Nitric oxide attenuates leukocyte-endothelial interaction via P-selectin in splanchnic ischemia-reperfusion

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Gauthier, Theresa W., Kelly L. Davenpeck, and Allan M. Lefer. Nitric oxide attenuates leukocyte-endothelial interaction via P-selectin in splanchnic ischemia-reperfusion. *Am. J. Physiol.* 267 (*Gastrointest. Liver Physiol.* 30): G562–G568, 1994.—We studied the effects of exogenous nitric oxide (NO) on leukocyte-endothelial interaction after 60 min of splanchnic artery ischemia and 120 min of reperfusion (SAO/R) in pentobarbital sodium-anesthetized rats via intravital microscopy. Treatment with the NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP, 20 μg/kg bolus followed by infusion at 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$), beginning 10 min before reperfusion, resulted in significantly decreased leukocyte-endothelial interaction. This was manifested by a significant decrease in leukocyte rolling and adherence in the postcapillary venules. Tissue protection was demonstrated by a significantly lower plasma free amino-nitrogen concentration in the SNAP-treated SAO/R rats compared with those receiving NO-depleted SNAP ($P < 0.05$). Immunohistochemical localization of P-selectin showed significantly decreased P-selectin expression on the venular endothelium after SAO/R in rats given SNAP 10 min before reperfusion (23.0 ± 3.2% vs. 54.9 ± 12.1% positive staining, respectively, $P < 0.01$). From these data, we conclude that the effects of exogenous NO on leukocyte-endothelial interaction after ischemia-reperfusion appear to be at least partially mediated through the endothelial adhesion molecule P-selectin.

leukocyte rolling; leukocyte adherence; adhesion molecules; immunohistochemistry

SPLANCHNIC ISCHEMIA AND REPERFUSION results in a severe form of circulatory shock, which is characterized by endothelial dysfunction (i.e., loss of endothelium-derived nitric oxide) and leukocyte accumulation, which contribute to intestinal tissue injury (1). Nitric oxide (NO), produced by endothelial cells, not only regulates vascular tone, but has recently been shown to play a significant role in leukocyte-endothelial interaction (18). The importance of endothelial dysfunction in ischemia-reperfusion has been demonstrated by the protective effects of administration of exogenous NO. In this regard, NO gas (1) and organic nitrates, which act as NO donors (6), have been shown to attenuate the severity of reperfusion injury, resulting in functional preservation of vascular endothelium and diminution in tissue injury (33). Moreover, reduced endothelial NO production results in increased neutrophil adherence and enhanced microvascular permeability in the postcapillary venule (17, 19).

Leukocyte-endothelial interaction involves a complex interplay among adhesion glycoproteins (i.e., integrins, immunoglobulin superfamily members, and selectins). One member of the selectin family, P-selectin, is rapidly translocated from the Weibel-Palade bodies to the endothelial cell surface upon endothelial cell activation with thrombin, histamine, hypoxia-reoxygenation, or oxygen-derived free radicals (23, 28, 31). P-selectin promotes rolling of leukocytes on the endothelium. Leukocyte rolling is the first step in leukocyte-endothelial interaction and facilitates polymorphonuclear leukocyte activation and adherence (23, 24).

The relationship between endothelium-generated NO and P-selectin-mediated leukocyte-endothelial adhesion is not clear. The objectives of this study were *1*) to examine leukocyte-endothelial interactions under intravital microscopy in the setting of splanchnic artery occlusion and reperfusion (SAO/R), *2*) to determine if these interactions are influenced by the exogenous NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP), and *3*) to ascertain whether the antiadhesive effects of NO are related to P-selectin.

METHODS

Experimental protocol. Male Sprague-Dawley rats, weighing 275–300 g, were anesthetized with pentobarbital sodium (35 mg/kg ip). A tracheostomy was performed to maintain a patent airway throughout the experiment. A polyethylene catheter was inserted in one carotid artery to monitor mean arterial blood pressure (MABP), and a second catheter was placed in the contralateral external jugular vein for infusion of drug and maintenance of anesthesia. MABP was recorded on a Grass model 7 oscillographic recorder (Grass Instruments, Quincy, MA), using Statham P23AC pressure transducers (Gould, Cleveland, OH). The abdominal cavity was opened via a midline laparotomy, and a second incision through the skin and abdominal muscle was performed on the right flank. The celiac artery and superior mesenteric artery (SMA) were isolated near their aortic root, and a 2.0 silk suture enclosed in a plastic drawstring was placed around each artery. The drawstring was exteriorized through the right flank incision to allow for easy occlusion and reperfusion of both arteries without moving the rat once it was positioned for intravital microscopy.

A loop of ileal mesentery was exteriorized through the midline incision and placed in a chamber for observation of the mesenteric microcirculation by intravital microscopy. The mesentery was placed over a Plexiglas pedestal in a superfusion chamber, and the ileum was secured for stabilization of the viewing field. The ileum and mesentery were superfused throughout the experiment with a modified Krebs-Henseleit solution [(in mM) 118 NaCl, 4.74 KCl, 2.45 $CaCl_2$, 1.19 KH_2PO_4, 1.19 $MgSO_4$, and 12.5 $NaHCO_3$] heated to 37°C and bubbled with 95% N_2-5% CO_2. A Microphot microscope, (Nikon, Tokyo, Japan) with a ×40 objective lens, and a ×1 ocular was used to visualize the mesenteric microcirculation. The image was projected by a videocamera (Hamamatsu Photonics, Hamamatsu, Japan) to a black and white Sony high-resolution video monitor, and the image was recorded with a videocassette recorder. Red blood cell velocity was determined on-line

using an optical Doppler velocimeter (4) obtained from the Microcirculation Research Institute (College Station, TX).

The rats were allowed to stabilize for 20 min after surgery. After stabilization, a 30- to 50-μm diameter postcapillary venule was chosen for observation. A baseline recording was made initially to establish basal values for leukocyte rolling and adherence. The celiac artery and SMA were then completely occluded by tightening the drawstrings. After 60 min of ischemia, the drawstrings were reopened and removed, allowing reperfusion of the splanchnic organs. Starting at the time of reperfusion, recordings were made at 30-min intervals for 2 h or until the MABP fell below 45 mmHg, at which time the experiment was terminated. Rats in which the vessel under observation did not reperfuse were excluded from further study. At the time of profound circulatory collapse (i.e., MABP < 45 mmHg) or at the end of the 2-h reperfusion period, 2 ml of arterial blood were drawn for measurement of plasma free amino-nitrogen concentration, an indicator of plasma proteolysis.

Rats were randomly assigned to one of three groups as follows: sham-operated control rats infused with SNAP (in which all surgical procedures were performed, but the mesenteric arteries were not occluded), SAO/R rats infused with NO-depleted SNAP, or SAO/R rats infused with freshly prepared SNAP. Both the SNAP and NO-depleted SNAP were administered as a 20 μg/kg bolus 10 min before reperfusion, followed by an infusion at 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for the remainder of the study period. Sham-operated control rats received the same amount of SNAP over the same time interval. As an additional control, three sham-operated control rats were given NO-depleted SNAP over the same time course, to ensure that NO-depleted SNAP alone did not result in increased leukocyte adherence or activation.

SNAP was depleted of NO after exposure to room temperature and light for >72 h (22). To document loss of functional NO activity after this time, relaxation of isolated rat SMA rings was examined with this compound and compared with freshly prepared SNAP (Fig. 1). SMA rings relaxed 92 ± 4% (n = 9) to 5 μM SNAP, whereas NO-depleted SNAP relaxed SMA rings only 11 ± 2% (n = 10; $P < 0.001$), confirming the loss of NO.

The number of rolling and adhered leukocytes, as well as the leukocyte rolling velocity, was determined off-line by playback of the videotape. Leukocytes were considered to be rolling if they were moving at a velocity slower than that of red blood cells. The rolling rate is expressed as the number of cells moving past a designated point per minute (i.e., leukocyte flux). A leukocyte was judged to be adherent if it remained stationary for >30 s (12). Adherence is expressed as the number of leukocytes per 100 μm of vessel length. Leukocyte rolling velocity was determined by measuring the time required for a leukocyte to travel 50 μm along the venular endothelium. The velocity for each time point represents the average velocity of 10 leukocytes per recording and is expressed in micrometers per second. To assess fracture stress, the ratio of leukocyte rolling velocity to red blood cell velocity (V_{wbc}/V_{rbc}) was calculated (36). V_{rbc} and venular diameter (D) were used to calculate venular wall shear rate (γ), employing the formula $\gamma = 8\,[(V_{mean}/D)$, where $V_{mean} = V_{rbc}/1.6]$.

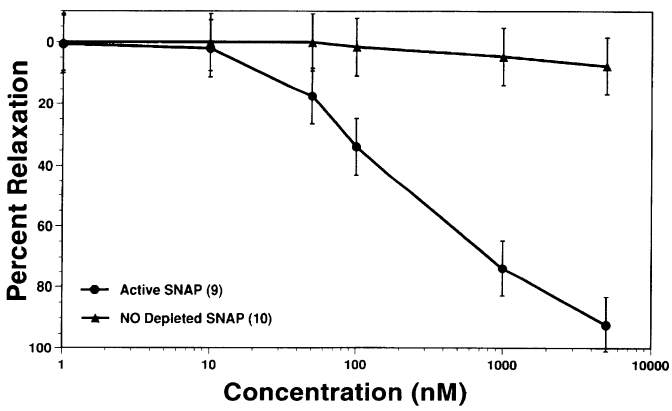

Fig. 1. Concentration-response curve for superior mesenteric artery ring relaxation to freshly prepared S-nitroso-N-acetylpenicillamine (SNAP) vs. nitric oxide (NO)-depleted SNAP. Values are means ± SE.

Immunohistochemistry. Immunohistochemical localization of P-selectin was investigated after SAO/R in the presence and absence of exogenous NO. Rats were anesthetized with pentobarbital sodium, and surgery was performed as described above. Both the celiac artery and SMA were occluded, using nontraumatic arterial clamps. Rats received either NO-depleted SNAP or SNAP as a 20-μg/kg bolus 10 min before reperfusion, followed by a 20-$\mu g \cdot kg^{-1} \cdot h^{-1}$ infusion over the 30-min reperfusion period. After 60 min of ischemia, the clamps were removed, allowing for reperfusion of the splanchnic vasculature. After 30 min of reperfusion, a time previously shown to result in maximal P-selectin expression in the cat (38), the SMA and celiac arteries were again occluded. At this time, both the SMA and superior mesenteric vein were rapidly cannulated for perfusion fixation of the tissue. Sham-operated control rats underwent similar procedures, but the SMA and celiac arteries were not occluded.

The ileal tissue was first washed free of blood by perfusion with modified Krebs-Henseleit buffer warmed to 37°C and bubbled with 95% O_2–5% CO_2. Once the venous perfusate was free of red blood cells, perfusion with iced 4% paraformaldehyde mixed in phosphate-buffered saline was initiated. The ileal tissue was perfusion fixed in this manner for 5 min. Two 3- to 4-cm-long segments of ileum were isolated from the perfused intestine and further fixed in 4% paraformaldehyde for 90 min at 4°C. The ileum was then cut into rings, and the tissue was dehydrated, using graded acetone washes at 4°C. Tissue sections were imbedded in plastic (Immunobed, Polysciences, Warrington, PA), and 4-μm-thick sections were cut and transferred to Vectabond-coated slides (Vector Laboratories, Burlingame, CA).

Immunohistochemical localization of P-selectin was accomplished, using a modified avidin-biotin immunoperoxidase technique (Vectastain ABC Reagent, Vector Laboratories), as previously described by Beckstead et al. (3). Tissue sections were incubated with the primary antibody (i.e., PB1.3) for 24 h at a dilution of 1:100. Control preparations consisted of omission of the primary antibody or omission of the secondary antibody. Positive staining for P-selectin was determined by detection of the brown peroxidase reaction product. Fifty venules per tissue section were examined, and the percentage of positive staining venules was determined.

Data analysis. All data are presented as means ± SE. Data were compared by analysis of variance (ANOVA), using post hoc analysis with Fisher's corrected t-test. $P \leq 0.05$ was considered statistically significant.

RESULTS

Immunohistochemistry. Endothelial-specific staining of P-selectin was confirmed, using a modified avidin-biotin immunoperoxidase technique. Ileal tissue sections were obtained from rats undergoing 60 min of SMA and celiac artery occlusion followed by 30 min of reperfusion. Sections obtained from rats given NO-depleted SNAP showed intense positive staining for

P-selectin on venular endothelial cells. Both the intensity and degree of endothelial staining were markedly reduced in tissue sections obtained from SNAP-treated SAO/R rats (Fig. 2). Tissue sections from sham-operated control rats demonstrated little or no specific staining on the venular endothelium. Additionally, no nonspecific staining was observed in sections in which either the primary or secondary antibody was omitted. These data were quantified by determining the number of positive staining venules on each tissue section. The percent of positive staining venules was significantly different among the three groups (Fig. 3). Very few venules stained positive for P-selectin in sham-operated control rats (6.7 ± 2.2%) whereas significant numbers of stained venules were observed in the SAO/R rats given NO-depleted SNAP (54.9 ± 12.1%, $P < 0.01$). The number of positively staining venules was significantly decreased in SAO/R rats given SNAP (23.0 ± 3.2%, $P < 0.01$). Thus P-selectin is expressed on rat mesenteric venules after ischemia and reperfusion, and treatment with SNAP 10 min before reperfusion significantly attenuated this expression.

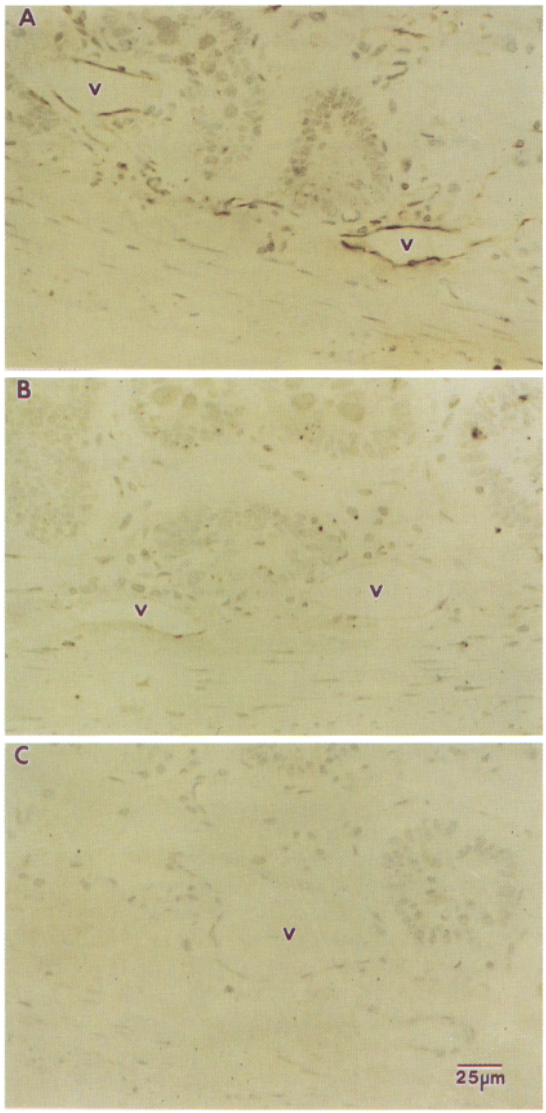

Fig. 2. Photomicrographs of rat ileal tissue incubated with monoclonal antibody PB1.3 and labeled with peroxidase substrate solution. Brown reaction product indicates positive antigen localization on the vascular endothelium. *A*: 2 venules (v) from a splanchnic artery occlusion/reperfusion (SAO/R) rat given NO-depleted SNAP. *B:* 2 venules from a SAO/R rat given freshly prepared SNAP. *C:* negative control venule from a SAO/R rat given NO-depleted SNAP, but incubated with secondary antibody only.

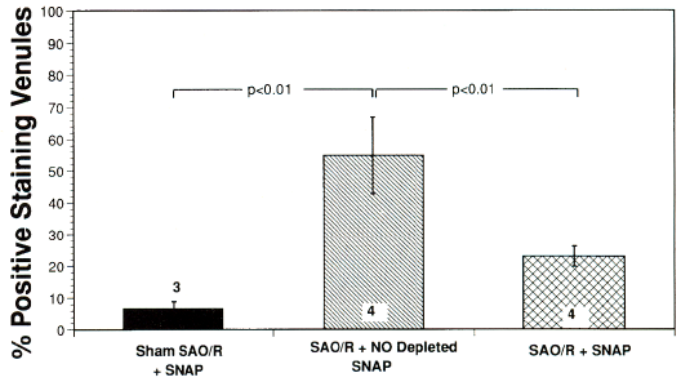

Fig. 3. Percent of positive staining venules for sham SAO/R + SNAP rats, SAO/R + NO-depleted SNAP, and SAO/R + SNAP. Values are means ± SE. Numbers in bars, no. of rats studied.

Intravital microscopy. Leukocyte-endothelial interaction was quantified using intravital microscopy, employing leukocyte rolling, leukocyte adherence, and leukocyte rolling velocity as measures of this interaction. Figure 4 illustrates the leukocyte rolling results. The number of rolling leukocytes under control conditions was not significantly different among the three groups. The overall initial mean for all rats was 19.3 ± 4.8 leukocytes/min. Leukocyte rolling was significantly increased after reperfusion in the SAO/R rats given NO-depleted SNAP with peak rolling occurring at 60 min postreperfusion. This increased rolling was significantly reduced after reperfusion in SNAP-treated SAO/R rats ($P < 0.05$).

Leukocyte adherence to the endothelium under control conditions was also similar for the three groups. The overall initial mean for all rats was 2.0 ± 0.5 cells.

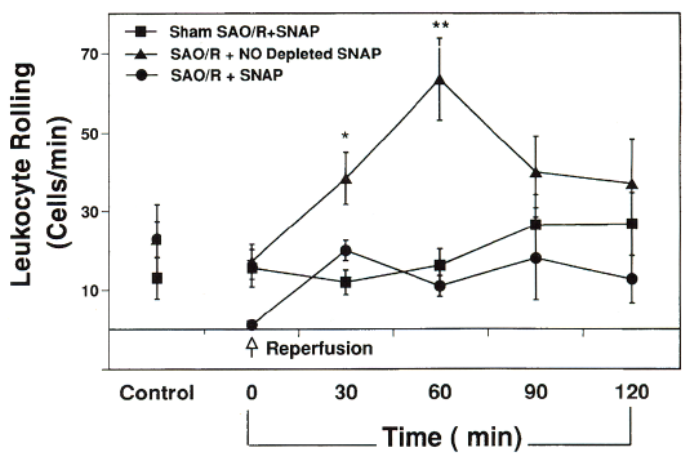

Fig. 4. No. of rolling leukocytes per minute. Values are means ± SE for no. of rolling cells for each group. Sham SAO/R + SNAP, $n = 5$; SAO/R + NO-depleted SNAP, $n = 7$; and SAO/R + SNAP, $n = 7$. Values for SAO/R + NO-depleted SNAP significantly different from SAO/R + SNAP, $^{*}P < 0.05$; $^{**}P < 0.01$.

However, adherence significantly increased 30 min postreperfusion and remained significantly elevated throughout the entire 120-min observation period in the SAO/R rats given NO-depleted SNAP ($P < 0.01$; Fig. 5). In the SNAP-treated SAO/R rats, adherence was virtually abolished, achieving values that were not significantly different from the sham-operated control group. Administration of NO-depleted SNAP for 120 min in the absence of ischemia-reperfusion had no effect on leukocyte adherence [0.67 ± 0.4, not significant (NS) from sham SAO/R + SNAP rats].

There was no significant difference in mean vessel diameter or control venular shear rates among the three groups of rats studied (Table 1). The addition of SNAP to either control or ischemic-reperfused rats did not have any significant effect on mean venular diameter in the mesentery (data not shown). After reperfusion, γ-values were significantly decreased in both ischemia-reperfusion groups as the result of reduced V_{rbc} ($P < 0.05$ from sham-operated control group). The mean initial V_{wbc} for all rats was 70.5 ± 2.4 μm/s. Moreover, rolling velocity in sham SAO/R rats did not change significantly throughout the experimental protocol (data not shown). V_{wbc} at the time corresponding to 120 min postreperfusion was not significantly different between sham-operated controls receiving SNAP vs. NO-depleted SNAP (72.4 ± 12.2 vs. 84.9 ± 23.4 μm/s, respectively). Rolling velocity for the SAO/R rats given NO-depleted SNAP decreased to 16.6 ± 4.4 by 30 min postreperfusion and remained low throughout the reperfusion period ($P < 0.01$ compared with sham-operated control group). In contrast, administration of SNAP significantly attenuated this decrease in rolling velocity at 30 min postreperfusion (56.6 ± 8.3) and throughout the postreperfusion period ($P < 0.05$, compared with SAO/R + NO-depleted SNAP), despite similar γ-values for both ischemia-reperfusion groups. Comparison of the ratio between V_{wbc} and V_{rbc} indicates that the difference in V_{wbc} is due to differences in leukocyte behavior, as the ratio between

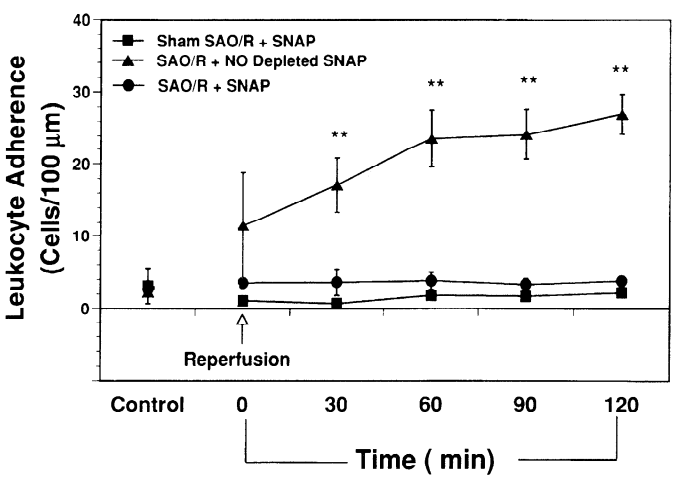

Fig. 5. No. of adherent leukocytes on 100-μm postcapillary venular endothelium. Values are means ± SE for no. of adherent cells for each group. Sham SAO/R + SNAP, $n = 5$; SAO/R + NO-depleted SNAP, $n = 7$; SAO/R + SNAP, $n = 7$. **Values in NO-depleted SNAP group significantly different from both the SNAP and Sham SAO/R + SNAP groups ($P < 0.01$).

Table 1. *Vessel diameter and shear rate for sham-operated control rats and rats subjected to SAO/R*

	Sham SAO/R	SAO/R + NO-Depleted SNAP	SAO/R + SNAP
Mean vessel diameter, μm	39.0 ± 1.5	37.5 ± 1.9	36.0 ± 2.2
Shear rate, s^{-1}			
Baseline	409 ± 59	556 ± 93	375 ± 89
30 min	464 ± 106	152 ± 11*	132 ± 12*
60 min	431 ± 96	187 ± 25*	120 ± 15*
90 min	414 ± 106	157 ± 27*	167 ± 42*
120 min	340 ± 111	129 ± 17*	134 ± 21*

Values are means ± SE. SNAP, *S*-nitroso-*N*-acetyl penicillamine; NO, nitric oxide. *Significantly different from sham-operated control values. SAO/R, splanchnic artery occlusion followed by reperfusion.

V_{rbc} and V_{wbc} was nearly doubled in SNAP-treated rats compared with rats given NO-depleted SNAP (Table 2).

Hemodynamics and tissue injury. MABP was continuously recorded for all three groups throughout the observation period (Fig. 6). Control MABP was not significantly different among the three groups of rats. The overall initial MABP was 121 ± 1.4 mmHg for all rats. MABP increased after occlusion of the SMA and celiac arteries. Addition of 20 μg/kg of SNAP as a bolus resulted in an abrupt decrease in MABP (i.e., ~35 mmHg) in control and ischemic rats, but MABP returned to baseline before reperfusion (data not shown). No significant change in MABP was observed during the 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$ infusion of SNAP in the sham-operated control rats. However, MABP decreased on reperfusion by ~60 mmHg in both ischemia-reperfusion groups. MABP remained significantly below that of sham-operated control rats throughout reperfusion ($P < 0.01$). Of interest, SNAP-treated SAO/R rats tended toward higher MABP during the reperfusion period compared with the SAO/R rats receiving NO-depleted SNAP, reaching significance at 40, 60, and 120 min postreperfusion ($P < 0.05$ to $P < 0.01$).

Additionally, plasma free-amino nitrogen concentration, a marker of plasma proteolysis that correlates to the severity of tissue injury, was significantly increased in the SAO/R rats given NO-depleted SNAP ($P < 0.01$ compared to sham-operated control rats). This increase in amino-nitrogen concentration was significantly attenuated in SNAP-treated SAO/R rats ($P < 0.05$; Fig. 7), indicating a tissue protective effect of SNAP. These data provide strong support for the beneficial effects of a NO donor in ischemia-reperfusion of the splanchnic viscera.

DISCUSSION

Leukocyte-endothelial cell interaction involves a complex system of adhesion molecules including the selectins, β_2 integrins and the immunoglobulin superfamily (5, 10, 39). Leukocyte interaction with the endothelium begins with leukocyte rolling, followed by adherence and transendothelial migration. One important adhesion molecule involved in early leukocyte-endothelial interaction is P-selectin. P-selectin allows

Table 2. *Ratio of white blood cell to red blood cell velocity*

Group	Time, min					
	Baseline	0	30	60	90	120
Sham SAO/R	0.049 ± 0.01	0.063 ± 0.02	0.049 ± 0.02	0.045 ± 0.02	0.045 ± 0.01	0.058 ± 0.02
SAO/R + NO-depleted SNAP	0.039 ± 0.01	0.023 ± 0.01	0.022 ± 0.007	0.015 ± 0.004	0.016 ± 0.005	0.020 ± 0.004
SAO/R + SNAP	0.045 ± 0.01	0.115 ± 0.04*	0.086 ± 0.02*	0.081 ± 0.03*	0.082 ± 0.05	0.075 ± 0.03

Values are means ± SE. *Values in SAO/R + SNAP group are significantly different from SAO/R + NO-depleted SNAP group ($P < 0.05$).

for direct contact between the stimulated endothelium and unactivated neutrophils by tethering neutrophils to the endothelial surface (24). Tethering of the unactivated neutrophil to the endothelial surface facilitates neutrophil interaction with endothelial-derived leukocyte activating substances such as platelet-activating factor (PAF) and results in neutrophil activation and subsequent adherence (8, 24, 27). Once firmly adhered through the interaction of the β_2 integrins (i.e., CD11/CD18) with their endothelial counter-receptor intercellular adhesion molecule-1 (ICAM-1), neutrophils can then undergo further activation, release of free radicals and proteolytic enzymes, and eventual transendothelial migration (5, 20, 21).

Ischemia-reperfusion is a stimulus for leukocyte-endothelial interaction (12). Loss of endothelial-derived NO is known to be one of the earliest manifestations of ischemia-reperfusion injury (37), and this reduced NO is believed to play a significant role in leukocyte-endothelial interaction (26). Administration of NO (1) or complex organic molecules releasing NO (6, 33) attenuate reperfusion-induced loss of endothelial-derived NO (6) and polymorphonuclear leukocyte accumulation in postischemic tissue (15). The NO donor SNAP, used in the present study, has been demonstrated to be a potent inhibitor of leukocyte-endothelial interaction in vitro (25). Conversely, inhibition of NO synthesis with NO synthase inhibitors, such as N^G-nitro-L-arginine methyl ester (L-NAME), promotes leukocyte adherence to the mesenteric microvascular endothelium in rat (2) and cat (18).

In the present study, 60 min of splanchnic ischemia followed by reperfusion resulted in a significant increase in leukocyte rolling and adherence in mesenteric postcapillary venules. We have previously demonstrated in an identical model of rat splanchnic ischemia-reperfusion that leukocyte-endothelial interaction in rat mesenteric venules after ischemia-reperfusion is largely mediated by P-selectin (8). In this regard, ischemia-reperfusion resulted in a marked upregulation of P-selectin on rat splanchnic venular endothelium as determined by immunohistochemistry (8). The P-selectin neutralizing monoclonal antibody PB1.3 was utilized in intravital microscopy to demonstrate a significant role for P-selectin in ischemia-reperfusion-induced leukocyte-endothelial interaction. When PB1.3 was given just before reperfusion, both leukocyte rolling and adherence were significantly attenuated in the mesenteric venules, thus demonstrating that P-selectin does play a primary role in leukocyte-endothelial interaction in the rat mesenteric microcirculation after ischemia-reperfusion (8).

Similarly, the NO donor SNAP given just before reperfusion significantly attenuated leukocyte rolling along the postcapillary venular endothelium after SAO/R. This decrease in leukocyte rolling correlated with a substantial decrease in leukocyte adherence. In fact, there was no significant difference in leukocyte adherence between SNAP-treated SAO/R rats and sham-operated controls. Interestingly, there were no significant differences between leukocyte rolling and adherence observed in SNAP-treated rats compared with rats given the P-selectin neutralizing monoclonal antibody PB1.3 in the previous study (8) (data not shown). SNAP

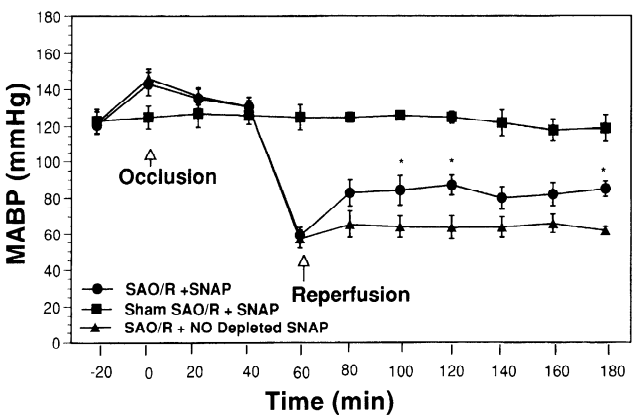

Fig. 6. Mean arterial blood pressure (MABP) for rats subjected to sham SAO/R + SNAP, SAO/R + NO-depleted SNAP, and SAO/R + SNAP. Values are means ± SE for surviving animals at each time point. *Values in SNAP group significantly different from NO-depleted SNAP ($P < 0.05$).

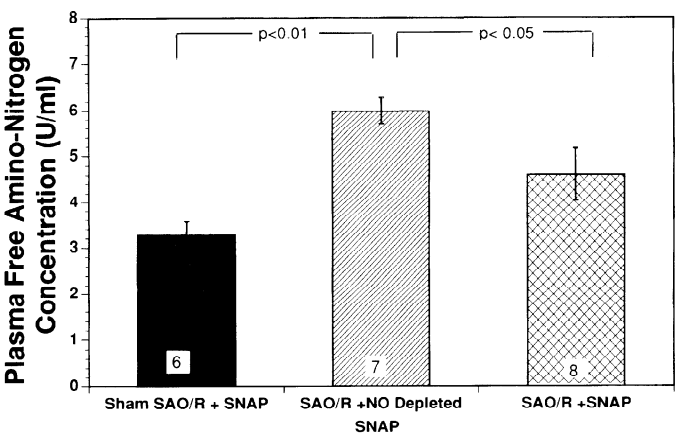

Fig. 7. Plasma free amino-nitrogen concentration for sham SAO/R + SNAP, SAO/R + NO-depleted SNAP, and SAO/R + SNAP groups. Values are means + SE., and no. in bar indicates no. of rats studied.

also significantly attenuated the decrease in V_{wbc} throughout the reperfusion period compared with NO-depleted SNAP. From these data, we can conclude that exogenous NO inhibits leukocyte rolling after SAO/R, and this reduced rolling results in a significant decrease in leukocyte adherence to the venular endothelium.

Immunohistochemical localization of P-selectin supports the intravital microscopy data and provides a valid mechanism for the leukocyte-endothelial interaction. In this regard, a high percentage of ileal venules from SAO/R rats treated with NO-depleted SNAP stained positively for P-selectin after 60 min of ischemia and 30 min of reperfusion, whereas treatment with SNAP significantly attenuated the number of positive staining venules as well as the intensity of this staining. Thus the decreased leukocyte rolling seen with intravital microscopy could be the result of decreased P-selectin expression after treatment of the reperfused mesentery with SNAP. These data represent the first evidence that the antiadhesive and tissue protective effects of exogenous NO after ischemia/reperfusion may be due to decreased expression of the endothelial adhesion molecule P-selectin.

Although our data demonstrate a significant inverse relationship between NO and expression of P-selectin after ischemia-reperfusion, the mechanism(s) by which NO mediates these effects remain unclear. Recent data suggest that one potential mechanism of the antiadhesive effects of NO is through its role as a free-radical scavenger (9). Oxygen-derived free radicals are generated by the endothelium via the xanthine-oxidase pathway immediately on reperfusion (11). Cultured endothelial cells exposed to hypoxia/reoxygenation or oxygen-derived free radicals rapidly express P-selectin, leading to neutrophil adherence (30, 31). Enhanced leukocyte adherence due to ischemia and reperfusion or oxygen radicals can be blocked both in vivo and in vitro by administration of superoxide dismutase, a scavenger of superoxide radicals (13, 35). Similarly, exogenous NO may scavenge free radicals generated at the time of reperfusion, thus preventing significant leukocyte-endothelial interaction. However, the possibility that the reduced expression of P-selectin on the endothelial surface may be the result of some direct effect of NO on P-selectin expression or the result of preservation of endogenous NO can not be excluded.

NO may also decrease leukocyte-endothelial interaction, specifically leukocyte adherence, by decreasing the production or release of stimuli, which can activate either the endothelium or the leukocyte. In this regard, Heller et al. (14) have demonstrated that NO donors directly inhibit the production of PAF by human endothelial cells exposed to thrombin. As noted previously (24), PAF is believed to play a pivotal role in activating the rolling leukocyte. Therefore, inhibiting PAF expression could significantly alter leukocyte activation and adherence. Decreased endothelial expression of PAF could contribute to the large reduction in leukocyte adherence in SNAP-treated rats despite a moderate degree of leukocyte rolling after reperfusion. Moreover, Salvemini et al. (32) have demonstrated that an NO-like factor stabilizes rat serosal mast cells. There is also some evidence that mast cell activation and subsequent release of mediators, such as histamine, contribute to ischemia-reperfusion injury (16, 34). Thus the NO administered in these experiments may have contributed to the stabilization of mast cells, thereby decreasing the release of histamine, a known stimulus for P-selectin expression.

Alternately, NO may directly inhibit the activation of polymorphonuclear cells (PMN). Moilanen et al. (29) suggest that exogenous NO donors increase guanosine 3′,5′-cyclic monophosphate within the PMN. These investigators (29) found that NO donors inhibited chemotaxis and reduced the oxidative burst of PMN. This reduced activation of PMN could affect firm adhesion to the endothelium via reduced CD18-ICAM-1 interaction. Additionally, Clancy et al. (7) demonstrated that NO inhibits superoxide production within PMN by acting directly on the assembly of NADPH oxidase within the neutrophil. Although these data suggest a direct effect of NO on neutrophils, our data imply that exogenous NO plays an earlier role in leukocyte-endothelial interaction after ischemia-reperfusion, since we observed that NO decreased the rolling of unactivated leukocytes along the endothelial surface.

Finally, plasma proteolysis, as measured by plasma free amino-nitrogen concentration, although significantly increased after ischemia-reperfusion in the NO-depleted SNAP group, was significantly reduced in the SNAP-treated SAO/R rats. This finding suggests that NO reduces the metabolic consequences of shock and its resulting tissue injury, in accordance with earlier observations of other means of administering NO (1, 6).

In summary, SAO/R in the rat resulted in expression of P-selectin on intestinal venules and enhanced leukocyte-endothelial interaction in these vessels. These interactions included augmented leukocyte rolling and subsequent adherence to the postcapillary venular endothelium. The addition of the NO donor SNAP significantly attenuated leukocyte-endothelial interaction and reduced plasma proteolysis. The immunohistochemical data provide the first evidence that the antiadhesive effects of exogenous NO may be mediated through a decreased expression of P-selectin.

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