The metabolic effects of endotoxin are differentially affected by the pattern of GH administration in the rat

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Abstract

GH treatment can increase the mortality and morbidity of critically ill patients. The mechanisms of these harmful effects of GH are unknown but have been, in part, ascribed to interactions between GH and the immune system. Because GH has pattern-dependent actions we have now compared the dose-related effects of continuous and intermittent GH treatment given with or without an endotoxin (lipopolysaccharide; LPS) challenge. Male Wistar rats ($n=6$ per group) were treated for 5 days with recombinant human GH (0, 10, 100 or 1000 $\mu\text{g/kg}$ per day) using either continuous s.c. infusion by osmotic minipump or intermittent twice daily s.c. injections. On day 4, endotoxin (5 mg/kg, i.p.) was injected and the animals monitored for a further 16 h. LPS administration

alone led to neutrophilia and lymphopenia, with increased plasma concentrations of urea, cholesterol, triglyceride, insulin and leptin, and decreased levels of IGF-I. High dose GH infusion (1000 $\mu\text{g/kg}$ per day) followed by LPS caused greater increases in plasma urea, cholesterol, triglyceride, sodium and magnesium, but lower plasma glucose and insulin levels, than treatment with LPS alone. In contrast, twice daily injections of GH did not enhance these effects of endotoxin. In conclusion, the effects of endotoxin on plasma electrolytes, lipids, urea, glucose and insulin are differentially affected by the pattern of GH administration in the rat.

Journal of Endocrinology (2001) **171**, 173–181

Introduction

Growth hormone (GH) is a key endocrine modulator of postnatal growth and metabolism, with potent anabolic and lipolytic effects (Moller *et al.* 1995). Recombinant human (rh) GH is currently approved for treatment of paediatric and adult GH deficiency, and several other paediatric growth disorders (Vance & Mauras 1999).

Because of the well-known anabolic effects, GH therapy has been approved as a treatment for the protein-wasting disorder of AIDS, and has been proposed as a possible treatment for the wasting of critical illness (Jenkins & Ross 1996). However, recent large, multicentre randomised, placebo-controlled clinical trials in critically ill adults have shown significant increases in morbidity and mortality with GH treatment (Takala *et al.* 1999).

The mechanisms of these lethal actions of GH are unknown, but lethality seems to be associated with severe infections, shock and development of multiple organ failure (Ruokonen & Takala 2000). This does not necessarily demonstrate that the harmful effects of GH are caused by an interaction with the immune response, such as GH synergistically elevating cytokine levels. GH has many other actions, such as its metabolic effects (Moller

et al. 1995), which could also adversely affect survival following shock or critical illness.

Although it is not yet understood why GH treatment has lethal effects in critically ill patients, there is little doubt that GH therapy to treat growth disorders and GH deficiency is a safe treatment. It has been argued that the increased mortality was due to the high dose of rhGH (0.1 mg/kg per day) used in the clinical trial, which is 10–20 times higher than that given as replacement therapy to treat adult GH deficiency (Bengtsson 1999). However, since there was a doubling of mortality it is unclear what doses of GH would be safe in critically ill patients. We have therefore performed dose-response studies in a previously characterised rat model of sepsis (Liao *et al.* 1996).

In the rat, many of the physiological effects of GH are affected by the pattern of GH administration, such as growth, insulin-like growth factor-I (IGF-I) responses and the lipolytic effects (Clark *et al.* 1995, 1996). These different responses to patterns of GH exposure appear to be mediated by intracellular signal transducer and activator of transcription (STAT) signalling proteins (Udy *et al.* 1997). It is possible that pattern-dependent effects of GH induce different biological responses in sepsis. However, there are no published studies comparing the effects of

continuous and intermittent GH exposure on the responses to a septic insult. We have therefore studied the effects of continuous and intermittent GH delivery on responses to endotoxin (lipopolysaccharide; LPS) challenge in adult male rats.

The overall aim of this study was to characterise haematological, biochemical, hormonal and histological changes known to be affected by GH administration or septic shock.

Materials and Methods

Animals

All experimental procedures were approved by the Institutional Animal Ethics Committee. Adult male Wistar rats aged 70–80 days were obtained from the Animal Resources Unit, University of Auckland. The rats were housed in a controlled environment (12 h light:12 h darkness cycle; 19–21 °C) with free access to food and water. They were allowed to adapt to this environment for at least 1 week before commencement of experiments. Rats were weighed daily throughout the experiment.

Materials

rhGH (Genotropin, 36 IU; reconstituted in 0.02 M glycine buffer, pH 9.6) was donated by Pharmacia & Upjohn (Stockholm, Sweden). *Escherichia coli* endotoxin (Serotype 055:B5; LPS) was obtained from Sigma (St Louis, MO, USA).

Experiment 1: continuous GH administration

For the treatment of rats with GH, Alzet mini-osmotic pumps (Alza Corporation, Palo Alto, CA, USA) were subcutaneously implanted under brief halothane anaesthesia, delivering rhGH at 10, 100 or 1000 µg/kg per day for 5 days. Control groups received a sham ('dummy') minipump. On day 4, the rats received an injection of either LPS (5 mg/kg, i.p.) or saline. Because LPS is known to reduce food intake, food was withdrawn from all groups at the time of LPS injection to avoid any confounding nutritional effects between groups. The animals were killed 16 h after LPS injection and trunk blood was collected for analysis. Liver tissue was collected for histological analysis and immediately fixed in freshly prepared 4% (w/v) paraformaldehyde before processing and embedding in paraffin wax. No blood and tissue samples were collected from any animals that died prior to sampling.

Experiment 2: intermittent GH administration

Rats received twice daily (1000 and 2200 h) injections of rhGH (10, 100 or 1000 µg/kg per day, s.c.) or vehicle injections for 5 days. Control groups received injections of

excipient. The total amount of GH administered per 24 h was the same as in the continuous infusion experiment (experiment 1). On day 4, rats received an injection (1600 h) of either LPS (5 mg/kg, i.p.) or saline. All rats received one final GH or vehicle injection 6 h after the injection of LPS. As in experiment 1, food was withdrawn at the time when LPS was injected, and the animals were killed 16 h later. Trunk blood and tissues were collected for analysis as described for experiment 1. No blood and tissue samples were collected from any animals that died prior to sampling.

Assays

Blood counts for neutrophils and lymphocytes, and plasma levels of urea, creatinine, alanine-amino transferase (ALT), aspartate-amino transferase (AST), albumin, triglyceride, cholesterol, lactate dehydrogenase (LDH), sodium, potassium and calcium were determined by routine clinical techniques using an autoanalyser (A⁺; Auckland Healthcare Laboratory Services, Auckland, New Zealand). Plasma levels of glucose and lactate were determined using standard colorimetric enzyme reactions modified for assay using a 96-well microplate reader as previously described (Liu *et al.* 1994). Plasma IGF-I levels were measured using the IGF-binding protein-blocked radioimmunoassay (RIA) (Blum & Breier 1994). Plasma insulin and leptin levels were measured by RIAs as described previously (Vickers *et al.* 2000).

Histology

Liver tissue sections (4 µm) were deparaffinised in xylene and rehydrated with graded alcohols. The tissue was then stained for 5 min in Harris haematoxylin and eosin before being dehydrated, cleared in xylene and coverslipped. Slides were examined using light microscopy.

Statistical analysis

Experimental values are presented as means ± S.E.M. No data from animals that died before sampling were included in the statistical analysis. Statistical analyses were carried out using a SPSS V10.05 statistical package (SPSS Inc., Chicago, IL, USA). Differences between the effect of high dose GH (1000 µg/kg per day) and LPS administration were determined by two-way ANOVA. The dose-response to GH was determined by one-way ANOVA in LPS-treated rats only. Mortality data were analysed by Fisher's Exact test. Statistical significance was accepted at the $P < 0.05$ level.

Results

Experiment 1: continuous GH treatment

Injection of LPS rapidly induced clinically evident illness, as indicated by decreased activity, ruffled fur and

Table 1 Haematological, biochemical and hormonal responses to GH and LPS in the rat. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 μ g/kg) minipump+LPS group ($n=3$) and the 1000 μ g/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 μ g/kg GH minipump) and one rat (1000 μ g/kg GH injections) died before sampling

	No LPS		LPS (5 mg/kg)				P value
	Dose of GH (µg/kg)		Dose of GH (µg/kg)				
	0	1000	0	10	100	1000	
Neutrophils							
M	1.14 ± 0.65	1.43 ± 0.18	4.97 ± 1.14	5.68 ± 1.76	6.25 ± 0.81	5.10 ± 1.24	#
I	1.55 ± 0.63	1.60 ± 0.65	6.96 ± 1.94	5.84 ± 2.97	6.77 ± 1.25	5.77 ± 2.77	#
Lymphocyte							
M	5.54 ± 2.26	5.27 ± 1.76	2.11 ± 1.76	1.35 ± 0.65	1.21 ± 0.53	1.92 ± 1.33	#
I	4.40 ± 0.76	4.42 ± 1.06	1.02 ± 0.74	1.52 ± 0.54	1.94 ± 0.77	1.52 ± 0.38	#
Lactate							
M	1.64 ± 0.31	1.24 ± 0.21	2.10 ± 0.32	2.48 ± 0.71	1.96 ± 0.27	1.48 ± 0.14	#
I	1.24 ± 0.1	1.57 ± 0.6	2.02 ± 0.37	1.98 ± 0.36	1.81 ± 0.44	1.79 ± 0.57	#
Sodium							
M	148.5 ± 1.4	143 ± 2.0	144 ± 3.0	150 ± 14.1	151 ± 0.8	146 ± 1.0	§
I	147 ± 2.6	147.8 ± 1.7	145 ± 2.8	143 ± 2.5	147 ± 2.5	147 ± 1.7	
Magnesium							
M	0.82 ± 0.04	0.74 ± 0.02	0.75 ± 0.03	0.83 ± 0.02	0.84 ± 0.07	0.91 ± 0.06	#,§
I	0.81 ± 0.09	0.69 ± 0.05	0.82 ± 0.11	0.83 ± 0.06	0.90 ± 0.10	0.71 ± 0.41	
Creatinine							
M	0.041 ± 0.004	0.043 ± 0.005	0.053 ± 0.008	0.048 ± 0.004	0.056 ± 0.008	0.066 ± 0.010	#, *
I	0.045 ± 0.005	0.042 ± 0.004	0.050 ± 0.00	0.048 ± 0.004	0.058 ± 0.011	0.056 ± 0.011	#
AST							
M	140 ± 16.6	116 ± 26.1	170 ± 33.5	177 ± 30.1	175 ± 55.8	152 ± 28.1	#
I	143 ± 42.7	134 ± 29.7	203 ± 39.4	195 ± 56.1	209 ± 94.5	205 ± 95.8	#
ALT							
M	32.3 ± 9.4	26.2 ± 5.6	50.83 ± 10.6	44.3 ± 13.6	48.2 ± 46.5	24.0 ± 22.7	
I	42.3 ± 8.8	45.6 ± 6.4	77.3 ± 41.7	114.0 ± 71.3	75.8 ± 59.9	97.0 ± 66.1	#
Leptin							
M	5.5 ± 2.4	4.3 ± 1.27	20.2 ± 5.6	11.0 ± 3.5	15.8 ± 9.4	17.2 ± 8.4	#
I	14.4 ± 8.4	15.6 ± 6.6	52.5 ± 13.1	51.8 ± 13.18	49.2 ± 27.8	39.9 ± 18.9	#

M=minipump, continuous infusion group; I=injection, intermittent group.

LPS effect ($P<0.05$, two-way ANOVA); § interaction of high dose GH (1000 μ g/kg) and LPS ($P<0.05$, two-way ANOVA); * dose-response of GH in LPS-treated rats ($P<0.05$, one-way ANOVA).

diarrhoea. No visible differences were observed between LPS-injected rats pretreated with rhGH compared with rats that received LPS alone. As expected, GH treatments induced a dose-dependent increase in body weight ($P<0.01$, data not shown). Weight loss occurred in all groups as a result of food withdrawal at the time of LPS challenge and was not prevented by continuous GH treatment at any dose. Three out of six rats pretreated with high dose continuous infusion GH (1000 μ g/kg per day) died within 16 h after LPS injection, whereas no rats died when treated with LPS alone or LPS plus low dose GH (10 or 100 μ g/kg per day). Gross post-mortem inspection did not provide any specific clues as to the cause of death of these animals.

LPS challenge was associated with a systemic inflammatory response as reflected by decreased ($P<0.001$) blood lymphocyte counts and increased ($P<0.001$) blood neutrophil counts (Table 1). LPS was also associated with changes in lipid metabolism, and renal and liver function,

as shown by increased plasma levels of cholesterol ($P<0.001$) (Fig. 1A), triglyceride ($P<0.001$) (Fig. 2A), urea ($P<0.001$) (Fig. 3A), creatinine ($P<0.01$) (Table 1) and AST ($P<0.05$) (Table 1). Plasma levels of lactate ($P<0.05$) (Table 1), insulin ($P<0.05$) (Fig. 6A) and leptin ($P<0.001$) (Table 1) were also increased in response to LPS (Table 1). In contrast, plasma levels of IGF-I were decreased after injection of LPS ($P<0.001$) (Fig. 4A). Plasma levels of glucose (Fig. 5A), albumin and LDH were unaffected by LPS challenge (data not shown).

In the groups that received rhGH+LPS there was a dose-dependent increase in the plasma levels of cholesterol ($P<0.001$), triglyceride ($P<0.01$) and urea ($P=0.05$) (Figs 1A, 2A and 3A). Continuous treatment with GH was also associated with a dose-related increase in plasma levels of IGF-I ($P<0.05$) (Fig. 4A) and a decrease in plasma levels of insulin ($P<0.001$) (Fig. 6A).

High dose continuous GH (1000 μ g/kg per day) treatment was associated with a marked increase in

Figure 1 Effect of GH and LPS on plasma cholesterol levels. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 μ g/kg) minipump+LPS group ($n=3$) and the 1000 μ g/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 μ g/kg GH minipump) and one rat (1000 μ g/kg GH injections) died before sampling. (A) Minipump=continuous GH infusion groups; (B) injections=intermittent GH groups. # LPS effect ($P<0.05$, two-way ANOVA); § interaction of high dose GH (1000 μ g/kg) and LPS ($P<0.05$, two-way ANOVA); * dose-response of GH in LPS-treated rats ($P<0.05$, one-way ANOVA).

Figure 2 Effect of GH and LPS on plasma triglyceride levels. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 μ g/kg) minipump+LPS group ($n=3$) and the 1000 μ g/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 μ g/kg GH minipump) and one rat (1000 μ g/kg GH injections) died before sampling. (A) Minipump=continuous GH infusion groups; (B) injections=intermittent GH groups. # LPS effect ($P<0.05$, two-way ANOVA); § interaction of high dose GH (1000 μ g/kg) and LPS ($P<0.05$, two-way ANOVA); * dose-response of GH in LPS-treated rats ($P<0.05$, one-way ANOVA).

Figure 3 Effect of GH and LPS on plasma urea levels. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 μ g/kg) minipump+LPS group ($n=3$) and the 1000 μ g/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 μ g/kg GH minipump) and one rat (1000 μ g/kg GH injections) died before sampling. (A) Minipump=continuous GH infusion groups; (B) injections=intermittent GH groups. # LPS effect ($P<0.05$, two-way ANOVA); § interaction of high dose GH (1000 μ g/kg) and LPS ($P<0.05$, two-way ANOVA).

several responses to LPS. Significant interactions between rhGH+LPS were observed with increased plasma levels of cholesterol ($P<0.05$) (Fig. 1A), triglyceride ($P<0.01$) (Fig. 2A), urea ($P<0.05$) (Fig. 3A), sodium ($P<0.01$) (Table 1) and magnesium ($P<0.001$) (Table 1).

Treatment with rhGH+LPS significantly decreased the plasma levels of glucose ($P<0.01$) and insulin ($P<0.001$) when compared with LPS alone (Figs 5A and 6A). There was no significant interaction between high dose continuous GH+LPS for potassium, calcium (data

Figure 4 Effect of GH and LPS on plasma IGF-I levels. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 μ g/kg) minipump+LPS group ($n=3$) and the 1000 μ g/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 μ g/kg GH minipump) and one rat (1000 μ g/kg GH injections) died before sampling. (A) Minipump=continuous GH infusion groups; (B) injections=intermittent GH groups. # LPS effect ($P<0.05$, two-way ANOVA); * dose-response of GH in LPS-treated rats ($P<0.05$, one-way ANOVA).

Figure 5 Effect of GH and LPS on plasma glucose levels. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 µg/kg) minipump+LPS group ($n=3$) and the 1000 µg/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 µg/kg GH minipump) and one rat (1000 µg/kg GH injections) died before sampling. (A) Minipump=continuous GH infusion groups; (B) injections=intermittent GH groups. § interaction of high dose GH (1000 µg/kg) and LPS ($P<0.05$, two-way ANOVA).

not shown), AST and ALT, and plasma leptin levels (Table 1).

Experiment 2: intermittent GH treatment

Twice daily injections of rhGH resulted in a dose-dependent increase in body weight ($P<0.001$) (data not

shown). Injection of LPS had similar inflammatory and catabolic effects to those seen in the first experiment (Table 1 and Figs 1B, 2B and 3B). Following LPS challenge, one rat died in the group pretreated with high dose GH (1000 µg/kg per day). There was no mortality in rats receiving LPS alone, or LPS+low dose GH injections (10 and 100 µg/kg per day).

Figure 6 Effect of GH and LPS on plasma insulin levels. Values are means $\pm$ S.E.M. of six animals per group with the exception of the high dose GH (1000 µg/kg) minipump+LPS group ($n=3$) and the 1000 µg/kg GH injection group ($n=5$) because, after LPS injection, three rats (1000 µg/kg GH minipump) and one rat (1000 µg/kg GH injections) died before sampling. (A) Minipump=continuous GH infusion groups; (B) injections=intermittent GH groups. # LPS effect ($P<0.05$, two-way ANOVA); § interaction of high dose GH (1000 µg/kg) and LPS ($P<0.05$, two-way ANOVA); * dose-response of GH in LPS-treated rats ($P<0.05$, one-way ANOVA).

Following LPS challenge, twice daily rhGH injections increased IGF-I levels in a dose-related manner ($P < 0.001$) (Fig. 4B). However, in contrast to the first experiment, no interactions or dose-related effects of intermittent GH were observed on plasma levels of cholesterol (Fig. 1B), triglyceride (Fig. 2B), urea (Fig. 3B), glucose (Fig. 5B) and insulin (Fig. 6B) levels or plasma electrolytes (Table 1).

Histological observations

As compared with control liver, treatment with both continuous and intermittent high dose GH (1000 µg/kg per day) with or without LPS was associated with very fine vacuolisation of hepatocytes, indicating reversible cell injury. In addition, a small number of inflammatory cells were observed in the sinusoids of animals treated with LPS.

Discussion

The present study is the first to show that plasma electrolytes, lipids, urea, glucose and insulin responses to LPS challenge in rats pretreated with GH are dependent on the pattern and dose of GH administration. Enhanced metabolic derangements were observed in rats treated with continuous GH followed by LPS administration. Rats pretreated with continuous high dose GH plus LPS exhibited increased levels of plasma triglycerides, cholesterol and urea, and decreased plasma levels of insulin and glucose. It remains to be established if this markedly altered energy and protein metabolism directly contributed to the 50% increase in mortality observed in this group. However, administration of the same amount of GH by twice daily injection did not result in these metabolic changes. Previous studies in rats have shown that continuous GH infusion enhances the biological effects of endotoxin (Liao *et al.* 1996, Unneberg *et al.* 1997), but there has been no comparison of different patterns of GH administration or dose of GH on the effects of endotoxin.

GH has both direct and indirect effects on target tissues and in several tissues these actions of GH are dependent on its pattern of secretion or administration (Clark & Robinson 1996, Sjogren *et al.* 1999, Yakar *et al.* 1999). For example, the different patterns of endogenous GH secretion in male and female rats may contribute to the sexual dimorphism in many aspects of growth and metabolism (Clark & Robinson 1996). The administration of exogenous GH to rats by either intermittent injection ('male' pattern) or continuous infusion ('female' pattern) can replicate these responses (Clark *et al.* 1985). These pattern-dependent effects of GH seem to be mediated downstream from the GH receptor by STAT proteins, particularly STAT5b (Udy *et al.* 1997).

Our observation of an enhanced lipolytic effect of continuous GH administration compared with intermittent GH administration in the presence of endotoxin is consistent with studies in obese GH-deficient rats (Clark *et al.* 1996). Other studies have shown that continuous GH exposure exacerbates the LPS-induced hyperlipidaemia (Liao *et al.* 1996, 1997). Hyperlipidaemia in response to LPS could represent a beneficial response, by decreasing the toxicity of LPS and redistributing nutrients to important cells and organs (Hardardottir *et al.* 1995). However, in the present study, enhanced hyperlipidaemia in animals treated with continuous high dose GH (1000 µg/kg per day) plus LPS was associated with a trend to higher mortality. The accompanying findings of an increased uraemia and decreased plasma glucose and insulin in the group treated with continuously infused GH plus LPS also suggest an exacerbation of the responses to LPS by continuous GH administration. A larger study would be needed to confirm an effect on mortality (Liao *et al.* 1996, 1997).

The acute phase of sepsis in both man and rodents is associated with decreased plasma IGF-I concentrations (Fan *et al.* 1994). However, in contrast to man, GH secretion in rats is decreased in response to sepsis (Fan *et al.* 1994, Van den Berghe 2000). There is a consensus that continuous GH treatment is associated with an enhanced plasma IGF-I response compared with intermittent daily injections of GH (Jorgensen *et al.* 1990). In the present study, pretreatment with both continuous and intermittent twice daily GH injections partly reversed the fall in plasma IGF-I caused by endotoxin, although the effect of twice daily GH injections was greater. This suggests that the observed interactions of continuous GH with LPS treatment were independent of changes of circulating IGF-I, confirming the observation of Liao and others (Liao *et al.* 1997, Unneberg *et al.* 1997, Balteskard *et al.* 1998). Treatment with IGF-I appears to be without lethal effects in rats when given with endotoxin (Liao *et al.* 2000).

In contrast to the clinical study in critically ill patients, the results of Liao *et al.* (1996) and our own data show a decrease in plasma glucose and insulin concentration in rats pretreated with continuous GH in combination with LPS. Our data now show that intermittent GH injections do not lower insulin concentrations in LPS-treated rats. In rats, GH usually increases insulin and glucose levels, especially in the presence of adrenal steroids (Clark *et al.* 1997). We have no explanation for this paradoxical 'insulin-like' effect of continuous GH when given in the presence of endotoxin. We think that it is unlikely that IGF-I levels could account for this effect. The low insulin levels could help to explain the massive lipolysis that occurs following continuous GH plus endotoxin.

The liver is a key target organ for GH action. It is therefore possible that the hepatic effects of GH may be implicated in its lethality. However, liver histology

demonstrated only very mild hepatocyte injury after exposure to high dose GH, LPS alone or GH plus LPS. Liver transaminases were also not significantly affected by GH after LPS challenge, thus making it unlikely that hepatocyte injury played a major role in the adverse effects of continuous GH administration during an LPS challenge in the rat. This is in contrast to that which has been observed in sheep, where GH pretreatment resulted in marked histological liver damage and elevation of transaminases (Oliver *et al.* 2001).

GH may also have more subtle hepatic effects that could interact with the livers' responses to septic shock. For example, in the rat, GH has pattern-dependent effects on the expression of liver enzymes, such as carbonic anhydrase III and several cytochrome P450 (CYP) enzymes (Jeffery *et al.* 1990, Shapiro *et al.* 1995). These enzymes are involved in the metabolic clearance of drugs and toxic substances, lipid peroxidation and anti-pyresis (Stegeman & Livingstone 1998, Kozak *et al.* 2000). It has been demonstrated in hypophysectomised rats that a pulsatile pattern of GH secretion (the 'male' pattern) results in an enhanced CYP activity, compared with continuous ('female' pattern) GH release (Shapiro *et al.* 1995). Thus, pattern-dependent effects of GH on liver enzymes may explain some of the observed differences in response to LPS.

Several effects of GH on the immune system are also pattern dependent (Clark 1997). For example, it has been demonstrated that continuous GH infusion is much more potent than GH injections at stimulating lymphoid tissue growth (Clark *et al.* 1995). GH may also be an endocrine as well as an autocrine modulator of inflammatory responses; both GH and the GH receptor are expressed in peripheral blood mononuclear cells (Clark 1997). However, a role for GH in inflammatory responses remains controversial. In the rat, LPS administration following GH priming increases serum interferon γ but not tumour necrosis factor (Liao *et al.* 1997). However, a recent *in vitro* study suggested that there was no effect of GH therapy on proinflammatory cytokines in response to endotoxin (Zarkesh-Esfahani *et al.* 2000). It is possible that the effects of GH on cytokines are also pattern dependent. Together with the use of different experimental models this may account for some of the discrepancies in the literature.

GH also affects thermoregulation (Juul *et al.* 1995): administration of GH enhances hyperthermia duration heat stress (Elvinger *et al.* 1992) and reduces heat shock protein expression in the liver (Deane *et al.* 1999). Thus, potentially, GH treatment can exacerbate the fever associated with septic shock. An experiment directly measuring the body temperature of endotoxin-treated rats given GH would be of interest.

The interactions of GH with other endocrine hormones, such as insulin, thyroid hormones tri-iodothyronine and thyroxine, and leptin may also influence the biological responses to septic shock. A regulatory linkage between GH and leptin has been suggested

(Dieguez *et al.* 2000, Fain & Bahouth 2000), but the interactions during septic shock are unknown. This study confirms that plasma leptin levels are significantly increased after LPS injection, but our results do not demonstrate an effect of GH treatment on plasma leptin, or an interaction between GH and LPS.

It has been stated that the pathogenesis of the deleterious effects of GH in critically ill patients and experimental models of septic shock cannot be explained by a single mechanism of action (Takala *et al.* 1999). As GH interacts with many metabolic, endocrine and immune processes, it does seem likely that their combined effects negatively affect the body's many defences during critical illness. However, this argument does not help to identify markers to alert clinicians to situations where GH-treated patients are at risk. This is important as in both the rat and humans the lethal effects of GH can occur within hours of its administration. The rapid nature of these lethal effects would appear to rule out some mechanisms based on metabolic, electrolyte and fluid balance changes. It is our opinion that no single parameter that we measured in GH-treated rats was changed sufficiently to account for the lethal effects of GH.

The idea that there is a 'single cause' to explain GH lethality in critical illness remains attractive for both theoretical and practical reasons. The dose, pattern and timing of GH treatment during critical illness also seem to be implicated in the outcome. Our study shows that several of the metabolic effects of GH occurred in a dose-related manner, making it unlikely that a lower dose of GH would have beneficial effects during critical illness.

In conclusion, responses to endotoxin, especially on plasma lipids, urea, glucose and insulin, are differentially affected by the pattern of GH pretreatment in the rat. These pattern-dependent effects of GH appear crucial for the observed deleterious effects in septic rats. The use of microarrays to look at gene expression may be a fruitful way to discover the genes and pathways involved. This will not be simple and may involve the use of many different tissues; we do not yet know which are the key tissues and organs involved in these lethal effects. The liver, however, seems to be a good candidate organ deserving further study.

Acknowledgements

This work was supported by funding from Pharmacia & Upjohn, Stockholm, Sweden. The authors would like to acknowledge Christine Keven and Janine Street for their technical assistance. Part of this work was presented at the 11th International Congress of Endocrinology, Sydney 2000.

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Received 29 March 2001

Accepted 11 June 2001