Inhibition of TNF-α production by pentoxifylline does not prevent endotoxin-induced decrease in serum IGF-I

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Abstract

Sepsis and endotoxin (LPS or lipopolysaccharide) injection induce a state of growth hormone (GH) resistance leading to decreased circulating insulin–like growth factor (IGF)-I. Because the proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β inhibit the GH–stimulated IGF-I expression *in vitro*, it was tempting to speculate that these two cytokines might play an important role in the reduction of circulating IGF-I levels caused by LPS. Pentoxifylline, a methylxanthine usually used in the treatment of peripheral arterial circulatory disorders, has been reported to inhibit TNF-α synthesis. The goal of our study was to investigate whether inhibition of TNF-α production by pentoxifylline could prevent the decrease in IGF-I and the GH resistance caused by LPS injection. Because previous studies demonstrated that pentoxifylline can reduce muscle catabolism induced by sepsis, we also assessed whether pentoxifylline could exert its anti-catabolic effect by preventing the decrease in circulating IGF-I. LPS injection in rats decreased serum IGF-I (-45% at 12 h; $P < 0.01$ vs time 0) and its liver mRNA (-67% at 12 h; $P < 0.01$ vs time 0) while it induced circulating TNF-α and IL-1β and their hepatic expression ($P < 0.01$). Pretreatment of LPS-treated animals by pentoxifylline abolished the LPS-induced rise in serum TNF-α (-98% at 90 min; $P < 0.001$ vs LPS alone) and to a lesser extent in serum IL-1β (-44% at 3 h; not significant vs LPS alone). Despite its dramatic inhibitory effect on TNF-α induction, however, pentoxifylline failed to suppress both the decrease in IGF-I and the GH resistance induced by LPS in rats. These results suggest that mediators other than TNF-α, in particular IL-1β or IL-6, could contribute to the GH resistance induced by LPS. They also suggest that the anticatabolic effect of pentoxifylline is not due to prevention of the decline of circulating IGF-I.

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Introduction

Protein hypercatabolism and muscle wasting characterize catabolic states induced by sepsis and trauma (Hasselgren & Fischer 2001). The mechanisms responsible for these changes have not been completely elucidated. Reduced caloric intake does not seem to play a major role, as adequate nutritional support usually fails to prevent muscle loss (Cerra *et al.* 1980). The etiology of muscle wasting is probably multifactorial, but several lines of evidence suggest that alterations of the growth hormone (GH)–insulin-like growth factor (IGF)-I axis contribute to the development of these catabolic situations. IGF-I is a GH-dependent anabolic hormone that stimulates protein synthesis and strongly reduces protein breakdown (Fryburg 1994). Decrease in circulating IGF-I has been reported in most catabolic situations (Ross *et al.* 1991, Frost *et al.* 1996, Timmins *et al.* 1996). Furthermore, exogenous GH and IGF-I have also been reported to improve nitrogen balance in some of these situations (Mulligan *et al.* 1993, Berneis *et al.* 1997). However, the mechanisms responsible for the decline in circulating IGF-I in sepsis are not yet established. Previous reports have demonstrated that proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α can decrease circulating IGF-I (Fan *et al.* 1995, 1996). Furthermore, recent experimental observations from our laboratory (Thissen & Verniers 1997, Defalque *et al.* 1999) and others (Wolf *et al.* 1996, Liao *et al.* 1997, Mao *et al.* 1999) have shown that the decrease in the IGF-I liver production associated with sepsis results from a state of GH resistance. Although IL-1β and TNF-α inhibit GH stimulation of the IGF-I gene in cultured hepatocytes and therefore mimick *in vitro* the *in vivo* effect of lipopolysaccharide (LPS) (Thissen & Verniers 1997, Defalque *et al.* 1999), the role of these cytokines in the GH resistance induced *in vivo* by LPS is still disputed.

Pentoxifylline, a methylxanthine which is usually used in the treatment of peripheral arterial circulatory disorders, has been reported to inhibit the synthesis of TNF-α in several inflammatory states, in particular in LPS-treated animals (Schade 1990, Lundblad *et al.* 1995, Staudinger

et al. 1996, Voisin *et al.* 1998). Therefore, pentoxifylline represents a useful tool for exploring the role of TNF-α in the decline of IGF-I induced by LPS. The aim of this study was to investigate whether inhibition of TNF-α production by pentoxifylline could prevent the decrease in IGF-I and the GH resistance caused by LPS injection in rats. Because previous studies demonstrated that pentoxifylline can reduce muscle catabolism induced by sepsis (Breuillé *et al.* 1993), we also assessed whether pentoxifylline could exert its anticatabolic effect by preventing the decrease in IGF-I.

Materials and Methods

Endotoxin and hormone preparations

Lipopolysaccharide of *Escherichia coli* (LPS, serotype 0127:B8) was obtained from Sigma Chemical Inc. (St Louis, MO, USA) and was diluted at 1 mg/ml in sterile endotoxin-free saline buffer. Rat growth hormone (rGH) (AFP-87401; B-13) was kindly provided by NIDDK (Torrance, CA, USA) and was diluted at 2 mg/ml in sterile saline solution containing 0·04% BSA. Pentoxifylline (TorentalR) was kindly provided by Hoechst Marion Roussel (Brussels, Belgium) (077H0892) and was diluted at 50 mg/ml in sterile saline solution.

Animals

Eight-week-old male Wistar rats weighing 197 ± 9 g (mean ± S.D.) were obtained from the Katholieke Universiteit of Leuven (Leuven, Belgium). They were housed for 7 days under controlled conditions of lighting (12 h light, from 0700 h to 1900 h). Food was only available between 1800 h and 0900 h while access to water was unrestricted. The experiments were carried out with the approval of the Animal Care and Use Committee of the Institution.

Experimental design

Experiment 1: time-course of the effect of LPS on IGF-I, IL-1β and TNF-α. After a 7-day adaptation period, rats received on the morning of the 8th day one i.p. injection of LPS (750 µg/100 g body weight (BW)) and were killed at different times after injection (0, 1, 2, 3, 6 and 12 h) (three rats per group).

Experiment 2: effect of pentoxifylline on LPS-induced TNF-α and IL-1β. After a 7-day adaptation period, rats were divided into five groups (four rats/group). Two i.p. injections were administered at 1-h intervals to each rat: two saline injections in the first group; one saline and one LPS (750 µg/100 g BW) in the second group; one pentoxifylline (10 mg/100 g BW) and one saline in the third group; one pentoxifylline and one LPS in the fourth group.

Rats were killed 90 and 180 min after LPS injection, except for the baseline group rats which were killed just before LPS injection.

Experiment 3: effect of pentoxifylline on LPS-induced decrease in IGF-I. After a 7-day adaptation period, rats were divided into nine groups (five to seven rats/group). In the first group, rats were killed without any injection (baseline rats); the second group received three saline injections; the third group received one s.c. rGH injection (200 µg/100 g BW) and two i.p. saline injections; the fourth group received one i.p. injection of LPS (750 µg/100 g BW) and two saline injections; the fifth group was given one i.p. injection of pentoxifylline (10 mg/100 g BW) and two saline injections. In the last four groups, the injected solutions were combined (pentoxifylline/LPS/saline, pentoxifylline/saline/rGH, pentoxifylline/LPS/rGH, saline/LPS/rGH). In each group, the injection of pentoxifylline was given 1 h before LPS and rGH injections.

Rats were killed 10 h after LPS injection, except for the baseline group rats which were killed just before injections. In each experiment, blood was collected into glass tubes, centrifuged (1800 *g* for 10 min at 4 °C) and serum was stored at -20 °C until analysis. Livers were removed and stored at -80 °C until analysis.

Northern blot analysis

Total RNA was prepared by the guanidine isothiocyanate-cesium chloride method. RNA (20 µg) was denatured in formaldehyde-MOPS, subjected to gel electrophoresis in 1% agarose and transferred to nylon membranes (Hybond, Amersham, Bucks, UK) by capillary transfer overnight. The level of IGF-I mRNA was determined by hybridization with a specific riboprobe including only the coding region for the mature IGF-I peptide. A 194-bp AvaII-HinfI cDNA rat IGF-I exon 4 fragment was ligated into the plasmid vector Bluescript (Stratagene, La Jolla, CA, USA) and linearized by HindIII. IGF-I mRNA transcripts, as visualized by Northern blot analysis, presented a complex picture consisting of a large (7·5 kb) transcript, a group of transcripts ranging from 0·8 to 1·2 kb, and two additional minor transcripts of 1·7 and 4 kb. Because all these transcripts may potentially be translated into IGF-I precursors, we performed a densitometric analysis of the four bands visible on the blot. The densitometric results corresponded to the sum of all IGF-I mRNA transcripts. The level of TNF-α mRNA was determined by hybridization with a random-primed [^{32}P]dCTP-labeled TNF-α cDNA probe (p6 rTNF-α) encompassing the rat TNF-α coding sequence (amino acids 44–231) (Pampfer *et al.* 1995). The level of IL-1β mRNA was determined by hybridization with a random-primed [^{32}P]dCTP-labeled IL-1β cDNA probe encompassing the rat IL-1β coding sequence (amino acids 241–781). To verify uniform loading, control hybridization was performed

with a 23-mer 18S oligonucleotide synthesized on a DNA synthesizer and end-labeled with adenosine-γ [^{32}P]triphosphate by T4 polynucleotide kinase (Amersham). The mRNA levels were quantified by densitometric scanning of the hybridization signal (LKB Ultroscan XL laser densitometry; LKB, Bromma, Sweden) with the use of software (Gel Scan, Pharmacia, Brussels, Belgium).

Serum IGF-I extraction and RIA

Serum (100 μl) was extracted using ODC silicic acid columns (Waters C18 Sep-Pak cartridges; Millipore, Milford, MA, USA) and binding proteins were eluted with 7% acetic acid and IGF-I with 100% methanol (Davenport *et al.* 1988, Defalque *et al.* 1999). IGF-I in serum was determined by RIA as previously described (Defalque *et al.* 1999). The RIA had a lowest detectable level of 0·2 ng/ml, and intra– and interassay coefficients of variation were respectively 5 and 13% for a concentration of 0·5 ng/ml.

Serum TNF-α and IL-1β assays

TNF-α and IL-1β concentrations in serum were assayed with specific enzyme-linked immunoabsorbant assay (ELISA) kits purchased from R&D Systems (Abingdon, Oxon, UK) with a sensitivity of 5 ng/ml.

Statistical analysis

Experimental data are presented as means ± S.E.M. Data were analyzed by ANOVA followed by Newman-Keuls' test except for time-course experiments which were analyzed by ANOVA followed by Dunnett's test. Statistical significance was set at $P < 0·05$.

Results

Effects of LPS on TNF-α and IL-1β

TNF-α and IL-1β were not detected in the serum of control rats (time 0) (Fig. 1A and D), but LPS injection induced the appearance of TNF-α and IL-1β in the circulation. The increase in serum TNF-α was already apparent at 1 h, peaked at 2 h (11 000-fold increase at 2 h vs time 0; $P < 0·01$) before slowly declining (at 12 h: not significant (NS)) (Fig. 1A). The increase in serum IL-1β was slower than TNF-α, peaking at 3 h (4000-fold increase at 3 h vs time 0; $P < 0·01$) and then slowly declining (at 12 h: $P < 0·01$) (Fig. 1D). In the absence of any stimulation, baseline levels of TNF-α and IL-1β gene expression were very low in the liver of rats. LPS administration markedly increased hepatic TNF-α gene expression. TNF-α mRNA levels peaked 1 h after LPS injection (22-fold increase at 1 h vs time 0; $P < 0·01$) and rapidly decreased to return to basal levels at 12 h (Fig. 1B

and C). IL-1β mRNA was also induced by LPS, peaked 1 h after LPS injection (80-fold increase at 1 h vs time 0; $P < 0·01$) and returned to basal levels at 12 h (Fig. 1E and F).

Effects of LPS on IGF-I

In agreement with our previous data (Defalque *et al.* 1999), one injection of LPS rapidly decreased serum IGF-I concentrations. Three hours after injection, serum IGF-I had already declined by 24% vs non-injected rats ($P < 0·05$). After 12 h, we observed a 45% decrease compared with control rats ($P < 0·01$) (Fig. 2A). This decrease in serum IGF-I was associated with reduced liver IGF-I gene expression. By comparison with control rats, the liver IGF-I mRNA levels were decreased by 25% ($P < 0·01$) 3 h after injection and by 67% 12 h after injection ($P < 0·01$) (Fig. 2B and C).

Effects of pentoxifylline on TNF-α and IL-1β

Injection of pentoxifylline abolished the basal serum levels of TNF-α in control animals (control: 28 ± 13 pg/ml vs pentoxifylline: non-detectable) (Fig. 3). Similarly, TNF-α liver expression was reduced, albeit non significantly, by pentoxifylline injection in control animals. When injected 60 min prior to LPS administration, pentoxyphylline inhibited serum TNF-α measured 90 min after LPS injection. Pentoxifylline reduced serum TNF-α to less than 2% of the levels seen in the LPS-treated animals (98% decrease vs LPS; $P < 0·001$) (LPS/pentoxifylline: 423 ± 132 pg/ml vs LPS: 20 448 ± 4 519 pg/ml; $P < 0·001$) (Fig. 3A). This inhibition of serum TNF-α was caused by reduced liver production, as suggested by the profound inhibition of liver TNF-α mRNA expression. Indeed, pretreatment with pentoxifylline prior to LPS decreased hepatic TNF-α mRNA to less than 13% of the levels observed in LPS-treated animals (86% decrease vs LPS; $P < 0·001$), to levels similar to those of the control group (control vs LPS/pentoxifylline; NS) (Fig. 3B and C). Similarly, hepatic IL-1β mRNA was reduced by pretreatment with pentoxifylline prior to LPS injection but to a lesser extent than TNF-α mRNA. In pretreated animals analyzed 3 h after LPS administration, IL-1β mRNA was reduced to 48% ($P < 0·05$: LPS vs pentoxifylline/LPS) (Fig. 4B and C). However, this reduction in IL-1β mRNA was not associated with a significant reduction in serum IL-1β. Indeed, in pretreated animals, the 50% decrease in serum IL-1β observed 180 min after LPS injection was not significant (Fig. 4A). Although serum TNF-α was still slightly increased 10 h after LPS injection (LPS: 84 ± 20 vs control: 26 ± 14 pg/ml; $P < 0·05$), it was not significantly different from control values in animals pretreated with pentoxifylline (LPS+pentoxifylline: 47 ± 13 vs control: 26 ± 14 pg/ml, NS).
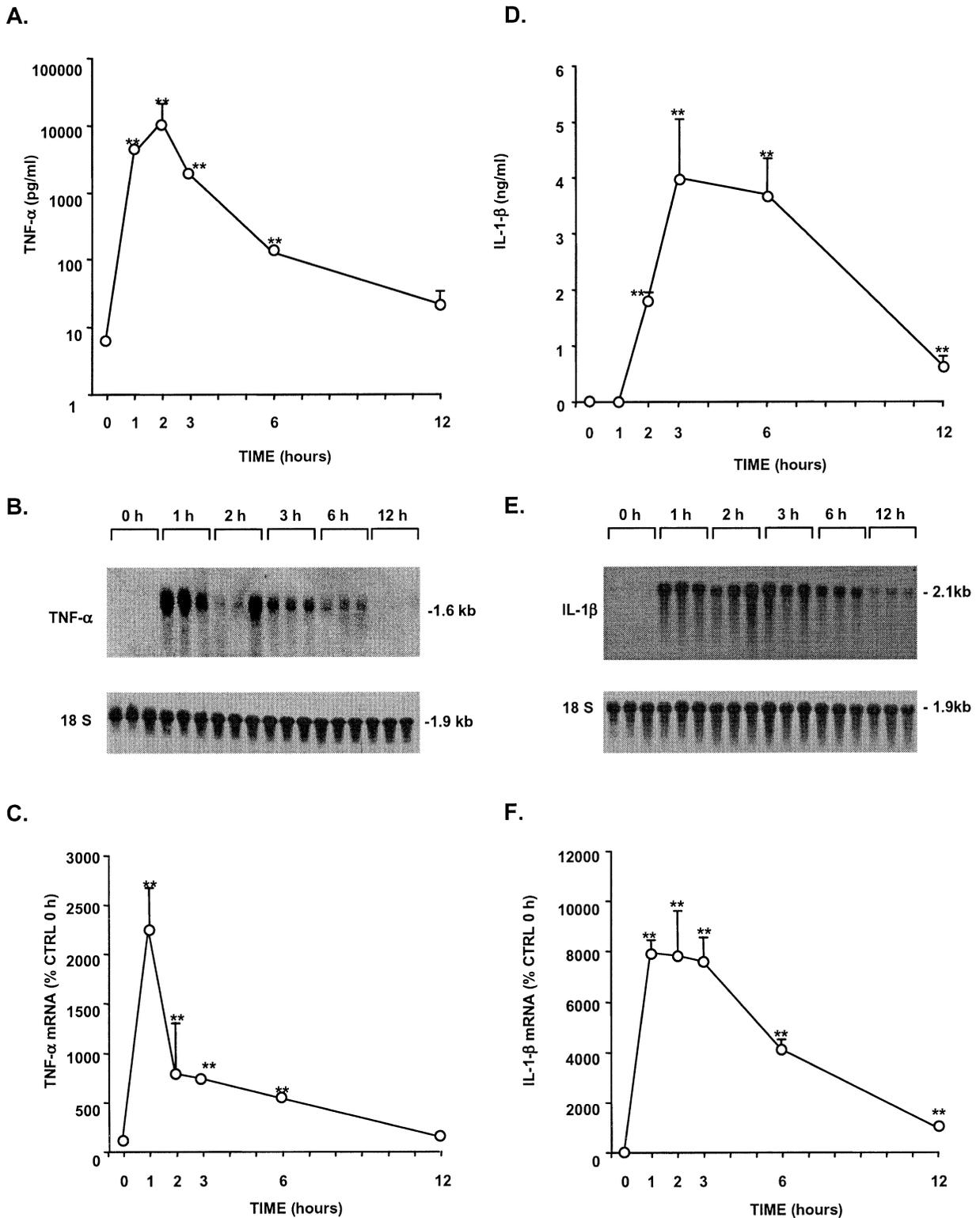
A.

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B.

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Figure 1 Time-course of the response of serum TNF-α and IL-1β and their hepatic mRNAs to LPS. (A, D) Time-course of serum TNF-α and IL-1β concentrations after LPS. These data are presented as means ± S.E.M. (B, E) Northern blot analysis of hepatic TNF-α and IL-1β mRNAs (top row) and 18S RNA (bottom row) respectively. (C, F) Densitometric analysis of the induction of TNF-α and IL-1β mRNAs by LPS in rat liver. These data are presented as the mean ± S.E.M., expressed as a percentage of the mean observed at time 0. **$P < 0.01$ compared with control values (time 0).

A.

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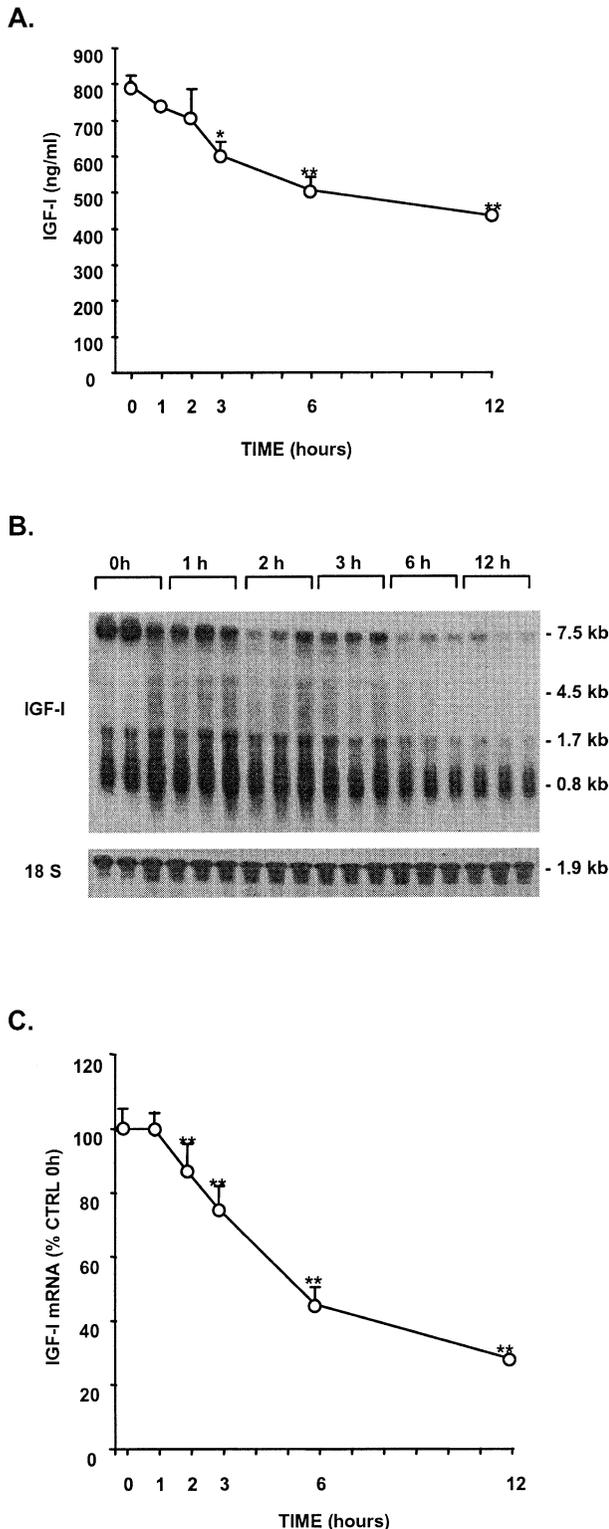
Effects of pentoxifylline on IGF-I in LPS-treated rats

Ten hours after LPS injection, serum IGF-I levels had declined by 40% compared with saline-injected rats ($P<0.001$) (Fig. 5A). However, pretreatment of LPS-treated rats with pentoxifylline, despite its inhibitory effect on TNF-α production, did not prevent the reduction in plasma IGF-I caused by LPS. To investigate the possibility that a state of GH deficiency may be responsible for the persistent low levels of IGF-I, we compared the IGF-I response between LPS-injected rats pretreated with pentoxifylline and LPS-injected rats pretreated with pentoxifylline and a single GH injection. Combined injections of GH and pentoxifyllline did not prevent the endotoxin-induced decrease in circulating IGF-I. Indeed, the IGF-I concentrations in these rats (362 ± 18 ng/ml) averaged those observed in rats treated with LPS alone (379 ± 16 ng/ml) or LPS rats pretreated with pentoxifylline alone (391 ± 22 ng/ml) (Fig. 5A). The liver IGF-I mRNA changes paralleled changes in circulating IGF-I peptide (Fig. 5B and C).

Discussion

This study demonstrates that inhibition of TNF-α production by pentoxifylline fails to prevent both the decrease in circulating IGF-I and the GH resistance caused by LPS injection in rats. Since TNF-α and IL-1β induction by LPS occurred before the decline in IGF-I and since these two cytokines inhibit GH-stimulated expression of the IGF-I gene *in vitro* (Thissen & Verniers 1997), it was tempting to speculate that these two pro-inflammatory cytokines might play an important role in the reduction of circulating IGF-I levels and in the induction of GH resistance caused by LPS. The levels of circulating TNF-α and IL-1β reached after LPS injection are clearly high enough to exert biological effects on hepatocytes, since they are close to the affinity of TNF-α and IL-1β receptors on these cells, and quite similar to those that have been shown to inhibit the IGF-I gene in cultured hepatocytes (Wolf *et al.* 1996, Thissen & Verniers 1997). Although IL-6 is also induced in this endotoxemia model (Ghezzi *et al.* 2000), it failed to suppress GH-induced IGF-I expression at least in cultured hepatocytes (Thissen & Verniers 1997). The appearance of TNF-α and IL-1β in the circulation was associated with increased expression of

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Figure 2 Time-course of the response of serum IGF-I and its hepatic mRNA to LPS. (A) Time-course of serum IGF-I concentrations after LPS. These data are presented as means ± S.E.M. (B) Northern blot analysis of hepatic IGF-I mRNA (top row) and 18S RNA (bottom row). (C) Densitometric analysis of hepatic IGF-I mRNA after LPS in rat liver. These data are presented as the mean ± S.E.M., expressed as a percentage of the mean observed at time 0. **$P<0.01$, *$P<0.05$ compared with control values (time 0).
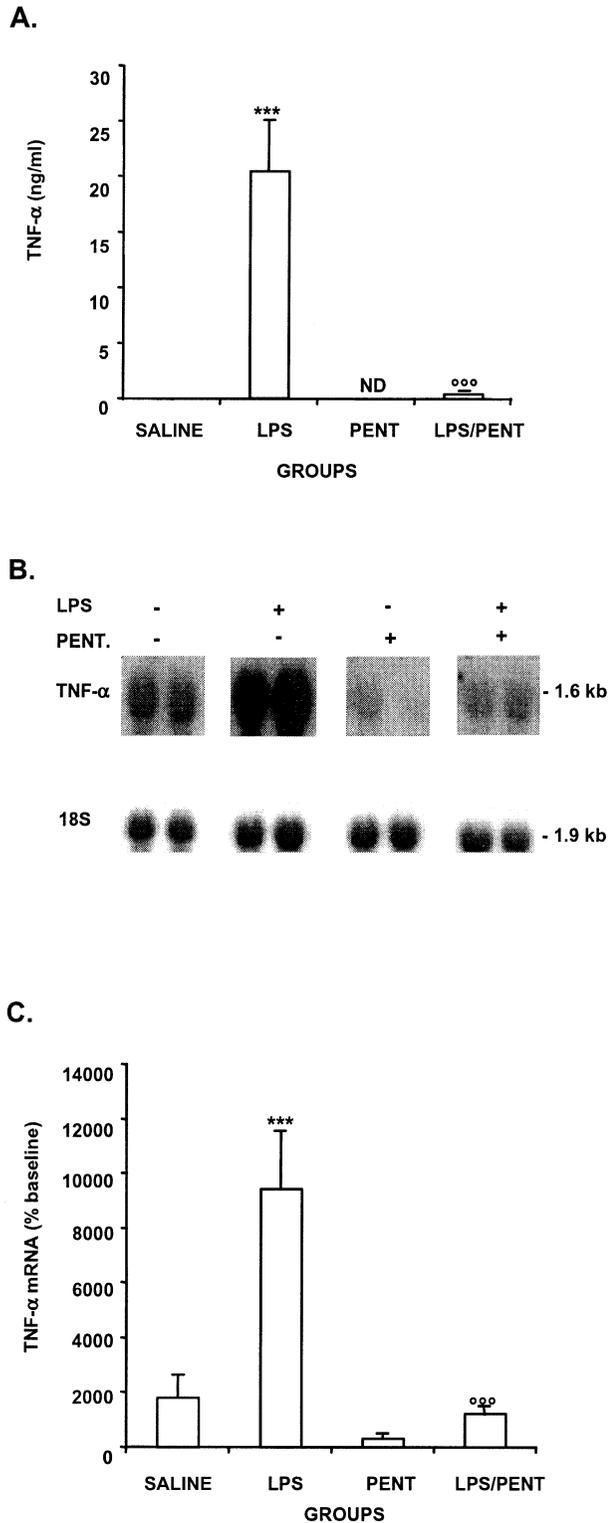
their transcripts in the liver. This suggests that LPS administration stimulates the production of these cytokines by Kupffer cells. These cells, which represent the major population of resident macrophages in the body, are indeed potential target cells for LPS in the liver since they express LPS receptors such as Toll-like receptor-4 (Aderem 2001). Moreover, the liver is the first organ encountered by the blood stream draining the peritoneal cavity where LPS has been injected. Thus, induction of TNF-α and IL-1β by LPS in Kupffer cells might result in a suppression of IGF-I expression in hepatocytes through paracrine activity since these cells are in close contact in the liver. This study demonstrates that injection of pentoxifylline completely inhibits circulating TNF-α induction after LPS injection. This inhibition involves a transcriptional mechanism in the liver, probably in Kupffer cells, since the induction of liver TNF-α mRNA by LPS was also suppressed by administering this drug. In addition, pentoxifylline inhibits IL-1β synthesis in the liver. However, IL-1β induction was not suppressed to the same extent as TNF-α. IL-1β was thus only reduced by a half at 180 min after LPS, since preliminary experiments showed that IL-1β levels peaked at this time point after LPS injection, in contrast to TNF-α which peaked at 90 min. Because TNF-α, which appeared before IL-1β, is a classical inducer of this later cytokine, the inhibition of IL-1β production by pentoxifylline might be related to TNF-α suppression. Thus, in a baboon model of sepsis induced by *E. coli*, monoclonal antibodies against TNF-α inhibit the production of IL-1β and other cytokines (Emerson *et al.* 1992). Nevertheless, the fact that IL-1β production is only partly inhibited by pentoxifylline suggests that TNF-α-independent pathway(s) controlling IL-1β synthesis might exist in this model, such as a direct stimulation of IL-1β gene transcription by LPS. In fact, a previous study has shown that the production of IL-1α, the cytokine closest to IL-1β, can still be stimulated by LPS in TNFR-p55 knock-out animals, suggesting that TNF-α is not necessary for IL-1β induction in response to LPS (Fantuzzi & Dinarello 1996). The most striking result of this study is that, despite a complete suppression of TNF-α production by pentoxifylline, the inhibition of liver IGF-I production caused by LPS has not been prevented. In addition to inducing GH resistance, LPS injection has also been shown to decrease GH secretion, mainly by

Figure 3 Serum TNF-α concentrations and hepatic TNF-α mRNA levels 90 min after LPS injection in rats pretreated with pentoxifylline (PENT). (A) Serum TNF-α concentrations after LPS and/or pentoxifylline. These data are presented as means ± S.E.M. (B) Northern blot analysis of hepatic TNF-α mRNA (top row) and 18S RNA (bottom row). (C) Densitometric analysis of hepatic TNF-α mRNA after LPS and/or pentoxifylline in rat liver. These data are presented as the mean ± S.E.M., expressed as a percentage of the mean observed in the baseline group (time 0). ***$P < 0.001$ compared with control (CTRL) values; °°°$P < 0.001$ compared with LPS values. ND, non detectable.

A.

B.

C.
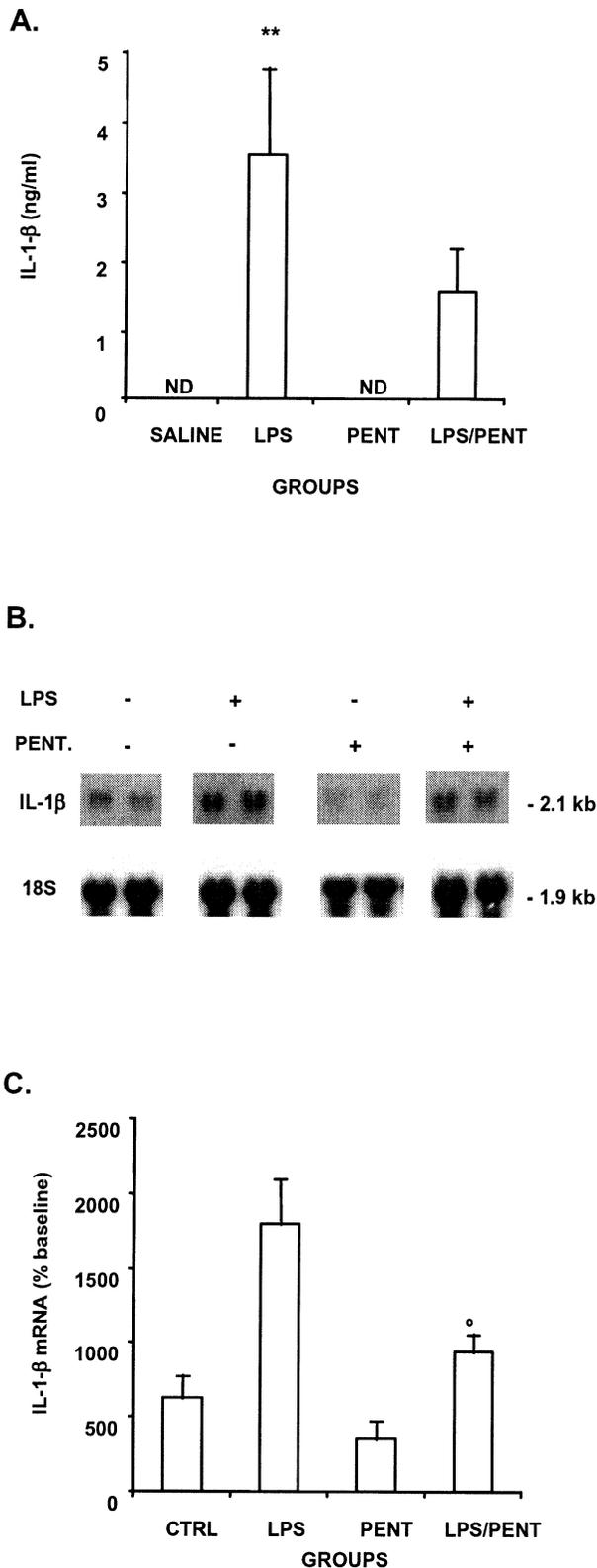
stimulating IL-1β synthesis (Peisen *et al.* 1995). Thus, we performed additional experiments using exogenous GH, to assess the ability of pentoxifylline to restore the GH sensitivity. Despite combined administration of pentoxifylline and GH in LPS-treated rats, IGF-I levels remained unchanged by comparison with animals injected with LPS alone. This observation indicates that the inhibition of GH secretion by LPS does not explain the persistence of reduced concentrations of IGF-I in this model. Inhibition of IGF-I production is therefore primarily related to GH resistance in our model. However, the failure of pentoxifylline to restore GH sensitivity despite TNF-α suppression remains unexplained. Thus, this observation does not agree with the predicted role of TNF-α in GH resistance associated with sepsis, as illustrated by the study of Fan *et al.* (1995) showing that the administration of antibodies neutralizing TNF-α activity restored by 50% the IGF-I levels after LPS injection, despite the persistence of reduced GH secretion. One hypothesis is that other factors implicated in the control of IGF-I production have been modulated by anti-TNF-α antibodies in the study by Fan *et al.*, or by drug treatment in our study. One possibility to explain the persistence of decreased IGF-I, despite the inhibition of TNF-α, is that the residual IL-1β levels (about 2 ng/ml) might have been sufficient to inhibit liver IGF-I production. This hypothesis is supported by the observation that IL-1β at 1 ng/ml still markedly inhibits GH-stimulated IGF-I gene expression in cultured hepatocytes (Thissen & Verniers 1997). The fact that IL-1β neutralization prevents IGF-I inhibition caused by LPS further strengthens this interpretation (Lang *et al.* 1996). Another possibility could be that IL-6 levels, which are less affected by pentoxifylline (Voisin *et al.* 1998), might be able to inhibit IGF-I expression. This hypothesis is suggested by recent observations showing that IL-6 is able to inhibit basal IGF-I expression *in vitro* (Lelbach *et al.* 2001) and to stimulate gene expression of SOCS-3 which exerts a negative feed-back loop controlling GH action (Ram & Waxman 1999, Colson *et al.* 2000). Previous studies documented that muscle proteolysis induced by *E. coli* injection in rats is reduced by pentoxifylline pretreatment (Vary *et al.* 1999). Our data indicate that this anti-catabolic effect of pentoxifylline does not result from the prevention of the decline of circulating IGF-I. However, in those studies, it is possible that pentoxifylline

Figure 4 Serum IL-1β concentrations and hepatic IL-1β mRNA levels 180 min after LPS injection in rats pretreated with pentoxifylline (PENT). (A) Serum IL-1β concentrations after LPS and/or pentoxifylline. These data are presented as means ± S.E.M. (B) Northern blot analysis of hepatic IL-1β mRNA (top row) and 18S RNA (bottom row). (C) Densitometric analysis of hepatic IL-1β mRNA after LPS and/or pentoxifylline in rat liver. These data are presented as the mean ± S.E.M., expressed as a percentage of the mean observed in the baseline group (time 0). **$P < 0.01$ compared with control (CTRL) values; °$P < 0.05$ compared with LPS values. ND, non detectable.
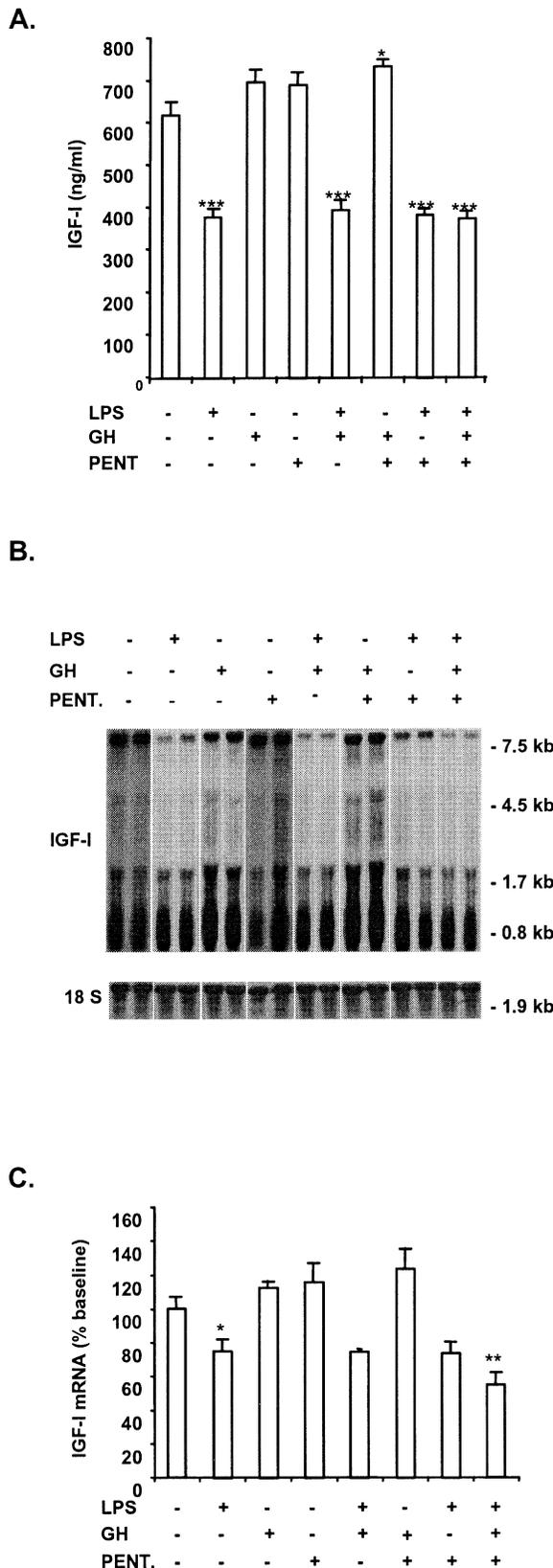
might have exerted its anti-catabolic action by preventing TNF-α induction into the muscle. Indeed, as recently observed in our laboratory, LPS injection can induce TNF-α expression into the muscle itself (Fernández–Celemín *et al.* 2002) and hence may cause an increase in proteolysis (Li *et al.* 1998). This finding implies that other studies are needed to clarify the effects of pentoxifylline prior to its potential use in clinical trials devoted to prevent lean mass loss associated with sepsis in humans.

In conclusion, this study demonstrates that suppression of TNF-α by pentoxifylline fails to restore both circulating IGF-I levels and GH sensitivity in rats challenged with LPS. Moreover, our data suggest that mediators other than TNF-α, such as IL-1β and IL-6, might play a major role in the pathogenesis of GH resistance associated with sepsis.

Acknowledgements

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Figure 5 Serum IGF-I concentrations and hepatic IGF-I mRNA 10 h after LPS and GH injections in rats pretreated by pentoxifylline (PENT). (A) Serum IGF-I concentrations after LPS, pentoxifylline and/or GH. These data are presented as means ± S.E.M. (B) Northern blot analysis of hepatic IGF-I mRNA (top row) and 18S RNA (bottom row). (C) Densitometric analysis of hepatic IGF-I mRNA after LPS, pentoxifylline and/or GH. These data are presented as the mean ± S.E.M., expressed as a percentage of the mean observed in the baseline group (time 0). *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with control values.

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