Investigation of Adrenergic and Prostaglandin Influences in the Endotoxin Alteration of Hepatic Heme Oxygenase, Microsomal Mixed-Function Oxidase, and Glucocorticoid-induced Tryptophan Oxygenase Activities

Joseph F. Williams and Andor Szentivanyi

Abstract: *The possible role for adrenergic influences or prostaglandins in the effects of endotoxin to inhibit the glucocorticoid induction of hepatic tryptophan oxygenase (TO) activity, to decrease the hepatic microsomal cytochrome P–450-dependent drug-metabolizing activity, and to induce heme oxygenase activity was examined. Administration of the α-adrenergic blocking agents phenoxybenzamine or phenotolamine attenuated the inhibitory effect of the bacterial lipopolysaccharide on the induction of TO activity by dexamethasone. Injection of a β-adrenergic blocker, propranolol, or of indomethacin, an inhibitor of prostaglandin biosynthesis, accentuated the effect of endotoxin to inhibit TO induction. Neither phenoxybenzamine, propranolol, nor indomethacin altered the effect of endotoxin to decrease aniline hydroxylase activity, ethylmorphine N-demethylase activity, or the levels of cytochrome P–450. Also, dexamethasone administration did not significantly protect against the effects of endotoxin on the hepatic microsomal drug metabolizing enzyme system, and none of the pharmacological agents diminished the effects of endotoxin to induce hepatic heme oxygenase activity. Endotoxin administration was also shown to diminish, but not prevent, the induction of cytochrome P–450 and ethylmorphine N-demethylase activity produced by phenobarbital. The results indicate that α-adrenergic mechanisms are involved in the endotoxic inhibition of the glucocorticoid induction of TO activity and suggest that neither adrenergic influences nor prostaglandins play a significant role in the effect of endotoxin to decrease hepatic mixed-function oxidase activity.*

Key Words: Endotoxin; Adrenergic influence; Indomethacin; Tryptophan oxygenase; Mixed-function oxidase; Heme oxygenase

INTRODUCTION

Bacterial endotoxins are known to produce a wide variety of pathophysiological responses when injected into experimental animals (Berry, 1977). Among the various organ systems

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From the Department of Pharmacology and Therapeutics, College of Medicine, University of South Florida, Tampa, Florida.

Address requests for reprints to Dr. Joseph F. Williams, University of South Florida, Department of Pharmacology and Therapeutics, Box 9, 12901 North 30th Street, Tampa, FL 33612.

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affected by endotoxin, the liver appears to be an organ at great risk. The effects of endotoxin on hepatic function have been reviewed by Utili et al. (1977) and include alterations in hepatic hemodynamics, a decrease in hepatic P_{O_2}, an increased glycogenolysis and decreased gluconeogenesis, a decreased responsiveness to the glucocorticoid induction of hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity and tryptophan oxygenase (TO) activity, a decreased activity of the hepatic microsomal mixed-function oxidase system, and an increased heme oxygenase activity.

The mechanisms by which endotoxin elicits these myriad responses are not known, but various studies have indicated that certain of the hepatic dysfunctions may be the consequence of various endogenous physiologically active substances released during endotoxin challenge (Berry, 1977). Included among these released substances are catecholamines, prostaglandins, and other arachadonic acid metabolites, and soluble factors, such as interferon, the glucocorticoid antagonizing factor (GAF), and a substance with insulin-like activity, released from cells of the reticuloendothelial system.

The extent to which the released substances mediate the effects of endotoxin is not completely resolved, but it has been shown that administration of certain pharmacological agents can prevent or ameliorate some of the effects of injected endotoxin, presumably by either preventing the release or the effects of the mediators. α-Adrenergic blocking agents, such as phenoxybenzamine and phentolamine, have been shown to block certain of the hemodynamic alterations induced by endotoxin, improve hepatic P_{O_2}, prevent decreased hepatic gluconeogenic activity, and protect from endotoxin lethality (Filkins, 1979; Kaelin and Rink, 1980). Conversely, β-adrenergic blockade with propranolol sensitized rats to lethal endotoxin shock and to depression of hepatic gluconeogenesis (Filkins, 1979). Neither α nor β blockade prevented depletion of hepatic glycogen. Inhibitors of prostaglandin synthesis, such as indomethacin, have been shown to decrease the release of arachadonic acid metabolites into the plasma, as well as from macrophages exposed in vitro to endotoxin (Cook et al., 1980; Rietschel et al., 1980). Indomethacin has also been shown to prevent the inhibitory effect of endotoxin on the glucocorticoid induction of PEPCK activity by blocking the release of glucocorticoid antagonizing factor (Goodrum et al., 1978; Goodrum and Berry, 1979).

Recently, several studies have appeared that suggest a possible role for catecholamines and/or prostaglandins in certain of the hepatic activities known to be affected by bacterial lipopolysaccharide, namely the inhibition of the glucocorticoid induction of hepatic TO activity and the decrease in hepatic microsomal drug-metabolizing enzyme activity. Sitaraman and Ramasarma (1975) and Sitaraman et al. (1979) have shown that treatment of rats with α-adrenergic agents, in particular norepinephrine, inhibited the glucocorticoid induction of hepatic TO activity, but not that of tyrosine aminotransferase. A similar selective inhibition of induction of these two hepatic enzyme activities is seen following treatment with endotoxin (Berry, 1977). Sitaraman and coworkers also demonstrated that the selective inhibition was specific to norepinephrine, was potentiated by other α-adrenergic agonists, and was blocked by treatment with α-adrenergic blocking agents but not with β-blockers. Catecholamines have also been shown to inhibit the activity of the hepatic microsomal mixed function oxidase system (Boobis and Powis, 1973). This effect may be due to the ability of these agents to increase cellular cyclic AMP, since Weiner et al. (1972a,b) demonstrated that dibutyryl cyclic AMP inhibits the metabolism of several drugs. Endotoxin has been reported to cause activation of adenylate cyclase (Bitensky et al., 1971; Gimpel et al., 1974). Thus, endotoxin inhibition of hepatic mixed-function oxidase activity could be due to either released catecholamines or to effects caused by adenylate cyclase and increased cyclic AMP levels. Finally, prostaglandins have been

Abbreviations. TO: tryptophan oxygenase; PEPCK: phosphoenolpyruvate carboxykinase; GAF: glucocorticoid antagonizing factor; cyclic AMP: adenosine 3',5'-monophosphate.

shown to inhibit the rate of hepatic microsomal drug-metabolizing activity (Weiner and Olson, 1980) and, as stated above, have been implicated in the release of the glucocorticoid antagonizing factor that is apparently involved in the endotoxin inhibition of the glucocorticoid induction of hepatic PEPCK activity.

The present study examines whether a role for adrenergic influences and/or prostaglandins may be implicated in the effect of endotoxin to inhibit the glucocorticoid induction of TO activity, to cause the decrease in hepatic drug-metabolizing enzyme activity, and to increase heme oxygenase activity. For this purpose, the relative effects of the administration of α- or β-adrenergic blocking agents and an inhibitor of prostaglandin syntehsis prior to endotoxin challenge have been evaluated with regard to these hepatic metabolic activities. A preliminary report of these studies has been presented (Williams and Szentivanyi, 1982).

METHODS

Male Sprague–Dawley rats (200–250g) were used throughout the study. Animals were maintained in stainless steel cages in a constant temperature room on a 12 hr light-dark cycle. Animals were kept for a minimum of 5 days after arrival in our animal care facilities before being used. Food and water were provided ad libitum except that all animals were fasted for the 24 hrs before killing.

The following compounds and dosages were used: *Eschericha coli* endotoxin 026:B6 (Difco Lab., Detroit, MI, Westphal extracted), 2 mg/kg; dexamethasone phosphate (Merck, Sharp & Dohme Lab., West Point, PA), 750 μg/kg; phenoxybenzamine (Smith, Kline & French, Philadelphia, PA), 2 mg/kg; propranolol (Sigma Chemical Co., St. Louis, MO), 4 mg/kg; indomethacin (Sigma Chemical Co.), 5 mg/kg; phentolamine HCl (CIBA-Geigy, Ardsley, NY) 5mg/kg; and sodium phenobaribtal (Ganes Chemical Co., Carlstadt, NJ), 80 mg/kg. All drugs were dissolved in sterile pyrogen-free isotonic saline solution (Travenol Lab. Inc., Deerfield, IL) except phenoxybenzamine, which was suspended in propylene glycol. All drugs were administered by intraperitoneal injection according to the schedule indicated in the text. Control animals received either saline or propylene glycol as dictated by the particular experimental procedures. Preliminary experiments indicated that propylene glycol did not effect any of the enzyme activities tested.

Tryptophan oxygenase activity was determined in fresh liver homogenate either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of 2 mM hematin, according to the method of Badaway and Evans (1975).

For the determination of the activity of the hepatic mixed-function oxidase system, the hepatic microsomal fraction was prepared as previously described (Williams and Karler, 1971). Microsomal protein was determined by the method of Lowry et al., (1951). Aniline hydroxylase activity and ethylmorphine N-demethylase activity were assayed as previously described by Imai et al. (1966) and Anders and Mannering (1966), respectively. Cytochrome P–450 was determined according to the method of Omura and Sato (1964) using 91 $cm^{-1}mM^{-1}$ as the extinction coefficient for the reduced CO minus oxidized absorption difference between 450 nm and 490 nm.

Microsomal heme oxygenase was determined by the method of Tenhunen et al. (1969) at a protein concentration of 2 mg/ml. The 105000 g supernatant was used as the source of biliverdin reductase. Methemalbumin was prepared as described by Bissell and Hammaker (1976) and used at a final concentration of 17 μM. Heme oxygenase activity was determined using an extinction coefficient of 30 cm^{-1} mM^{-1} for the absorption difference between 468 nm and 530 nm.

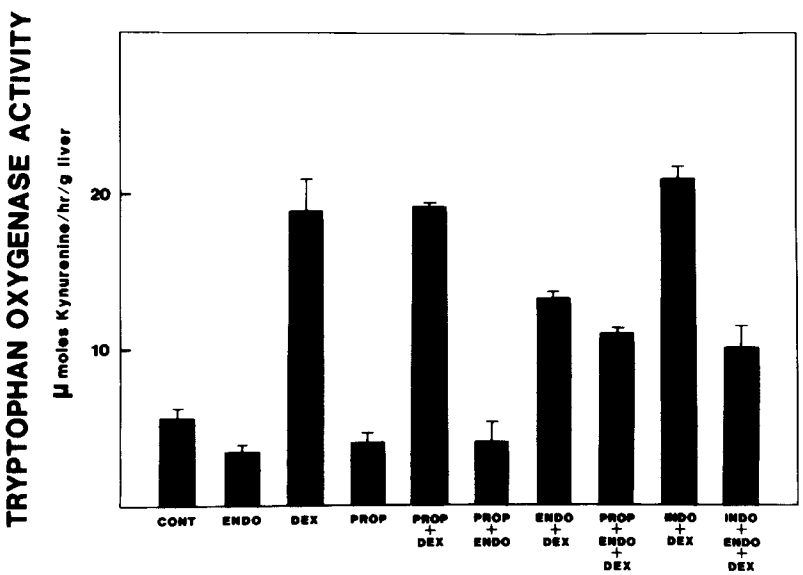
Statistical analysis was performed using the one-way analysis of variance and Tuckey's Honestly Significant Difference Test for multiple comparison, adopting $P<0.05$ as the level for significant difference.

RESULTS

Initially, consideration was given to the appropriate experimental protocol to effectively examine the possible adrenergic and prostaglandin influences in the effects of endotoxin to alter the activity of the hepatic enzymes to be studied. In particular, the appropriate time schedule and dosage for the pretreatment with the pharmacological antagonists, and the administration of dexamethasone relative to injection of the lipopolysaccharide, was deemed pertinent. Other investigators using propranolol, phenoxybenzamine, phentolamine, indomethacin, and dexamethasone to study various responses elicited by endotoxin have employed various doses and pretreatment schedules, ranging from 60 min prior, to simultaneously with, the lipopolysaccharide (Figlewicz and Filkins, 1978; Filkins, 1979; Kaelin and Rink, 1980; Kuttner et al., 1982). After some preliminary experiments examining a few slightly different injection protocols, the results of which did not markedly differ, the following protocol was adopted for studying the influence of the pharmacological antagonists on the inhibition by endotoxin of the dexamethasone induction of TO activity: propranolol and indomethacin were injected 30 min and 60 min, respectively, prior to the lipopolysaccharide; phenoxybenzamine was injected 10 min before endotoxin; dexamethasone was injected 2 hrs after the administration of endotoxin; and the animals were killed 4 hrs after the steroid administration, a time previously shown to be that at which the interaction between the lipopolysaccharide and steroid induction of TO activity is evident (Berry et al., 1966; Williams, unpublished observations). If dexamethasone and endotoxin were administered simultaneously and animals killed 4 hrs later, similar results were obtained (data not shown).

Figures 1 and 2 show the effect of pretreatment of rats with the pharmacological antagonists, using the adopted injection schedule, on the endotoxin inhibition of the dexamethasone induction of hepatic TO activity. Treatment of animals with propranolol or indomethacin did not

Figure 1 *Effect of pretreatment of rats with propranolol (PROP) or indomethacin (INDO) on the endotoxin (ENDO) inhibition of the dexamethasone (DEX) induction of hepatic TO activity. Animals received PROP or INDO 30 min and 60 min, respectively, prior to ENDO; DEX was administered 2 hrs after ENDO. All animals were killed 4 hrs after DEX. Values are mean ± SE of 4 animals per group.*



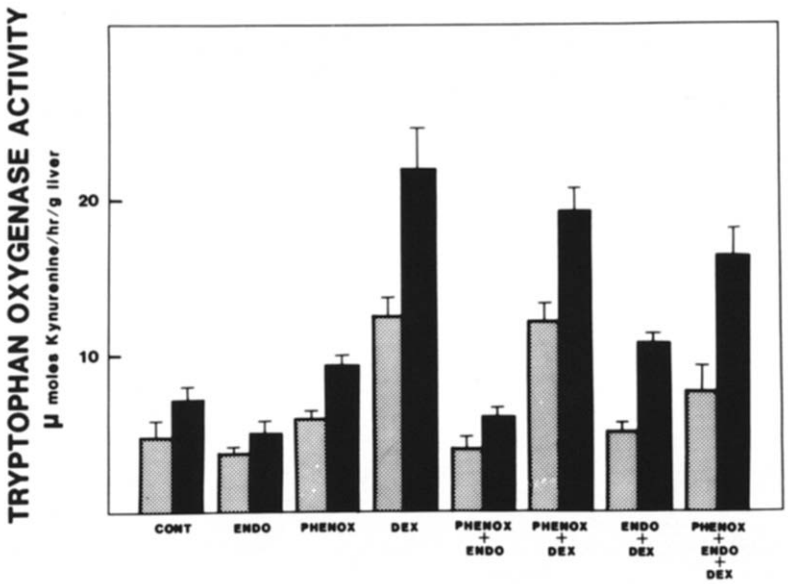


Figure 2 *Effect of pretreatment of rats with phenoxybenzamine (PHENOX) on the endotoxin (ENDO) inhibition of the dexamethasone (DEX) induction of hepatic holo (stippled bar) and total (solid bar) TO activity. Animals received PHENOX 10 min prior to ENDO; DEX was administered 2 hrs after ENDO and all animals were killed 4 hrs after DEX. Values are mean ± SE of 9 animals per group.*

alter levels of TO activity, nor did the pretreatment with these agents affect the induction of TO activity seen following dexamethasone injection (Figure 1). Endotoxin caused a significant decrease in TO activity and also significantly decreased the dexamethasone induction of enzyme activity. The endotoxic inhibition of the glucocorticoid induction of TO was slightly, but significantly, accentuated by pretreatment of the animals with either propranolol or indomethacin. Figure 2 shows the effect of pretreatment of animals with phenoxybenzamine on the endotoxin inhibition of dexamethasone induction of TO activity. In these experiments, both holo and total TO activity were determined. Endotoxin caused a slight decrease in total TO activity, but not in holo enzyme activity. Phenoxybenzamine alone caused a small increase in total, but not holo, enzyme activity. Dexamethasone significantly increased both holo and total TO activity, and this effect was inhibited by endotoxin. Pretreatment of animals with phenoxybenzamine did not significantly alter the effects of endotoxin or dexamethasone when the agents were administered singly; however, pretreatment of animals with phenoxybenzamine significantly attenuated the endotoxin inhibition of dexamethasone induction of total enzyme activity. Holo enzyme activity was slightly, but not significantly, increased when compared to the activity of an animal receiving endotoxin and dexamethasone. Table 1 shows that pretreatment of rats with phentolamine, another α-adrenergic blocking agent, 15 min prior to the lipopolysaccharide also caused the attentuation of the endotoxin effect to inhibit the glucocorticoid induction of TO activity.

The effect of the various pharmacological blocking agents, as well as dexamethasone and phenobarbital, on the endotoxin depression of hepatic mixed function oxidase activities is shown in Figures 3a and b. Previous studies have shown that the effect of endotoxin on these hepatic enzyme activities is first manifest between 12–24 hrs postinjection (Bissell and Hammaker, 1976; Williams et al., 1980b). The results shown were obtained from animals injected with propranolol 30 min prior to and 12 hrs after endotoxin; indomethacin was administered 60 min prior to and 12 hrs after endotoxin; phenoxybenzamine was given 15 min

Table 1 *Effect of pretreatment of rats with phentolamine on endotoxin inhibition of dexamethasone induction of tryptophan oxygenase activity*[a]

Treatment	*Tryptophan oxygenase activity*	
	Holo	Total
	(μmole kynurenine/hr/gm liver)	
Dexamethasone	15.68 ± 1.25	25.46 ± 2.01
Endotoxin + dexamethasone	3.52 ± 0.20[b]	13.86 ± 0.81[b]
Phentolamine + endotoxin + dexamethasone	5.89 ± 0.40[b,c]	21.86 ± 2.46[c]

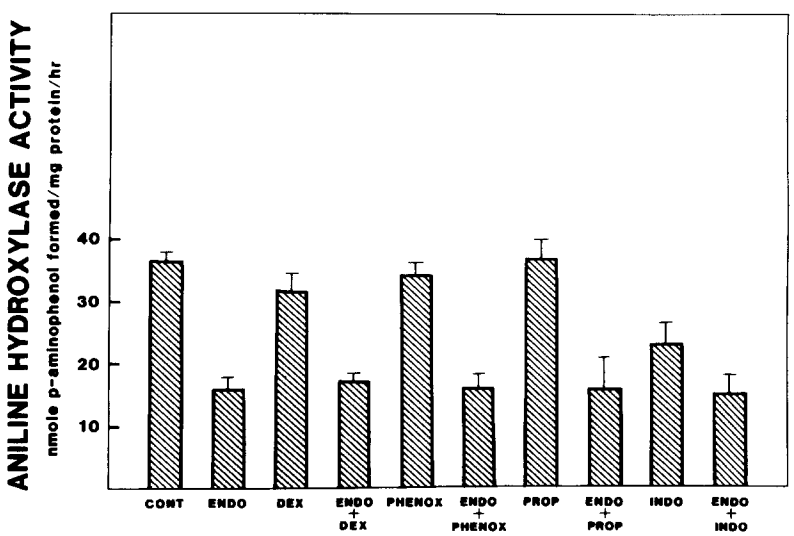
[a]Phentolamine given 15 min prior to endotoxin; dexamethasone given 2 hrs after endotoxin, all animals killed 4 hrs after dexamethasone. Tabular values are mean ± SE, N=4. Control values were Holo: 2.37 ± 0.17; Total: 6.72 ± 0.12.

[b]Significantly different from dexamethasone value, $P<0.05$.

[c]Significantly different from endotoxin-dexamethasone value, $P<0.05$.

before the lipopolysaccharide; dexamethasone and phenobarbital were given simultaneously with the injection of endotoxin; all animals were killed 24 hrs after injection of the lipopolysaccharide. This is a slightly different pretreatment schedule than that used in the studies of the TO activity. The major difference is the multiple injections of propranolol and indomethacin. Initial experiments (data not shown) using the previous protocol for these two agents showed that they did not alter the effect of endotoxin to decrease hepatic drug-metabolizing enzyme activity. However, because of the longer experimental time, it was thought that the effect of propranolol, a competitive β-adrenergic blocking agent, and indomethacin might diminish. Thus, the additional administration of these agents 12 hrs after endotoxin was instituted. Phenoxyben-

Figure 3 *Effect of endotoxin on hepatic mixed-function oxidase enzyme activity in rats treated with dexamethasone (DEX), phenoxybenzamine (PHENOX), propranolol (PROP), indomethacin (INDO), or phenobarbital (PB). Animals were killed 24 hrs after ENDO. Values are mean ± SE 5 to 7 animals per group. A. Aniline hydroxylase activity.*



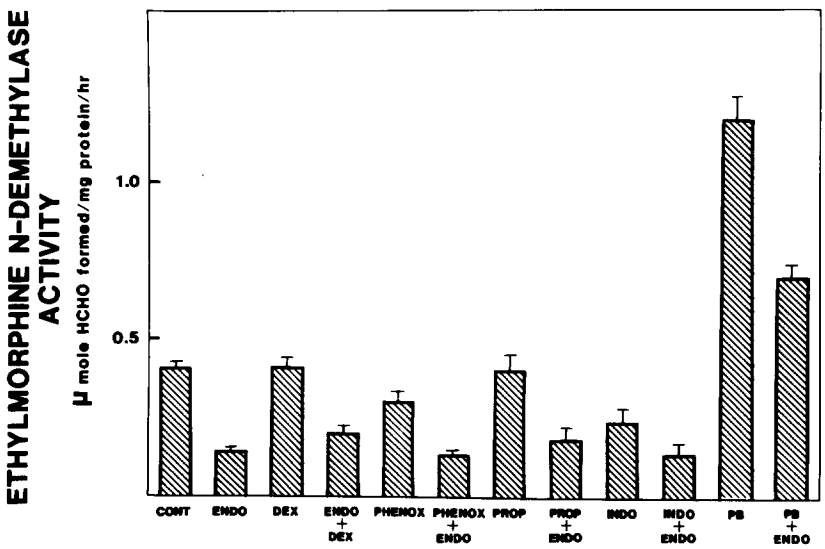


Figure 3B *Ethylmorphine N-demethylase.*

zamine is a long-acting, noncompetitive α-adrenergic antagonist whose effect persists for at least 24 hrs, and so was given only as the single dose. Additionally, in other experiments, dexamethasone was administered 2 hrs after the lipopolysaccharide; the results of these experiments did not markedly differ from the ones shown. The results indicate that injection of rats, according to the protocol indicated, with dexamethasone, phenoxybenzamine, or propranolol did not affect the basal levels of aniline hydroxylase activity or ethylmorphine N-demethylase activity, nor did the agents prevent the loss of drug-metabolizing activity effected by endotoxin. Pretreatment with indomethacin caused a significant loss of both aniline hydroxylase activity and ethylmorphine N-demethylase activity, and a further decrease in each of these enzyme activities was seen after endotoxin administration. Phenobarbital, an inducer of the hepatic drug-metabolizing enzyme system, caused a significant increase in ethylmorphine N-demethylase activity and endotoxin partially prevented this increase in drug-oxidation rate.

Table 2 shows the cytochrome P−450 levels in animals treated with dexamethasone, phenoxybenzamine, or phenobarbital alone, and with endotoxin; the animals were killed at 12 or 24 hrs after endotoxin. No changes in cytochrome P−450 levels were observed in any of the groups killed at the 12-hr time point except for those receiving phenobarbital alone and in combination with endotoxin. Phenobarbital alone caused a significant increase in cytochrome P−450 values. In the animals receiving both phenobarbital and endotoxin, the level of cytochrome P−450 was significantly decreased from the value observed in the phenobarbital-treated group, as well as from the value obtained for control animals. At 24 hrs, all endotoxin-treated animals had significantly less cytochrome P−450 than the corresponding treatment groups not receiving the lipopolysaccharide. In the phenobarbital-endotoxin group, the cytochrome P−450 level observed at 24 hrs was significantly increased from the value obtained at 12 hrs, but was still significantly less than the value obtained at this time point for animals receiving phenobarbital alone.

Table 3 shows the heme oxygenase activity from the animals treated with phenoxybenzamine and phenobarbital. Endotoxin caused a significant increase in heme oxygenase activity at both 12 and 24 hrs postinjection. Neither phenoxybenzamine nor phenobarbital affected

Table 2 *Cytochrome P-450 levels of animals treated with endotoxin alone or in combination with various agents*[a]

Group/treatment	Cytochrome P-450 (nmole/mg)	
	(12 hrs)	(24 hrs)
Control	0.86 ± 0.03	0.89 ± 0.03
Endotoxin	0.74 ± 0.04	0.53 ± 0.03[b]
Dexamethasone	0.75 ± 0.03	0.76 ± 0.02
Dexamethasone + endotoxin	0.78 ± 0.03	0.64 ± 0.03[b]
Phenoxybenzamine	0.85 ± 0.05	0.75 ± 0.04
Phenoxybenzamine + endotoxin	0.75 ± 0.04	0.48 ± 0.03[b]
Phenobarbital	1.17 ± 0.01	2.26 ± 0.04
Phenobarbital + endotoxin	0.67 ± 0.04[b]	0.86 ± 0.02[b]

[a]Agents administered according to schedule indicated in text. Animals killed either 12-24 hrs after endotoxin. Values are mean ± SE, N=5 to 7 animals per group.

[b]Significantly different from corresponding treated or control animal not receiving endotoxin, $P<0.05$.

Table 3 *Heme oxygenase activity of animals treated with endotoxin alone or in combination with phenoxybenzamine or phenobarbital*[a]

Group/treatment	Heme oxygenase activity (nmole bilirubin/10mg/min)	
	(12 hrs)	(24 hrs)
Control	0.37 ± 0.05	0.42 ± 0.03
Endotoxin	0.84 ± 0.08[b]	1.14 ± 0.11[b]
Phenoxybenzamine	0.39 ± 0.02	0.35 ± 0.04
Phenoxybenzamine + endotoxin	1.50 ± 0.17[b]	0.93 ± 0.18[b]
Phenobarbital	0.30 ± 0.04	0.30 ± 0.04
Phenobarbital + endotoxin	1.03 ± 0.09[b]	1.08 ± 0.04[b]

[a]Phenoxybenzamine administered 15 min prior to endotoxin. Phenobarbital injected simultaneously with endotoxin. Animals killed either 12 or 24 hrs after endotoxin. Values are mean ± S.E., N=5 to 7 animals per group.

[b]Significantly different from corresponding treated or control animal not receiving endotoxin, $P<0.05$.

heme oxygenase activity, and they did not decrease the induction of heme oxygenase activity effected by endotoxin. In fact, the heme oxygenase activity determined at 12 hrs for animals receiving phenoxybenzamine and endotoxin was significantly greater than the values for the animals receiving endotoxin alone. At 24 hrs, the heme oxygenase activity for these two treatment groups was still elevated above control values, but they were not significantly different from each other.

In other experiments (not shown) it was determined that neither dexamethasone, propranolol, nor indomethacin altered the effects of endotoxin on cytochrome P-450 or heme oxygenase activity.

Briefly recapitulating, various pretreatment schedules have been examined for the investigation of adrenergic and prostaglandin influences in the effect of endotoxin to inhibit the dexamethasone induction of TO activity, to decrease cytochrome P-450-dependent drug

oxidation, and to induce heme oxygenase activity. Pretreatment of animals with α-adrenergic antagonists reversed the inhibitory effect of endotoxin on the glucocorticoid induction of TO activity. None of the agents examined modified the effect of endotoxin to decrease drug-metabolizing activity or to induce heme oxygenase activity.

DISCUSSION

The results of the present study indicate a protective effect of α-adrenergic blocking agents against the inhibitory effect of endotoxin on the glucocorticoid induction of hepatic TO activity. This inhibition-reversal effect was seen using either phenoxybenzamine, a nonequilibrium α blocker, or phentolamine, a competitive α-adrenergic antagonist. In contrast, administration of either propranolol, a β-adrenergic blocker, or indomethacin, an inhibitor of prostaglandin synthesis, prior to endotoxin did not prevent, and even slightly potentiated, the endotoxin inhibition of the induction of enzyme activity. These latter results would suggest that the inhibitory effects of endotoxin are probably not mediated through β-adrenergic mechanisms or by prostaglandins that are known to be released by the lipopolysaccharide. Furthermore, since indomethacin blocks the release of GAF, the inhibitory mediator of endotoxin effect on PEPCK induction (Goodrum et al., 1978; Goodrum and Berry, 1979), this failure of indomethacin to modify the effect of endotoxin on TO induction suggests that GAF may also not be involved.

α-Adrenergic mechanisms have been implicated in the effects of bacterial lipopolysaccharide by several previous investigations. The results of the present study are similar to those of Filkins (1979) who showed a protective effect of α-, but not β-adrenergic blockade against endotoxin shock lethality and the decrease in hepatic gluconeogenic activity. The exact mode by which α-adrenergic blockade is beneficial during endotoxin shock is not shown. Kaelin and Rink (1980) suggested that the effect might be due to blocking the vasoconstrictor effects of released catecholamines and, thus, preventing the marked hepatic hypoxia seen during endotoxin shock. Although similar interpretations could be expounded to explain the results of the current study, recent experiments in our laboratory utilizing isolated hepatic cell preparations are difficult to reconcile with this interpretation, and other explanations may need to be investigated. Using isolated parenchymal cells, we have shown (Lowitt et al., 1981) that the effect of endotoxin on the dexamethasone induction of TO activity is not a direct effect, but is effected through interactions of the lipopolysaccharide with cellular constituents of the hepatic nonparenchymal cell fraction. These results suggest that endotoxin releases a mediator from this cell fraction that is responsible for the inhibition of the enzyme induction in the parenchymal cells. The identity of this substance is unknown, but, as stated above, it is probably not identical to GAF. The nonparenchymal cell fraction used contains primarily Kupffer cells as the major cell type. It seems unlikely that this cell fraction could contain significant levels of norepinephrine to be released and to inhibit the induction of TO activity. Hikawyj-Yevich and Spitzer (1977) have shown that endotoxin can directly alter the α receptor activity of the isolated adipocytes. Thus, it is conceivable that α blockade may either alter the effect of endotoxin to release the mediator or interfere with the interaction of the mediator with the parenchymal cell. Studies are currently in progress to evaluate the effects of α-adrenergic blockers on the effect of endotoxin on the glucocorticoid induction of TO activity using isolated hepatic parenchymal and nonparenchymal preparations.

The mechanism of endotoxin action that causes a decrease in hepatic mixed-function oxidase activity and induces heme oxygenase activity is apparently distinct from that involved in the endotoxin inhibition of TO activity. The decrease in ethylmorphine N-demethylase activity, aniline hydroxylase activity, the level of cytochrome P−450, and the increase in heme oxygenase activity was not prevented by pretreatment with an α-adrenergic antagonist. The ineffectiveness of pretreatment with propranolol or indomethacin to moderate the effect of the lipopolysaccharide on these hepatic microsomal activities suggests that neither β-adrenergic

mechanisms, such as the production of cyclic AMP, nor prostaglandins participate in the response. Dexamethasone administration, in doses up to 30mg/kg (data not shown), also did not ameliorate these hepatic effects of bacterial lipopolysaccharide. Finally, the administration of the bacterial cell wall constituent was shown to markedly reduce the level of cytochrome P−450 induced by phenobarbital administration, suggesting interference with synthesis of the hemoprotein.

It is now well recognized that many interferon-inducing agents, including endotoxin, can depress the level of cytochrome P−450 and the associated drug-metabolizing enzyme activities (Mannering et al., 1980). Indeed, the administration of interferon preparations has been shown to result in the loss of cytochrome P−450 (Sonnenfeld et al., 1980; Harned et al., 1982). Preliminary experiments in our laboratory have shown that endotoxin added directly to suspensions of isolated hepatic parenchymal cells does not cause any loss of cytochrome P−450 or aniline hydroxylase activity over an 8-hr incubation period (unpublished observation). These results suggest that the effect of endotoxin on hepatic cytochrome P−450, like other responses, is probably mediated by factors, perhaps interferon, released from other cell types. Indeed, Egawa et al. (1981) have shown that administration of serum from endotoxin-treated animals contains a substance that can cause a decrease in hepatic cytochrone P−450. They, however, question whether the inhibitory factor is interferon. Endotoxin-induced interferon is reportedly heat-labile, whereas the serum factor was heat-stable. Also, we have previously shown that the C3H/HeJ mouse, which is unresponsive to many of the biological effects of endotoxin, including the production of interferon, did show a decrease in hepatic microsomal drug metabolizing enzyme activity following endotoxin injection (Williams et al., 1980a). Additional studies are necessary to further characterize the effects of interferon and interferon-inducing agents on the hepatic drug-metabolizing activity.

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