially, as reviewed by Morgan [50]. It is probably the case that proinflammatory cytokines do not directly regulate hepatic P450 expression, but cause alterations by eliciting various inflammatory signal pathways. A role for the sphingomyelinase pathway in interleukin-1β suppression of rat hepatic CYP2C11 has been proposed [51, 52].

In conclusion, the current study confirmed that the acute inflammatory response elicited by the Gram-positive inflammatory stimulant SEB in a murine model is associated with alterations in hepatic cytochromes P450. Based on these results, it is likely that patients who are septic due to infections with Gram-positive organisms are just as likely to have significant depression of hepatic cytochrome P450-mediated drug metabolism as patients with Gram-negative sepsis and also to be at risk for adverse drug interactions and toxicities.

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