Increased intestinal protein synthesis during sepsis and following the administration of tumour necrosis factor α or interleukin-1 α

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The influence of sepsis on intestinal protein synthesis was studied in rats. Sepsis was induced by caecal ligation and puncture (CLP); control rats were sham-operated. Protein synthesis was measured *in vivo* in the jejunum and ileum following a flooding dose of [14 C]leucine. At 8 h after CLP the protein synthesis rate was increased by approx. 15% in jejunal mucosa, and at 16 h after CLP, the protein synthesis rate was increased by 50–60% in the mucosa and seromuscular layer of both jejunum and ileum. In a second series of experiments, rats were treated with recombinant tumour necrosis factor α (rTNF α) or recombinant interleukin-1 α (rIL-1 α) administered at a total dose of 300 μ g/kg body weight over 16 h. Control rats received corresponding volumes of solvent. Treatment with rTNF α resulted in an approx. 25% increase in mucosal protein synthesis in jejunum. Following treatment with rIL-1 α , protein synthesis increased by 25% in jejunal mucosa and almost doubled in ileal mucosa. The results suggest that sepsis stimulates intestinal protein synthesis and that this response may, at least in part, be mediated by TNF and/or IL-1.

INTRODUCTION

Sepsis and trauma are associated with changes in protein metabolism in various organs and tissues, such as muscle, liver and lungs [1–3]. In contrast, the influence of sepsis on intestinal protein metabolism is not known. This is notable because the intestine has one of the highest protein turnover rates in the body, accounting for 10–15% of total body protein production [4] and, consequently, changes in intestinal protein turnover could have an important impact on whole-body protein economy. Furthermore, the intestine is the production site of various proteins with important biological functions, such as gut hormones, digestive enzymes and immunoreactive proteins.

The purpose of the present study was two-fold. First, we examined the influence of sepsis on intestinal protein synthesis. This was done by measuring protein synthesis rates *in vivo* in the jejunum and ileum of rats that had undergone sham-operation or caecal ligation and puncture (CLP). Secondly, because cytokines, in particular tumour necrosis factor (TNF) and interleukin-1 (IL-1), have been implicated as regulators of various sepsis-induced metabolic changes [5,6], we tested the hypothesis that TNF or IL-1 can induce changes in intestinal protein synthesis similar to those seen during sepsis.

Some of the results of this study were reported previously in an abstract [7].

MATERIALS AND METHODS

Experimental design

Male Sprague–Dawley rats (150–200 g) were housed in a room with a 12 h-light/12 h-dark cycle and a temperature of 22 °C. Animals were allowed to acclimatize for 4–5 days before experiments.

Two series of experiments were performed. In the first series of experiments the influence of sepsis on intestinal protein synthesis was studied. Sepsis was induced by CLP as described previously [8,9]. Control rats were sham-operated. All animals were resusci-

tated with 5 ml 0.9% NaCl/100 g body weight administered subcutaneously at the time of surgery. In order to avoid any influence on protein metabolism of differences in food intake between the two groups of rats, all animals were fasted but had free access to water after the operative procedures. Groups of rats were studied 8 h or 16 h after CLP or sham-operation. This septic model, which resembles the situation in many surgical patients with sepsis caused by intra-abdominal abscess and devitalized tissue, was characterized regarding mortality rates, haemodynamic and haematological changes and bacteriological findings in blood and peritoneal fluid in previous studies both from our [9] and other [8] laboratories.

In the second series of experiments, the effect of TNF α or IL-1 α on intestinal protein synthesis was studied. Rats were treated with 300 μ g of human recombinant TNF α (rTNF α ; kindly provided by Dr. Patricia Olson, Chiron Co., Emeryville, LA, U.S.A.)/kg, or with the same amount of human rIL-1 α (kindly donated by Dr. Peter Lomedico, Hoffman–LaRoche, Inc., Nutley, NJ, U.S.A.), divided into three equal intraperitoneal doses administered at 16:00 h, 24:00 h and 08:00 h. The rTNF α preparation had a specific activity of $(2\text{--}4) \times 10^8$ units/mg of protein in the L929 cell cytolytic assay [10], and contained less than 5 endotoxin units (EU)/mg of protein, as determined by the limulus lysate assay [10a]. The rIL-1 α preparation had a specific activity of 3×10^8 units/mg of protein in the D10 cell assay [11], and contained less than 0.75 EU/mg of protein by the limulus lysate assay. The cytokine preparations were diluted in sterile water to a final concentration of 10 μ g/ml before administration. Control animals received corresponding volumes of sterile water intraperitoneally. All rats had free access to drinking water but were fasted after the first injection to eliminate any influence on protein metabolism of anorexia caused by the cytokines. Intestinal protein synthesis rates were determined 16–18 h after the first injection of cytokine.

In separate experiments, corresponding amounts of heat-inactivated (100 °C for 60 min) cytokine preparations were administered to rats, using a protocol identical to that described

Abbreviations used: CLP, caecal ligation and puncture; TNF α , tumour necrosis factor α ; IL-1 α , interleukin-1 α ; EU, endotoxin units; K_s , protein synthesis rate; VIP, vasoactive intestinal peptide.

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above, and protein synthesis was measured in the jejunal mucosa. The heat inactivation destroys the cytokine activity without affecting endotoxin.

Intestinal protein synthesis

Protein synthesis was measured *in vivo* using the flooding dose technique described by McNurlan *et al.* [12,13], with minor modifications. Rats were anaesthetized with pentobarbital (45 mg/kg intraperitoneally) and following a midline abdominal incision, 10 μ Ci of [U- 14 C]leucine (New England Nuclear, Boston, MA, U.S.A.) and 100 μ mol of unlabelled leucine/100 g body weight were injected in a volume of 1 ml of saline/100 g body wt. into the inferior vena cava. Exactly 2 and 10 min after injection of the isotope (in different groups of rats) the small intestine was removed from Treitz' ligament to the ileocaecal valve. The intestinal lumen was immediately flushed with ice-cold saline to retard protein synthesis. A 10 cm segment of the proximal jejunum and a 10 cm segment of the distal ileum were excised while stretching the intestine with a 3.5 g weight to standardize measurements. The intestinal segments were opened along the anti-mesenteric border and spread on a piece of glass on ice. The mucosal and seromuscular layers were separated by scraping the luminal side with a microscope slide. During these procedures, which took 1–2 min, the tissue was repeatedly rinsed with ice-cold saline to keep protein synthesis to a minimum. The mucosal and seromuscular layers were weighed, frozen in liquid nitrogen and stored at -70°C until analysis.

The tissue was homogenized in 10% trichloroacetic acid, and after centrifugation at 2000 *g* for 20 min the supernatant was saved for determination of specific radioactivity in the free leucine fraction. The protein pellet of the sample obtained 10 min after isotope injection was washed twice with 5 ml of cold 10% trichloroacetic acid and once with ether/ethanol (1:1, v/v). The pellet was hydrolysed for 24 h in 6 M-HCl at 110°C for determination of specific radioactivity of protein-bound leucine.

Dowex 50W X4, a cation-exchange resin which binds positively charged molecules such as amino acids, was used to separate leucine from other amino acids and from metabolites of leucine (e.g. α -oxoisocaproic acid) prior to measuring leucine specific radioactivity. A column of the resin in the H^{+} -form was prepared (5 cm $\times$ 0.6 cm diameter) with an approximate bed volume of 3 ml. A portion of the supernatant of the tissue homogenate or of the hydrolysate was applied to the column which was then washed with 10 ml of deionized water (6–7 times the column volume). This volume was adequate to remove from the column any radioactive metabolites of leucine, while the amino acids (including leucine) remained bound to the column. The amino acids were then eluted from the column with 1 M-NaOH. In preliminary studies, in which we counted 1 ml fractions after adding 15 ml of 1 M-NaOH to the column, we found that leucine was eluted in fractions 6–8. Therefore, in all subsequent experiments, we routinely discarded the first 5 ml of NaOH applied to the column and then collected the subsequent 3 ml, which were used for determination of specific radioactivity of leucine. An aliquot of the eluate was used for measurement of radioactivity in a scintillation spectrometer (LKB R17 Rackbeta Liquid Scintillation Counter, LKB, Stockholm, Sweden). The leucine concentration was determined in the remainder of the eluate by h.p.l.c. using pre-column derivatization with *o*-phthalaldehyde and *t*-butyl thiol by the method of Peinado *et al.* [14].

The protein synthesis rate, K_s , expressed as percentage per day, was calculated from the equation:

$$K_s = \frac{S_B}{S_A \times t} \times 100$$

where S_B is the specific radioactivity of protein-bound leucine, S_A is the mean specific radioactivity of tissue free leucine (using groups of rats at 2 and 10 min to assess the change in specific radioactivity over 0–10 min), and t is the period of incorporation expressed in days. Determining S_A at zero time in this way assumes that the rate of decline between 0 and 2 min is the same as the rate between 2 and 10 min. The accuracy of this assumption was verified by McNurlan *et al.* [12,13].

Wet and dry weight and protein content were measured in segments of the intestine adjacent to the segments that were used for measurement of protein synthesis *in vivo*. Dry weight was determined after drying a piece of intestinal wall (approx. 200 mg) at 80°C for 48 h. Protein was determined according to Lowry *et al.* [15].

Statistics

Results are presented as means $\pm$ S.E.M. Student's *t* test or analysis of variance followed by Tukey's test was used for statistical analysis.

RESULTS

The mortality rate 16 h after CLP was approx. 30%, similar to previous reports [9]. Surviving animals showed signs of sepsis in the form of piloerection, exudate around the eyes and nostrils, and diarrhoea.

In initial experiments the specific radioactivity of free [14 C]leucine was determined in the mucosa and seromuscular layer of control and septic rats at 2 min and 10 min after isotope injection. Results showed that the precursor specific radioactivity was almost identical in the mucosa and seromuscular layer from both control and septic jejunum (Fig. 1) and ileum (results not shown), and that the specific radioactivity decreased only slightly during 10 min. In subsequent experiments, therefore, tissue free

Fig. 1. Specific radioactivity of tissue free leucine 2 and 10 min after an intravenous flooding dose of [14 C]leucine

Determinations were made in (a) mucosa and (b) serosa (seromuscular layer) of sham-operated (○) and septic (●) rats. Each data point represents results from six or seven animals. When standard error bars are not shown, they were smaller than the symbols.

Table 1. Protein synthesis rates in mucosa and serosa of rat jejunum and ileum 8 h and 16 h after sham-operation or CLP

Protein synthesis rates in jejunal and ileal mucosa and serosa were determined following a flooding dose of [14 C]leucine as described by McNurlan *et al.* [12,13]. Groups of rats were studied 8 h or 16 h after sham-operation or CLP. Results are means $\pm$ S.E.M. The number of rats in each group was eight; * $P < 0.05$ versus sham-operation by ANOVA.

	Protein synthesis (%/day)			
	8 h		16 h	
	Sham	CLP	Sham	CLP
Jejunum				
Mucosa	87 $\pm$ 7	94 $\pm$ 4*	101 $\pm$ 5	162 $\pm$ 6*
Serosa	62 $\pm$ 4	68 $\pm$ 4	69 $\pm$ 3	108 $\pm$ 5*
Ileum				
Mucosa	64 $\pm$ 3	60 $\pm$ 3	75 $\pm$ 8	111 $\pm$ 6*
Serosa	42 $\pm$ 1	40 $\pm$ 4	46 $\pm$ 4	68 $\pm$ 6*

Table 2. Protein synthesis rates in mucosa and serosa of rat jejunum and ileum following control injections or treatment with rTNF α or rIL-1 α

Protein synthesis rates in jejunal and ileal mucosa and serosa were determined following a flooding dose of [14 C]leucine as described by McNurlan *et al.* [12,13]. Groups of rats were treated with 300 μ g of rTNF α or IL-1 α /kg divided into three intraperitoneal doses. Control rats received corresponding volumes of solvent intraperitoneally. Protein synthesis rates were determined 16–18 h after the first control or cytokine injection. Results are means $\pm$ S.E.M. The number of rats in each group was eight; * $P < 0.05$ versus control, by ANOVA.

	Protein synthesis (%/day)			
	TNF experiment		IL-1 experiment	
	Control	rTNF α	Control	rIL-1 α
Jejunum				
Mucosa	98 $\pm$ 6	122 $\pm$ 5*	88 $\pm$ 2	121 $\pm$ 5*
Serosa	65 $\pm$ 4	68 $\pm$ 3	54 $\pm$ 3	55 $\pm$ 3
Ileum				
Mucosa	90 $\pm$ 4	100 $\pm$ 5	60 $\pm$ 5	116 $\pm$ 11*
Serosa	55 $\pm$ 7	63 $\pm$ 3	36 $\pm$ 3	39 $\pm$ 3

leucine specific radioactivity was determined only at 10 min. A similar modification of the flooding dose technique was described by Jepson *et al.* [16]. Even if a small error was introduced by this modification, the almost identical specific radioactivity of the precursor amino acid in control and septic rats (Fig. 1) suggests that the relationship between synthesis rates in control and septic rats was not altered by this simplification of the technique.

Protein synthesis rates in the mucosa and seromuscular layer of the jejunum and ileum from control and septic rats are shown in Table 1. At 8 h after CLP the protein synthesis rate was increased by approx. 15% in jejunal mucosa, and at 16 h after CLP the protein synthesis rate was increased by approx. 50–60% in the mucosa and seromuscular layer of both jejunum and ileum. The increase in protein synthesis rate was somewhat more pronounced in jejunum than in ileum, a pattern that was observed in repeated experiments. The basal protein synthesis rates, as well as the difference between mucosa and serosa observed here, are in line with previous reports [12,13].

Tissue wet and dry weight and protein content were determined 16 h after CLP or sham-operation. There was a slight oedema in the jejunum of septic rats compared with control rats, with an increase in water content from 78.6 $\pm$ 0.5 to 81.9 $\pm$ 0.6% of wet weight ($P < 0.05$), but no other differences in wet weight, water or protein content were noted between control and septic animals. The protein content of the intestinal wall was 6.45 $\pm$ 0.20 and 6.18 $\pm$ 0.32 mg/cm of jejunum from control ($n = 7$) and septic ($n = 7$) rats respectively. The corresponding values for ileum were 5.17 $\pm$ 0.43 and 5.41 $\pm$ 0.40 mg/cm.

In the second series of experiments, rats showed clinical evidence of sickness in the form of piloerection and small amounts of exudate around the eyes and nostrils following injection of rTNF α or rIL-1 α . Loose stool, but not diarrhoea, was seen after the cytokine injections. No gross pathological lesions were found in the jejunum or ileum of control or cytokine-injected rats. There was no mortality in any of the groups.

Protein synthesis rates in the second series of experiments are shown in Table 2. Treatment with rTNF α resulted in an approx. 25% increase in mucosal protein synthesis in the jejunum. Protein synthesis in the mucosa of the ileum and in the seromuscular layer of the jejunum and the ileum was not affected by rTNF α . The wet and dry weights of the jejunum and ileum were not influenced by rTNF α (results not shown). Protein content was reduced from 4.80 $\pm$ 0.52 mg/cm in the ileal mucosa of control rats ($n = 8$) to 3.46 $\pm$ 0.20 mg/cm ($P < 0.05$) in rats treated with rTNF α ($n = 8$), but was unchanged in the jejunal mucosa and in the seromuscular layer of both jejunum and ileum following treatment with rTNF α (results not shown).

Treatment with rIL-1 α resulted in an approx. 40% increase in the jejunal mucosal protein synthesis rate and an almost doubled synthesis rate in the ileal mucosa (Table 2). The rIL-1 α treatment did not affect protein synthesis in the seromuscular layer of either jejunum or ileum. Despite the increased protein synthesis rates, the protein content of the intestinal wall was not different between control and rIL-1 α treated animals (results not shown).

The protein synthesis rate in the jejunal mucosa following administration of heat-inactivated rTNF α ($n = 6$) or heat-inactivated rIL-1 α ($n = 6$) was 101 $\pm$ 13%/day and 88 $\pm$ 13%/day respectively. The corresponding value for control rats ($n = 6$) was 90 $\pm$ 8%/day. The differences between these three groups of rats were not statistically significant. This result suggests that the increased protein synthesis rates noted following administration of rTNF α or rIL-1 α were not caused by the small amounts of endotoxin present in the cytokine preparations.

DISCUSSION

The present study shows that sepsis, induced by CLP in rats, was associated with increased intestinal protein synthesis. The earliest and most pronounced changes were noted in the jejunal mucosa, but as sepsis progressed protein synthesis increased in both jejunal and ileal mucosa and seromuscular layer. Administration of rTNF α or rIL-1 α to normal rats resulted in increased mucosal protein synthesis, suggesting that the response to sepsis may at least partly be mediated by TNF and/or IL-1.

The flooding dose technique used here to measure protein synthesis *in vivo* was described previously by McNurlan *et al.* [12,13]. With this technique, the specific radioactivity of the precursor amino acid equilibrates rapidly in different compartments (e.g. plasma and tissue), and the specific radioactivity of the tissue free amino acid can be used for the calculation of protein synthesis rate. More recent studies suggest that it may be more accurate to use the plasma, rather than the tissue, specific radioactivity under certain circumstances [17]. One potential problem with the flooding dose technique is that protein synthesis

rates may be affected by the large amount of leucine. However, previous studies [12,13] demonstrated that protein synthesis in the jejunal mucosa was not altered by the high dose of leucine during the 10 min of incorporation. Similar results were observed when the flooding technique was used to measure protein synthesis in human skeletal muscle [18].

Measurement of protein synthesis in the gut is associated with a number of specific problems [19]. The intestinal wall is not a homogeneous tissue, and evidence for different protein synthesis rates in the mucosa and seromuscular layer, as observed here, has been reported by others [12,13]. In addition, the present results suggest that the different layers of the intestinal wall respond differently to various stimuli, since the cytokines increased protein synthesis in the mucosa but not in the seromuscular layer. Even within the different layers of the intestinal wall, different cell populations may have different protein turnover rates and may respond individually to various stimuli.

The present study does not provide information regarding the specific cell type(s) that is responsible for the increased protein synthesis during sepsis or following the administration of TNF α or IL-1 α . Because enterocytes have a high metabolic rate [20] and synthesize a large number of proteins, including digestive enzymes and various secreted proteins such as gut hormones and immunoreactive proteins, it is likely that the increased intestinal protein synthesis noted in the current study at least partly reflects stimulated enterocyte protein synthesis. This was supported by recent studies in our laboratory in which protein synthesis was increased in isolated enterocytes from septic rats (T. Higashiguchi, P.-O. Hasselgren, J. Frederick, O. Zamir & J. E. Fischer, unpublished work). It should be noted, however, that the increased protein synthesis in the seromuscular layer of the jejunum and ileum 16 h after CLP noted in the present study suggests that cells other than enterocytes, possibly smooth muscle cells, participate in the response to sepsis.

Although the effect of sepsis on intestinal protein metabolism has not been reported previously, the influence of infection or intestinal bacteria has been studied in a small number of previous reports, and results were contradictory. Muramatsu *et al.* [21] observed increased intestinal protein synthesis in chicks with normal gut microflora compared with germ-free controls. In other studies, intestinal protein synthesis was increased in guinea pigs infected with the parasite *Trichostrongylus colubriformis* [22]. In contrast, infection with *Pseudomonas aeruginosa* of burn wounds in rats resulted in a decreased protein content of the jejunal wall, compared with both non-infected burned rats and pair-fed control animals [23]. Intestinal protein turnover rates were not measured in that study, but reduced intestinal RNA content was interpreted as indicating that enterocytes were unable to maintain protein synthesis activity in rats with infected burn wounds.

The finding that the protein content of the intestinal wall was not increased during sepsis or following administration of IL-1 α or TNF α , despite increased protein synthesis, may have several explanations. Increased cell loss and stimulated cell proliferation could give rise to such results. The intestinal epithelium has a rapid cell turnover [24], and it has been estimated that about half of the total intestinal protein synthetic activity reflects replacement of exfoliated enterocytes [12]. Previous studies suggest that the enterocyte proliferation rate is under the regulation of a number of different factors, such as nutritional status [25,26], intestinal bacterial flora [27] and various hormones [19]. The effects of sepsis and cytokines on the intestinal epithelial proliferation rate remain to be determined.

Other explanations for unchanged or decreased intestinal wall protein content may be stimulated degradation and/or secretion of proteins during sepsis or following treatment with the cyto-

kines. There is evidence that enterocytes produce both endogenous and secreted proteins [28]. Gastrointestinal hormones are one important group of secreted proteins which are synthesized in the gut. In this context, it is interesting to note that in previous studies circulating levels of vasoactive intestinal peptide (VIP) were increased during endotoxaemia and sepsis [29,30]. In recent studies in our laboratory, plasma levels of VIP and other gut peptides were markedly elevated in septic and cytokine-treated rats, and plasma concentrations were higher in the portal vein than in the peripheral blood [31]. Thus it may be speculated that increased intestinal protein synthesis in sepsis and following administration of TNF or IL-1 at least partly reflects increased production of gastrointestinal hormone(s).

Because in the first part of the current study the metabolic changes were induced by septic peritonitis, it may be argued that the increased intestinal protein synthesis was a local, rather than systemic, response to sepsis. Although this cannot be ruled out, the fact that the response was different in the jejunum and the ileum suggests that the increased protein synthesis was not caused by local factors only. Furthermore, in recent studies in our laboratory, subcutaneous administration of endotoxin to rats resulted in increased protein synthesis in both mucosa and serosa of the small intestine (T. Higashiguchi, P.-O. Hasselgren, J. Frederick, O. Zamir & J. E. Fischer, unpublished work), further supporting the concept that increased intestinal protein synthesis is a systemic response to sepsis.

It is not apparent from the present study whether the increased intestinal protein synthesis observed following administration of rTNF α or rIL-1 α was the direct effect of the cytokines or required a second mediator. Previous studies have shown that several of the metabolic effects induced by TNF are mediated by glucocorticoids [32–34]. The interaction between the cytokines also needs to be considered. TNF induces release of IL-1, and during sepsis an early increase in TNF levels is followed by release of IL-1 [35]. Thus it is possible that the increased intestinal protein synthesis noted following administration of TNF was the result of IL-1 production, or a third regulator may have been released by both cytokines. An additional factor that needs to be taken into account is fever induced by the cytokines. Body temperature was not measured in the present experiments, but previous studies have shown that both TNF and IL-1 can produce fever when injected into animals [36–38]. It should be noted, however, that CLP in small rats is not associated with hyperthermia [39], suggesting that fever is not necessary for the increase in intestinal protein synthesis.

A number of previous reports support the concept that cytokines may be important for the regulation of gut metabolism. For example, evidence for cytokine production in the gut was reported in a recent study in which enhanced production of IL-1 β by mucosal mononuclear cells was observed in patients with inflammatory bowel disease [40]. Enterocytes themselves may also produce cytokines [41]. Release of TNF from the splanchnic bed following administration of endotoxin in man was reported recently, although it is not known from that study which splanchnic organ(s) accounted for the response [42]. When segments of mouse duodenum were incubated with IL-1, secretion of goblet cell mucus increased in a dose-dependent fashion, suggesting that at least this enterocyte function is regulated by a cytokine [43].

In summary, the present study describes a novel metabolic consequence of sepsis, i.e. increased intestinal protein synthesis. The data suggest that part of this response is mediated by TNF α or IL-1 α . The results are important because they imply that the small intestine may influence whole body protein economy during sepsis, and because they may reflect synthesis of proteins with important biological functions, such as gastrointestinal hormones

and immunoreactive proteins. Further studies are necessary to identify which specific proteins are synthesized at an increased rate in the intestinal wall during sepsis and following the administration of TNF α or IL-1 α .

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