PRELIMINARY REPORT

Interleukin-6 Stimulates Gluconeogenesis in Primary Cultures of Rat Hepatocytes

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Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1 have been shown to stimulate the synthesis of acute-phase proteins; however, few studies have examined the effect of these cytokines on gluconeogenesis. We investigated the effects of these cytokines on gluconeogenesis in primary cultures of rat hepatocytes. Incubation of hepatocytes for 24 hours with TNF-α or IL-1α did not affect gluconeogenesis. Hepatocytes incubated with 100 pmol/L and 1 nmol/L IL-6 had a dose-dependent increase ($P < .05$) in gluconeogenesis (2.6 ± 0.1 and 3.2 ± 0.1 pmol/10^6 cells/min, respectively) as compared with controls (2.0 ± 0.1).

THE SYSTEMIC RESPONSE to stress is characterized by a classic pattern of metabolic and hormonal alterations collectively known as the acute-phase response. During this response, the liver produces acute-phase proteins and glucose. Acute-phase proteins are important opsonins of bacteria. Glucose, which is produced primarily by hepatic gluconeogenesis, is a vital energy source for lymphocytes during stress.[1] Although tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1α have all been shown to stimulate hepatocyte acute-phase proteins,[2,3] studies examining the direct effect of these cytokines on hepatocyte gluconeogenesis are sparse. We examined the effect of TNF-α, IL-6, and IL-1α on gluconeogenesis in primary cultures of rat hepatocytes.

MATERIALS AND METHODS

Chemicals

L-[U-^{14}C]lactic acid sodium salt (5.92 GBq/mmol) and D-[U-^{14}C]glucose (10.8 GBq/mmol) were purchased from Amersham (Arlington Heights, IL). Collagenase D (>0.15 Wunsch U/mg) was from Boehringer Mannheim (Indianapolis, IN). Anion exchange resin (AG 1 × 8, 200 to 400 mesh, formate form) was obtained from BioRad Laboratories (Richmond, CA). rhIL-6 (ED_{50} = 0.2 ng/mL, for thymidine incorporation in mouse B9 hybridoma cells), rhIL-1α (ED_{50} = 0.1 ng/mL, for thymidine incorporation in C3H/HeJ thymocytes), and type I collagen were purchased from Upstate Biotechnology (Lake Placid, NY). rhTNF-α (5×10^7 U/mg) was kindly supplied by Genentech (San Francisco, CA).

Animals

Male Fischer 344 rats (Charles River Laboratories, Wilmington, MA) weighing 190 to 300 g were used for all experiments.

Preparation of Rat Hepatocyte Primary Cultures

Hepatocytes were isolated by in situ collagenase liver perfusion and purified by percoll sedimentation.[4] Hepatocytes (1×10^6 cells per well) were plated on type I collagen in 2 mL RPMI 1640 + 5% fetal calf serum and maintained for 24 hours at 37°C in the presence of 5% CO_2.

Stimulation of Hepatocytes and Determination of Gluconeogenesis

After 2 hours in culture, hepatocytes (n = 4 wells per group) were stimulated for 24 hours with either rhTNF-α (30, 300 pmol/L), rhIL-1α (10, 100 U/mL), rhIL-6 (100 pmol/L; 1nmol/L), rhTNF-α + rhIL-1α, rhTNF-α + rhIL-6, or media alone (control [CTL]). After 24 hours, media were removed and hepatocytes were incubated for 4 hours in 2 mL Hanks balanced salt solution containing 1 mmol/L lactate and 0.23 μCi L-[U^{14}C]lactic acid. Supernatants were removed at 2 and 4 hours, and glucose was separated from lactate by passing 500 μL supernatant through 1.5-mL beds of anion exchange resin that was hydrated with 0.5 mmol/L boric acid 1:1 wt/wt. The efficiency of lactate removal was 98% and the efficiency of glucose recovery was 100% as determined by ^{14}C-lactate and ^{14}C-glucose standardization. Radioactive glucose was counted in a Beckman LS 6000IC Scintillation Counter, (Beckman Instruments, Fullerton, CA), and the ^{14}C disintegrations per minute were determined. From the counts obtained, the moles of glucose were determined for each sample. Rates of gluconeogenesis were calculated by linear regression from the glucose values obtained at 2 and 4 hours from each well. In separate experiments, hepatocytes were incubated for 1 and 4 hours with 1 nmol/L IL-6 or media alone (CTL). Rates of gluconeogenesis over 4 hours were also measured in these experiments.

Data Analysis

Rates of gluconeogenesis (pmoles of glucose per 10^6 cells per minute) are expressed as the mean ± SEM. Statistical analysis was performed by comparing all rates of gluconeogenesis by one-way ANOVA, and significance was defined as P less than .05.

RESULTS

Hepatocyte viability as determined by trypan blue exclusion at 28 hours ranged from 95% to 100%, and gluconeogenesis rates were linear (Spearman R^2 = .96). Rates of gluconeogenesis of hepatocytes (6 wells per group) incubated for 1 and 4 hours with rhIL-6 (8.9 ± 0.1 and 7.0 ± 0.2 pmol/10^6 cells/min, respectively) were no different than CTL (7.9 ± 0.3 and 6.2 ± 0.3). In 24-hour incubations, rhTNF-α and rhIL-1α alone or in combination had no effect on hepatocyte gluconeogenesis. However, rhIL-6 (100 pmol/L, 1 nmol/L) caused a dose-dependent increase in

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Table 1. Rates of Gluconeogenesis (pmol/10^6 cells/min) of Hepatocytes Incubated Either With rhTNF-α, rhIL-6, rhIL-α, or Media Alone (CTL)

Cytokine	Gluconeogenesis Rate With Cytokine (pmol/10^6 cells/min)		Gluconeogenesis Rate of Control (pmol/10^6 cells/min)	
rhTNF-α				
30 pmol/L	2.2 ± 0.1	(n = 8)	2.0 ± 0.1	(n = 8)
300 pmol/L	2.2 ± 0.1	(n =8)		
rhIL-1α				
10 U/mL	2.1 ± 0.1	(n = 4)		
100 U/mL	2.0 ± 0.1	(n = 4)	1.9 ± 0.1	(n = 4)
rhIL-1α, rhTNFα				
100 U/mL, 30 pmol/L	2.1 ± 0.1	(n = 4)		
100 U/mL, 300 pmol/L	2.1 ± 0.1	(n = 4)		
rhIL-6				
100 pmol/L	2.6 ± 0.1*	(n = 8)		
1.0 nmol/L	3.2 ± 0.1†	(n = 8)	2.0 ± 0.1	(n = 8)
rhIL-6, rhTNFα				
100 pmol/L, 300 pmol/L	3.1 ± 0.1‡	(n = 4)		

*$P < .05$ *v* control.

†$P < .05$ *v* control or 100 pmol/L rhIL-6.

‡$P < .05$ *v* control or 100 pmol/L rhIL-6 or 300 pmol/L TNF-α.

gluconeogenesis from 30% to 60% above control. In addition, hepatocytes incubated with 100 pmol/L rhIL-6 in combination with 300 pmol/L rhTNF-α had a greater rate of gluconeogenesis as compared with either control, 100 pmol/L rhIL-6, or 300 pmol/L TNF-α (Table 1).

DISCUSSION

Since gluconeogenic capacity is a critical determinant of survival in stress states[5] and since cytokines are elevated in many inflammatory states, our laboratory has investigated the effects of cytokines on hepatic metabolism. We have previously shown that in vivo administration of TNF-α (Blumberg et al, unpublished data, June 1992) or IL-1 to rats stimulates hepatic gluconeogenesis.[6] However, incubation of these cytokines with hepatocytes did not affect gluconeogenesis. This disparate effect of TNF-α and IL-1 on gluconeogenesis in vivo as compared with in vitro may be related to in vivo elevation of plasma glucagon, a direct stimulant of gluconeogenesis.[7,8]

Although IL-6 is a major stimulant of hepatic acute-phase protein production, few studies have examined the effect of IL-6 on hepatocyte gluconeogenesis. Our results are in agreement with previous studies in which 1-hour incubations of hepatocytes with IL-6 did not alter gluconeogenesis.[9] We also observed that short-term incubations of hepatocytes with IL-6 had no effect on gluconeogenesis. However, incubation of hepatocytes for 24 hours with IL-6 caused a marked stimulation of gluconeogenesis, which occurred in a dose-dependent fashion. This differential effect seen with long-term incubations of IL-6 as compared with short-term incubations suggests that IL-6 may regulate gluconeogenesis by transcription of mRNA for gluconeogenic enzymes as opposed to alteration of enzyme kinetics.

Similar to studies demonstrating that TNF-α may exert a permissive effect on hepatocyte metabolism, we observed that TNF-α increased the stimulatory effect of IL-6 on gluconeogenesis.[7] Since TNF-α is known to elevate serum IL-6 levels,[10] it is likely that these two cytokines act together to stimulate gluconeogenesis in vivo.

Similar to TNF-α, lipopolysaccharide administered in vivo increases serum IL-6 levels. Rats challenged with a sublethal injection of endotoxin maintain serum IL-6 levels of approximately 1 to 3 nmol/L for 22 hours.[11] Since we have shown that equivalent concentrations of IL-6 stimulate hepatocyte gluconeogenesis in vitro, IL-6 may have a significant role in stimulating hepatic gluconeogenesis during stress states.

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