Dexamethasone administration attenuates the inhibitory effect of lipopolysaccharide on IGF-I and IGF-binding protein-3 in adult rats

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Abstract

The aim of this study was to investigate whether glucocorticoid administration had a beneficial effect on serum concentrations of insulin-like growth factor I (IGF-I) and on IGF-binding protein 3 (IGFBP-3) in rats injected with lipopolysaccharide (LPS). Adult male rats were injected with LPS or saline and pretreated with dexamethasone or saline. Dexamethasone administration decreased growth hormone (GH) receptor and IGF-I mRNA levels in the liver of control rats. LPS decreased GH receptor and IGF-I gene expression in the liver of saline-treated rats but not in the liver of dexamethasone-pretreated rats. In the kidney, GH receptor mRNA levels were not modified by dexamethasone or LPS treatment. However, LPS decreased renal IGF-I gene expression and dexamethasone pretreatment prevented this decrease. Serum concentrations of IGF-I were decreased by LPS, and dexamethasone pretreatment attenuated this effect. The gene expression of

IGFBP-3 in the liver and kidney and its circulating levels were decreased by LPS. In control rats dexamethasone increased circulating IGFBP-3 and its gene expression in the liver, and decreased the proteolysis of this protein. Dexamethasone pretreatment attenuated the LPS-induced decrease in IGFBP-3 gene expression in the liver and prevented the LPS-induced decrease in IGFBP-3 gene expression in the kidney. Moreover, dexamethasone pretreatment attenuated the LPS-induced decrease in serum concentrations of IGFBP-3 and decreased the LPS-induced IGFBP-3 proteolysis in serum. In conclusion, dexamethasone pretreatment partially attenuates the inhibitory effect of LPS on serum IGF-I by blocking the decrease of its gene expression in the kidney as well as by attenuating the decrease in serum concentrations of IGFBP-3.

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Introduction

Catabolism and growth impairment are complications of several situations such as sepsis and inflammatory diseases. These situations are associated with neuroendocrine changes that contribute to the catabolic state and that include an increase in serum concentrations of glucocorticoids together with a decrease in serum insulin-like growth factor I (IGF-I). Sepsis can be induced experimentally by administering lipopolysaccharide (LPS), a component of the wall of gram-negative bacteria. LPS administration decreases circulating levels of IGF-I both in humans (Lang *et al.* 1997) and in experimental animals (Fan *et al.* 1994, Soto *et al.* 1998). We have previously observed that the decrease in serum IGF-I is associated with a decrease in growth hormone (GH) receptor and IGF-I synthesis in the liver (Priego *et al.* 2002a, 2003a).

IGF-I is an important mitogen and acts in an endocrine, paracrine or autocrine manner. The bioactivity of this growth factor is regulated by IGF-I binding proteins

(IGFBPs), where IGFBP-3 is the main carrier of circulating IGF-I. This binding protein can increase the half-life of circulating IGF-I and control the access of IGF-I to extravascular target tissues. IGFBP-3 also plays an important role in the cellular environment, where it may both inhibit and potentiate IGF-I-stimulated DNA synthesis (De Mellow & Baxter 1988). The mechanisms by which IGFBP-3 activity can be regulated are through its organ synthesis or through its proteolysis. This last modulation generates fragments that bind IGF with lower affinity compared with intact IGFBP-3, resulting in an increased bioavailability of IGF (Blat *et al.* 1994). We have previously reported that in LPS-treated rats there was a decrease in serum concentrations of IGFBP-3 and in its synthesis in the liver (Priego *et al.* 2003b).

LPS administration to rats induces an activation of the hypothalamic–pituitary–adrenal axis, shown by an increase in serum adrenocorticotropin and corticosterone concentrations (Fan *et al.* 1994, Priego *et al.* 2002a). The increased levels of glucocorticoids after acute endotoxin exposure

could be the mediator of depressed serum and liver IGF-I levels, since the glucocorticoid, dexamethasone, inhibits IGF-I synthesis in the liver (Luo & Murphy 1989) and decreases GH receptor (Gabrielsson *et al.* 1995). However, we have recently reported that glucocorticoids are not responsible for the inhibitory effect of LPS on circulating IGF-I and IGFBP-3, as well as their gene expression in the liver (Priego *et al.* 2002a,b). Moreover, prevention of the increase in glucocorticoid secretion after LPS by adrenalectomy potentiates the inhibitory effect of LPS on serum and liver IGF-I (Priego *et al.* 2002a). This fact suggests that glucocorticoids have a beneficial effect, probably preventing the release of an IGF-I inhibitory factor. Nitric oxide (NO) could be one of the possible inhibitory factors. We have observed that administration of an inducible NO synthase (iNOS) inhibitor prevented the increase in serum nitrites and nitrates as well as the decrease in both IGF-I and IGFBP-3 serum levels and their hepatic mRNA levels induced by LPS (Priego *et al.* 2004). Furthermore, it has been reported that dexamethasone decreases hepatocyte iNOS mRNA levels *in vitro* (Geller *et al.* 1993). On the other hand, corticosteroid therapy has been considered as beneficial in the treatment of septic shock patients in recent years. Several studies have shown that corticosteroid therapy appears to aid in the treatment of the shock state and lead to a more rapid reversal of the hemodynamic alterations. As a consequence of the hemodynamic improvement, there was also a benefit to overall mortality (Annane *et al.* 2002, Yildiz *et al.* 2002).

Taking into account the fact that in the last few years glucocorticoid therapy has been considered beneficial in the treatment of septic shock patients, reducing mortality, the present study was conducted in order to analyze if dexamethasone pretreatment is able to prevent the effect of LPS on the serum IGF-I–IGFBP-3 system and in this way counteract the catabolic response associated with the sepsis situation. Although the liver is the main source of serum IGF-I (Sjogren *et al.* 1999), the kidney has also been reported as another source (Li *et al.* 1997, Ibáñez de Cáceres *et al.* 2002b). For this reason, hepatic and renal IGF-I and IGFBP-3 mRNA levels were measured. Moreover, we wanted to analyze if the effects of dexamethasone pretreatment in LPS-treated rats could be mediated by NO. For this purpose, serum concentrations of nitrite and nitrate, as an index of NO production, were also analyzed.

Materials and Methods

Animals and experimental design

Male Wistar rats (250 g; Charles River, Barcelona, Spain) were used for the experiment. They were housed three or four per cage with free access to food and water, under constant conditions of temperature (20–22 °C) and light (lights on from 0730 to 1930 h). The procedures followed the guidelines recommended by the European Union for

the care and use of laboratory animals. Forty rats were injected intraperitoneally with LPS (250 µg/kg) or saline at 1800 h on one day and at 1000 h on the following day. Rats were killed by decapitation 3 h after the second LPS or saline injection, between 1300 h and 1345 h. This LPS administration protocol has been shown to decrease levels of serum IGF-I and its mRNA in the liver (Priego *et al.* 2003a). In order to study if glucocorticoid administration is able to prevent the effect of LPS on IGF-I, rats were pretreated with dexamethasone. Dexamethasone (Sigma-Aldrich, Madrid, Spain) was injected subcutaneously (200 µg/kg) two hours before each LPS injection (at 1600 h on the first day and at 0800 h on the second day). Control rats were injected with saline. Trunk blood was collected in cooled tubes, allowed to clot, centrifuged and the serum stored at – 20 °C until nitrites and nitrates, IGF-I and IGFBP-3 analyses were performed. Immediately after decapitation the liver and kidney were removed, frozen in liquid nitrogen and stored at – 80 °C until RNA extraction was performed.

Determination of nitrite and nitrate concentrations

Nitrite and nitrate concentrations in serum were measured by a modified method of the Griess assay, described by Miranda *et al.* (2001). Serum was deproteinized to reduce turbidity by centrifugation through a 30-kDa molecular mass filter using a Centrifree Micropartition device with a YM-30 ultrafiltration membrane (Amicon Division, Millipore Corporation, Bedford, TX, USA), at 15 000 r.p.m. for 1 h at 37 °C for 300 µl samples. One hundred microliters filtered serum were mixed with 100 µl vanadium chloride, quickly followed by the addition of the Griess reagents. The determination was performed after incubation at 37 °C for 30 min. The absorbance was measured at 540 nm. Nitrite and nitrate concentrations were calculated using a NaNO₂ standard curve and were expressed as µM.

Hormone measurements

Serum IGF-I was removed from IGFBPs by an acid-ethanol extraction and was measured by a double-antibody RIA (Soto *et al.* 1998). The IGF-I antiserum (UB-2495) was a gift from Drs Underwood and Van Wyk, and was distributed by the Hormone Distribution Program of NIDDK (Bethesda, MD, USA) through the National Hormone and Pituitary Program. Levels of IGF-I were expressed in terms of IGF-I from Gropep (Adelaide, Australia). The sensitivity of the assay was 20 pg/ml. The intra-assay coefficient of variation was 8%. All samples were run in the same assay.

Western ligand blot

Western blots were prepared as previously described (Soto *et al.* 1998). Blood serum proteins were separated by

12.5% SDS-PAGE under non-reducing conditions and blotted onto nitrocellulose membranes (Hybond-C-extra; Amersham, Little Chalfont, Bucks, UK) by means of a semidry electrophoretic transfer cell (Bio-Rad, Hercules, CA, USA). The blots were dried and blocked for 1 h with 5% nonfat dry milk and 0.1% Tween (Sigma) in Tris-buffered saline and incubated overnight at 4 °C with ^{125}I -labeled IGF-I (5×10^5 c.p.m./ml). The nitrocellulose sheets were washed and dried, and blots were exposed at -80 °C to X-ray film (Kodak X-Omat AR; Eastman Kodak, Rochester, NY, USA) and two intensifying screens for 1–3 days according to the signal obtained. The signals of the film were quantified by densitometric scanning using a Gengenius (Syngene, Cambridge, Cambs, UK). The density of the IGFBP-3 band in each lane was expressed as the percentage of the mean density of sera from control rats injected with saline.

Proteolysis assay

To measure the IGFBP-3 proteolysis in serum, we used the Lamson *et al.* (1991) method. Serum samples (5 μl) were mixed with 15 000 c.p.m. ^{125}I -rhIGFBP-3 (glycosylated recombinant human IGFBP-3 (GroPep) iodinated using the chloramine T method) in a total volume of 30 μl in 0.05 M phosphate buffer, pH 7.4. The mixture was incubated for 18 h at 37 °C, and the reaction was stopped by adding 10 μl non-reducing sample buffer and then boiling. Ten microliters of the mixture were subjected to 12.5% SDS-PAGE and run at 160 mA for 2 h. Gels were fixed and dried (Bio-Rad Gel Drying System-543) and exposed to X-ray film at -80 °C for 2–3 days according to the signal obtained. The intact ^{125}I -rhIGFBP-3 (42- to 45-kDa band) from each sample was measured by densitometry. Proteolysis was expressed as the percentage of the total optical density from heat inactivated serum (56 °C for 30 min).

RNA extraction and Northern blot analysis

Total RNA extraction was performed with a kit (Ultraspec RNA; Biotecx Laboratories, Houston, TX, USA) following the manufacturer's instructions. For Northern blotting, 30 μg denatured RNA from livers and kidneys were separated by formaldehyde-agarose gel electrophoresis, transferred to nylon membranes (Hybond-N+; Amersham) by overnight capillary blotting and fixed by UV crosslinking (Fotodyne, Hartland, WI, USA).

Hepatic and renal levels of IGF-I and GH receptor mRNA were measured by Northern blot hybridization using riboprobes (Roberts *et al.* 1987, Baumbach *et al.* 1989). The rat IGF-I and GH receptor probes were derived from a HindIII fragment of the pGEM-3 plasmid vector (Promega, Madison, WI, USA). ^{32}P -labeled RNA antisense probes were generated from linearized plasmid with [α - ^{32}P]CTP (Nuclear Ibérica, Madrid, Spain) and

T7 RNA polymerase (Roche Molecular Biochemicals, Barcelona, Spain).

Prehybridization was performed for 30 min at 68 °C in ULTRAhyb buffer (Ambion, Austin, TX, USA) followed by hybridization for 16 h at the same temperature with 1×10^6 c.p.m./ml IGF-I-labeled riboprobe or 3×10^6 c.p.m./ml GH receptor-labeled riboprobe, in the same buffer. The membranes were washed twice with $2 \times \text{SSC}$, 0.1% SDS at 68 °C for 10 min, and twice with $0.1 \times \text{SSC}$, 0.1% SDS at 68 °C, also for 10 min.

The rat IGFBP-3 cDNA probe encodes the IGFBP-3 protein mRNA (Albiston & Herington 1990). The probe was obtained by cutting the PEGEM 4Z plasmid vector using EcoRI and HindIII. Prehybridization was performed for 30 min in ULTRAhyb buffer, followed by hybridization at 42 °C for 16 h with 3×10^6 c.p.m./ml IGFBP-3 labeled with a random priming DNA labeling kit (DECAprimeTM II; Ambion). The membranes were washed twice with $2 \times \text{SSC}$, 0.1% SDS at 42 °C for 10 min, and twice with $0.1 \times \text{SSC}$, 0.1% SDS, also for 10 min at 42 °C. To verify loading, control hybridization was performed with a 28S DNA probe labeled with [^{32}P]dCTP by random primer. The membranes were exposed at -80 °C for 1–4 days depending on the intensity of the signal obtained. To obtain semiquantitative data for statistical analysis, each gel was run with 5 RNA samples from each experimental group. The density of the each band was normalized with the 28S hybridization and expressed as the percentage of the mean of the control group injected with saline.

Statistical analysis

Data were analyzed using the program STATGRAPHICS plus (Manugistic Inc., Rockville, MD, USA) for Windows. Data are presented as means $\pm$ S.E.M. Comparisons between means were made by two-way ANOVA and subsequent Duncan's multiple range test. Significance was assumed at $P < 0.05$.

Results

Dexamethasone administration significantly decreased GH receptor mRNA levels in the liver of control rats ($P < 0.01$) (Fig. 1A). LPS decreased GH receptor mRNA in saline-treated rats ($P < 0.01$) (Fig. 1A), but not in dexamethasone-pretreated rats (Fig. 1A). In contrast, renal GH receptor mRNA levels were not modified by dexamethasone or by LPS treatment (Fig. 1B).

Similar to the effects found on hepatic GH receptor, dexamethasone administration significantly decreased ($P < 0.01$) IGF-I gene expression in the liver of control rats. LPS decreased IGF-I mRNA levels in saline-treated rats ($P < 0.01$), but not in dexamethasone-pretreated rats (Fig. 2A). Nevertheless, the effects of dexamethasone and LPS

injections on the renal IGF-I gene expression are different to the effect found on renal GH receptor (Fig. 2B). Dexamethasone administration did not modify the IGF-I gene expression in the kidney of control rats. LPS significantly decreased ($P<0.01$) the IGF-I mRNA levels in the kidney of saline-treated rats but not in dexamethasone-pretreated rats (Fig. 2B).

After administration of LPS, serum concentrations of IGF-I were significantly decreased in saline-treated rats ($P<0.01$) (Fig. 3). Dexamethasone pretreatment did not modify serum concentrations of IGF-I in control rats,

whereas it clearly attenuated the decrease in serum concentrations of IGF-I caused by LPS injections (Fig. 3).

LPS injections significantly decreased liver IGFBP-3 gene expression ($P<0.01$) (Fig. 4A). Dexamethasone pretreatment increased the IGFBP-3 gene expression in the liver in control rats ($P<0.01$) and attenuated the decrease on IGFBP-3 induced by LPS. Similarly, LPS injections significantly decreased ($P<0.01$) IGFBP-3 mRNA levels in the kidney (Fig. 4B) and dexamethasone pretreatment prevented this decrease (Fig. 4B).

LPS injections significantly decreased serum concentrations of IGFBP-3 (Fig. 5). Dexamethasone pretreatment increased serum IGFBP-3 concentrations in control rats ($P<0.01$) and attenuated the decrease in serum IGFBP-3 induced by LPS.

IGFBP-3 proteolysis in the serum of rats treated with dexamethasone and LPS is shown in Fig. 6. Proteolysis of IGFBP-3 was not significantly decreased by LPS in saline-treated rats. However, dexamethasone pretreatment clearly decreased the proteolysis of IGFBP-3 in the serum of control rats ($P<0.01$) and decreased LPS-induced IGFBP-3 proteolysis ($P<0.01$) (Fig. 6).

As shown in Fig. 7, in control rats dexamethasone did not modify serum nitrite and nitrate concentrations. LPS increased serum nitrite and nitrate concentrations in saline-treated rats ($P<0.01$), whereas in dexamethasone-pretreated rats LPS was not able to increase the nitrite and nitrate levels.

Discussion

In this study, control and LPS-treated rats were pretreated with dexamethasone to study the effect of glucocorticoid administration on serum IGF-I and IGFBP-3 in the situation of acute inflammation. The results indicate that dexamethasone pretreatment in LPS-treated rats attenuated the decrease in serum IGF-I concentrations by blocking the decrease in its gene expression in the kidney. Moreover, dexamethasone pretreatment in LPS-treated

Figure 1 Hepatic (A) and renal (B) growth hormone receptor (GHR) mRNA expression in rats injected with dexamethasone (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg). The upper panels show GHR mRNA abundance determined by densitometry and expressed as a percentage of the mean value in control rats treated with saline. Both dexamethasone and LPS decreased GH receptor mRNA in the liver ($F_{1,36}=40.91$, $P<0.001$ and $F_{1,36}=5.64$, $P<0.05$ respectively). Data represent the mean $\pm$ S.E.M. for 9–10 rats per group. ** $P<0.01$ vs control group injected with saline; ## $P<0.01$ vs LPS group injected with saline (two-way ANOVA and Duncan's multiple comparison test). The lower panels show a representative Northern blot analysis of GHR mRNA hybridization. RNA was hybridized with an RNA probe for rat GHR mRNA as described in Materials and Methods. The size of the hybridization bands (kb) and ribosomal 28S RNA are indicated on the right; each lane corresponds to an individual animal from the indicated group. C, control.

rats attenuated the decrease in serum IGFBP-3 by increasing its gene expression in the liver and kidney and by decreasing its serum proteolysis.

As previously reported, LPS injections decreased GH receptor and IGF-I mRNAs in the liver (Priego *et al.* 2002a, 2003a). However, in the kidney, LPS did not affect

Figure 3 Serum concentrations of insulin-like growth factor I (IGF-I) in rats treated with dexamethasone (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg). Dexamethasone pretreatment increased serum IGF-I concentrations ($F_{1,35}=14.49$, $P<0.001$), whereas LPS decreased the serum concentrations of IGF-I ($F_{1,35}=75.55$, $P<0.001$). Each bar represents the mean $\pm$ S.E.M. of 8–10 rats per group. ** $P<0.01$ vs control group injected with saline; ## $P<0.01$ vs LPS group injected with saline; ++ $P<0.01$ vs control group injected with dexamethasone (two-way ANOVA and Duncan's multiple comparison test).

GH receptor gene expression, although it decreased IGF-I gene expression, as in the liver. These data indicate that, in the kidney, the inhibitory effect of LPS on IGF-I could be mediated by post-receptor GH signaling. We have previously observed that the effects of LPS are stronger on IGF-I than on GH receptor gene expression (Priego *et al.* 2003a). In fact, with doses of LPS lower than those used in the present study (Priego *et al.* 2003a) or in arthritic rats (López-Calderón *et al.* 2001) we found a decrease in hepatic IGF-I mRNA without changes in the GH receptor. Li *et al.* (1997) reported that LPS increased renal IGF-I mRNA. Discrepancies with our findings could be due to

Figure 2 Hepatic (A) and renal (B) insulin-like growth factor I (IGF-I) gene expression in dexamethasone- (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg)-treated rats. Upper panels: liver and kidney IGF-I mRNA expression. Two-way ANOVA revealed that dexamethasone decreased hepatic IGF-I mRNA ($F_{1,36}=16.63$, $P<0.001$) as did LPS ($F_{1,36}=13.64$, $P<0.001$). There was an interaction between the effect of dexamethasone and LPS administration on renal IGF-I mRNA ($F_{1,35}=5.06$, $P<0.05$), as LPS treatment decreased renal IGF-I in saline-pretreated rats but not in dexamethasone-pretreated rats. Data (means $\pm$ S.E.M.) from 5–10 individual rats were quantified by densitometry and expressed as a percentage of the mean value in control rats treated with saline. ** $P<0.01$ vs control group injected with saline; # $P<0.05$ vs LPS group injected with saline. A representative Northern blot of IGF-I mRNA hybridization is shown in the lower panels of the figure. The size of the hybridization bands (kb) are indicated on the right; each band corresponds to an individual rat from the indicated group. C, control; 28S, 28S ribosomal RNA.

the different form of LPS administration. In the Li *et al.* study, the analysis was performed 4 h after a single LPS injection, while we administered two injections of LPS and analyzed the kidney 19 h after the first injection. In these conditions, we found that LPS decreased IGF-I gene expression in the kidney. Furthermore, in chronic inflammation we also found a decrease in renal IGF-I mRNA (Ibáñez de Cáceres *et al.* 2002a).

In agreement with previous data (Luo & Murphy 1989) dexamethasone administration to control rats decreased hepatic IGF-I mRNA. This seems to be due to a direct

Figure 5 Effect of dexamethasone (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg) administration on the serum IGF-I-binding protein levels of IGFBP-3. IGFBP-3 protein levels were measured by the IGF-I binding activity of the sera. A representative Western ligand blot of IGFBP-3 in 2.5 µl pools of serum from the different groups is shown in the lower panel. IGFBPs were separated by a 12.5% SDS-PAGE gel, transferred to nitrocellulose, ligand blotted with ^{125} I-IGF-I, and visualized via autoradiography; the approximate molecular mass of the band is indicated on the right. In the upper panel, data (means $\pm$ S.E.M.) from 10 individual rats were quantified by densitometry and expressed as a percentage of the mean value of control rats treated with saline. Dexamethasone increased serum concentrations of IGFBP-3 ($F_{1,39}=23.60$, $P<0.001$), whereas LPS decreased it ($F_{1,39}=16.21$, $P<0.001$). ** $P<0.01$ vs control group injected with saline; ### $P<0.01$ vs LPS group injected with saline; +++ $P<0.01$ vs control group injected with dexamethasone (two-way ANOVA and Duncan's multiple comparison test). C, control.

Figure 4 Hepatic (A) and renal (B) IGFBP-3 mRNA content in dexamethasone- (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg)-treated rats. A representative Northern blot of IGFBP-3 mRNA hybridization is shown in the lower panels. RNA was hybridized with a cDNA probe for rat IGFBP-3 mRNA as described in Materials and Methods. The size of the hybridization band (kb) and ribosomal 28S RNA are indicated on the right; each lane corresponds to an individual animal from the indicated group. Quantitative analyses are expressed as percentages of data from control rats injected with saline (upper panels). There was no interaction between the effect of dexamethasone and LPS treatment on hepatic and renal IGFBP-3 mRNA. Dexamethasone increased IGFBP-3 mRNA in the liver ($F_{1,37}=38.77$, $P<0.001$) and in the kidney ($F_{1,31}=14.18$, $P<0.001$), whereas LPS treatment decreased hepatic ($F_{1,37}=41.32$, $P<0.001$) and renal ($F_{1,31}=2.36$, not significant) IGFBP-3 mRNA. Each bar represents the mean $\pm$ S.E.M. for 5–10 rats per group. ** $P<0.01$ vs control group injected with saline; ### $P<0.01$ vs LPS group injected with saline; +++ $P<0.01$ vs control group injected with dexamethasone. C, control.

Figure 6 IGFBP-3 proteolysis in serum of dexamethasone- (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg)-treated rats. ^{125} I-rhIGFBP-3 was incubated for 18 h at 37 °C with sera and submitted to SDS-PAGE as described in Materials and Methods. A representative autoradiograph of IGFBP-3 protease assay of rat serum samples from control and experimental groups treated with saline or dexamethasone and heat-inactivated (HI) serum are shown in the lower panel. Heat-inactivated serum was used as a control, as no proteolytic activity is found in the serum after being heated. Molecular mass is shown on the right. IGFBP-3 proteolysis was expressed as a percentage decrease in ^{125} I-rhIGFBP-3 in each sample relative to ^{125} I-rhIGFBP-3 in control samples incubated with heat-inactivated serum (upper panel). Dexamethasone decreased IGFBP-3 proteolysis ($F_{1,35}=22.62$, $P<0.001$), whereas LPS treatment did not modify it ($F_{1,35}=0.52$, not significant). Each point represents the mean $\pm$ S.E.M. for 9 rats per group. ** $P<0.01$ vs control group injected with saline; ### $P<0.01$ vs LPS group injected with saline (two-way ANOVA and Duncan's multiple comparison test). C, control.

effect of dexamethasone on the liver, since this glucocorticoid decreased hepatic GH receptor mRNA levels, as described by other authors both *in vivo* (Gabrielsson *et al.* 1995, Kritsch *et al.* 2002) and *in vitro* (Beauloye *et al.* 1999). However, we did not find any modifications in GH receptor or IGF-I mRNA levels in the kidney of control rats after dexamethasone administration as previously described by other authors (Luo & Murphy 1989, Heinrichs *et al.* 1994). It is possible that the liver is an organ which is more sensitive to the effects of glucocorticoids than is the kidney. In this respect, Luo & Murphy (1989) reported a decrease in hepatic IGF-I mRNA levels after administering doses of dexamethasone similar to those used in the present study, but only found a decrease in renal IGF-I mRNA with a higher dose.

The LPS-induced decrease in GH receptor and IGF-I mRNAs in the liver was not bigger in rats pretreated with dexamethasone. Furthermore, this glucocorticoid was able to prevent the decrease in renal IGF-I mRNA caused by

Figure 7 Effect of dexamethasone (200 µg/kg) and lipopolysaccharide (LPS, 250 µg/kg) injections on serum concentrations of nitrites and nitrates. There was an interaction between the effect of dexamethasone and LPS administration on serum nitrite and nitrate concentrations ($F_{1,40}=13.38$, $P<0.001$) as LPS increased serum nitrites and nitrates in saline-pretreated rats but not in dexamethasone-pretreated rats. Results are expressed as means $\pm$ S.E.M., $n=10$ rats per group. ** $P<0.01$ vs control group injected with saline; ### $P<0.01$ vs LPS group injected with saline (two-way ANOVA and Duncan's multiple comparison test).

LPS. These results contrast with others previously reported (Li *et al.* 1997), in which the LPS-induced decrease in hepatic IGF-I gene expression and the increase in renal IGF-I gene expression were attenuated by pretreatment with the antiglycorticoid RU-486. However, in this study, the antagonistic action of RU-486 was not effective enough, since it did not increase corticosterone levels in rats treated with LPS. As dexamethasone pretreatment inhibits or does not modify IGF-I mRNAs in the liver and kidney of control rats, but does not modify or prevents the decrease in both organs in LPS-treated rats, our results suggest that dexamethasone pretreatment can inhibit the mechanism by which LPS decreases these two parameters. This mechanism could involve NO, since dexamethasone pretreatment prevents the increase in serum nitrite and nitrate concentrations in LPS-treated rats. Moreover, we have previously reported (Priego *et al.* 2004) that inhibition of iNOS by aminoguanidine administration prevented the effect of LPS on circulating IGF-I and its gene expression in the liver as well as on circulating nitrite and nitrate concentrations.

There are inconsistent reports of the effect of glucocorticoids on serum IGF-I concentrations, with some studies reporting a reduction in circulating levels (Canalis 1997) and others reporting no effect (Ward *et al.* 1999). In our study, serum IGF-I was not significantly modified by dexamethasone in control rats, although IGF-I gene expression in the liver was decreased, and it was unchanged in the kidney. Discrepances between serum IGF-I and

hepatic IGF-I mRNA have previously been described in rats injected with dexamethasone (Luo & Murphy 1989, Kritsch *et al.* 2002). According to our results, dexamethasone had little stimulatory effect on serum IGF-I but decreased IGF-I gene expression in the liver (Luo & Murphy 1989). Dexamethasone pretreatment attenuated the LPS-induced decrease in serum IGF-I in spite of not modifying the decrease in hepatic IGF-I mRNA. The effect of dexamethasone pretreatment on serum IGF-I in LPS-treated rats might, in part, be the result of blocking the decrease in renal IGF-I mRNA. The discordance between serum IGF-I and hepatic IGF-I mRNA in rats that received dexamethasone could also be explained by the fact that glucocorticoids affect production of IGFBPs and thereby may alter the metabolic clearance of IGF-I. Our results are consistent with this hypothesis, since dexamethasone pretreatment increased serum IGFBP-3 in control rats and attenuated the decrease in LPS-treated rats. This increase in serum IGFBP-3 induced by pretreatment with dexamethasone might be contributing, in part, to the increase in serum IGF-I in control and in LPS-treated rats, although hepatic IGF-I gene expression was decreased in both groups of rats. In this respect, it has been reported that the steroid, prednisolone, partially normalizes serum IGF-I and IGFBP-3 levels in inflammatory bowel disease (Gronbek *et al.* 2002).

The increase in serum IGFBP-3 induced by pretreatment with dexamethasone in control and LPS-treated rats is a consequence of the increase in hepatic and renal IGFBP-3 mRNAs. Regulation of IGFBP-3 by dexamethasone has been studied *in vitro* and *in vivo* in both adult rats and man. In cocultured hepatic parenchymal and nonparenchymal cells, dexamethasone reduced the production of IGFBP-3 by inhibiting IGFBP-3 gene transcription (Villafuerte *et al.* 1995). In contrast to this *in vitro* study, Luo & Murphy (1990) reported increases in rat serum IGFBP-3 and hepatic IGFBP-3 expression after a single dose of dexamethasone. In humans, Miell *et al.* (1994) reported an increase in serum IGFBP-3 levels after short-term dexamethasone treatment. Moreover, in Cushing's syndrome increased circulating IGF-I and IGFBP-3 have been reported (Bang *et al.* 1993). According to our results, dexamethasone also increased IGFBP-3 in organs different from the liver (Koedam *et al.* 2000). The increase in IGFBP-3 mRNA in liver and kidney caused by dexamethasone pretreatment was higher in LPS-treated than in control rats. This effect might be due, in part, to the inhibitory effects of dexamethasone on NO production, since dexamethasone decreased the expression of iNOS in hepatocytes (Geller *et al.* 1993). In addition, in the present study we found that dexamethasone pretreatment blocked the increase in circulating nitrite and nitrate concentrations in LPS-treated rats, and furthermore, we have shown that iNOS inhibition attenuated the inhibitory effect of LPS on hepatic IGFBP-3 mRNA levels (Priego *et al.* 2004).

To our knowledge, this is the first study in which IGFBP-3 proteolysis was measured in serum of rats treated with dexamethasone. Our findings show that dexamethasone pretreatment decreases IGFBP-3 proteolysis in the serum of control and LPS-treated rats. Dexamethasone can induce a catabolic state and although this state has been characterized by an increased IGFBP-3 proteolysis (Davenport *et al.* 1992), this does not always occur, as has been observed in arthritic rats (Ibáñez de Cáceres *et al.* 2002a) and in anorexia nervosa (Stoving *et al.* 1999). NO has been reported to be involved in the activation of proteolytic enzymes (Trachtman *et al.* 1996). However, the effects of dexamethasone on serum proteases seems to be independent of NO, since dexamethasone pretreatment decreased IGFBP-3 proteolysis both in control and in LPS-treated rats. Moreover, serum nitrite and nitrate concentrations were only decreased by dexamethasone pretreatment in LPS-treated rats. This hypothesis is supported by our previous studies in LPS-treated rats (Priego *et al.* 2004) and in experimental arthritis (Ibáñez de Cáceres *et al.* 2003) after aminoguanidine administration. The inhibition of IGFBP-3 fragmentation occurred concomitantly with the described increase in serum IGFBP-3 in both groups of rats pretreated with dexamethasone. Our findings also show that the increase in hepatic and renal IGFBP-3 gene expression after dexamethasone pretreatment also contributes to the increase in serum IGFBP-3 observed in control and LPS-injected rats.

From the present study, we can conclude that the increase in serum concentrations of IGFBP-3 together with the block of the decrease in renal IGF-I mRNA induced by dexamethasone pretreatment contribute to the attenuation of the inhibitory effect of LPS on serum IGF-I concentrations.

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