Induced nitric oxide promotes intestinal inflammation following hemorrhagic shock

Christian Hierholzer,1 Jörg C. Kalff,1 Timothy R. Billiar,1 Anthony J. Bauer,2 David J. Tweardy,3 and Brian G. Harbrecht1

1 Departments of Surgery and Medicine and 2 Division of Gastroenterology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213; and 3 Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

Submitted 18 October 2002; accepted in final form 15 August 2003

Hemorrhagic shock is commonly viewed as a whole body ischemia-reperfusion (I/R) insult. The intestine is highly sensitive to I/R injury and experiences a marked reduction in blood flow during circulatory shock due to a disproportionate constriction of the splanchnic circulation (18). We have shown (11) that isolated I/R injury of the gut is associated with decreased gut motility and can result in postinjury atony and functional ileus. The intestine plays a critical role in the systemic inflammatory response due to its ability to secrete proinflammatory cytokines and release mediators into the systemic circulation following the breakdown of the mucosal barrier (25, 28, 32). These events may accentuate tissue damage in other organ systems and may contribute to organ failure and mortality (6). Despite the commonly held perception that hemorrhagic shock and I/R injury are equivalent, there are substantial differences in the body’s response to shock induced by loss of circulating volume compared with localized ischemia that suggest that hemorrhagic shock induces a unique physiological response (11).

Hemorrhagic shock stimulates the expression of the inducible nitric oxide (NO) synthase (iNOS), which is responsible for the sustained production of NO (10, 17). Recent studies (7, 15) provide evidence that iNOS plays a critical role in regulating smooth muscle contractility of the intestine. NO regulates intestinal smooth muscle function through iNOS-dependent upregulation of adhesion molecules and iNOS-dependent infiltration of polymorphonuclear cells (PMN) into the jejunal muscularis that subsequently affects intestinal smooth muscle function. We have previously shown (10, 20) that NO regulates the magnitude of the inflammatory response after hemorrhage and participates in proinflammatory cell signaling. In addition to regulating the inflammatory response, NO produced by iNOS may contribute to organ damage through both direct cytotoxic effects and the interaction with reactive oxygen intermediates (ROIs) under conditions of redox stress, although the mechanisms responsible for tissue injury may be organ specific (29, 30). NO has also been shown to directly impair intestinal barrier function at the cellular level (34). There are therefore several potential mechanisms through which NO may modulate intestinal function after hemorrhage.

We have previously demonstrated that NO from iNOS regulates proinflammatory activation, gene expression, and tissue injury in the liver after hemorrhage (10, 22). For this study, we hypothesized that excess NO derived from iNOS expression was, in part, responsible for the increased inflammatory response, impaired smooth muscle function, and structural damage of the intestine produced by hemorrhage. We report here that in hemorrhagic shock, iNOS inhibition with l-N6-(1-iminoethyl)-lysine (l-NIL) (23) or genetic deletion of the iNOS gene reduces the intestinal inflammatory response measured by PMN infiltration, cytokine production, and activation of proinflammatory transcription factors NF-κB and Stat3. Downregulation of inflammation is accompanied by improved intestinal smooth muscle function and reduced intestinal damage.

MATERIALS AND METHODS

Hemorrhagic shock protocol and organ isolation and preparation. This study was approved by the University of Pittsburgh Animal Care and Use Committee and conformed to National Institutes of Health...
specimen at a magnification of 1000x. The reaction was stopped with cold KRB. All histochemically stained circular muscle whole mounts were stained for MPO. Freshly prepared whole mounts were separated into two jejunal layers, muscularis (muscularis whole mounts) and mucosa, using surgical techniques as previously described (16). This was done by determining the cross-sectional area by the following equation (muscle density assumed to be 1.03 mg/mm³); mm² = [wet muscle weight (mg)/muscle length (mm) × muscle density (mg/mm³)]. Contractions were recorded, measured, and stored in a computer using a commercially available hardware and software package (Biopac Systems, Santa Barbara, CA).

RT-PCR. Total RNA (2.5 μg) was subjected to first-strand cDNA synthesis using oligo(dT) primer and Maloney murine leukemia virus RT (10). Primers were designed to amplify rat granulocyte colony-stimulating factor (G-CSF) and rat IL-6 with the assistance of a PCR primer design program, PCR Plan (IntelliGeneins, Mountain View, CA). The primers amplified products of 560 bp for G-CSF (13) and 339 bp for IL-6. The identity of the amplified DNA fragment obtained with RT-PCR and specific for G-CSF and IL-6 was confirmed using restriction site analysis as described (10). Varied quantities of total RNA (0.25–5 μg) were reverse transcribed for all primer pairs to obtain reproducible amplification in the exponential range, and 2.5 mg total RNA were demonstrated to be in the exponential range for all primers. PCR conditions were as follows: denaturation at 94°C for 1 min; annealing at 57°C for 1 min; and polymerization at 72°C for 2 min. PCR reactions were performed in a Perkin-Elmer 480 thermocycler using different numbers of cycles to detect a linear range of input RNA. The optimal cycle number was identified as 30 cycles. Rat peritoneal macrophages elicited with thiglycolate and RAW cells (RAW 264.7 macrophage cell line) stimulated in vitro with LPS served as positive controls for G-CSF and IL-6. The negative control for each set of PCR reactions contained water instead of DNA template. The 5′-primer was labeled with [32P]dATP. To control for RNA quality and cDNA synthesis, β-actin mRNA was also amplified (652-bp product). β-Actin was used to normalize loading of gels. A strong β-actin band indicated that adequate cDNA was obtained from all tissues. The relative radioactivity of the bands with correction to β-actin was determined by PhosphorImager analysis for all gels. Fifteen microliters of the PCR reaction were separated on a 10% polyacrylamide gel. After gel drying and exposing to a PhosphorImager screen (Molecular Dynamics PhosphorImager), the relative radioactivity of the bands was determined by volume integration using laser-scanning densitometry. Each gel contained the same positive control, which permitted normalization of samples and comparison between gels.

EMSA. EMSA was performed using whole tissue extracts from the experimental groups as described (10). Binding reactions were performed using 20 μg extracted protein and radiolabeled DNA-binding elements. The activation of NF-κB was determined using the duplex oligonucleotide based on the NF-κB binding site upstream of the murine iNOS promoter (5). Activation of Stat3 was assessed using the high-affinity serum-inducible element (hSIE) duplex oligonucleotide that preferentially binds Stat3 and Stat1 (33). EMSA was performed on a 4% polyacrylamide gel as described (4). Supershift analysis and cold competition assay were performed to confirm the identity of the protein-DNA complex and to demonstrate specific NF-κB binding. Antibodies specific for NF-κB p50, NF-κB p65, and Stat3α were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and were generated against amino acids 350–363 of human NF-κB p50 and amino acids 531–550 of human NF-κB p65 or in rabbits against the COOH-terminal amino acids of murine Stat3. The Stat3α-specific antibody was generated at Charles River Pharmaservices (South-
bridge, MA) by immunizing chickens with the COOH-terminal 10-amino acid residues of human Stat3β conjugated to thyroglobulin. The level of transcription factor activation was quantitated using Phospholimager analysis of gel shift band intensities.

**Statistics.** Unless otherwise indicated, data are presented as means ± SE. Comparisons of means were performed using ANOVA followed by comparison of individual pairs of means using Scheffé’s test. Both tests are contained within the StatView 4.1 program (Abacus Concepts, Berkeley, CA).

**RESULTS**

*Hemorrhagic shock results in increased expression of iNOS in the jejunum.* In previous studies (17), we have shown that iNOS (or NOS2) is upregulated in both the lungs and liver during shock. In this study, we measured iNOS mRNA levels in the jejunum of rats following resuscitation from hemorrhagic shock to determine whether iNOS was also generated at this site. With the use of semiquantitative RT-PCR, we found that levels of iNOS mRNA were increased 8.7-fold ($P < 0.03$) over sham controls 4 h following resuscitation from hemorrhage (Fig. 1). Treatment with the selective enzyme inhibitor L-NIL had no effect on iNOS mRNA levels in shock or sham animals.

Selective iNOS inhibition attenuates shock-induced morphologic injury of the intestine. We have shown that iNOS inhibition reduced lung injury and pulmonary PMN infiltration in shocked rats (10). Furthermore, in a rat model of I/R injury, the infiltration of PMN into the muscularis of jejunum was a critical mediator of intestinal smooth muscle dysfunction (11). We hypothesized that induced NO would contribute to jejunal damage and infiltration of PMN into the muscularis. Intestinal injury and muscularis PMN infiltration were assessed by histology using staining with hematoxylin and eosin and for MPO. Cross-sections of midjejunum from shocked animals demonstrated a broadening of the intestinal villi, edema, and infiltration of inflammatory cells (Fig. 2). Isolates of the muscularis demonstrated an 8.3-fold increase in MPO-positive PMN in shock animals compared with sham control animals. Jejunal cross sections of NIL-treated shock animals demonstrated a restoration of mucosal architecture and inhibition of PMN infiltration into the muscularis (Fig. 2).

Selective iNOS inhibition attenuates shock-induced muscular dysfunction of the intestine. Smooth muscle contractility of the intestine during sepsis or after surgical manipulation is regulated, in part, by iNOS activity (7,15). To test the hypothesis that induced NO contributes to the impairment of smooth muscle function following hemorrhagic shock, we generated dose-response curves of intestinal smooth muscle contractility in vitro using control (KRB) and betahanechol-stimulated (0.1–300 μM) preparations. Circular muscular strips from the midjejunum of rats subjected to shock demonstrated a 66% reduction in contraction amplitude in response to betahanechol (Fig. 3) 4 h after hemorrhage compared with control specimens ($P = 0.01$). With the use of the iNOS inhibitor L-NIL, the contractile response of the circular muscle following shock was almost completely restored ($P = 0.03$).

**iNOS regulates expression of ICAM-1.** PMN recruitment and diapedesis from the vasculature into the tissues requires adhesion of PMN to endothelial cells, and ICAM-1 regulates the PMN influx into the muscularis of the jejunum (14). To determine whether iNOS was involved in the regulation of intestinal ICAM-1 expression following shock, we measured ICAM-1 mRNA levels in shock rats treated without and with iNOS inhibition. With the use of semiquantitative RT-PCR, ICAM-1 mRNA levels from the jejunum of rats subjected to hemorrhagic shock (HS) and killed 4 h following resuscitation (A and B). RT-PCR reactions were performed using total RNA (2.5 μg) from the jejunum of shock and sham animals receiving L-Nω-(1-iminoethyl)-lysine (L-NIL; n = 5, gray bars) or saline (n = 5, black bars). Reaction products were separated on polyacrylamide gels, dried, and exposed to PhosphorImager (A). In B, the radioactive signal in the region corresponding to the predicted amplified fragment of rat iNOS mRNA was quantitated with correction to β-actin (C) using laser densitometry and ImageQuant software and plotted. Values shown represent means ± SE. #P < 0.03 compared with sham.

**iNOS regulates cytokine expression in the intestine after hemorrhage.** The inflammatory response following hemorrhagic shock includes the expression of proinflammatory cytokines such as IL-6 and G-CSF that regulate the accumulation of PMN in peripheral tissues (12,24). To determine whether the reduced inflammatory response following iNOS inhibition was accompanied by reduction in IL-6 and G-CSF expression, we measured IL-6 and G-CSF mRNA levels in the jejunum using semiquantitative RT-PCR. Levels of IL-6 and G-CSF mRNA were increased 4.1- and 5.7-fold in shock animals compared with.
sham animals (Figs. 5 and 6). After NIL treatment, this increase was reduced by 61 and 40%, respectively.

iNOS modulates the activation of transcription factors important for cytokine expression. Activation of transcription factors NF-κB and STAT contributes to the expression of cytokines such as TNF, IL-6, and G-CSF. There is iNOS-dependent activation of NF-κB and Stat3 DNA binding in the lungs following hemorrhage (10). To evaluate whether NF-κB activation in the jejunum of shock rats was iNOS dependent, we measured NF-κB activation after hemorrhage without and with iNOS inhibition. NF-κB activation was determined by EMSA using the duplex oligonucleotide based on a consensus NF-κB binding sequence. The jejunum of shock animals demonstrated a 3.4-fold increase in NF-κB activation at 4 h following resuscitation compared with sham controls (Fig. 7). Binding activity was completely inhibited by an excess of unlabeled oligonucleotide, and supershift analyses demonstrated the binding complex to consist of p50 and p65 subunits (data not shown). After L-NIL treatment, levels of NF-κB activation decreased 57% in shocked animals.

IL-6 and G-CSF both signal through the activation of STATs, proteins that serve the dual function of signal transducers and activators of transcription. IL-6 and G-CSF predominantly activate Stat3, one of seven STAT proteins identified and cloned so far (1, 3). To determine whether IL-6 and G-CSF expression were regulated by NO-mediated changes in Stat3 activation, EMSA was performed using protein extracts from the jejunum of shock and sham animals with and without L-NIL treatment and the hSIE, a duplex oligonucleotide probe known to form a complex with Stat3 and Stat1. Jejunal extracts from sham shock animals demonstrated low levels of activated SIF-A (Stat3 homodimer) complex. SIF-B (Stat3/Stat1 het-

![Image](http://ajpgi.physiology.org/)
erodimer) and SIF-C (Stat1 homodimer) complexes also were detected. Jejunal extracts from shock animals demonstrated a marked increase in all complexes, especially SIF-A. Binding of protein to the labeled hSIE binding element was completely inhibited by 25-fold excess of unlabeled hSIE oligonucleotide but not by up to 100-fold excess of unlabeled nonspecific oligonucleotide (data not shown). Incubation with the Stat3-specific antibody resulted in supershift, but incubation with both antibodies did not completely supershift the SIF-A complex with the residual, demonstrating mobility similar to Stat3.

DNA-affinity purification of whole tissue extracts of shock gut followed by immunoblotting with Stat3-specific monoclonal antibody that recognizes all Stat3 isoforms confirmed the activation of all Stat3 isoforms including Stat3 (data not shown). Quantitation of the SIF-A complex (Stat3 homodimer; Fig. 8) revealed a 3.4-fold increase 4 h following resuscitation in shock animals compared with sham controls. Treatment with the iNOS inhibitor L-NIL resulted in a 46% reduction of SIF-A binding activity.

Studies using iNOS knockout mice. To confirm that the results observed following L-NIL treatment were the consequence of selective inhibition of the inducible form of NOS, studies using iNOS-deficient mice were employed. We measured the activation of NF-κB and Stat3 in the jejunum of iNOS-deficient and wild-type mice 4 h following hemorrhagic shock. Analogous to the results detected in rats, hemorrhagic shock followed by resuscitation in wild-type control mice resulted in increased binding activity, 3.8-fold for NF-κB ($P = 0.01$) and 3.6-fold for Stat3 ($P = 0.02$), in the jejunum of sham shock mice (Fig. 9). The results of competition assays with unlabelled oligonucleotides and supershift analysis were similar.
to those seen in the rat model described above (data not shown). In mice lacking the iNOS gene, binding activity of NF-κB and Stat3 in the jejunum following hemorrhagic shock was reduced by 61% (\(P = 0.02\)) and 56% (\(P = 0.03\)), respectively, compared with wild-type animals.

DISCUSSION

The intestine is highly sensitive to I/R injury and experiences a marked reduction in blood flow during circulatory shock due to a disproportionate constriction of the splanchnic circulation. Intestinal injury and loss of gut barrier function have been implicated in systemic inflammation and may promote distant organ failure (6). The intestinal injury following shock is associated with an inflammatory response within the gut wall as well as impairment of smooth muscle contractility. Recent studies provide evidence that iNOS is involved in the regulation of intestinal smooth muscle function. With the use of rat models of smooth muscle dysfunction induced by sepsis (7) or surgical manipulation (15, 16), iNOS inhibition or iNOS deficiency resulted in a marked attenuation of the contractile response. We therefore investigated whether iNOS played a critical role in the impairment of smooth muscle function after hemorrhage. In this study, with the use of the selective iNOS inhibitor L-NIL and iNOS knockout mice, we demonstrate that induced NO plays a critical role in regulating the inflammatory response as well as morphological and functional damage in the jejunum after hemorrhagic shock. NO from iNOS decreased intestinal smooth muscle contractility and regulated the infiltration of PMN into the muscularis. We found that iNOS inhibition after hemorrhage restored intestinal contractility and reduced PMN influx into the muscularis. These results indicate that iNOS is involved in regulation of intestinal smooth muscle function after hemorrhagic shock. Inhibition of iNOS activity in our study not only resulted in amelioration of gut dysfunction but also dramatically reduced shock-induced intestinal injury.

We demonstrated that iNOS regulates the increased expression of the adhesion molecule ICAM-1 that regulates PMN influx into the intestine. Furthermore, the iNOS-dependent increase in the expression of the proinflammatory cytokines IL-6 and G-CSF after hemorrhage was associated with an iNOS-dependent increase in DNA binding activity of NF-κB.

**Fig. 7.** The activation of NF-κB in the jejunum of rats subjected to shock is decreased following selective iNOS inhibition in shock. A: EMSA was performed using radiolabeled NF-κB duplex oligonucleotide and 20 μg of protein extracts of shock and sham animals without or with L-NIL treatment. The position of NF-κB complex is indicated. B: the radioactive signal was quantitated by PhosphorImager analysis. The values shown are means ± SE; black bars represent untreated animals; gray bars represent L-NIL-treated animals. (#\(P = 0.02\) compared with sham; *\(P = 0.01\) compared with shock).

**Fig. 8.** The activation of Stat3 in the jejunum of rats subjected to shock is decreased following L-NIL treatment. A: EMSA was performed using radiolabeled high-affinity serum-inducible element (hSIE) and 20 μg of protein extracts of shock and sham animals without or with L-NIL treatment. The positions of the SIF-A, -B, and -C complexes are indicated. B: the radioactive signal was quantitated by PhosphorImager analysis. The values shown are means ± SE; black bars represent untreated animals; gray bars represent L-NIL-treated animals (\#\(P = 0.01\) vs. sham; *\(P = 0.03\) vs. shock).
and Stat3 in the jejunum. These data indicate not only an association between NO-dependent inflammation and organ injury but also suggest an important role for NO in cell signaling in the intestine after hemorrhage. These results demonstrate that the induction of iNOS in hemorrhagic shock is a key event that initiates the inflammatory response and results in organ injury in the intestine as well as in the lung and liver (10).

The activation of Stat3 is a critical step in the signaling cascade of several cytokines including IL-6 and G-CSF. G-CSF and IL-6 bind to their respective receptors and activate protein tyrosine kinases resulting in phosphorylation of the Stat complex. The receptor complex then phosphorylates STAT proteins causing their dimerization and translocation into the nucleus where they bind to specific DNA binding sequences in the promoter regions of genes and facilitate their transcription. STAT protein activation has been implicated in transcription. Studies have shown that at lower concentrations, NO may be an intermediate step in the upregulation of adhesion molecules, such as ICAM-1, that may be an intermediate step in the upregulation of adhesion molecules, such as ICAM-1, that finally result in PMN infiltration. With the use of the selective iNOS inhibitor L-NIL, we demonstrate that the iNOS-dependent increase in the expression of the proinflammatory cytokines IL-6 and G-CSF is associated with an iNOS-dependent increase in DNA binding activity of Stat3 in the jejunum. We have previously identified a dense network of macrophages within the muscularis layer of the jejunum and have demonstrated a temporal correlation between PMN infiltration and smooth muscle dysfunction in a rat model of intestinal ischemia (11). In this study, we demonstrate that the increased expression of ICAM-1 after hemorrhagic shock that regulates the PMN influx is iNOS dependent. The intestinal macrophage cell population has been shown to be a significant source of iNOS in the gut, and leukocytes infiltrating into the intestine as a result of iNOS upregulation may cause damage through the release of granular and lysosomal contents (15). Our data demonstrate that reduced PMN infiltration into the intestine is associated with decreased histological evidence of intestinal injury after hemorrhage. However, it remains unclear whether NO is capable of directly damaging the muscularis layer. Under conditions of redox stress, induced NO may also mediate organ damage through interaction with reactive radicals present during reperfusion, and this mechanism may also occur in the intestine after hemorrhage (29).

The effects of NO on intestinal function and injury range from beneficial to detrimental and may be dose dependent (34). Because intestinal blood flow is diverted to essential organs such as the brain and heart during shock, NO may play a beneficial role by facilitating oxygenation through vasodilation. Studies have shown that at lower concentrations, NO may protect the intestinal mucosa by maintaining perfusion and inhibiting adhesion of PMN and platelets (19). In support of this mechanism, nonspecific blockade of NOS has been shown to increase I/R-induced intestinal injury, whereas L-arginine administration and NO donors improved I/R-induced injury (15, 16). However, at high concentrations, NO promotes intestinal injury and loss of gut barrier function (34). Recent studies (34) demonstrate that increased iNOS activity is di-

---

**Fig. 9.** NF-κB and Stat3 activation in the jejunum of wild-type (WT) and iNOS knockout (KO) mice subjected to hemorrhagic shock. EMSA was performed using radiolabeled NF-κB duplex oligonucleotide (A) or radiolabeled hSIE (C) and 20 μg of protein extracts of shock and sham WT or KO mice. The positions of NF-κB and the SIF-A, -B, and -C complexes are indicated. B and D: the radioactive signal was quantitated by PhosphorImager analysis and plotted. The values shown are means ± SE; black bars represent WT mice; gray bars represent KO mice. In shocked WT mice, the activation of NF-κB (3.8-fold; P = 0.01) and the activation of Stat3 homodimer (SIF-A; 3.6-fold; P = 0.02) were increased significantly compared with sham WT mice. In shocked KO mice, NF-κB activation was reduced 61% (P = 0.02), and activation of Stat3 homodimer (SIF-A) was reduced 56% (P = 0.03) compared with shocked WT mice.

---

**A**

Sham WT  |  Sham KO

**B**

<table>
<thead>
<tr>
<th></th>
<th>NF-κB binding Activity (Arbitrary Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sha</td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td></td>
</tr>
</tbody>
</table>

**C**

Sham WT  |  Sham KO

**D**

<table>
<thead>
<tr>
<th></th>
<th>Stat3 binding Activity (Arbitrary Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sha</td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td></td>
</tr>
</tbody>
</table>

---

**References:**

1. AJP-Gastrointest Liver Physiol • VOL 286 • FEBRUARY 2004 • www.ajpgi.org
rectly involved in endotoxin-induced gut injury by reducing cellular viability, causing villous injury, and contributing to loss of intestinal barrier function by increasing intestinal permeability and promoting bacterial translocation. Increased permeability of cell monolayers produced by excessive NO was accompanied by ATP depletion and decreased tight junction integrity (26). In accordance with these data, in our study, selective iNOS inhibition was protective to the intestine and restored muscle contractility. Whether mucosal barrier function was altered in this model remains unresolved and will require further study.

An important contributor to tissue damage following gut ischemia is the generation of ROIs. Inducible NO contributes to intestinal injury not only through its direct effect on inflammatory signaling and smooth muscle inhibition, but NO may also interact with superoxide to form peroxynitrite, a much more powerful oxidative agent than either superoxide or NO alone (29). Studies using peroxynitrite scavengers demonstrated reduced NO-mediated permeability of intestinal monolayers, suggesting that the NO-induced increase in mucosal permeability may be mediated by peroxynitrite (31). ROIs can be produced by resident macrophages, infiltrating neutrophils, and monocytes. A decrease in peroxynitrite produced by iNOS inhibition may also contribute to the reduction of intestinal injury seen in these experiments.

The stimuli resulting in increased iNOS expression after hemorrhagic shock have not yet been defined. We have demonstrated that hemorrhagic shock results in activation of the transcriptional factor NF-κB and that some of the increased NF-κB binding after shock is iNOS independent (Fig. 7). Increased binding activity of NF-κB is important in the expression of iNOS. iNOS may potentially be induced after hemorrhage by the activation of NF-κB through redox-sensitive mechanisms or potentially through hypoxia-sensitive transcription factors that are NO independent (9). The resulting increased production of NO from iNOS may then produce intestinal injury and dysfunction directly or through the recruitment of leukocytes into the intestine.

In conclusion, this study demonstrates that the induction of iNOS contributes to the initiation of a complex inflammatory response in the gut wall, including activation of signaling proteins and cytokine expression, ultimately leading to morphological and functional intestinal injury. These results suggest that reducing bioavailability of induced NO in hemorrhagic shock by selective iNOS inhibition may be beneficial to the gut.

GRANTS
This work was supported by National Institutes of Health Grants P50-GM-53789, DK-55664, and GM-44100 and by Deutsche Forschungsgemeinschaft Grant HI-614/2-1.

REFERENCES


