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ENDOTOXIN INHIBITION OF DEXAMETHASONE INDUCTION OF TRYPTOPHAN OXYGENASE IN SUSPENSION CULTURE OF ISOLATED RAT PARENCHYMAL CELLS

INVOLVEMENT OF THE HEPATIC NONPARENCHYMAL CELL FRACTION

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Abstract—The effect of endotoxin on the induction of tryptophan oxygenase activity by dexamethasone has been studied in suspension cultures of isolated rat hepatic parenchymal cells incubated alone or mixed with hepatic nonparenchymal cells. Hepatic cellular fractions were isolated from untreated rats and from animals injected 2 hr prior to killing with 2 mg/kg of endotoxin. Endotoxin (400 μg/ml) added to suspensions of parenchymal cells from untreated animals did not decrease the induction of tryptophan oxygenase by dexamethasone. Endotoxin addition to cell suspension containing parenchymal and nonparenchymal cells from untreated rats caused a significant diminution in the induction of enzyme activity elicited by 0.1 μM, but not by 20 μM, dexamethasone. The nonparenchymal cell fraction from untreated animals had no effect on enzyme induction in the absence of added endotoxin. However, nonparenchymal cells isolated from rats pretreated *in vivo* with endotoxin diminished the induction of tryptophan oxygenase activity elicited by 0.1 and 20 μM dexamethasone even in the absence of added endotoxin. The inhibitory effect of nonparenchymal cells from endotoxin-pretreated rats was related to cell concentration and was accentuated by the *in vitro* addition of endotoxin. Parenchymal cells from the endotoxin-pretreated animals had an altered sensitivity to dexamethasone. The EC_{50} concentration for dexamethasone induction of tryptophan oxygenase was 40 and 500 μM for parenchymal cells from untreated and endotoxin-pretreated animals respectively. Parenchymal cells from the pretreated animals were more sensitive to the inhibitory effect of the nonparenchymal cells from the pretreated animals. These results are interpreted as indicating that endotoxin does not directly antagonize the induction of tryptophan oxygenase. The results suggest that this inhibition of tryptophan oxygenase induction is a mediated event involving an interaction of endotoxin with cellular constituents of the hepatic nonparenchymal cell fraction.

The administration of endotoxin, the lipopolysaccharide component of the cell wall of gram negative bacteria, to mice or rats causes a wide variety of biological effects, including alterations in an array of hepatocellular metabolic activities [1–4]. Recently, various reports [5–9] have indicated that some of the effects of endotoxin on hepatic metabolism are not direct effects but are mediated by substances released by endotoxin from cells of the reticuloendothelial system. The inhibitory effect of endotoxin on the glucocorticoid induction of mouse hepatic phosphoenolpyruvate carboxykinase (GTP: oxaloacetate carboxylase [transphosphorylating], EC 4.1.32) (PEPCK) has been demonstrated to be mediated by a factor (glucocorticoid antagonizing factor, GAF) released from murine peritoneal macrophages cultured in the presence of endotoxin [5–7]. Goodrum and Berry [8] have shown that GAF is also present in serum of endotoxin-treated mice. GAF, but not endotoxin, has been shown to inhibit the induction of PEPCK by hydrocortisone in cultures of Reuber H35 hepatoma cells. Studies by McCallum [9] have shown in the mouse that the hepatic macrophage (Kupffer cell) is the source of a mediator of endotoxin that directly decreases gluconeogenesis of isolated mouse hepatocytes.

Previous studies have indicated that, in addition to PEPCK, the induction by glucocorticoids of hepatic tryptophan oxygenase (L-tryptophan oxygenase 2,3-oxidoreductase [decyclizing] EC 1.13.11.11) is also inhibited by endotoxin [1, 2, 10]. Agarwal *et al.* [11] observed that the hydrocortisone induction of tryptophan oxygenase activity could be antagonized by endotoxin in the isolated perfused rat liver. Thorotrast, a reticuloendothelial system active substance, also blocked the induction of tryptophan oxygenase. It was suggested that endotoxin and thorotrast may both be inhibitory due to effects on hepatic nonparenchymal cellular constituents, particularly the Kupffer cells. However, a direct

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effect of endotoxin on hepatic parenchymal cell metabolism was not excluded. Indeed, McGivney and Bradley [12, 13] have shown recently that incubation of isolated mouse hepatocytes with endotoxin can decrease the activity of a number of mitochondrial enzymes.

In the present study we have examined the effect of endotoxin on the induction of tryptophan oxygenase by dexamethasone in suspension culture of isolated hepatic parenchymal cells. Parenchymal cells were incubated with the lipopolysaccharide and steroid in the absence and presence of the nonparenchymal cell fraction. The influence of the *in vivo* administration of endotoxin on the induction of tryptophan oxygenase by dexamethasone in the isolated parenchymal cells from the pretreated animal was also examined. Additionally, the dexamethasone induction of tryptophan oxygenase in parenchymal cells mixed with nonparenchymal cells from endotoxin-pretreated rats in the absence and presence of added endotoxin was determined. Preliminary reports of these investigations have been presented [14, 15].

MATERIALS AND METHODS

Dexamethasone phosphate was a gift from Merck Sharp & Dohme Laboratories (West Point, PA). *Escherichia coli* lipopolysaccharide 026:B6 (Westphal extraction) was purchased from Difco Laboratories (Detroit, MI). Sodium heparin was obtained from the Eastman Organic Chemicals Co. (Rochester, NY). Collagenase (type I), hyaluronidase (type I), hemin and L-tryptophan were purchased from the Sigma Chemical Co. (St. Louis, MO). Waymouth's medium MB 752/1 and Nitex monofilament nylon screening were obtained from the Grand Island Biological Co. (Grand Island, NY) and Tetko, Inc. (Elmsford, NY), respectively.

Treatment of animals. Male Sprague–Dawley rats (180–170 g) were used throughout the study. The animals were allowed food and water *ad lib.* *E. coli* endotoxin was dissolved in isotonic pyrogen-free NaCl solution and was administered i.p. at a dose of 2 mg/kg body weight 2 hr prior to killing the animals. All animals were killed between 10:00 a.m. and 1:00 p.m. to minimize diurnal variation in enzyme activity [16].

Preparation of isolated hepatic parenchymal and nonparenchymal cell fractions. Isolated hepatocytes were prepared by the method of Jeejeebhoy *et al.* [17] except that two 5-min gravity sedimentation steps were used for the recovery of the parenchymal cell fraction. The sedimented parenchymal cells were suspended in 100 ml of bicarbonate buffered (27 mM) modified Waymouth's medium MB 752/1, pH 7.4. The final cell concentration ranged from 2 to 6×10^6 parenchymal cells/ml. Portions (20 ml) of the isolated hepatocyte suspension were immediately distributed into 25 ml spinner culture flasks (Bellco, Vineland, NJ) and incubated under an atmosphere of humidified 95% O_2–5% CO_2 in a 37° warm room. The pH of the incubation medium was monitored frequently throughout the incubation period and maintained at pH 7.3 ± 0.1 by titration with 0.75 M $NaHCO_3$ solution [18].

The hepatic nonparenchymal cell fraction was obtained from the supernatant fluid of the second gravity sedimentation step. The supernatant fluid was divided into portions and centrifuged at 400 *g* for 4 min at 4°. The sedimented cells from individual tubes were suspended in the Waymouth's medium containing the parenchymal cells to give the desired parenchymal to nonparenchymal cell ratio. Isolation and combination of cell fractions from separate animals were accomplished within a 2-hr time interval.

Cell counts of the parenchymal and the mixed cell suspensions were performed using an improved Neubauer Chamber. Parenchymal and nonparenchymal cell viability was assessed at the beginning and at the end of the incubation by the ability of the cells to exclude trypan blue [14, 19, 20]. Initial cell viability ranged from 93 to 97 per cent and was 80 to 86 per cent after a 6-hr incubation. No significant difference in cell viability was seen between cell fractions obtained from control or endotoxin-pretreated animals.

Assay of tryptophan oxygenase activity in isolated hepatic parenchymal cells. A 5-ml sample of the cell suspension was taken at 0, 2, 4 or 6 hr of incubation and immediately centrifuged at 1000 *g* for 2 min at 4°. After removal of the supernatant fluid, the cell pellet was frozen by liquid nitrogen and stored overnight at −90°. Cell pellets were homogenized in 10 ml of an ice-cold solution containing 50 mM sodium phosphate buffer (pH 7.0), 2.5 mM L-tryptophan, 35 mM KCl, and 2 μM hemin. Tryptophan oxygenase activity was determined in portions (2.25 ml) of the cell homogenate according to the method of Badawy and Evans [21]. Enzyme activity was calculated using an extinction coefficient of 4.54 cm^{-1} mM^{-1} for kynurenine. Tryptophan oxygenase activity is expressed as nmoles of kynurenine formed per 10^6 parenchymal cells per hr. Tryptophan oxygenase activity was not affected significantly by freezing or the 24-hr storage of the sedimented cells. The enzyme activity of the frozen, stored cell samples (N = 6) was determined to be 98 ± 3 per cent of the activity determined for freshly prepared cell samples.

Analysis of data. Statistical analysis of the data was performed using the two-tailed Student's *t*-test for paired observations. $P < 0.05$ was adopted as the level for significant difference.

RESULTS

Tryptophan oxygenase activity of freshly prepared hepatic parenchymal cells from untreated animals was proportional to the cell concentration used and was 47 ± 2 nmoles of kynurenine formed per 10^6 parenchymal cells per hr (N = 20). Basal enzyme activity remained essentially unchanged throughout the 6–8 hr period of suspension culture. This tryptophan oxygenase activity for the isolated hepatocytes is comparable to that determined for fresh liver homogenates (5 μmoles of kynurenine formed per hr per g of liver), assuming 1.2×10^8 parenchymal cells per g of liver [20]. Basal tryptophan oxygenase activity for parenchymal cells prepared from endotoxin-pretreated animals was 89 ± 7 nmoles of kynurenine formed per 10^6 parenchymal cells per hr

(N = 13). Nonparenchymal cell fractions from either untreated or endotoxin-pretreated rats had no detectable basal or dexamethasone-inducible tryptophan oxygenase activity.

The concentration–response relationship of dexamethasone to tryptophan oxygenase activity for parenchymal cells isolated from untreated animals or endotoxin-pretreated rats is shown in Fig. 1A and Fig. 1B respectively. The parenchymal cell suspensions were incubated in the presence of 0.01 to 20 μM dexamethasone for 6 hr, an interval during which maximal enzyme induction occurs [14, 19, 22]. Dexamethasone caused a concentration-related increase in tryptophan oxygenase activity in suspensions of parenchymal cells from either untreated or endotoxin-pretreated animals. The percentage increases, over basal enzyme activity, elicited by 20 μM dexamethasone were 456 and 300 per cent for the parenchymal cells from untreated and endotoxin-pretreated animals respectively. The absolute values for tryptophan oxygenase activity of the parenchymal cells isolated from endotoxin-pretreated rats were significantly higher (about 2-fold) than the values obtained for parenchymal cells from untreated animals. The percentage increase, however, appears to be less. Assuming maximum enzyme induction is produced by 20 μM dexamethasone [22], the data in Fig. 1 indicate that the EC_{50} concentrations for dexamethasone induction of tryptophan oxygenase activity are 40 and 500 nM for parenchymal cells from untreated and endotoxin-pretreated animals respectively. Subsequent experiments utilized 0.1 and 20 μM dexamethasone when parenchymal cells from untreated animals were used and 1.0 and 20 μM dexamethasone when cells from endotoxin-pretreated animals were used.

Addition of endotoxin (50 μg/ml or 400 μg/ml) to hepatic parenchymal cell suspensions from untreated animals had no effect on basal tryptophan oxygenase activity or on the increased tryptophan oxygenase activity caused by either 0.1 or 20 μM dexamethasone (Table 1). Thus, in the presence or absence of 400 μg/ml endotoxin, parenchymal cell tryptophan oxygenase activity was increased approximately 136 and 372 per cent by 0.1 and 20 μM dexamethasone respectively. In other experiments (data not shown), hepatic parenchymal cell suspensions were preincubated with 400 μg/ml of endotoxin for 2 hr prior to the addition of either 0.1 or 20 μM dexamethasone. This preincubation of the cells with endotoxin also did not alter either basal enzyme activity or the magnitude of the induction elicited by the subsequent addition of the glucocorticoid.

The effect of endotoxin on the induction of tryptophan oxygenase activity in cell suspensions containing both hepatic parenchymal and nonparenchymal cells from untreated animals is shown in Table 2. In the absence of endotoxin, the nonparenchymal cell fraction had no effect on the induction of tryptophan oxygenase activity elicited by either 0.1 or 20 μM dexamethasone. In the absence of steroid, endotoxin also had no effect on the basal enzyme activity determined for the mixed-cell suspension. On the other hand, the addition of 400 μg/ml of endotoxin to the mixed-cell suspension caused a significant attenuation of the increase in tryptophan oxygenase activity caused by 0.1 μM dexamethasone. Dexamethasone (0.1 μM) alone increased trypto-

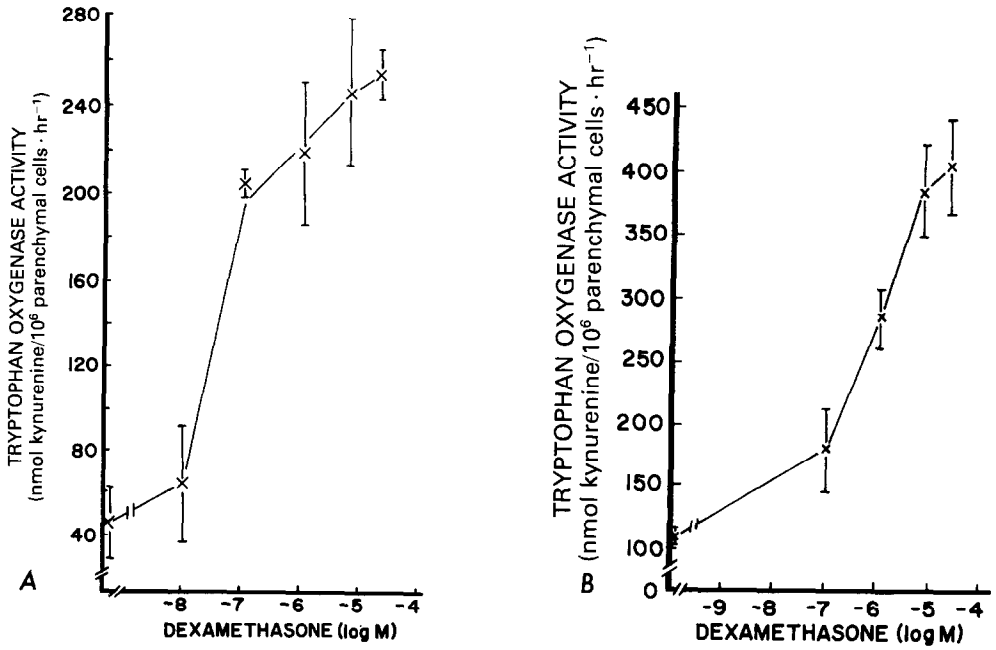

Fig. 1. Dexamethasone concentration–response relationship for the induction of tryptophan oxygenase activity in suspensions of isolated hepatic parenchymal cells (2–6 × 10^6 parenchymal cells/ml). All suspensions were incubated for 6 hr with various concentrations of dexamethasone. Enzyme activity was determined on homogenates of cell suspensions as described in Materials and Methods. Each value is the mean ± S.E. from three experiments. Panel A: parenchymal cells from untreated animals. Panel B: parenchymal cells from endotoxin-pretreated animals.

Table 1. Induction of tryptophan oxygenase activity by dexamethasone in suspensions of isolated hepatic parenchymal cells from untreated rats—Effect of endotoxin*

Incubation additions		Tryptophan oxygenase activity†
Dexamethasone (μM)	Endotoxin (μg/ml)	
None	None	44 ± 7
None	400	45 ± 10
0.1	None	116 ± 19
0.1	50	102 ± 15
0.1	400	106 ± 40
20	None	208 ± 36
20	400	216 ± 47

* Isolated hepatic parenchymal cell suspensions (2–6 × 10^6 cells/ml) were incubated for 6 hr after addition of endotoxin and/or dexamethasone.

† Tryptophan oxygenase activity is expressed as nmoles of kynurenine formed/10^6 parenchymal cells · hr^{-1}. Each value is the mean ± S.E. from three to four experiments.

phan oxygenase by 127 per cent over basal activity whereas that observed in the presence of endotoxin was increased only 60 per cent. In contrast, no significant effect of endotoxin on the increase in tryptophan oxygenase activity elicited by 20 μM dexamethasone in the mixed-cell suspension was observed. Preincubation of the mixed-cell suspension for 2 hr with 400 μg/ml of endotoxin gave results comparable to those shown in Table 2. In other experiments, a lower concentration of endotoxin (50 μg/ml) was shown to be ineffective in altering the increase in tryptophan oxygenase activity caused by 0.1 μM dexamethasone (data not shown).

The dexamethasone induction of tryptophan oxygenase activity in suspension culture of hepatic parenchymal cells alone and the mixed-cell suspension of parenchymal and nonparenchymal cells from endotoxin-pretreated animals is shown in Fig. 2. When the steroid was omitted from the incubation medium, basal tryptophan oxygenase activity of parenchymal cells (Fig. 2A) or parenchymal cells mixed with nonparenchymal cells (Fig. 2B) was unchanged over the 6 hr-incubation period. In the presence of 20 μM dexamethasone, tryptophan oxygenase activity was increased 237 per cent in suspensions of the parenchymal cells alone (Fig. 2A), whereas it was increased only 80 per cent in suspensions containing both the parenchymal and nonparenchymal cells (Fig. 2B). Thus, the presence of the nonparenchymal cell fraction (Fig. 2B) resulted in a 66 per cent attenuation of the induction of tryptophan oxygenase activity seen in suspensions of parenchymal cells obtained from endotoxin-treated animals.

Table 3 summarizes the effects of nonparenchymal cells from untreated and endotoxin-pretreated animals on the induction of tryptophan oxygenase activity in parenchymal cells from endotoxin-pretreated animals. Combination of nonparenchymal cells from untreated animals with the parenchymal cells from endotoxin-treated animals did not affect the increase in tryptophan oxygenase activity elicited by 1.0 or 20 μM dexamethasone. In contrast, the combination of the two cellular fractions, both obtained from endotoxin-pretreated animals, resulted in a significant attenuation of the increase in tryptophan oxygenase activity caused by either 1.0 or 20 μM dexamethasone, compared to the activity observed for the parenchymal cells in the absence of the nonparenchymal cells. In the latter case, hepatocyte tryptophan oxygenase activity was increased 200 and 350 per cent by 1.0 and 20 μM dexamethasone respectively. In the presence of the nonparenchymal cells from the endotoxin-treated animals, the increases in enzyme activity induced by 1.0 and 20 μM dexamethasone were 80 and 115 per cent respectively. Thus, the presence of the nonparenchymal cells from the treated animals prevented by approximately 60 per cent the increase in

Table 2. Induction of tryptophan oxygenase activity by dexamethasone in suspensions of hepatic parenchymal cells alone or mixed with nonparenchymal cells—Effect of endotoxin*

Cell suspension	Incubation additions		Tryptophan oxygenase activity†
	Dexamethasone (μM)	Endotoxin (μg/ml)	
Parenchymal cells	0.1	None	116 ± 19
Parenchymal cells + nonparenchymal cells	0.1	None	112 ± 11
Parenchymal cells + nonparenchymal cells	0.1	400	80 ± 7‡
Parenchymal cells	20	None	202 ± 46
Parenchymal cells + nonparenchymal cells	20	None	198 ± 26
Parenchymal cells + nonparenchymal cells	20	400	155 ± 19

* Suspensions of parenchymal cells (2–6 × 10^6 cells/ml) alone or combined with nonparenchymal cells at a cell ratio of 2:1 were incubated for 6 hr. In each experiment, both cellular fractions were obtained from the same untreated rat.

† Tryptophan oxygenase activity is expressed as nmoles of kynurenine formed/10^6 parenchymal cells · hr^{-1}. Each value is the mean ± S.E. from three to six experiments. Basal enzyme activity was 47 ± 5 nmoles kynurenine formed/10^6 parenchymal cells · hr^{-1}.

‡ Significantly different ($P < 0.05$) from dexamethasone alone.

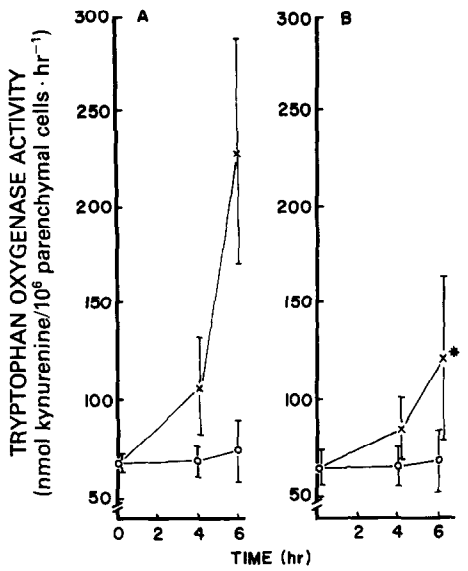

Fig. 2. Dexamethasone induction of tryptophan oxygenase activity in parenchymal cells from animals pretreated with endotoxin. Panel A: hepatic parenchymal cell suspensions ($2–6 \times 10^6$ cells/ml) were incubated in the presence (×——×) or absence (○——○) of 20 μM dexamethasone. Panel B: hepatic parenchymal and nonparenchymal cells from endotoxin-pretreated rats were mixed at a cell ratio of 2:1 and incubated in the presence of (×——×) or absence (○——○) of 20 μM dexamethasone. Tryptophan oxygenase activity was determined for duplicate samples of the cell homogenates. Values are expressed as nmoles kynurenine/10^6 parenchymal cells · hr^{-1} and each is the mean ± S.E. from five experiments. An asterisk indicates that activity was significantly different from that of hepatic parenchymal cells exposed to dexamethasone (Fig. 2A), $P < 0.05$.

tryptophan oxygenase activity affected by both of the steroid concentrations.

Table 4 compares the tryptophan oxygenase activity of isolated hepatic parenchymal cells from untreated animals incubated with 0.1 μM dexamethasone and with nonparenchymal cells from either untreated or endotoxin-pretreated animals. When both cellular fractions were obtained from untreated animals, a 221 per cent increase over basal enzyme activity was seen. However, when parenchymal and nonparenchymal cells, obtained from untreated and endotoxin-pretreated animals, respectively, were incubated together, tryptophan oxygenase activity was increased only 184 per cent above basal value. Similar results were obtained using 20 μM dexamethasone (data not shown).

Table 5 shows data from experiments in which endotoxin (400 μg/ml) was added to suspensions containing parenchymal cells from untreated animals and nonparenchymal cells from endotoxin-pretreated animals. Tryptophan oxygenase activity increased 208 and 153 per cent in the absence and presence, respectively, of the lipopolysaccharide. Thus, the addition of endotoxin *in vitro* to these suspensions attenuated the induction by 26 per cent. In one experiment the dexamethasone induction of tryptophan oxygenase activity was determined with a suspension of parenchymal and nonparenchymal cells from an untreated animal and with a suspension of parenchymal cells from the untreated animals mixed with nonparenchymal cells from an endotoxin-pretreated animal. The cell suspensions were incubated in the absence and presence of the *in vitro* addition of endotoxin. Tryptophan oxygenase activity for the mixed cell suspension from an untreated animal, for parenchymal cells mixed with nonparenchymal cells from an endotoxin-pretreated animal, and for the latter cell suspension plus endotoxin was 279, 226 and 153 nmoles of kynurenine formed per 10^6 parenchymal cells per hr respectively.

Evidence that the attenuation of the induction of tryptophan oxygenase was related to the nonparenchymal cell concentration is shown in Table 6. In these experiments the absolute ratio of hepatic parenchymal to nonparenchymal cells was 1:4. When

Table 3. Induction of tryptophan oxygenase activity by dexamethasone in isolated hepatic parenchymal cells from endotoxin-pretreated rats—Effect of hepatic nonparenchymal cells from untreated or endotoxin-pretreated rats*

Source of non-parenchymal cells	Dexamethasone (μm)	Tryptophan oxygenase activity† Incubation time 0 hr	6 hr
None	20	86 ± 11	382 ± 66
Untreated rats	20	86 ± 11	390 ± 67
Endotoxin-pretreated rats	20	86 ± 11	196 ± 53‡
None	1	115 ± 12	334 ± 31
Untreated rats	1	115 ± 12	319 ± 56
Endotoxin-pretreated rats	1	115 ± 12	218 ± 50‡

* Hepatic parenchymal cells from endotoxin-pretreated rats ($2–6 \times 10^6$ cells/ml) alone or with hepatic nonparenchymal cells from untreated or endotoxin-pretreated rats, in the ratio of 2:1, were incubated for 6 hr with the indicated concentration of dexamethasone.

† Tryptophan oxygenase is expressed as nmoles of kynurenine formed/10^6 parenchymal cells · hr^{-1}. Each value is the mean ± S.E. from three to five experiments.

‡ Significantly different from parenchymal cells alone and from mixed cell suspensions from untreated animals, $P < 0.05$.

Table 4. Induction of tryptophan oxygenase activity by 0.1 μM dexamethasone in isolated hepatic parenchymal cells from untreated rats—Effect of hepatic nonparenchymal cells from untreated or endotoxin-pretreated rats*

Cell suspension	Tryptophan oxygenase activity†
Parenchymal cells (untreated rats) + nonparenchymal cells (untreated rats)	221 ± 24
Parenchymal cells (untreated rats) + nonparenchymal cells (endotoxin-pretreated rats)	185 ± 21‡

* Isolated hepatic parenchymal + nonparenchymal cells, at a ratio of 2:1, were incubated for 6 hr with dexamethasone.

† Tryptophan oxygenase activity is expressed as nmoles of kynurenine formed/10^6 parenchymal cells · hr^{-1}. Each value is the mean ± S.E. from five experiments. Basal enzyme activity was 73 ± 7 nmoles kynurenine/10^6 parenchymal cells · hr^{-1}.

‡ Significantly different from parenchymal + nonparenchymal cells from untreated rats, $P < 0.05$.

all of the nonparenchymal cells were obtained from untreated animals, tryptophan oxygenase activity increased 210 per cent over basal activity. When one-half of the nonparenchymal cells were from animals pretreated *in vivo* with endotoxin, the enzyme activity increased 159 per cent. When all the nonparenchymal cells were from endotoxin-pretreated animals, tryptophan oxygenase increased 129 per cent.

DISCUSSION

The results of the present study indicate that endotoxin does not directly inhibit tryptophan oxygenase induction by dexamethasone in the isolated rat hepatic parenchymal cell suspensions. Incubation of the hepatocytes with two concentrations of the lipopolysaccharide did not result in a reduced level of either the basal or steroid-induced enzyme activity. Failure of endotoxin to directly affect certain hepatocellular enzymes has been reported previously by other investigators. Filkins and Cornell [23], Filkins and Buchanan [24], and McCallum [9] have shown that the addition of endotoxin to a suspension of rat or mouse hepatic parenchymal cells does not affect glycogenolysis or gluconeogenesis. Furthermore, addition of the lipopolysaccharide to Reuber H35 rat hepatoma cells in culture does not result in the inhibition of the corticosteroid induction of PEPCK [8].

Endotoxin, when added to suspensions of parenchymal and nonparenchymal cells, significantly inhibited the induction of tryptophan oxygenase caused by 0.1 μM, but not by 20 μM, dexamethasone. In the absence of endotoxin, the hepatic nonparenchymal cells from untreated rats did not affect enzyme induction elicited by either steroid concentration. On the other hand, incubation of hepatic parenchymal cells from untreated rats with nonparenchymal cells from animals pretreated with endotoxin resulted in an attenuation of induced enzyme activity compared to that observed with the parenchymal cells alone. This inhibitory effect of the nonparenchymal cells from the pretreated animals was seen with both 0.1 and 20 μM dexamethasone. Increasing the ratio of nonparenchymal to parenchymal cells, as well as adding endotoxin to the mixed-cell suspension, accentuated the inhibition of enzyme induction.

The results of the present study are interpreted as indicating that endotoxin activates, both *in vivo* and *in vitro*, hepatic cells of the nonparenchymal fraction to release a mediator antagonistic to the induction of tryptophan oxygenase by glucocorticoids. Thus, our results support the suggestion of Agarwal *et al.* [11] that hepatic nonparenchymal cells participate in the inhibitory effect of endotoxin and the work of others [5–9] demonstrating the mediation of endotoxin effects through cells of the reticuloendothelial system. In the present study, the inhibitory effect on tryptophan oxygenase activity, mediated through the hepatic nonparenchymal cell fraction, was similar in some respects to results of other studies with GAF on PEPCK activity [8]. In the present study and in the studies with GAF, basal enzyme

Table 5. Induction of tryptophan oxygenase activity by 0.1 μM dexamethasone in isolated hepatic parenchymal cells from untreated rats incubated with hepatic nonparenchymal cells from endotoxin-pretreated rats—Effect of *in vitro* addition of endotoxin*

Cell suspension	Endotoxin (μg/ml)	Tryptophan oxygenase activity†
Parenchymal cells (untreated rats) + nonparenchymal cells (endotoxin-pretreated rats)	None	208 ± 24
Parenchymal cells (untreated rats) + nonparenchymal cells (endotoxin-pretreated rats)	400	153 ± 15‡

* Isolated hepatic parenchymal plus nonparenchymal cells, at a ratio of 2:1, were incubated for 6 hr with dexamethasone.

† Tryptophan oxygenase activity is expressed as nmoles of kynurenine formed/10^6 parenchymal cells · hr^{-1}. Each value is the mean ± S.E. from four experiments. Basal enzyme activity was 56 ± 2 nmoles kynurenine/10^6 parenchymal cells · hr^{-1}.

‡ Significantly different from cells in the absence of endotoxin. $P < 0.05$.

Table 6. Induction of tryptophan oxygenase activity by 0.1 μM dexamethasone in isolated hepatic parenchymal cells from untreated rats—Effect of varying the concentration of hepatic nonparenchymal cells from endotoxin-pretreated rats

Cell suspension: Relative cell ratio*			
Parenchymal cells (untreated rats)	Nonparenchymal cells (untreated rats)	nonparenchymal cells (endotoxin-pretreated rat)	Tryptophan oxygenase activity†
1	4	0	226 ± 18
1	2	2	189 ± 16‡
1	0	4	167 ± 11‡

* Hepatic parenchymal cells (1 to 2.5 × 10^6 cells/ml) from untreated rats were mixed with nonparenchymal cells from untreated or endotoxin-pretreated rats at the indicated ratio and incubated for 6 hr with 0.1 μM dexamethasone.

† Tryptophan oxygenase activity is expressed as nmoles of kynurenine formed/10^6 parenchymal cells · hr^{-1}. Each value is the mean ± S.E. from six experiments. Basal enzyme activity was 73 ± 7 kynurenine/10^6 parenchymal cells · hr^{-1}.

‡ Significantly different from suspension of parenchymal and nonparenchymal cells, both from untreated rats, $P < 0.05$.

activity was unaffected and only inhibition of steroid induction of enzyme activity was seen. This is in contrast to the direct inhibition of PEPCK and gluconeogenesis of mouse hepatocytes observed by McCallum [9] with culture fluid from mouse Kupffer cells incubated with endotoxin. Moreover, like the effects of GAF [8], the inhibition of the induction of tryptophan oxygenase effected by the nonparenchymal cells from the endotoxin-pretreated rats was not overcome by steroid concentrations up to 20 μM. The inhibition seen when mixed-cell suspensions from untreated animals were incubated *in vitro* with endotoxin, however, was dependent on the steroid concentration used. This result suggests a difference may exist between the *in vivo* and *in vitro* effects of endotoxin on the nonparenchymal cells. It is also possible that mediators other than GAF are involved in this effect. The effective concentration of endotoxin in the present study was considerably higher than that used by others [5, 9]. GAF was released from mouse peritoneal macrophages cultured with 100 μg/ml of endotoxin for 17 hr [5], whereas the mouse Kupffer cell inhibitor of PEPCK activity was released by incubating adherent cells with 5 μg/ml of endotoxin for 2 hr [9]. Although the effective endotoxin concentrations may indicate the release of multiple substances, other differences between the various studies must also be considered. Thus, species differences (mouse vs rat), differences in cell type studied (peritoneal macrophage vs Kupffer cell), and differences in culture conditions (adherent cells vs cell suspensions) may explain some of the apparent discrepancies. Further work is necessary to resolve these differences and to further characterize the presumed inhibitor released from the rat nonparenchymal cell fraction. In this regard, preliminary results (unpublished observations) have indicated that an inhibitor of the induction of tryptophan oxygenase is present in medium obtained from some, but not all, cultures of nonparenchymal cells incubated with endotoxin.

The results of experiments utilizing parenchymal cells from endotoxin-pretreated rats suggest additional influences of endotoxin *in vivo* not elicited *in vitro*. After pretreatment of rats with endotoxin for 2 hr prior to killing, higher steroid concentrations were required for enzyme induction that were needed for induction in parenchymal cells from untreated animals. Thus, the EC_{50} concentration for dexamethasone induction of tryptophan oxygenase was 40 and 500 nM for the parenchymal cells isolated from untreated and endotoxin-pretreated animals respectively. Moreover, the per cent increase in tryptophan oxygenase activity elicited in the parenchymal cells from the pretreated animals was less than that obtained for cells from untreated animals. Incubation of parenchymal cells from untreated animals with endotoxin for 2 hr prior to the addition of dexamethasone did not affect the drug concentration needed or the magnitude of the induction of tryptophan oxygenase. Additionally, the parenchymal cells from the pretreated animals were more sensitive than parenchymal cells from untreated animals to the inhibitory activity of the nonparenchymal cells isolated from the pretreated animals. The reason for these differences is not known, but it may be related to *in vivo* effects of endotoxin on nonhepatic tissues to release either other inhibitory mediators, steroids, or substances affecting hepatocellular metabolism [1, 25].

Other biological effects of endotoxin administration to animals on hepatic metabolic activity include diminution of microsomal drug-metabolizing enzyme activity and alteration in heme synthesis and catabolism [26–30]. Studies similar to those presented may be of value in ascertaining whether these reported effects of endotoxin on hepatic parenchymal cell enzyme activities are due to direct or mediated events. Such information may be inportant when pharmacological agents are used in instances where alterations in the activity of the reticuloendothelial system may exist.

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REFERENCES

1. L. J. Berry, in *Microbial Toxins* (Eds. S. Kadis, G. Weinbaum and S. J. Ajl), Vol. 5, p. 165. Academic Press, New York (1971).
2. L. J. Berry, *CRC Crit. Rev. Toxic.* **5**, 238 (1977).
3. R. Utili, C. O. Abernathy and H. J. Zimmerman, *Life Sci.* **20**, 553 (1977).
4. D. C. Morrison and R. J. Ulevitch, *Am. J. Path.* **93**, 527 (1978).
5. R. N. Moore, K. J. Goodrum and L. J. Berry, *J. reticuloendothel. Soc.* **19**, 187 (1976).
6. R. N. Moore, K. J. Goodrum, R. E. Couch, Jr. and L. J. Berry, *J. reticuloendothel. Soc.* **23**, 321 (1979).
7. K. J. Goodrum and L. J. Berry, *Proc. Soc. exp. Biol. Med.* **159**, 359 (1978).
8. K. J. Goodrum and L. J. Berry, *Lab. Invest.* **41**, 174 (1979).
9. R. E. McCallum, *Microbiology, 1980* (Ed. D. Schlessinger), p. 87. American Society for Microbiology, Washington, DC (1980).
10. D. F. Rippe and L. J. Berry, *Infect. Immunity* **8**, 534 (1973).
11. M. K. Agarwal, W. W. Hoffman and F. Rosen, *Biochim. biophys. Acta* **177**, 250 (1969).
12. A. McGivney and S. G. Bradley, *J. reticuloendothel. Soc.* **26**, 307 (1979).
13. A. McGivney and S. G. Bradley, *Infect. Immunity* **25**, 664 (1979).
14. S. Lowitt, J. F. Williams and A. Szentivanyi, *Pharmacologist* **20**, 157 (1978).
15. S. Lowitt, J. F. Williams and A. Szentivanyi, *Fedn. Proc.* **38**, 366 (1979).
16. R. Hardeland and L. Rinsing, *Nature, Lond.* **219**, 619 (1968).
17. K. N. Jeejeebhoy, J. Ho, G. R. Greenberg, M. J. Phillips, A. Bruce-Robertson and U. Soditke, *Biochem. J.* **146**, 141 (1975).
18. L. L. Miller, in *Isolated Liver Perfusion and Its Applications* (Eds. I. Bartosek, A. Guaitami and L. L. Miller), p. 11. Raven Press, New York (1973).
19. T. Berg, D. Bowman and P. O. Seglen, *Expl. Cell Res.* **72**, 571 (1972).
20. P. O. Seglen, *Meth. Cell Biol.* **13**, 30 (1976).
21. A. A-B. Badawy and M. Evans, *Biochem. J.* **150**, 511 (1975).
22. T. Berg and J. Morland, *Biochim. biophys. Acta* **392**, 233 (1975).
23. J. P. Filkins and R. P. Cornell, *Am. J. Physiol.* **277**, 778 (1974).
24. J. P. Filkins and B. J. Buchanan, *Proc. Soc. exp. Biol. Med.* **155**, 216 (1977).
25. L. J. Berry and D. S. Smythe, *J. exp. Med.* **114**, 761 (1961).
26. R. Gorodischer, J. Krasner, J. J. McDevitt, J. P. Noland and S. J. Yaffe, *Biochem. Pharmac.* **25**, 351 (1976).
27. D. M. Bissell and L. E. Hammaker, *Archs Biochem. Biophys.* **176**, 91 (1976).
28. K. W. Renton and G. J. Mannering, *Biochem. biophys. Res. Commun.* **73**, 343 (1976).
29. J. F. Williams, S. Lowitt and A. Szentivanyi, *Pharmacologist* **21**, 232 (1979).
30. J. F. Williams, S. Lowitt and A. Szentivanyi, *Biochem. Pharmac.* **29**, 1483 (1980).