IGF-I/IGFBPs system response to endotoxin challenge in sheep

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Abstract

Endotoxin (LPS), a membrane component of gram-negative bacteria produces multiple endocrine and metabolic effects that mimic those seen in acute sepsis. It induces species-dependent alterations of the growth hormone (GH) axis that may participate in the shift of the metabolism towards catabolic events. Humans and sheep show increased GH secretion in response to LPS, as opposed to rats, which have been the most studied. The purpose of our work was to evaluate the effects in intact rams of an acute intravenous administration of a high dose of LPS on the insulin-like growth factor (IGF)-I/IGF-binding proteins (IGFBPs) system and to analyse the temporal relationship of GH axis changes with those of several hormonal and metabolic parameters such as somatostatin, cortisol, insulin, and glucose.

LPS induced a late moderate decrease of total IGF-I plasma levels following a 5-h steady-state period ($-26.6 \pm 4.2\%$, $P < 0.05$, 9 h after LPS), despite a biphasic and sustained increase of GH secretion in the same animals (2.48 ± 0.39 ng/ml 2 h after LPS and 2.7 ± 0.37 ng/ml 5 h after LPS vs 0.77 ± 0.10 before LPS; Briard *et al.* 1998a). Western ligand blot analysis in IGFBPs showed an

early short-lasting increase in IGFBP-1 ($188.8 \pm 39\%$ $P < 0.05$, 3 h after LPS). No significant change was seen for either IGFBP-2, -3 or -4. We observed a marked and sustained increase in cortisol (128.18 ± 7.21 ng/ml 3 h after LPS, vs 21.17 ± 4.22 before LPS). Insulin also increased (27.69 ± 3.90 μ U/ml 3 h after LPS, vs 13.48 ± 1.69 before LPS) and its burst coincided with that of IGFBP-1.

Moderately decreased IGF-I and increased IGFBP-1 plasma levels contrasted with the sustained increase in GH secretion that we recently described, thereby suggesting that endotoxin causes a state of resistance to GH. This may be exacerbated by reduced IGF-I bioavailability and/or action, and which may participate in the pathophysiology of the catabolic state seen in sepsis. The temporal analysis of hormone responses suggests that endotoxin-induced alterations of the IGF-I/IGFBPs system may involve the prolonged and substantial somatostatin rise that we recently demonstrated, together with an increase in glucocorticoid and cytokine as more generally assumed.

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Introduction

The interaction of the immune and neuroendocrine systems has recently been a matter of great interest. Septic shock, endotoxemia and sepsis as well as critical illness and inflammation are associated with complex hormonal changes that might affect both the immune and metabolic responses and contribute to a damaging catabolic state with weight loss, abnormal glucose regulation and severe muscle proteolysis (Mizock 1995, Cooney *et al.* 1997, Chang & Bistrian 1998). Among the endocrine effects of sepsis, incompletely characterised alterations in the somatotroph axis have been demonstrated that may contribute to hypercatabolism (Voerman *et al.* 1992, Botfield *et al.*

1997). Endotoxin (LPS), a lipopolysaccharide found as a principal component of gram-negative bacteria, is the main mediator of septic shock (Morrison & Ryan 1987). It has been shown to be a potent stimulator of the immune system as well as a modulator of several mediators and hormones. Schematically, LPS administration induces a rapid increase in plasma concentration of tumour necrosis factor alpha (TNF- α), interleukin (IL)-1 and IL-6. In turn, endotoxin, together with circulating cytokines, stimulates eicosanoid and interleukin production by various tissues including the hypothalamus and the pituitary (Tilders *et al.* 1994). LPS induces multiple endocrine and metabolic effects that mimic those seen in acute sepsis (Jenkins & Ross 1996a, Van den Berghe *et al.* 1998). It increases

activity of the hypothalamic-pituitary-adrenal (HPA) axis (Makara *et al.* 1971, Rivier *et al.* 1989, Tilders *et al.* 1994, Dadoun *et al.* 1997) and decreases thyrotrophin (TSH) (Elson *et al.* 1982) and luteinizing hormone (LH) (Peter *et al.* 1989, Battaglia *et al.* 1997) secretion.

Endotoxin effects upon GH-axis are species-dependent. In rats, the whole GH-axis is depressed: GH secretion and IGF-I plasma concentration are decreased after endotoxin challenge (Kasting & Martin 1982, Fan *et al.* 1994, Soto *et al.* 1998). Conversely, GH secretion is increased after endotoxin challenge in humans (Elin *et al.* 1981), pigs (Hevener *et al.* 1997) and sheep (Coleman *et al.* 1993). We recently confirmed these data in sheep and demonstrated the pituitary as the main site for LPS effect upon GH secretion (Briard *et al.* 1998a). In humans (Lang *et al.* 1997) and pigs (Hevener *et al.* 1997), total plasma IGF-I is nevertheless decreased. LPS effect upon IGF-I has not yet been characterised in sheep. This parameter demonstrates major functional significance since decreases in plasma IGF-I are strongly correlated with imbalanced nitrogen status and hypercatabolism (Cohick & Clemmons 1993, Donaghy & Baxter 1996). Moreover a number of studies suggest that IGF-I treatment may assist recovery from catabolic states related to sepsis (Jurasinski & Vary 1995, Balteskard *et al.* 1998).

Furthermore, LPS might alter IGF-I action through changes in circulating IGF binding proteins (IGFBPs) as demonstrated in several species: increased IGFBP-1, -2 and/or -3 in rats (Fan *et al.* 1994, Soto *et al.* 1998), decreased IGFBP-2 in cattle (Elsasser *et al.* 1995) and increased IGFBP-1 in humans (Lang *et al.* 1997). Although hypercatabolism seen in sepsis is certainly multifactorial, IGF-I decrease, and alterations of its bio-availability and/or actions through IGFBPs modulations, could be involved. The mechanism(s) leading to these alterations in the IGF-I/IGFBPs system remain(s) unclear.

Because of similarities between humans and sheep in LPS action, i.e. catabolic state contrasting with increased GH secretion, sheep appear to be an interesting model on which to assess the effect of acute LPS on the IGF-I/IGFBPs system, yet uncharacterised in this species. Indeed, high doses of endotoxin that lead to clinical features seen in severe sepsis cannot be used on humans for ethical reasons. Sheep, being a ruminant, are also an interesting model since large nutrient reserves in the rumen may limit the specific impact of endotoxin-induced anorexia (Elsasser *et al.* 1995) and, therefore, may dissociate indirect nutritional effects from more direct endotoxin effects.

We previously treated sheep with a high dose of endotoxin in order to determine the effect of this compound upon GH secretion (Briard *et al.* 1998a). The purpose of the current work was to evaluate the concomitant effect of endotoxin on the IGF-I/IGFBPs system in the same animals, and to analyse the temporal relationship of these changes with those of several hormonal

and metabolic parameters that might affect GH-axis regulation, such as somatostatin, cortisol, insulin, and glucose.

Materials and Methods

Animals

Four intact rams (9–11 months old, 40–45 kg body weight) from the Merino Alps breed were obtained from ENSA (Domaine du Merle, Salon de Provence, France). Two weeks prior to the onset of the study, rams were transferred to the animal room of the laboratory. All experimental procedures were performed in accordance with local animal-use regulations. Studies were approved by the Faculty Committee on the Use and Care of Animals.

Experimental procedure

The animals ($n=4$) were housed in individual pens placed immediately adjacent to each other. They were free to sit or to stand and exposed to natural lighting conditions. The day preceding the experiment, an indwelling catheter was inserted into the external jugular vein. On the following day, endotoxin was administered by an i.v. bolus injection (200 ng/kg) in 2 ml saline through the jugular cannula, followed by 5 ml saline as previously described (Coleman *et al.* 1993, Briard *et al.* 1998a). Endotoxin (*Escherichia coli* 055: B5; Sigma, St Louis, MO, USA) was diluted in phosphate-buffered saline with 0.1% bovine serum albumin to a stock concentration of 0.1 µg/µl. Anti-coagulation was achieved using heparin (bolus of 25 000 IU at the beginning of the sampling period, followed by infusion of 10 000 IU/h).

Body temperature and general welfare of the animals was evaluated regularly. Jugular blood was collected every 15 min during the 2 h period preceding endotoxin injection and during the subsequent 9 h. Blood samples were collected in chilled tubes and immediately centrifuged at 4 °C for 10 min. The resulting plasma was stored at -20 °C until assayed for IGF-I, IGFBPs, cortisol, insulin, and glucose.

Hormone and glucose assays

All assays were performed in plasma samples previously used for GH and somatostatin radioimmunoassay, the results of which have been reported in Briard *et al.* 1998a.

Total plasma IGF-I was measured using a well-established assay system (Hardouin *et al.* 1989) previously validated in sheep (Briard *et al.* 1998b). Briefly, samples were gel filtered in acetic acid on columns of Ultrogel AcA54 (Sepracor/IBF s.a., Villeneuve la Garenne, France)

in order to separate IGFs from their binding proteins. Recombinant human IGF-I was provided by Ciba-Geigy Ltd (Basel, Switzerland) and was used as cold standard and radioactive tracer after iodination by the chloramine-T method. IGF-I RIA was performed using anti-IGF-I antiserum prepared by Dr Closset (Liège, Belgium). Unknown samples were studied at three concentrations, each in duplicate plus one non-specific binding tube. Intra- and inter-assay coefficients of variation were 4.8 and 10% respectively. Because of disparities in individual IGF-I basal (pre-endotoxin) plasma levels, IGF-I was expressed as a percentage of mean basal plasma value.

IGFBPs serum profile was evaluated using Western ligand blotting as previously described (Hossenlopp *et al.* 1986, Baciuchka *et al.* 1998). Non reduced samples were submitted to SDS-PAGE on 12.5% polyacrylamide slab gels before electrotransfer onto nitrocellulose. Nitrocellulose membranes were probed with approximately 2.5×10^5 c.p.m./ml [125 I]-IGF-II (Amersham, Aylesbury, UK) and IGFBPs visualised by autoradiography. Autoradiographic films were quantified by densitometric scanning (Sharp JX-325 laser densitometer, Pharmacia Biotech., St Quentin en Yvelines, France) followed by computerised image analysis (Image Master Software; Pharmacia Biotech., St Quentin en Yvelines, France). Densitometric analyses were performed on films with different exposure times and those that gave linear signal curves were used for semiquantitative analysis. For each band, the signal level was expressed as a percentage of each animal value measured at time 0.

Cortisol was measured in plasma extracts according to a previously described RIA method (Dadoun *et al.* 1997). The limit of detection and the intra-assay coefficient of variations were 0.5 ng/ml and 4.7% respectively.

Insulin was measured using a commercially available radioimmunoassay kit (INSI-PR, Cis bio international ORIS, Gif-sur-Yvette, France) previously validated in sheep (Bocquier *et al.* 1998); assay sensitivity was 3.6 µU/ml; intra-assay and inter-assay coefficients of variations were respectively 6% and 5.3%.

Plasma glucose was measured by the glucose oxidase-peroxidase method, using a commercially available kit (Glucose enzymatique color liquide, Biotrol Merck diagnostic, Nogent sur Marne, France).

Statistical analysis

All data are reported as mean $\pm$ s.e.m. Mean plasma IGF-I, IGFBP-1, cortisol, insulin and glucose values were calculated during each hourly period of sampling. Paired Student's *t*-tests were used in all statistical analyses between hourly periods (designated H-2 to H9), using a computer software program (Statview 512, Brain Power, Inc, Calabasas, CA, USA). For IGF-I and IGFBP-1, additional statistical analyses were performed between different sampling times using paired Student's *t*-test, since

only one measurement was performed in hourly period H5 and H9. $P < 0.05$ was considered significant.

Results

Intravenous administration of 200 ng/kg endotoxin led to increased respiration, intermittent coughing and diarrhoea, and a lack of alertness to the surroundings. High body temperature (41–43 °C) was recorded in all animals and lasted for 6 h.

IGF-I/IGFBPs

During the 5 h period following endotoxin injection (H1 to H5), IGF-I plasma levels remained unchanged ($P > 0.2$); a subsequent moderate but significant decrease of IGF-I was seen during H9 (H9: $73.4 \pm 4.2\%$, $P < 0.05$ vs H-1, H-2) and each pre-endotoxin sampling time.

IGFBPs plasma profile consisted of five major bands that included a doublet of 39–41 kDa, and three smaller bands of approximately 34, 30, and 24 kDa (Fig. 1). It conforms to that described in human (Hardouin *et al.* 1989) and cow (Cohick *et al.* 1992) plasma using both ligand blotting and immunoblotting techniques, and matches with that previously described in sheep using a ligand blotting technique comparable to ours (McCann *et al.* 1997). The nomenclature derived from these studies was applied to our results and IGFBPs of 24, 30, 34 and 39–41 kDa were designated IGFBP-4, -1, -2 and -3 respectively. IGFBP-1 increased later than GH, approximately 105 min after endotoxin injection, remained significantly elevated until H4 and then decreased (H-1: $105.1 \pm 6.3\%$; H3: $188.8 \pm 39.0\%$, $P < 0.05$ vs H-1 and H-2; H4: $183.6 \pm 40.2\%$, $P < 0.05$ vs H-1, H-2 and each pre-endotoxin sampling time) (Figs 2 and 3). No significant change was seen for either IGFBP-2, -3 or -4 after endotoxin challenge.

In a previous study, approximately 75 min after the endotoxin injection, we observed in the same animals, a significant increase in plasma GH levels (H-1: 0.77 ± 0.10 ng/ml; H2: 2.48 ± 0.39 ng/ml, $P < 0.0005$ vs H-1 and H-2) (Briard *et al.* 1998a). A second and major sustained increase in GH concentration was observed during the fifth hourly period after endotoxin administration (H5: 2.7 ± 0.37 ng/ml, $P < 0.0005$ vs H-1 and H-2) and lasted until the end of the sampling period.

Other parameters

The HPA axis was rapidly and very strongly activated (Figs 2 and 3). Cortisol rise occurred earlier and was much more substantial than that of GH (H-1: 21.17 ± 4.22 ng/ml; H1: 57.83 ± 12.56 ng/ml, $P < 0.005$ vs H-1 and H-2; peak value during H3: 128.18 ± 7.21 ng/ml, $P < 0.0005$ vs H-1 and H-2). Cortisol levels remained significantly

Figure 1 Western ligand blot analysis (WLB) of IGFBPs in plasma from one representative animal before (–105 min, –45 min and 0) and after (45 min to 510 min) an i.v. injection of endotoxin. Serum samples were submitted to WLB as described in Materials and Methods. IGFBPs were probed with [125 I]-IGF-II and visualised by autoradiography. Molecular weights are shown on the left.

increased until the end of the sampling period ($P < 0.0005$ for each hourly period vs H-1 and H-2).

Glucose changes occurred later, leading to hypoglycaemia from 240 min until the end of the sampling period (H-1: 0.912 ± 0.025 g/l; H4: 0.767 ± 0.026 g/l, $P < 0.0005$ vs H-1 and H-2; H9: 0.580 ± 0.023 g/l, $P < 0.0005$ vs H-1 and H-2) (Figs 2 and 3).

Plasma insulin showed a significant rise starting at 120 min and lasting for 2 h (H-1: 13.48 ± 1.69 μ U/ml; H3: 27.69 ± 3.9 μ U/ml, $P < 0.05$ vs H-1 and H-2; H4: 28.29 ± 3.42 μ U/ml, $P < 0.005$ vs H-1 and H-2); this rise preceded the drop of glucose levels. Insulin levels then returned to baseline but remained high towards glycaemia (Figs 2 and 3).

In a previous study (Briard *et al.* 1998a), approximately 45 min after the endotoxin injection, we observed in the same animals a marked increase in somatostatin (SRIH) levels, culminating during H2 (H-1: 11.62 ± 1.19 pg/ml; H2: 66.9 ± 9.75 pg/ml, $P < 0.0005$ vs H-1 and H-2) (Briard *et al.* 1998a). This increase in peripheral plasma SRIH slightly preceded (15 min) the first GH rise. SRIH levels slowly returned to baseline (H4 to H6). A second sustained increase in SRIH levels was seen from H7 to H9 (H7: 33.00 ± 5.12 pg/ml, $P < 0.0005$ vs H-1 and H-2; H9: 34.94 ± 4.36 pg/ml, $P < 0.0005$ vs H-1 and H-2).

Discussion

As previously reported in the same species (Coleman *et al.* 1993), and also recently demonstrated in the animals used in the current study (Briard *et al.* 1998a), acute endotoxin administration evokes a sustained biphasic increase of GH

release. A strong stimulation of the HPA axis occurred, together with the activation of the somatotroph axis as previously observed in intact rams using a higher dose of LPS (Dadoun *et al.* 1997) and in other species (Makara *et al.* 1971, Rivier *et al.* 1989, Tilders *et al.* 1994). Interestingly, we demonstrate here that a late decrease in IGF-I plasma levels, following a 5 h steady-state period, and an early short-lasting increase in IGFBP-1, contrast with the increase of GH secretion.

A similar dissociation between GH and IGF-I responses to endotoxin has been recently demonstrated in pigs (Hevener *et al.* 1997), and interestingly also in humans (Lang *et al.* 1997). In rats (Fan *et al.* 1994, 1995a) and cattle (Elsasser *et al.* 1995), reduced GH levels may at least partly explain IGF-I decrease. Conversely the IGF-I decrease observed in sheep suggests that endotoxin causes a state of resistance to increased GH in humans and pigs, as previously proposed by others (Hevener *et al.* 1997, Lang *et al.* 1997). Such a hypothesis concords with the low effectiveness of GH therapy to restore IGF-I levels and to reverse the catabolic state in septic patients (Gottardis *et al.* 1991, Dahn *et al.* 1998). Moreover, GH-resistance is well-documented in cases of critical illness and catabolic states of diverse origins (reviewed in Jenkins & Ross 1996b and Donaghy & Baxter 1996).

Furthermore, our results showed increased IGFBP-1 plasma concentrations that nearly doubled 3 to 4 h after endotoxin injection. Indeed, we cannot eliminate the possibility that IGFBP-3 proteolytic fragments and/or N-glycosylated IGFBP-4 might migrate with the same mobility as IGFBP-1 using SDS-PAGE electrophoresis; however, these proteolytic fragments show much reduced affinity for IGF-II and are poorly detected by Western ligand blot (Lassarre & Binoux 1994). Endotoxin has

Figure 2 Effect of an i.v. injection of endotoxin (200 ng/kg; injection indicated by an arrow) on mean ($\pm$ S.E.M.) hourly IGF-I, IGF-BP1, cortisol, glucose and insulin plasma levels. Statistical significance vs basal values (both H-2 and H-1 hourly periods analysed separately): * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$. ▼, Hourly periods when plasma GH has been shown to be significantly increased in the same animals (P at least < 0.005 vs basal values; Briard *et al.* 1998a). □, Hourly periods when plasma somatostatin has been shown to be significantly increased in the same animals ($P < 0.0005$ vs basal values; Briard *et al.* 1998a).

previously been shown to modulate plasma IGFBPs. The increase in IGFBP-1 we observed in sheep shows similarities with data obtained in humans injected with small doses of LPS (Lang *et al.* 1997). In rats, in which GH plasma levels are suppressed by endotoxin, plasma IGFBP-1 changes match those seen in sheep (Fan *et al.* 1994, Soto *et al.* 1998).

The endotoxin-induced IGFBP-1 increase we observed in sheep may alter the peripheral response to changes in GH/IGF-I levels, through modulation of IGF-I bioavailability and/or action (Lee *et al.* 1993, Jones & Clemmons 1995). IGFBP-1 has been shown to inhibit IGF actions by binding to IGFs and preventing the binding of IGFs to IGF receptors, and especially to block the availability of IGFs for insulin-like actions (Lee *et al.* 1993, Lewitt 1994). Despite increased GH secretion, and initially preserved IGF-I levels, the early IGFBP-1 rise we observed

may participate in protein breakdown through reduced IGF-I bioavailability, as more generally assumed in catabolic states (Brismar *et al.* 1994, Donaghy & Baxter 1996, Jenkins & Ross 1996b). Raised GH levels, together with reduced IGF-I action, may also favour direct GH metabolic effects, such as resistance to insulin, neoglucogenesis and lipolysis. These effects are typical features of septic states (Mizock 1995), and participate in the increased substrate disposal for vital organs such as the brain and the immune system which do not require insulin action for glucose transport (Van de Berghe *et al.* 1998). This may also partly explain the delayed hypoglycaemia we observed, which appeared once IGFBP-1 decreased. Indeed, decreasing IGFBP-1 may favour IGF-I insulin-like actions leading to hypoglycaemia because of increased glucose utilisation (Lee *et al.* 1993, Lewitt 1994).

Figure 3 Effect of an i.v. injection of endotoxin (200 ng/kg; injection indicated by an arrow), in one representative animal, on IGF-I, IGFBP-1, cortisol, glucose and insulin plasma levels.

Another consequence of IGFBP-I rise and altered IGF-I bioavailability may be reduced feedback of IGF-I upon GH; this may explain at least in part the second increase in GH plasma concentration that we recently described in the same animals 4 h after endotoxin administration (Briard *et al.* 1998a), which shortly followed IGFBP-I zenith. The late decrease in IGF-I may also participate in sustaining this second GH rise. In sheep, IGF-I exerts its feedback action mainly at the pituitary level (Spencer *et al.* 1993, Fletcher *et al.* 1995). Indeed, we previously demonstrated that the second GH increase was not related to changes in GH RH and/or SRIH levels in hypothalamo-hypophysio-portal blood (Briard *et al.* 1998a).

Cytokines secreted in response to endotoxin such as TNF- α , IL-1 β and IL-6, may directly participate in the modulation of the IGF system. *In vivo* administration of TNF- α or IL-1 β decreases IGF-I plasma levels and hepatic mRNA and show opposite effects upon IGFBP-1 in rodents (Fan *et al.* 1995b, 1996, Samstein *et al.* 1996). Similar effects *in vitro* suggest a direct hepatic mechanism

(Samstein *et al.* 1996, Thissen & Verniers 1997). However multiple other factors may be involved and the temporal analysis of metabolic and hormonal changes facing the endotoxin-induced variations in IGF-I and IGFBP-1 may provide clues in understanding the mechanism(s) of these variations. Among potential regulatory factors, nutrition and insulin appear to be major regulators of IGF-I and IGFBP-1 (Lee *et al.* 1993, Jones & Clemmons 1995). Decreased nutrient intake leads to GH-resistance, decreased IGF-I and increased IGFBP-1 plasma levels (Lee *et al.* 1993, Thissen *et al.* 1994, Donaghy & Baxter 1996) and endotoxin-induced anorexia may participate in the IGF system changes we observed. However, in sheep, large nutrient reserves in the rumen may limit the specific impact of anorexia (Elsasser *et al.* 1995). This may also explain why in sheep IGF-I decrease was moderate and delayed, when compared with that seen in humans (Lang *et al.* 1997). In addition, decrease in glycaemia occurred after IGFBP-1. Therefore IGFBP-1 increase may not be ascribed to plasma glucose changes.

An inverse relationship between IGFBP-1 and insulin has been demonstrated in most physiological and pathological conditions (Lee *et al.* 1993, Thissen *et al.* 1994, Jones & Clemmons 1995). Interestingly, this inverse relationship is lacking in our model and changes in plasma insulin cannot account for those of IGFBP-1, since the rise in IGFBP-1 coincided with increased insulin levels. Insulin resistance has been demonstrated in endotoxemia and septic states (Mizock 1995) and may account at least in part for this loss of association between insulin and IGFBP-1 (Lee *et al.* 1993). Similarly the insulin changes we observed do not suggest involvement of insulin in IGF-I response to endotoxin, since insulin has been shown to increase IGF-I secretion both *in vivo* and *in vitro* (Pao *et al.* 1993, Brismar *et al.* 1994).

The IGF-I and IGFBP-1 changes we observed after endotoxin could be mediated by changes in other regulatory hormones such as glucocorticoids and somatostatin. In sheep, endotoxin-induced IGFBP-1 rise was preceded by an important burst of cortisol, and IGFBP-1 later decreased along with cortisol. Furthermore, the late IGF-I decrease we observed may be related to a suppressive effect of the prolonged cortisol rise. Indeed, glucocorticoid administration decreases hepatic IGF-I mRNA and increases IGFBP-1 gene expression leading to an important rise in the circulating levels of IGFBP-1 in both rats and humans (Luo *et al.* 1990, Conover *et al.* 1993). However, it has been proposed recently that in rats, endotoxin-induced IGFBP-1 increase is independent of glucocorticoids, as opposed to IGF-I changes, because of the differential effects of the glucocorticoid receptor antagonist RU-486 (Li *et al.* 1997).

SRIH involvement in IGF system modulation has received much less attention and its participation in endotoxin-induced effects has not been investigated. Interestingly, the increase in IGFBP-1 we describe here coincided or just followed the considerable increase in SRIH plasma concentration that we recently showed in the same animals (Briard *et al.* 1998a). This early rise in peripheral SRIH probably contributed to the increase in IGFBP-1. *In vivo*, octreotide, a long acting somatostatin analogue, has been shown to raise IGFBP-1 plasma levels and/or hepatic mRNA in both humans and rats (Ezzat *et al.* 1991, Flyvbjerg *et al.* 1995). SRIH and its analogues may increase IGFBP-1 through its inhibitory effect upon GH and/or insulin. However, in humans the effect of SRIH analogues upon IGFBP-1 has been shown to be at least partly independent of these hormones (Ezzat *et al.* 1992, Wolthers *et al.* 1994). Moreover, a direct effect has been reported in human hepatoma cells (Ren *et al.* 1992). Interestingly, in our study, the rise in IGFBP-1 and SRIH coincided with high levels of both insulin and GH. SRIH may also have participated in the late decrease in IGF-I, in response to endotoxin, since octreotide decreases IGF-I plasma levels and/or hepatic expression in GH-treated GH-deficient humans and rats (Laursen *et al.* 1993,

Ambler *et al.* 1996). The selective blockade of somatostatin action during endotoxin challenge should be useful in order to further characterise the involvement of SRIH in septic shock pathophysiology.

In conclusion, endotoxin induced complex effects upon the IGF-I/IGFBP system in sheep. Moderately decreased IGF-I and increased IGFBP-1 plasma levels faced a sustained GH increase, suggesting that endotoxin leads to a state of resistance to GH, which may be enhanced by reduced IGF-I bioavailability and/or action, and may participate in the pathophysiology of the catabolic state seen in sepsis. The temporal analysis of hormone responses suggests that the prolonged and substantial SRIH rise we recently described (Briard *et al.* 1998a), may be involved in endotoxin-induced alterations of the IGF/IGFBP system, together with glucocorticoids and cytokines as more generally assumed.

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