Influence of Sepsis and Endotoxemia on Polyamine Metabolism in Mucosa of Small Intestine in Rats

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We examined the influence of sepsis and endotoxemia in rats on the biosynthesis of polyamines in small-intestinal mucosa. Sepsis was induced by cecal ligation and puncture (CLP); control rats were sham-operated. In other experiments, rats were treated with two subcutaneous injections of endotoxin (1 mg/kg) or corresponding injections of sterile saline. Ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (SAMDC) activities and concentrations of putrescine, spermidine, and spermine were measured in jejunal mucosa at intervals during 16 hours. Sepsis stimulated ODC and SAMDC activities and increased putrescine and spermidine concentrations in jejunal mucosa. Injection of endotoxin resulted in metabolic changes similar to those observed following CLP. The results suggest that sepsis and endotoxemia stimulate polyamine biosynthesis in mucosa of small intestine. The role of polyamines in the regulation of cell proliferation and metabolic changes in the intestinal mucosa during sepsis remains to be determined.

THE POLYAMINES putrescine, spermidine, and spermine are present in virtually all eukaryotic cells.[1] Regulation of the biosynthesis of polyamines and their biological functions have been reviewed extensively elsewhere.[1-3] In short, putrescine is formed by decarboxylation of ornithine, a reaction catalyzed by ornithine decarboxylase (ODC). Formation of spermidine and spermine is regulated by the enzyme *S*-adenosylmethionine decarboxylase (SAMDC). Tissue concentrations of the polyamines are also influenced by cellular uptake and release of the substances[4] and by their degradation. In addition, spermidine and spermine can be converted back to putrescine by the enzymes spermidine/spermine acetyltransferase and polyamine oxidase.[1]

The polyamines have been implicated in a number of cellular functions, including membrane stabilization and synthesis of DNA, RNA, and proteins.[1,5,6] In particular, polyamines are essential for cellular proliferation, and increased ODC activity is usually seen in conditions characterized by high cell turnover.[3] Equally important is the regulatory effect on protein synthesis, and there is evidence to suggest that the influence on cell proliferation is secondary to the regulation of protein synthesis.[1] Polyamines regulate protein synthesis both at the level of tRNA acetylation[7] and by affecting the rate of translation.[5] In addition, polyamines may be involved in posttranslational modification of proteins.[1]

Intestinal mucosa is characterized by rapid cell turnover and has one of the highest protein turnover rates in the body.[8] There is evidence that polyamines are necessary for the growth of gastrointestinal mucosa, and that they are essential for repair of mucosal injury.[3] Increased ODC activity and polyamine levels have been reported in various conditions characterized by mucosal damage, such as water-immersion–induced stress in rats,[9] ischemia-reperfusion,[10] and burn injury.[11]

Sepsis is another condition associated with severe metabolic stress in various organs and tissues, including intestinal mucosa.[12] In recent studies in our laboratory, protein synthesis was substantially increased in intestinal mucosa of septic rats,[13,14] a metabolic response that, in part, may reflect increased cell loss and cell turnover in the intestinal epithelium.[15] Increased production of putative gut hormonal peptides may also contribute to the stimulated mucosal protein synthesis seen during sepsis.[16] Considering the role of polyamines in the regulation of cell proliferation and protein synthesis, the results observed in the intestine of septic rats may be consistent with stimulated polyamine biosynthesis. However, the influence of sepsis on intestinal polyamine metabolism has not been reported.

The purpose of the present study was to test the hypothesis that sepsis stimulates biosynthesis of polyamines in the mucosa of small intestine. This was achieved by measuring ODC and SAMDC activities and polyamine levels in jejunal mucosa following induction of sepsis in rats by cecal ligation and puncture (CLP). To rule out the possibility that metabolic changes seen after CLP merely reflected the local effects of septic peritonitis rather than the systemic response to sepsis, the effect of subcutaneously administered endotoxin on mucosal polyamine biosynthesis was also tested.

MATERIALS AND METHODS

Sepsis was induced in male Sprague-Dawley rats (body weight, 140 to 160 g) by CLP as described previously.[17,18] Control rats were sham-operated. All rats were resuscitated with saline (10 mL/100 g body weight) administered subcutaneously on the back at the time of surgery. Animals had free access to water but were fasted after the surgical procedures to avoid any influence of different food intake between the groups on metabolic changes. Metabolic studies were performed in groups of rats at intervals up to 16 hours after induction of sepsis. This time point was chosen on the basis of previous studies in which protein synthesis[13,14,16] and cellular proliferation[15] were increased in mucosa of small bowel 16 hours after CLP. CLP is a clinically relevant model of sepsis, since it

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resembles the situation in surgical patients with sepsis caused by intraabdominal abscess and devitalized tissue. The model has been characterized with respect to mortality rates and hemodynamic, hematologic, and metabolic changes in previous reports from our laboratory[18] and other.[17]

In another set of experiments, endotoxemia was induced in rats by subcutaneous injection of 1 mg/kg endotoxin (lyophilized lipopolysaccharide from *Escherichia coli* 0111:B4; Calbiochem, La Jolla, CA) dissolved in 1 mL sterile saline. This dose of endotoxin was repeated after 8 hours, and animals were studied 16 hours after the first injection of endotoxin. Control rats were injected with corresponding volumes of sterile saline. All rats were fasted but had free access to water after the first endotoxin or saline injection. The dose and time interval for endotoxin injections were based on a previous report in which this endotoxin protocol produced changes in cellular proliferation in jejunal mucosa of rats similar to those seen following CLP.[15]

Enzyme Activities and Polyamine Levels

With rats under pentobarbital anesthesia (45 mg/kg administered intraperitoneally), a 10-cm segment of the jejunum, starting at the ligament of Treitz, was excised. Length of the intestinal segment was determined with a 3.5-g weight attached to the distal end of the small intestine. The intestine was immediately opened longitudinally, and the mucosa was rinsed with ice-cold saline. The mucosa was scraped from the underlying seromuscular layer using a glass microscope slide, and was immediately frozen at −70°C until analysis of enzyme activities and polyamine levels.

The activity of ODC was determined by a radiometric method in which the amount of $^{14}CO_2$ liberated from L-[1-^{14}C]-ornithine (58 mCi/mmol; New England Nuclear, Boston, MA) was determined as described previously[9,10] with minor modifications. In short, tissue samples were placed in 3 mL 0.1-mol/L Tris (hydroxymethyl)-aminomethane hydrochloride buffer (pH 7.4) containing 1 mmol/L EDTA, 50 μmol/L pyridoxal 5-phosphate, and 5 mmol/L dithiothreitol. The tissues were homogenized twice with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) for 15 seconds and then centrifuged at 30,000 × *g* for 30 minutes. Protein content was determined according to the method of Lowry et al.[19] A 100-μL aliquot of the supernatant was incubated in stoppered vials in the presence of 1.7 nmol L-[1-^{14}C]-ornithine for 15 minutes at 37°C. $^{14}CO_2$ liberated during incubation by decarboxylation of ornithine was trapped on filter paper impregnated with 20 μL 2N NaOH suspended in a hanging well above the reaction mixture. The reaction was stopped by addition of 0.3 mL 10% trichloroacetic acid, and the incubation vials were placed on ice for 2 hours. Radioactivity of $^{14}CO_2$ trapped in the filter paper was measured in a liquid scintillation spectrophotometer (LKB 1217; Rackbeta, Stockholm, Sweden). Enzyme activity was expressed as picomoles of $^{14}CO_2$ per milligram protein per hour. It should be noted that in previous studies we found that mucosal protein content was not influenced by sepsis.[13,14] Thus, expressing enzyme activity (and polyamine levels, see below) per milligram protein should not give rise to false high or low levels.

SAMDC activity was determined in a similar way by measuring release of $^{14}CO_2$ from *S*-[carboxyl-^{14}C]adenosyl-L-methionine as described by Wang et al.[20] Except that 30 μmol/L putrescine was added to the buffer to stabilize SAMDC, the method was identical to that described above for ODC activity.

Polyamine levels were measured by high-performance liquid chromatography (HPLC) as described previously by Wang et al,[21] with minor modifications. In short, tissue samples were placed in 0.4N perchloric acid and homogenized for 20 seconds, ultrasonicated for 15 seconds, and centrifuged at 1,600 × *g* for 10 minutes. The supernatant was collected and neutralized to pH 7.0 with 6N KOH and centrifuged to remove the precipitate. A 0.2-mL aliquot of the solution was delivered to a 3.5-mL vial equipped with a Teflon-lined screw cap. After addition of 100 μL saturated Na_2CO_3 and 200 μL dansyl chloride solution (10 mg/mL acetone), the reaction was allowed to proceed by heating at 70°C for 30 minutes. Five tenths of a milliliter of glass-distilled water and 2.5 mL toluene were then added. After mixing and centrifuging, the organic portion was collected and evaporated to dryness under a stream of N_2. The residue was dissolved in 500 μL acetone and filtered. An aliquot of 200 μL was used for HPLC analysis. A Waters (Milford, MA) HPLC system was used, which included a Spectra-Physics SP8775 autosampler injector, two 510 solvent delivery units, a 680 solvent programer, and a Novapack C_{18} column (length, 15 cm; inner diameter, 3.9 mm) in a radial compression module. The fluorescence detector was a Shimadzu RF-535 fluorescence HPLC monitor (Shimadzu Scientific Instruments, Columbia, MD). Solvent A and solvent B were composed of acetonitrile, water, glacial acetic acid, and triethylamine in the proportions of 40:60:0.02:0.001 and 95:5:0.02:0.005, respectively. The mobile phases used in this separation consisted of a program of a linear gradient run over 15 minutes starting with 65% solvent A and 35% solvent B and increasing solvent B linearly to 100%. Each sample was run for 13 minutes, and the equilibration delay between injections was 2 minutes. The mobile phases (A and B) were prepared fresh before starting the automatic injector. Measurements of polyamines were made by comparing ratios of polyamines to 1,10-diaminodecane peak heights with a standard curve. Samples for a calibration curve were obtained by adding known amounts of standards in 1 mL glass-distilled water followed by extraction and dansylation as previously described.

Statistics

Results are the mean ±SEM. Student's *t* test or ANOVA followed by Duncan's test were used for statistical comparisons.

RESULTS

Sixteen hours after CLP, the mortality rate was approximately 30%, similar to a previous report from our laboratory.[18] There was no mortality among sham-operated control rats. Surviving septic rats showed signs of illness in the form of piloerection, exudate around the eyes and nostrils, and mild diarrhea. In rats that had undergone CLP, the cecum was engorged and gangrenous and the peritoneal cavity contained 3 to 4 mL brownish, foul-smelling fluid. In previous studies from our laboratory[18] and other,[17] cultures of blood and peritoneal fluid grew a mixed aerobic and anaerobic bacterial flora following CLP in rats.

Sepsis resulted in increased mucosal ODC activity in the jejunum, and this increase was noted as early as 4 hours after CLP (Fig 1). At the end of the experiment, mucosal ODC activity was approximately five times higher in septic than in sham-operated control rats. Activity of SAMDC was also higher in small-intestinal mucosa of septic rats than in mucosa of control rats, but the difference between the two groups was less pronounced than that observed for ODC activity (Fig 1). The difference in SAMDC activity between control and septic rats noted at 16 hours reflected a decline in SAMDC activity in control rats rather than an increase in SAMDC activity in septic rats.

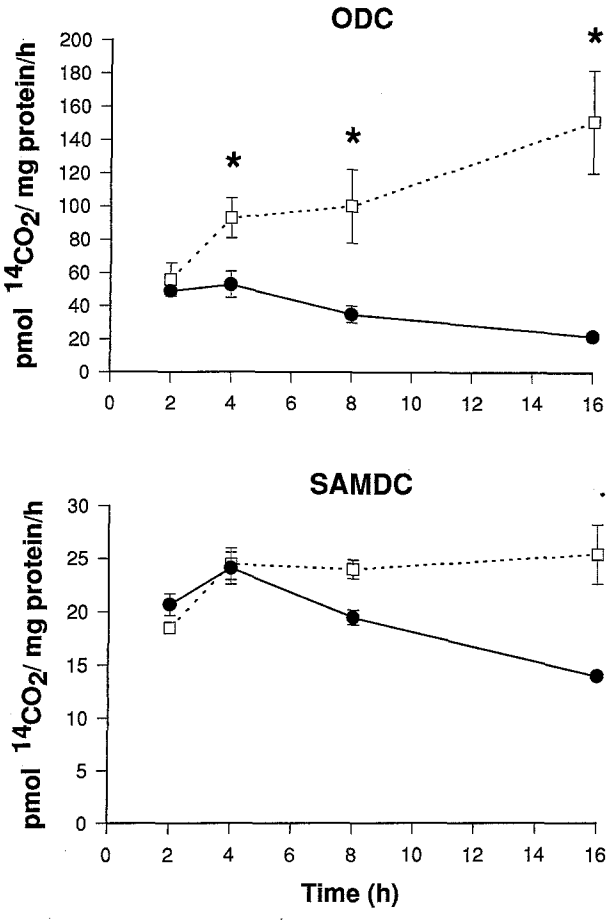

Fig 1. ODC and SAMDC activities in jejunal mucosa of rats following sham-operation (●) or CLP (□). n = 6 to 8 at each time point for both groups. *$P < .05$ *v* sham.

Putrescine levels in intestinal mucosa were higher in septic than in sham-operated rats, but the difference did not become statistically significant until 16 hours after CLP (Fig 2). The higher putrescine levels in septic rats at this time point reflected a decline of putrescine levels in control rats between 4 and 16 hours rather than an increase in putrescine levels in septic rats. Spermidine levels were slightly increased 16 hours after CLP, whereas no effects on spermine levels were noted during the 16-hour study period (Fig 2).

Because CLP results in septic peritonitis, it could be argued that changes in intestinal polyamine metabolism reflected the local response to intraabdominal infection rather than the systemic response to sepsis. To address this question, we next examined the effect of endotoxemia on mucosal polyamine metabolism. Although injection of endotoxin in healthy rats does not necessarily mimic the septic situation, endotoxemia is frequently used as a model to study sepsis-related metabolic alterations.

The response to endotoxemia was similar but not identical to that seen following CLP; 16 hours after the first endotoxin injection, ODC and SAMDC activities and putrescine levels in jejunal mucosa were increased (Fig 3). In contrast to sepsis, spermidine levels were not altered but spermine levels were slightly increased in mucosa of endotoxemic rats (Fig 4).

DISCUSSION

The present study showed that sepsis induced by CLP in rats increased ODC and SAMDC activities and polyamine levels in mucosa of small intestine. Because a similar metabolic response (albeit not identical) was induced by subcutaneous injections of endotoxin, it is likely that the increased polyamine biosynthesis noted following CLP was part of the systemic response to sepsis rather than the local effect of septic peritonitis. Changes in polyamine levels were less pronounced than changes in ODC activity, possibly because polyamine levels are influenced by changes in

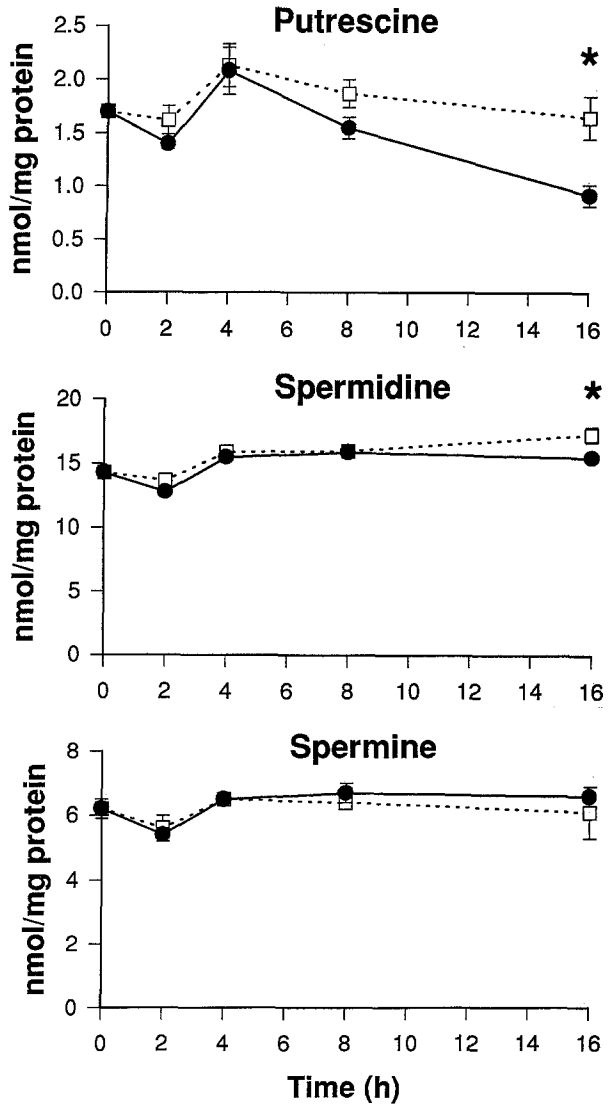

Fig 2. Polyamine levels in jejunal mucosa following sham-operation or CLP in rats. Symbols and number of animals are the same as in Fig 1.

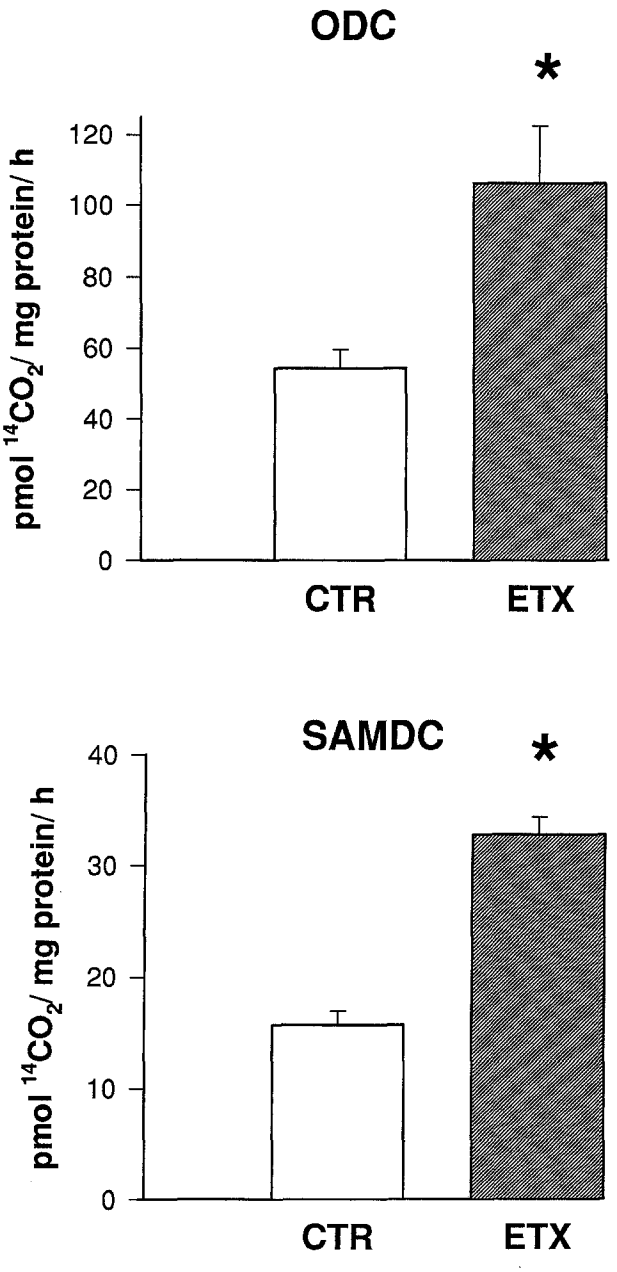

Fig 3. ODC and SAMDC activities in jejunal mucosa of saline (control [CTR])- and endotoxin (ETX)-treated rats. Rats were injected subcutaneously with two doses of ETX (1 mg/kg each) with an 8-hour interval, or corresponding volumes of sterile saline. Metabolic studies were performed 16 hours after the first injection.

cellular uptake and release in addition to changes in intracellular polyamine biosynthesis.[3,4]

The intestinal mucosa is a heterogenous tissue containing a number of different cell types. Although the specific cell(s) in which changes in polyamine metabolism took place was not determined in the present study, other studies suggest that the enterocytes account for at least part of the ODC and SAMDC activity in mucosa of small intestine.[22]

To our knowledge, this is the first report on the influence of sepsis on mucosal polyamine metabolism. In a study by Wang et al,[21] the effect of local inflammation in the small bowel caused by the parasitic nematode *Trichinella spiralis* was examined. Results in that study showed that activity of ODC in mucosa of small intestine was increased in rats that had been inoculated with *T. spiralis* larvae, and this increase in ODC was accompanied by increases in mucosal putrescine, spermidine, and spermine content. Thus, polyamine metabolism in the mucosa of small intestine may be similarly affected by local inflammation[21] and systemic sepsis as observed in the present study following CLP or injection of endotoxin.

In several previous reports, increased polyamine biosynthesis was associated with mucosal injury or dysfunction, and was essential for the restoration of mucosal damage caused by various stimuli, such as water immersion of rats,[9] burn injury,[11] and ischemia/reperfusion.[10] In a recent study, we observed that CLP in rats resulted in increased cell loss from the tips of the villi, shortened villi, elongated crypts, and increased incorporation of ^{3}H-thymidine into mucosal DNA in small intestine.[15] Thus, it is possible that the stimulated biosynthesis of polyamines during sepsis is a

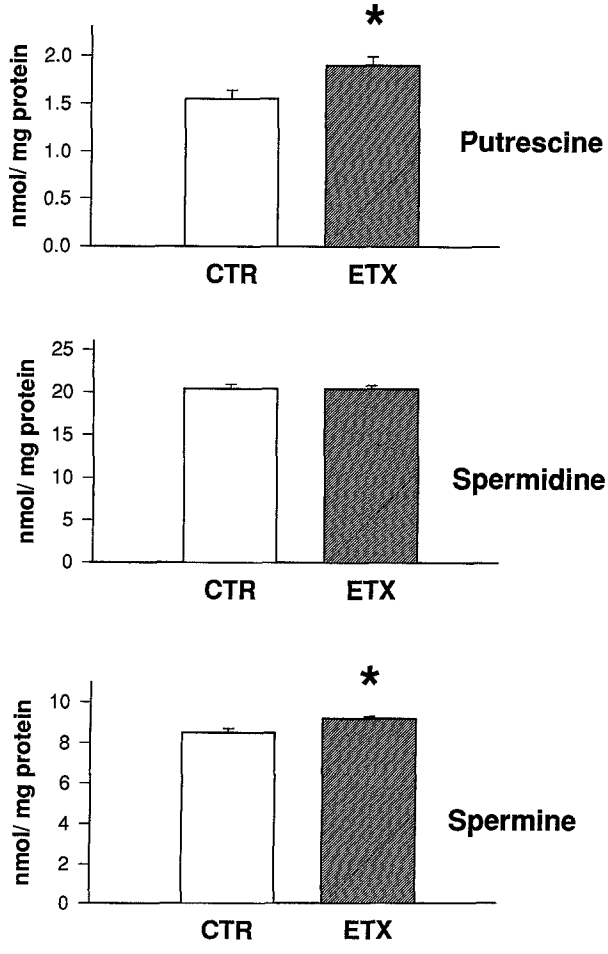

Fig 4. Polyamine levels in jejunal mucosa of saline (control [CTR]) and endotoxin (ETX)-treated rats. The experimental protocol was identical to that in Fig 3.

response to stress-induced mucosal injury. However, it should be noted that increased ODC activity does not necessarily reflect mucosal damage or repair of such damage.[3,23] The exact relationship between increased mucosal polyamine biosynthesis and morphologic changes during sepsis remains to be determined.

In most previous reports on the effects of various pathophysiological conditions on mucosal polyamine metabolism, ODC level was measured. We are not aware of previous studies in which stress-induced changes in SAMDC activity were examined. In the present study, both ODC and SAMDC activities were increased during sepsis and endotoxemia. A similar parallel regulation of the two enzymes was noted following dietary manipulation of fasted rats.[24] The finding of increased activities of both ODC and SAMDC during sepsis and endotoxemia suggests that both enzymes may be regulated by the same factor(s). Alternatively, the results may reflect an initial increase in ODC activity, followed by increased putrescine levels, which in turn may be responsible for the stimulated SAMDC activity. Evidence of a stimulating effect of putrescine on SAMDC activity in intestinal epithelial cells was reported previously.[20] A more detailed time course for changes in putrescine levels and SAMDC activity than was established in the current study would be required to further test the role of increased putrescine levels in the upregulated SAMDC activity.

In the present study, putrescine and spermidine levels in mucosa were increased during sepsis, whereas spermine concentrations were not significantly altered. This is in contrast to the local inflammatory response to *T. spiralis,* which was characterized by increased mucosal concentrations of all three polyamines.[21] Whether this reflects a different degree of stimulation or different mediators and mechanisms of polyamine biosynthesis in systemic versus local inflammation remains to be determined. It should be noted that mucosal polyamine levels are regulated not only by ODC and SAMDC activities but also by uptake of luminal polyamines, from both bacteria and exfoliated intestinal epithelial cells.[3] Thus, differences in polyamine levels during different experimental conditions may reflect differences in intestinal bacterial flora and/or rates of cell loss in addition to differences in enzyme activities.

Although the mediator(s) of sepsis-induced polyamine biosynthesis in intestinal mucosa was not determined here, a recent study by Chung et al[25] suggests that interleukin-1 may regulate intestinal ODC activity. Increased mucosal interleukin-1 production during endotoxemia[26] further supports the role of this cytokine as a potential mediator of sepsis-induced metabolic changes in mucosa of the small intestine.

The biological role of increased mucosal polyamine levels during sepsis is not known from the present study, but in previous reports, evidence was found that polyamines are essential for cell proliferation and various metabolic activities, including protein synthesis.[1-3] In a recent study, DNA, RNA, and protein synthesis rates in cultured IEC-6 cells were stimulated by putrescine, whereas no evidence for a role of spermidine or spermine was found in the same study.[6] From the present results and those reported previously from our laboratory,[13-15] it may be speculated that increased ODC and SAMDC activities and polyamine concentrations, particularly putrescine, are involved in the stimulation of cell proliferation and protein synthesis in mucosa of small intestine during sepsis.

REFERENCES

1. Pegg AE: Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 234:249-262, 1986
2. Tabor CW, Tabor H: Polyamines. Annu Rev Biochem 53:749-790, 1984
3. McCormack SA, Johnson LR: Role of polyamines in gastrointestinal mucosal growth. Am J Physiol 260:G795-G806, 1991
4. Kumagai J, Johnson LR: Characteristics of putrescine uptake in isolated rat enterocytes. Am J Physiol 254:G81-G86, 1988
5. Atkins JF, Lewis JB, Anderson CW, et al: Enhanced differential synthesis of proteins in a mammalian cell-free system by addition of polyamines. J Biol Chem 250:5688-5695, 1975
6. Ginty DD, Osborne DL, Seidel ER: Putrescine stimulates DNA synthesis in intestinal epithelial cells. Am J Physiol 257:G145-G150, 1989
7. Cohen SS, Morgan S, Streibel E: The polyamine content of the tRNA of *E. coli.* Proc Natl Acad Sci USA 64:669-676, 1969
8. Fauconneau G, Michel MC: Role of the gastrointestinal tract in regulation of protein metabolism, in Munro HN (ed): Mammalian Protein Metabolism, vol 4. New York, NY, Academic, 1970, pp 480-522
9. Wang JY, Johnson LR: Induction of gastric and duodenal mucosal ornithine decarboxylase during stress. Am J Physiol 257:G259-G265, 1989
10. Fujimoto K, Granger DN, Price VH, et al: Ornithine decarboxylase is involved in repair of small intestine after ischemia-reperfusion in rats. Am J Physiol 261:G523-G529, 1991
11. Chung DH, Evers BM, Townsend CM, et al: Burn-induced transcriptional regulation of small intestinal ornithine decarboxylase. Am J Surg 163:157-163, 1992
12. Wilmore DW, Smith RJ, Sarah T, et al: The gut: A central organ after surgical stress. Surgery 104:917-923, 1988
13. von Allmen D, Hasselgren PO, Higashiguchi T, et al: Increased intestinal protein synthesis during sepsis and following the administration of tumor necrosis factor α or interleukin-1α. Biochem J 286:585-589, 1992
14. Higashiguchi T, Noguchi Y, O'Brien W, et al: Effect of sepsis on mucosal protein synthesis in different parts of the gastrointestinal tract in rats. Clin Sci 87:207-211, 1994
15. Rafferty JA, Noguchi Y, Fischer JE, et al: Sepsis in rats stimulates cellular proliferation in the mucosa of the small intestine. Gastroenterology 107:121-127, 1994
16. Higashiguchi T, Noguchi Y, Noffsinger A, et al: Sepsis increases production of total secreted proteins, vasoactive intestinal peptide, and peptide YY in isolated rat enterocytes. Am J Surg 168:251-256, 1994
17. Chaudry IH, Wichterman KA, Baue AE: Effect of sepsis on tissue adenine nucleotide levels. Surgery 85:205-211, 1979
18. Pedersen PV, Warner BW, Bjornson HS, et al: Hemodynamic and metabolic alterations during experimental sepsis in young and adult rats. Surg Gynecol Obstet 168:148-156, 1989
19. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

20. Wang JY, Viar MJ, McCormack SA, et al: Effect of putrescine on *S*-adenosylmethionine decarboxylase in a small intestinal crypt cell line. Am J Physiol 263:G494-G501, 1992

21. Wang JY, Johnson LR, Tsai YH, et al: Mucosal ornithine decarboxylase, polyamines, and hyperplasia in infected intestine. Am J Physiol 260:G45-G51, 1991

22. Fitzpatrick LR, Wang P, Eikenburg BE, et al: Effect of refeeding on polyamine biosynthesis in isolated enterocytes. Am J Physiol 250:G709-G713, 1986

23. Thirumalai CHR, Tseng CC, Tabata K, et al: Relationship between ornithine decarboxylase and gastric damage. Am J Physiol 253:G1-G6, 1987

24. Moore P, Swendseid ME: Dietary regulation of the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in rats. J Nutr 113:1927-1935, 1983

25. Chung DH, Evers BM, Townsend CM, et al: Cytokine regulation of gut ornithine decarboxylase gene expression and enzyme activity. Surgery 112:364-369, 1992

26. Mester M, Tompkins RG, Gelfand JA, et al: Intestinal production of interleukin-1α during endotoxemia in the mouse. J Surg Res 54:584-591, 1993