Effect of sepsis on mucosal protein synthesis in different parts of the gastrointestinal tract in rats

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1. In a previous study we found that the protein synthesis rate was increased by 50–60% in the mucosa of the jejunum and ileum during sepsis in rats. It is not known if sepsis affects protein turnover in other parts of the gastrointestinal tract as well.

2. In the present study, the influence of sepsis on mucosal protein synthesis in different parts of the gastrointestinal tract, from the stomach to the rectum, was determined in rats.

3. Sepsis was induced by caecal ligation and puncture; control rats underwent sham-operation. Protein synthesis rate was measured in vivo after administration of a flooding dose of [14C]leucine.

4. Basal mucosal protein synthesis rates were lower in the colon than in the rest of the gastrointestinal tract. Sixteen hours after caecal ligation and puncture, the protein synthesis rates were increased by 40–85% in the mucosa of the small and large intestine and the rectum, whereas in the gastric mucosa, the protein synthesis rate was reduced by approximately 40%.

5. The results suggest that mucosal protein synthesis rates differ in the various regions of the gastrointestinal tract, and that the metabolic response to sepsis is different in the stomach than in the rest of the gastrointestinal tract. The finding of a reduced protein synthesis rate in the gastric mucosa may partly explain the tendency to gastric stress ulcers and bleeding seen clinically in sepsis.

INTRODUCTION

Sepsis results in pronounced changes in protein metabolism in various organs and tissues, including skeletal muscle, liver and lungs [1–3]. In recent studies, we found that sepsis affects protein turnover in the small intestine as well. Thus, 16 h after the induction of sepsis in rats, the protein synthesis rate was increased by 50–60% in the mucosa of the jejunum and ileum [4]. Because basal protein turnover rates are high in the gastrointestinal tract [5], changes in intestinal protein metabolism may have a significant impact on whole-body protein economy during sepsis.

A recent study demonstrated that protein synthesis rates vary in different parts of the gastrointestinal tract: the highest protein synthesis rates were noticed in the mucosa of the small intestine and the antrum of the stomach, whereas protein synthesis rates were lower in the oesophagus, colon and rectum [6]. In the same study, the response to acute ethanol toxicity was different in the various parts of the gastrointestinal tract: the ethanol-induced reduction in protein synthesis was greatest in the most proximal part of the gastrointestinal tract, intermediate in the small intestine and least pronounced in the caecum and colon.

It is not known if sepsis influences protein turnover differently in the various parts of the gastrointestinal tract. In the present study, we therefore examined the effect of sepsis in rats on protein synthesis rates in different regions of the gastrointestinal tract, from the stomach to the rectum. The results suggest that mucosal protein synthesis is increased during sepsis in all sections of the small and large bowel, whereas in the gastric mucosa, protein synthesis is markedly reduced.

MATERIALS AND METHODS

Animals and protocol

Male Sprague-Dawley rats (120–150 g) were housed in a room with a 12 h light/12 h dark cycle (light 06.00 hours to 18.00 hours) and with a temperature of 21°C. Sepsis was induced by caecal ligation and puncture (CLP) as described previously [7, 8]. Control rats were sham-operated, i.e. they underwent laparotomy and manipulation, but no puncture or ligation, of the caecum. The surgical procedures were performed between 16.00 hours and 18.00 hours. All animals were resuscitated with 5 ml of 0.9% NaCl/100 g body weight administered subcutaneously on the back 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 they had free access to water after the operative procedures. Metabolic studies were performed 16 h after CLP or sham-operation. We recently found that jejunal and ileal mucosal protein synthesis was increased at this time point after the induction of sepsis in rats [4]. The status of the animals was checked on a regular basis during the 16 h study period, and rats that appeared moribund were killed. The septic model used here is clinically relevant because it resembles the situation in many surgical patients with sepsis caused by intra-abdominal abscess and devitalized tissue. Mortality rates after CLP in rats and haemodynamic and haematological changes, as well as bacteriological findings in blood and peritoneal fluid, were described previously [7, 8]. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Cincinnati, and all experiments adhered to the National Institutes of Health guidelines for the use of experimental animals.

Measurement of protein synthesis

Mucosal protein synthesis was measured in vivo using the flooding dose technique described by McNurlan and co-workers [9, 10] with minor modifications [4]. Sixteen hours after CLP or sham-operation, rats were anaesthetized with pentobarbital (45 mg/kg body weight intraperitoneally) and after a midline abdominal incision, 5 μCi of [U-14C]leucine (New England Nuclear, Boston, MA, U.S.A.) and 100 μmol of unlabelled leucine/100 g body weight were injected in a volume of 1 ml of 0.9% NaCl/100 g body weight into the inferior vena cava. Exactly 10 min after injection of the isotope, the gastrointestinal tract was removed from the gastro-oesophageal junction to the anus. The lumen was immediately flushed with ice-cold saline to halt protein synthesis. The whole stomach and segments of the duodenum, jejunum, ileum, ascending and descending colon, and rectum was harvested by scraping the luminal side with the edge of a microscope slide. The junction between the jejunum and ileum was defined as the mid-point between the ligament of Treitz and the ileocaecal valve. The midpoint of the transverse colon was used to separate the right and left colon. The length of the different segments of the gastrointestinal tract was determined with a 3.5 g weight attached to the distal end of the intestine in order to standardize measurements. The mucosa was weighed, and protein was determined as described by Lowry et al. [11]. For determination of dry weight, a portion of the mucosa (approximately 50–60 mg wet weight) was dried at 85°C for 48 h.

Statistics

Results are presented as means ± SEM. Student's t-test was used for statistical comparisons.

RESULTS

The mortality rate 16 h after CLP was approximately 30%, similar to that in previous reports [8]. Most of these animals died spontaneously. A few animals that looked moribund on inspection (lying still on their side with no spontaneous activity) were killed before the end of the 16 h study period, and these animals were included in the 30% mortality rate. Animals that survived 16 h after CLP showed signs of sepsis in the form of lethargy, piloerection, exudate around the eyes and nostrils, and diarrhoea. No sham-operated rats died.

Sixteen hours after CLP, mucosal wet and dry tissue free leucine and t is the time in days [4]. The validity of basing measurement of the protein synthesis rate on the tissue free leucine specific radioactivity from one time point only (10 min after injection of the isotope) rather than two time points (2 min and 10 min), as was originally described [9, 10], was reported previously [4]. It should be noted that protein synthesis rates were measured in anaesthetized animals in the present experiments. This was done to make it possible to compare the results with those in our previous report on mucosal protein synthesis in septic rats [4]. Protein turnover rates reported in that study were similar to those usually observed in awake rats. Because identical protocols were followed when protein synthesis was measured both in control and septic rats, it is not likely that the differences noticed between the groups were influenced by the fact that animals were anaesthetized when the flooding dose of [U-14C]leucine was administered.

Mucosal weight and protein content

For the determination of mucosal wet and dry weight and protein content, mucosa from the whole stomach and segments of the duodenum, jejunum, ileum, ascending and descending colon, and rectum was harvested by scraping the luminal side with the edge of a microscope slide. The junction between the jejunum and ileum was defined as the mid-point between the ligament of Treitz and the ileocaecal valve. The midpoint of the transverse colon was used to separate the right and left colon. The length of the different segments of the gastrointestinal tract was determined with a 3.5 g weight attached to the distal end of the intestine in order to standardize measurements. The mucosa was weighed, and protein was determined as described by Lowry et al. [11]. For determination of dry weight, a portion of the mucosa (approximately 50–60 mg wet weight) was dried at 85°C for 48 h.

Statistics

Results are presented as means ± SEM. Student's t-test was used for statistical comparisons.
weight, protein concentration and protein content were reduced in the stomach (Table 1). With the exception of a reduced wet weight of rectal mucosa, no significant differences in wet or dry weight or protein concentration and content were noticed in the other parts of the gastrointestinal tract between the two groups of rats. A slight, but statistically non-significant, increase in tissue water was noted in the mucosa of the ileum and right colon of septic rats, whereas no differences in tissue water were seen in the other segments of the gastrointestinal tract between control and septic rats. The specific radioactivity of protein-bound (S_{P}) and tissue free (S_{A}) leucine and protein synthesis rate (K_{S}) in the mucosa of different parts of the gastrointestinal tract were determined after a flooding dose of [^{14}C]leucine as described previously [4]. The studies were performed 16h after sham-operation or CLP. Results are means ± SEM. The number of rats in each group was eight. Statistical significance: *P < 0.05 compared with sham-operated rats.

Table 2. Specific radioactivity of protein-bound (S_{P}) and tissue free (S_{A}) leucine and protein synthesis rate (K_{S}) in the mucosa of different parts of the gastrointestinal tract of sham-operated and septic rats. Protein synthesis rates in the mucosa of different parts of the gastrointestinal tract were determined after a flooding dose of [^{14}C]leucine as described previously [4]. The studies were performed 16h after sham-operation or CLP. Results are means ± SEM. The number of rats in each group was eight. Statistical significance: *P < 0.05 compared with sham-operated rats.

<table>
<thead>
<tr>
<th>Region</th>
<th>Wet wt. (mg)</th>
<th>Dry wt. (mg)</th>
<th>Tissue water (% of wet wt.)</th>
<th>Protein concn. (% of wet wt.)</th>
<th>Protein content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham CLP</td>
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<td>Sham CLP</td>
</tr>
<tr>
<td>Stomach</td>
<td>336 ± 11</td>
<td>207 ± 10*</td>
<td>47.5 ± 2.4</td>
<td>28.6 ± 1.7*</td>
<td>85.9 ± 0.5</td>
</tr>
<tr>
<td>Duodenum</td>
<td>43.6 ± 4.1</td>
<td>36.6 ± 1.9</td>
<td>6.5 ± 0.4</td>
<td>5.9 ± 0.3</td>
<td>84.2 ± 1.3</td>
</tr>
<tr>
<td>Jejunum</td>
<td>37.5 ± 2.3</td>
<td>32.4 ± 2.5</td>
<td>6.9 ± 0.4</td>
<td>5.9 ± 0.4</td>
<td>81.5 ± 0.3</td>
</tr>
<tr>
<td>Ileum</td>
<td>27.1 ± 2.2</td>
<td>28.9 ± 1.6</td>
<td>4.1 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>82.5 ± 0.2</td>
</tr>
<tr>
<td>Right colon</td>
<td>30.2 ± 1.4</td>
<td>33.9 ± 3.2</td>
<td>6.0 ± 0.4</td>
<td>5.6 ± 0.5</td>
<td>80.3 ± 0.7</td>
</tr>
<tr>
<td>Left colon</td>
<td>20.7 ± 1.9</td>
<td>19.6 ± 1.9</td>
<td>3.2 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td>84.2 ± 0.6</td>
</tr>
<tr>
<td>Rectum</td>
<td>31.0 ± 2.0</td>
<td>23.4 ± 2.1*</td>
<td>4.0 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>87.0 ± 0.8</td>
</tr>
</tbody>
</table>

The specific radioactivity of protein-bound (S_{P}) and tissue free (S_{A}) leucine and protein synthesis rate (K_{S}) in the mucosa of different parts of the gastrointestinal tract of sham-operated and septic rats. Protein synthesis rates in the mucosa of different parts of the gastrointestinal tract were determined after a flooding dose of [^{14}C]leucine as described previously [4]. The studies were performed 16h after sham-operation or CLP. Results are means ± SEM. The number of rats in each group was eight. Statistical significance: *P < 0.05 compared with sham-operated rats.

<table>
<thead>
<tr>
<th>Region</th>
<th>Sham CLP</th>
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<th>Sham CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>0.43 ± 0.03</td>
<td>98 ± 6</td>
<td>65 ± 5</td>
<td>0.22 ± 0.03*</td>
<td>77 ± 5</td>
<td>41 ± 4*</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.56 ± 0.03</td>
<td>87 ± 3</td>
<td>94 ± 4</td>
<td>0.76 ± 0.04*</td>
<td>80 ± 3</td>
<td>139 ± 5*</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.52 ± 0.03</td>
<td>86 ± 5</td>
<td>89 ± 7</td>
<td>0.64 ± 0.07*</td>
<td>81 ± 4</td>
<td>115 ± 9*</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.40 ± 0.05</td>
<td>87 ± 3</td>
<td>67 ± 6</td>
<td>0.53 ± 0.05*</td>
<td>87 ± 6</td>
<td>91 ± 7*</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>0.26 ± 0.03</td>
<td>103 ± 4</td>
<td>40 ± 5</td>
<td>0.56 ± 0.07*</td>
<td>139 ± 13*</td>
<td>61 ± 6*</td>
</tr>
<tr>
<td>Descending colon</td>
<td>0.29 ± 0.04</td>
<td>91 ± 4</td>
<td>45 ± 6</td>
<td>0.65 ± 0.05*</td>
<td>130 ± 19*</td>
<td>74 ± 8*</td>
</tr>
<tr>
<td>Rectum</td>
<td>0.41 ± 0.04</td>
<td>95 ± 4</td>
<td>66 ± 8</td>
<td>1.10 ± 0.20*</td>
<td>131 ± 16*</td>
<td>123 ± 15*</td>
</tr>
</tbody>
</table>

The protein synthesis rate was increased during sepsis by 30–40% in the mucosa of the jejunum and ileum (Table 2), confirming a recent report from our laboratory [4]. Increased protein synthesis during sepsis was noted also in the mucosa of the duodenum, ascending and descending colon, and rectum, and the relative increase was even more pronounced in these segments of the gastrointestinal tract during sepsis since a higher $S_{A}$ would reduce the calculated synthesis rate by the formula of McNurlan et al. [9].

The protein synthesis rate was increased during sepsis by 30–40% in the mucosa of the jejunum and ileum (Table 2), confirming a recent report from our laboratory [4]. Increased protein synthesis during sepsis was noted also in the mucosa of the duodenum, ascending and descending colon, and rectum, and the relative increase was even more pronounced in these segments of the gastrointestinal tract than in the jejunum and ileum: the mucosal protein synthesis rate was increased in septic rats by 48%, 53%, 64% and 86% in the duodenum, ascending and descending colon and rectum, respectively. In sharp contrast, the protein synthesis rate in the gastric mucosa was reduced by 37% in septic rats (Table 2).

DISCUSSION

In the present report, the flooding dose technique described by McNurlan et al. [9, 10] was used to
measure protein synthesis rates. This method has been used in a number of previous studies on the influence of injury and sepsis on protein synthesis in muscle [12, 13], liver [14] and intestine [4]. The flooding dose technique has several major advantages. It is technically simple to perform and the problem of selecting the proper precursor pool is avoided since the specific radioactivity equilibrates in different pools. Errors due to loss of radioactivity from protein with possible reincorporation of labelled amino acids are avoided since measurements are performed over a short period of time.

It should be noted that controversy exists regarding the use of the flooding dose technique [15, 16]. Recent studies by Rennie et al. [16] suggest that a flooding dose of leucine may stimulate protein synthesis when compared with a constant infusion technique, at least in human skeletal muscle. In the report of McNurlan et al. [9], however, evidence was provided that the large dose of leucine did not affect protein synthesis in rat intestinal mucosa. Even if absolute protein synthesis rates are slightly different from one method to another, in many studies, including the present report, the main objective is to determine differences in protein synthesis between experimental groups with different pathological conditions.

The present result of variable protein synthesis rates in different parts of the gastrointestinal tract is similar to that of a recent study by Marway et al. [6]. In that study as well, the lowest synthesis rates were observed in colonic mucosa. The same authors [6] found evidence that protein synthesis in the stomach is heterogeneous, with higher protein synthesis rates in the antrum than in the cardia. In the present report, protein synthesis was measured in the mucosa of the entire stomach, and this probably explains why the basal synthesis rate observed here (65%/day) was intermediate between the synthesis rate in the cardia (47%/day) and the mucosa of the antrum (106%/day) as reported by Marway et al. [6]. The differences in protein synthesis rates between the various regions of the gastrointestinal tract may reflect different functional roles and/or different cell proliferation rates in each anatomical region.

Increased mucosal protein synthesis in the jejunum and ileum of septic rats, as observed here, is similar to the findings of a recent study from our laboratory [4]. The present experiments extended our previous report by examining the influence of sepsis on protein synthesis in other parts of the gastrointestinal tract in addition to the jejunum and ileum. Sepsis resulted in increased protein synthesis not only in these parts of the gastrointestinal tract, but in the duodenum, colon and rectum as well. In sharp contrast, protein synthesis in the gastric mucosa was reduced, suggesting that the metabolic response to sepsis is different in the stomach than in the rest of the gastrointestinal tract. A similar differential metabolic response to sepsis was reported by Lang et al. [17], who found that sepsis in rats increased glucose uptake in vivo in all sections of the gastrointestinal tract except the stomach. Although it is possible that gastric emptying is delayed during sepsis, it should be noted that the stomach was empty, with no food residues present 16h after CLP or sham-operation, indicating that the present results were not influenced to a great extent by delayed gastric emptying in septic rats.

Protein synthesis was measured in whole mucosa in the different regions of the gastrointestinal tract in the present report. Thus, it is not known in which cell type the increase in protein synthesis took place during sepsis. Although the enterocyte is the predominant cell type in the mucosa, it is possible that results were influenced by protein synthesis in other cells as well, such as macrophages and lymphocytes present in the lamina propria or submucosal layer. In a recent study we observed that protein synthesis was increased in enterocytes isolated from the jejunum of septic rats [18]. Thus, the present results may, at least in part, reflect stimulated enterocyte protein synthesis.

Because the gastrointestinal tract has one of the highest protein turnover rates in the body [5], changes in gastrointestinal protein turnover rates may significantly influence whole-body protein economy. One important implication of the present study, therefore, is that whole-body protein balance during sepsis may be influenced by metabolic changes in the gastrointestinal tract.

The finding of an unchanged protein content in the intestinal mucosa, despite increased protein synthesis, is similar to the results of our previous study [4], in which protein turnover rates were measured in the jejunum and ileum. This result may have several explanations, including increased cell loss and stimulated cell proliferation. The intestinal epithelium has a rapid cell turnover [19] and in previous studies it was estimated that approximately half of the total intestinal protein synthesis reflects replacement of exfoliated enterocytes [9]. The influence of sepsis on cell replication in various parts of the gastrointestinal tract remains to be determined.

Another explanation for the unchanged intestinal mucosal protein content, despite increased protein synthesis, may be stimulated production of secretory proteins. Previous studies [20] provided evidence that enterocytes produce both endogenous and secretory proteins. We recently observed that plasma levels of several gastrointestinal hormones were elevated in septic rats, and plasma levels were higher in portal than in peripheral blood [21]. In a subsequent study, production of total secreted proteins, vasoactive intestinal peptide and peptide YY was increased in isolated enterocytes from septic rats [18]. Thus, it is possible that the increased protein synthesis observed in septic rats, at least that in the small intestine, partly reflects stimulated
production of gut hormones and/or other secretory proteins.

In contrast to the small and large intestine, the mucosal protein content in the stomach was reduced in septic rats. This finding was consistent with the inhibited protein synthesis in the gastric mucosa during sepsis observed here, but increased cell loss without a compensatory increase in proliferation may be an additional factor. So-called stress ulcers with sometimes life-threatening bleeding frequently develop in the stomach of septic and other critically ill patients [22]. It is not known from the present study whether inhibited gastric protein synthesis plays a role in the development of this complication in septic patients, but it may be speculated that reduced protein synthesis during sepsis reflects inhibited production of proteins that are important for the protection of the gastric mucosa.

The mediators and mechanisms of altered protein turnover in the gastrointestinal tract during sepsis are not known. However, we [4] recently found that administration of tumour necrosis factor or interleukin-1 to normal rats resulted in increased jejunal and ileal protein synthesis, suggesting that these cytokines may be involved in the regulation of intestinal protein synthesis during sepsis.

The present results are important from a clinical standpoint because they suggest that whole-body protein economy may be influenced by metabolic changes in the gastrointestinal tract during sepsis. The study underscores the central role of the gut in the metabolic response to sepsis and other critical illness [23]. Reduced metabolic activity in the mucosa of the stomach during sepsis may have far-reaching clinical implications and deserves further investigation.

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REFERENCES