Effects of endotoxin lipopolysaccharide administration on the somatotrophic axis

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Abstract

The aim of this work was to study the effect of chronic activation of the immune system on the somatotrophic axis. Accordingly, the changes in growth hormone (GH) secretion, circulating insulin-like growth factor-I (IGF-I) and IGF binding proteins (IGFBPs) in response to endotoxin lipopolysaccharide (LPS) administration were examined in adult male Wistar rats. Acute LPS injection (2.5, 25 or 250 µg/kg) increased serum corticosterone in a dose-dependent manner and decreased serum levels of insulin and IGF-I, serum GH concentration declined linearly as the LPS dose increased. Western ligand blot showed an increase in the 33 kDa band (corresponding to IGFBP-1 and IGFBP-2) in the rats that received the highest dose of

LPS (250 µg/kg). Chronic LPS administration (250 µg/kg daily for 8 days) significantly decreased body weight, serum levels of IGF-I and pituitary GH content, whereas it increased circulating IGFBP-3 (47 kDa band), IGFBP-1 and IGFBP-2 (33 kDa band) and the 24 kDa band (which possibly corresponds to IGFBP-4). Serum concentration of corticosterone and hypothalamic somatostatin content were also increased by chronic LPS treatment. These data suggest that the decrease in GH and IGF-I secretion and the increase in circulating IGFBPs are important mechanisms in body weight loss during chronic inflammation.

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Introduction

It is well known that the immune and the neuroendocrine systems communicate bidirectionally. Hormones modulate the immune system, and in turn, cytokines, the soluble factors secreted by the immune cells, modify the neuroendocrine secretion (for reviews see Madden & Felten 1995, Besedovsky & Del Rey 1996). Acute infection induces adrenal hypertrophy (Pinchot *et al.* 1949) and immunization with sheep red blood cells activates the hypothalamic–pituitary–adrenal axis (Besedovsky *et al.* 1975). These effects seem to be mediated by the released cytokines, and it has been shown that interleukin (IL)-1, IL-2, IL-6 and γ -interferon increase glucocorticoid secretion (Besedovsky & Del Rey 1996).

However, immunostimulation not only activates the adrenal axis, but also modifies other hormones. Acute endotoxin administration in rats increases plasma concentration of corticosterone whereas it decreases circulating growth hormone (GH) and insulin-like growth factor-I (IGF-I) (Egdahl 1959, Kastin & Martin 1982, Fan *et al.* 1994). In humans, growth retardation has been described in children suffering from chronic infectious illnesses (McCaffery *et al.* 1970). A decrease in serum concentration of IGF-I in septic patients has also been observed

(Dahn *et al.* 1988). However, the effect of cytokines on GH secretion is not well known. IL-1 administration has been reported both to increase (Rettori *et al.* 1987) and decrease (Peisen *et al.* 1995) GH secretion. Similarly, tumor necrosis factor- α (TNF α) has also been reported to increase (Rettori *et al.* 1989, Elsasser *et al.* 1991) and decrease (Walton & Cronin 1989) GH secretion. Thus, several mechanisms by which chronic immune activation decreases circulating IGF-I have been hypothesized: increased glucocorticoid secretion, decreased GH secretion and GH resistance.

The aim of this work was to analyze the effect of chronic inflammation on the somatotrophic axis. We studied the effect of lipopolysaccharide (LPS) on IGF-I and GH secretion as important components of the somatotrophic axis. Since IGF-I action can be modulated by changes in the IGF-binding proteins (IGFBPs), which can increase or inhibit the IGF-I effects, we also analyzed serum IGFBPs. Serum concentrations of corticosterone and insulin were determined.

Materials and Methods

Adult male Wistar rats weighing 250–300 g were obtained from Charles River (Barcelona, Spain) and maintained

Figure 1 Changes in serum concentrations of GH and IGF-I three hours after an injection of varying doses of LPS. Each point represents the mean $\pm$ S.E.M. for at least 9 rats per group. * $P < 0.05$, ** $P < 0.01$ vs control group (Duncan's multiple range test).

Figure 2 Effect of a single LPS injection at different doses on serum levels of corticosterone and insulin. Each point represents the mean $\pm$ S.E.M. for at least 6 rats per group. * $P < 0.05$, ** $P < 0.01$ vs control group (Duncan's multiple range test).

under controlled temperature (20–22 °C) and light conditions (lights on from 0730 to 1930 h). Food and water were available *ad libitum*. The rats were used at least 8 days after arrival. The procedures followed the guidelines recommended by the European Union for the care and use of laboratory animals.

Acute LPS administration

The animals were injected with 0, 2.5, 25 or 250 µg/kg LPS (serotype 055:B5; Sigma Chemical Co., St Louis, MO, USA) in 250 µl saline i.p. at 1000 h. Acute LPS administration induces transient hypotension and hyperglycemia which disappear at 3 h (Virkamäki & Yki-Jörvinen 1994); therefore, 3 h after LPS injection the rats were killed by decapitation and trunk blood was collected in tubes at 4 °C. The blood was allowed to clot, centrifuged and the serum was stored at –20 °C until GH, IGF-I, IGFBPs, insulin and corticosterone assays were performed. Immediately after decapitation the pituitary gland was removed and stored at –20 °C for GH assay. The medial basal hypothalamus were dissected as previously described (López-Calderón *et al.* 1990) and

quickly frozen in a dry-ice/acetone mixture and stored at –80 °C until the somatostatin assay was carried out.

Chronic LPS

Rats were injected i.p. once daily for 8 days with 0 or 250 µl/kg LPS in 250 µl saline. At 1300 h, three hours after the last injection, the animals were killed and trunk blood, pituitary and hypothalamus were removed and stored until hormone assays were carried out. The thymus and spleen were dissected and weighed.

Serum concentrations of glucose were measured with a commercial kit from Boehringer Mannheim (Barcelona, Spain).

Hormone determination

Serum IGF-I concentrations were measured by a double-antibody RIA (Daughaday *et al.* 1980). The IGF-I anti-serum (UB2-495) was a gift from Drs Underwood and Van Wik, and distributed by the Hormone Distribution Program of NIDDK through the National Hormone and Pituitary Program (University of Maryland, School

Figure 3 A representative Western ligand blot of IGFBPs in 2 µl serum from rats injected with different doses of LPS or saline three hours before being killed. IGFBPs were separated by a 12.5% SDS-PAGE gel, transferred to nitrocellulose, ligand blotted with ^{125}I -IGF-I, and visualized via autoradiography. Approximate molecular weight of each band is indicated on the right.

of Medicine, Baltimore, MD, USA). Levels of IGF-I were expressed in terms of IGF-I A52-EPD-186 standard (Eli Lilly & Company, Indianapolis, USA). The intra-assay coefficient of variation was 8%. Samples from one experiment were run in the same assay. To confirm the elimination of IGFBPs, extracted and non-extracted serum fractions were incubated with ^{125}I -IGF-I at 4 °C for 24 h. The separation of bound and free tracers was carried out with dextran charcoal. Extracted fractions showed 98% free IGF-I whereas non-extracted fractions showed only 42% free IGF-I.

Concentrations of GH were measured by a double-antibody RIA using reagents kindly provided by Dr Parlow of the NIDDK's National Hormone and Pituitary Program. Levels of GH were expressed in terms of

Figure 4 The effect of acute LPS administration on serum IGFBP. Data from 8 or 9 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 saline group (Student's *t*-test).

NIDDK rat-GH-RP-2 standard. The GH detection level was 10 pg, and the intra-assay coefficient of variation was 3%. All necessary comparisons between test and control animals were made within one assay run.

Hypothalamic somatostatin content and serum insulin were measured by radioimmunoassays previously described (Tamarit-Rodriguez *et al.* 1985, Vara & Tamarit-Rodriguez 1988). Serum concentrations of corticosterone were determined by a competitive protein-binding assay (Millán *et al.* 1996).

Figure 5 Effect of treatment with 250 µg/kg LPS for 8 days on serum concentrations of corticosterone and insulin and on body weight. Values are means ± S.E.M. for at least 9 rats per group. **P*<0.05 vs control group, ***P*<0.01 vs saline group (Student's *t*-test).

Western ligand blot of IGF-BPs

Western blots were prepared as previously described (Hossenlopp *et al.* 1986). Two microliters sera were diluted in sample buffer, boiled for 2 min at 100 °C, and submitted to electrophoresis on 1% SDS-12.5% acrylamide gels under nonreducing conditions. Prestained molecular weight standards (Bio-Rad, CA, USA) were run in parallel lanes. Following electrophoresis, proteins were transferred onto nitrocellulose sheets (Hybond-C extra, Amersham International, Amersham, Bucks, UK) using a semi-dry electrophoretic transfer cell (Bio-Rad). After transfer, the nitrocellulose sheets were dried and blocked for 1 h with 5% non-fat dry milk, 0.1% Tween (Sigma) in Tris-buffered saline. The nitrocellulose membranes were incubated overnight at 4 °C with ¹²⁵I-labeled IGF-I (5 × 10⁵ c.p.m./ml). The nitrocellulose sheets were washed, dried and blots were exposed at - 80 °C to X-ray

Table 1 Effect of LPS administration (250 µg/kg) over 8 days on the relative spleen, thymus and adrenal weights. Values are means ± S.E.M.

	Spleen (mg/100 g)	Thymus (mg/100 g)	Adrenal (mg/100 g)
Saline (n=10)	337 ± 12	154 ± 9.4	17.8 ± 0.4
LPS (n=10)	550 ± 20**	157 ± 8	20.4 ± 0.72**

***P*<0.01 compared with saline group (Student's *t*-test)

film (Kodak X-Omat AR, Eastman Kodak, Rochester, NY, USA) and two intensifying screens for 1–3 days according to the signal obtained. Autoradiographs were analyzed by densitometric scanning using a PC-Image VGA24 program for Windows. To obtain semiquantitative data for statistical analysis two gels were run, with 5 serum samples from one dosage of LPS and 4 control samples in each gel. The density of the IGF-BP bands in each lane was expressed as the percentage of the mean density of control sera.

Statistical analysis

All data are presented as the mean ± S.E.M. Simple linear regression was used to determine dose-related responses to treatments. Comparisons between means were made by one-way analysis of variance and subsequent Duncan's multiple range test. Comparisons between two groups were performed using Student's *t*-test. Significance was assumed when *P*<0.05. GH data were subjected to log transformation since variances showed a log-normal distribution.

Results

Acute LPS administration decreased serum concentration of GH in a dose-dependent manner (*F*_{1,36}=6.4, *P*<0.05,

Figure 6 Effect of LPS administration for 8 days on serum concentrations of IGF-I, GH, pituitary GH content and hypothalamic somatostatin content. Values are means $\pm$ S.E.M. for at least 9 rats per group. * $P < 0.05$, ** $P < 0.01$ vs control group (Student's *t*-test).

Fig. 1). Serum concentrations of both IGF-I and insulin were also decreased in rats which received 25 and 250 $\mu\text{g/kg}$ LPS ($P < 0.05$, Figs 1 and 2). However, serum concentrations of glucose were not modified 3 h after LPS administration (6.6 ± 0.23 mM in the control group compared with 6.5 ± 0.25 and 6.4 ± 0.38 in the rats that received 25 and 250 $\mu\text{g/kg}$ LPS respectively). In contrast, serum corticosterone levels increased depending on the dose ($F_{1,36} = 17.8$, $P < 0.01$, Fig. 2). Pituitary GH and hypothalamic somatostatin contents were not significantly modified 3 h after a single LPS injection (data not shown).

Western ligand blot of rat serum IGFBP showed a major band of 47 kDa corresponding to IGFBP-3, a 33 kDa band which most likely represents IGFBP-1 and IGFBP-2, and a 24 kDa band identified as IGFBP-4 (Fig. 3). The densitometric quantification (Fig. 4) showed an increase in serum IGFBP-1 and IGFBP-2 in the rats that received an LPS injection of 250 $\mu\text{g/kg}$, whereas no modifications in the IGFBPs were observed in the rats injected with 25 or 2.5 $\mu\text{g/kg}$ LPS.

Since IL-1 or LPS treatments have been shown to induce anorexia, we examined body weight on days 4 and 8 of LPS treatment. As shown in Fig. 5, body weight was decreased in the rats treated with 250 $\mu\text{g/kg}$ LPS ($P < 0.01$), whereas, spleen and adrenal weight were significantly increased ($P < 0.01$) and thymus weight was not modified by chronic LPS administration (Table 1).

Administration of 250 $\mu\text{g/kg}$ LPS for 8 days did not modify serum insulin levels, but resulted in an increase in both serum concentration of corticosterone ($P < 0.05$) (Fig.

5) and in adrenal weight ($P < 0.01$) (Table 1). Although serum GH was not significantly modified by chronic LPS treatment, pituitary GH content and serum concentration of IGF-I were significantly decreased ($P < 0.05$) (Fig. 6), whereas hypothalamic somatostatin content was increased in LPS-treated rats ($P < 0.01$). As shown in Fig. 7, rats treated chronically with LPS showed a significant increase in the three IGFBP bands identified by ligand blot in serum. This increase does not seem to be due to hemodynamic alterations, since serum concentrations of proteins were not modified by chronic LPS treatment (51 ± 1.06 g/l in the control group compared with 49.5 ± 1.7 g/l in the rats treated with LPS).

Discussion

As expected, 3 h after the first LPS injection a marked catabolic response was observed in serum hormones, with an increase in serum corticosterone together with a significant decrease in serum concentrations of insulin, IGF-I and GH. Acute LPS administration at the higher dose also modified the IGFBPs of low molecular weight, IGFBP-1 and IGFBP-2. The increase in these serum binding proteins is in accordance with other data previously described (Fan *et al.* 1994) and may be due to hepatic modifications, since a decrease in the hepatic IGF-I output and an increase in the hepatic release of IGFBP-1 and IGFBP-2 four hours after a single LPS injection have been described (Fan *et al.* 1995a). The

Figure 7 Effect of chronic LPS treatment on serum IGFBPs. The lower panel is a representative Western ligand binding of ^{125}I -IGF-I to 2 μl serum from LPS-treated and control rats treated with saline. Approximate molecular weights of each band are shown on the right. Densitometric measurements of bands from the Western ligand blot are shown as a percentage of controls (upper panel). Values are means $\pm$ S.E.M. for at least 8 rats per group. * $P < 0.05$, ** $P < 0.01$ vs control group (Student's *t*-test).

increase in these IGFBPs might be secondary to the increased release of cytokines, since TNF and IL-6 induce hepatic IGFBP-1 production (Fan *et al.* 1995b, Samstein *et al.* 1996). Another possibility is that LPS-induced modifications in serum concentrations of insulin, IGF-I, GH and corticosterone stimulate hepatic IGFBPs synthesis, since corticosterone stimulates whereas insulin, IGF and GH inhibit the hepatic expression of the IGFBP1 gene (Ooi *et al.* 1990, Thissen *et al.* 1994). In our study insulin secretion was decreased after acute, but not during chronic LPS administration. Several alterations in carbohydrate metabolism have been described during acute endotoxemia, such as insulin resistance (Virkamäki & Yki-Järvinen 1994), reduced or increased plasma insulin and increased secretion of glucagon (Yki-Järvinen *et al.* 1989, Bundz *et al.* 1995, Lang *et al.* 1996). However, insulin and glycemia alterations last only 2–3 h.

The effect of chronic LPS treatment on serum concentrations of corticosterone and GH was notably less evident than the effect of acute administration of LPS. However, LPS administered at a dose of 250 $\mu\text{g}/\text{kg}$ over 8 days significantly decreased body weight and increased the serum concentration of corticosterone. The different adrenal response to acute or chronic LPS administration can be explained by a tolerance phenomenon. Different data have been reported depending on the times of

administration and the doses used – from a lowered to an almost absent adrenal response to LPS (Mefford *et al.* 1991, Mengozzi & Ghezzi 1991, Hadid *et al.* 1995). The tolerance to chronic LPS administration seems to be related to decreased macrophage responsiveness to LPS, rather than to a decreased neuroendocrine response to cytokines (Mefford *et al.* 1991, Hadid *et al.* 1996).

In chronically LPS-treated rats, the decrease in serum IGF-I concentration is concomitant with an increase in the serum binding capacity of this hormone. Although it has been reported that IGFBP-3 is GH and IGF-I dependent (Spagnoli & Rosenfeld 1997), acute LPS administration, in a higher dose than in the present study (1 mg/kg), increases hepatic IGFBP-3 levels together with a significant decrease in serum GH and IGF-I levels (Fan *et al.* 1994). These results suggest that other factors may modulate the serum concentration of IGFBP-3, and are in accordance with other data previously reported showing that IL-1, TNF and transforming growth factor (TGF- β) induce IGFBP-3 synthesis (Olney *et al.* 1995, Hembree *et al.* 1996, Han *et al.* 1997). Since LPS administration has been shown to increase not only IL-1 and TNF but also hepatic TGF β mRNA levels (Masuhara 1995), these can be possible mechanisms by which LPS increases circulating IGFBP-3. Thus, the increase in IGFBP-3 levels may decrease the bioavailability of IGF-I in the peripheral tissues as has been shown in arthritic human cartilage (Neidel *et al.* 1997).

The mechanism by which chronic activation of the immune system inhibits the GH-IGF system is not well known. It has been postulated that the decrease in circulating IGF-I in response to infection may be caused by a direct effect of the cytokines IL-1 and/or TNF at the hepatocyte level (Thissen & Verniers 1997), since TNF administration decreases circulating and hepatic IGF-I (Fan *et al.* 1995b). In our data, the difference in serum GH levels between the control group and the group receiving LPS for 8 days was not significant, but chronic LPS administration significantly decreased the serum concentration of IGF-I and the pituitary GH content. The decrease in both pituitary GH content and serum concentration of IGF-I suggests a decrease in pituitary GH secretion in LPS-treated rats. In fact, hypothalamic somatostatin content increased significantly in chronically LPS-treated rats. An increase in somatostatin release induced by acute LPS treatment has been described both *in vivo* and *in vitro* (Fukata *et al.* 1985, Peisen *et al.* 1995). Furthermore, the suppression of GH secretion by acute endotoxin administration has been reversed by antisomatostatin serum (Kastin & Martin 1982). These data suggest that the inhibitory effect of chronic LPS administration on the somatotrophic axis can be mediated, at least in part, by an activation of the hypothalamic somatostatin release.

The effects of LPS on the neuroendocrine system seem to be mediated by the cytokines released by LPS-stimulated macrophages and monocytes (Michalek *et al.*

1980). It has recently been reported that the acute LPS-induced suppression of GH secretion is mediated by IL-1 β and corticotropin releasing hormone (Peisen *et al.* 1995). However, another study showed that i.c.v. administration of IL-1 stimulated GH secretion (Rettori *et al.* 1987). In contrast, a continuous i.v. or i.c.v. administration of IL-1 β and to a lesser extent IL-1 α , inhibited GH secretion (Wada *et al.* 1995). These discrepancies may be due to the dosage, since other authors have described a biphasic effect of central IL-1 administration on GH secretion, with a stimulatory effect at low doses and an inhibitory effect at higher doses of IL-1 (Payne *et al.* 1992). In conclusion these data suggest that the catabolic state observed in chronic inflammation or endotoxic shock may be secondary to the decrease in GH and IGF-I secretion together with an increase in the circulating IGF-BPs.

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