Nitric oxide increases fluid extravasation from the splenic circulation of the rat

PETER S. ANDREW, YIMING DENG, RICHARD SULTANIAN, AND SUSAN KAUFMAN

Departments of Physiology and Medicine, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Received 23 August 2000; accepted in final form 17 November 2000

Nitric oxide (NO) biosynthesis is known to be elevated during septic shock (23). We hypothesized that NO would increase intrasplenic fluid efflux and that this would be due to an increase in net PC resulting from relatively greater dilatation of the splenic resistance arterioles (termed hilar arteries) than the venules (termed hilar veins). Splenic arterial and venous blood flows were measured during infusion of the NO donor S-nitroso-N-acetylpenicillamine (SNAP) into the splenic artery of anesthetized male rats. It was reasoned that a significant increase in the arteriovenous flow differential (A-V) would confirm increased NO-induced fluid efflux from the splenic vasculature into the lymphatic system. Changes in intrasplenic P_C in response to close arterial infusion of SNAP were measured in an isolated, blood-perfused spleen (36, 38). Isometric tension, measured with the use of a wire myograph system, was used to determine the vascular reactivity of isolated splenic hilar arteries and veins to the NO donors SNAP and sodium nitroprusside (SNP). We hypothesized that differential vasoreactivity between isolated hilar arteries and veins to these NO donors would be in a manner consistent with an NO-induced increase in intrasplenic P_C and subsequent intrasplenic fluid efflux i.e., that the maximal relaxation of hilar veins would be less than that of hilar arteries and/or that the hilar arteries would be more sensitive to NO-induced vasorelaxation.

METHODS

The experiments described in this paper were examined by the local Animal Welfare Committee (University of Alberta) and found to be in compliance with the guidelines issued by the Canada Council on Animal Care. At the completion of an experiment, all animals were killed with an anesthetic overdose (0.3 ml iv Euthanyl; 240 mg/ml pentobarbital sodium; MTC Pharmaceuticals, Cambridge, Ontario, Canada).

Animal Model

Male Long-Evans rats (450–600 g) were obtained from Charles River (St. Foy, Quebec, Canada). They were held in...
the University Animal Facility for at least 1 wk before experimental procedures, exposed to light of a 12:12-h cycle, maintained in a humidity- and temperature-controlled environment, and maintained on a 0.3% sodium diet and water ad libitum.

Figure 1 illustrates the vasculature of the rat spleen. This diagram indicates the relative anatomic position of blood vessels that are relevant to in vivo and in vitro experimental preparations.

Experiment A: Effect of SNAP on Mean Arterial Pressure, Splenic Blood Flow, Fluid Extravasation, Hematocrit, and Splenic Tissue Weight

Surgery. Anesthesia was induced with pentobarbital sodium (60 mg/kg body wt ip) and maintained with Inactin [ethyl-(1-methyl-propyl)-malonyl-thio-urea; 80 mg/kg body wt sc]. Body temperature was maintained by placing the rat on a heating pad (Deltaphase Isothermal pad, Braintree Scientific, Braintree, MA). Access to the abdominal organs was through a midline laparotomy. To ensure that the artery and vein supplied and drained only the spleen, all accessory branches running from the splenic vessels to the pancreas, stomach, and surrounding tissue were ligated. At the end of the experiment, vascular isolation was verified by infusing dye into the splenic artery (9).

Cannulations. The spleen was carefully cleared from its attachments to the stomach and replaced in its natural position in the abdominal cavity. The stomach was then delivered through the abdominal incision and laid on the thorax of the rat. The gastric artery, gastric vein, splenic artery, and splenic vein were cleared carefully, and with minimal handling, they were given their extreme vasoreactivity. The gastric artery was cannulated with drawn out Tygon tubing [nominal dimensions: 0.25-mm internal diameter (ID), 0.5-mm outer diameter (OD)] and the gastric vein with Silastic (0.30-mm ID, 0.64-mm OD; Dow Corning). Polyethylene (0.58-mm ID, 0.97-mm OD) and Silastic (0.51-mm ID, 0.94-mm OD; Dow Corning) cannulas were placed in the femoral artery and vein, respectively. The arterial cannula was used for measuring blood pressure. The venous cannula was used for saline infusion.

Measurement of hematocrit. Blood samples (50 μl) were taken from the gastric vein cannula (which represents blood flow out of the spleen) and femoral artery cannula (which represents blood composition that would flow into the spleen) and then transferred to heparinized microhematocrit tubes. They were then centrifuged and read immediately after collection.

Implantation of blood flow probes. Flow probes (1RB series, Transonic Systems, Ithaca, NY) were attached to micro manipulators and aligned to receive the splenic artery and vein. The use and calibration of these probes have been previously described (9). The probes were then positioned under the splenic vascular arcade, and the splenic artery and vein were slipped into the probe windows. The stomach was replaced over the splenic vessels and probes, and the incision was closed with stainless steel sutures covered with moist sponges and plastic film. The stomach was covered with moist sponges and plastic film.

The blood flow probe (1RB series) is capable of measuring instantaneous and average volume flow in arteries and veins (0.25 to 8 mm in diameter). The flow probes are factory-calibrated to meet Transonic Flowprobe Specifications (Transonic Systems). A zeroed blood flow reading from the 1RB flow sensor was confirmed before each implantation by placement of the probe in a nonturbulent saline bath. Validation of the accuracy of blood flow measurements by the 1RB series flow sensor has been reported (40).

Data acquisition and analysis. The femoral artery cannula was connected to a Statham pressure transducer. Blood pressure and flow were recorded online with the use of a data-acquisition board (DI-400, DATAQ Instruments, Akron, OH). The data were collected and analyzed with the use of DATAO’s own software (WINDAQ). Significance was accepted at P < 0.05.

Protoc. After cannulation of the femoral vein, saline infusion was started (3 ml/h) and continued over the duration of the experiment. The femoral artery was then cannulated, and blood pressure was recorded. The arterial cannula was also the site for blood sampling. The flow probes were positioned around the splenic artery and vein, after which the preparation was allowed to stabilize for 45 min. Baseline blood flows and pressure were then recorded for 30 min after this stabilization period. Femoral artery and gastric vein blood samples were taken after the stabilization period for hematocrit measurements. Mean arterial pressure (MAP) was determined by averaging blood pressure measurements over the 5-min periods immediately before SNAP infusion (baseline value) and before the end of the SNAP infusion period (experimental value). The baseline mean splenic blood flows were averaged over 15 min. SNAP was then infused (10 μl/min) for 15 min at 0.3 μg/ml through the splenic artery, during which time splenic blood flows were averaged over 1-min intervals throughout the infusion of SNAP. At the end of this SNAP infusion period, blood samples were again taken from the femoral artery and gastric vein. The splenic vessels were then ligated, and the spleen was removed and weighed.

Statistical analysis. MAP before and during SNAP infusion into the splenic artery was compared using a paired t-test. The splenic arterial and venous blood flows were analyzed using a one-way repeated-measures ANOVA on ranks because data were not normally distributed. The splenic A-V difference in blood flow (fluid extravasation) was calculated for each animal. The change in mean (A-V) difference was
Experiment B: Effect of SNAP on Splenic $P_C$

Surgery. Anesthesia was induced with isoflurane (2.5%; IsoFlo, Abbott Laboratories) and continued until the femoral vein was cannulated, at which time pentobarbital sodium (65 mg/ml iv; MTC Pharmaceuticals) was infused (50 mg/kg). Inactin (80 mg/kg sc; BYK), given at the end of surgery, was used to maintain the rat under a surgical plane of anesthesia (no paw-pinch response) for the duration of the experiment.

Vascular isolation of the spleen was implemented and then verified as previously described (9). Silastic (0.51-mm ID, 0.94-mm OD; Dow Corning) and PE-50 (0.58-mm ID, 0.965-mm OD; Intramedic) cannulas were placed in the femoral vein and artery, respectively. MAP was monitored at the femoral artery, and pentobarbital sodium was administered through the femoral vein. The venous line was also used to infuse saline (3 ml/h) to maintain euvolemic hydration of the animal throughout the duration of the experiment. The right common carotid artery was occlusively cannulated with the use of PE-90 (0.86-mm ID, 1.27-mm OD) tubing to provide the source of oxygenated blood for splenic perfusion.

The spleen was carefully cleared from the stomach and replaced in its natural position in the abdominal cavity. The stomach was then placed on top of the thoracic cavity and retracted, thereby exposing the gastric artery and vein, which were used to access the splenic artery and vein. The gastric artery was cannulated with drawnout PE-50 tubing (0.58-mm ID, 0.965-mm OD), whereas the gastric vein was cannulated with microrenethane (0.30-mm ID, 0.64-mm OD; Braintree Scientific). The gastric artery cannula was connected via a three-way adapter to a pressure transducer (which monitored splenic arterial perfusion pressure) and to a peristaltic pump. The venous cannula was advanced to the junction of the gastric vein and the splenic vein, and it was connected to a pressure transducer (which monitored venous pressure of the blood-perfused spleen). When the surgery was completed, splenic perfusion was started. At the start of perfusion, heparin (0.15 ml; 10,000 IU/ml iv) was injected. The splenic perfusion consisted of oxygenated blood from the carotid artery and perfused into the splenic artery via the peristaltic perfusion pump (1.0 ml/min). Systemic pressure and splenic arterial and venous perfusion pressures were monitored online with the use of a data-acquisition system (D1–400, DATAQ Instruments) and recorded using DATAQ's own software (WINDAQ).

$P_C$. In the blood-perfused spleen, $P_C$ was determined with the use of the double vascular occlusion technique (38). After stabilization, both inflow and outflow cannulas were simultaneously occluded. Arterial pressure ($P_A$) and venous pressure ($P_V$) equilibrated rapidly to a value reflective of $P_C$. If $P_A$ and $P_V$ did not exactly equilibrate to the same pressure on double occlusion, then the mean of both pressures was determined and defined as $P_C$ (6). Results of previous studies have shown that $P_C$ measured by double vascular occlusion is equivalent to those measured by other classical means, such as the micropuncture technique (12).

Protocol. Animals were allowed to stabilize for 30 min before any hemodynamic variables were measured. SNAP was then infused into the splenic artery at a rate of 0.1 $\mu$g·10 $\mu$l·min$^{-1}$; this dose achieved approximately the same plasma concentration as in experiment A, given the difference in arterial blood flow. Double vascular occlusions were conducted at 5, 10, and 20 min after the infusion of SNAP was started. The $P_C$ is reported as the mean of these three readings, as there was no significant difference among the values. The double occlusion was performed by simultaneously tightening a snare placed around the splenic vein, whereas the perfusion pump was stopped and the tubing clamped, so that arterial inflow was blocked for a period of $\sim$5 s (12). Control animals, implanted with the same cannulas and treated in the same manner as the experimental animals, were infused with saline and subjected to the same protocol.

Statistical analysis. The significance of the NO-induced alterations in intrasplenic $P_C$ was assessed using a paired $t$-test. The level of statistical significance was defined at $P < 0.05$.

Experiment C: Effect of SNAP on Vasoreactivity of Isolated Splenic Resistance Arteries and Veins

Vessel preparation. Rats were decapitated. The spleen and its associated vascular arcade were rapidly removed through a left thoracotomy and placed in ice-cold HEPES-buffered phosphate saline solution (PSS). Hilar arteries (125–200 $\mu$m) and veins (350–450 $\mu$m) that led directly into or out from the splenic tissue itself were dissected free from surrounding adipose tissue, cut into $\sim$2-mm lengths, and mounted on an isometric tension myograph system (Kent Scientific, Litchfield, CA). Splenic resistance arteries and veins were matched by location in all animals used i.e., selected hilar arteries and veins lay adjacent to each other and immediately pierced the body of splenic tissue. The blocks were positioned in an organ bath with 5 ml of HEPES-PSS solution kept at 37°C. Two separate organ baths were used to study matched arterial and venous segments, and changes in isometric force were recorded on a data-acquisition system (WINDAQ, DATAQ Instruments).

Determination of the optimum tension setting. To our knowledge, there are no previous studies of the splenic vascular bed with the use of a wire myograph. The optimum tension setting in our design related to the internal circumference of the blood vessel at which maximum active tension plus least passive tension were generated; this measured internal circumference would equate to a percentage of the theoretical internal circumference for hilar arteries ($L_{100}$) or veins ($L_5$) that would exist in vivo. The percentage of either $L_{100}$ or $L_5$ was determined by construction of an internal circumference-tension characteristics curve for both hilar arteries ($n = 10$) and veins ($n = 9$). Our data from these experiments showed that the mean $R^2$ value for the exponen- tial function was 0.93 ($\pm$0.02 SE) for hilar arteries ($n = 10$) and 0.91 ($\pm$0.02 SE) for veins ($n = 9$). The internal circumference of a blood vessel that gave maximal active tension and least passive tension was selected from graphic analysis of the internal circumference-tension characteristics curve (Fig. 2). The passive internal circumference-tension characteristics were used to determine the theoretical internal circumference at the transmural pressures denoted for hilar artery ($L_{100}$, 100 mmHg) or vein ($L_5$, 5 mmHg); the basis for these calculations has been previously described (27). The percentage of $L_{100}$ or $L_5$ that would produce the maximal active tension and least passive tension was then calculated. The mean percentage value derived for hilar arteries and veins was 65% (0.65) of the $L_{100}$ and 80% (0.8) of the $L_5$, respectively.

From these preliminary experiments to determine the calculations of 0.65 for $L_{100}$ and 0.8 for $L_5$, all subsequent vessel experiments involving vasorelaxant responses to the NO donor agents only required the construction of a passive
curv. From the passive curve data, we could calculate the tension at which to set the vessel to achieve an optimum tension setting. Once set to an optimum tension, each vessel was allowed to stabilize for 30 min in HEPES-PSS buffer, with the buffer changed at 10-min intervals, before generation of dose-response curves.

Resting length-tension curve. After being mounted, vessels were allowed to stabilize for 30 min in HEPES-PSS buffer under no tension, during which time the buffer solution was changed at 10-min intervals. This was followed by a preconditioned stretch, after which vessels were rested at 0.1–0.2 mm/mm and allowed to stabilize in HEPES-PSS buffer for a further 10 min. This was followed by construction of a passive internal circumference-tension curve. From the exponential curve fit of passive tension generated vs. internal vessel circumference, the law of Laplace was used to calculate where the optimal tension setting of each individual vessel should be set, based on a calculation of 0.65 for $L_{100}$ for hilar arteries and 0.8 for $L_{100}$ for hilar veins.

Solutions and drugs. The HEPES-PSS solution that was maintained at a pH of 7.4 contained (in mM) 142 sodium chloride, 4.7 potassium chloride, 1.17 magnesium sulphate, 1.56 calcium chloride, 1.18 potassium phosphate, 10 HEPES, and 5.5 glucose. Stock solutions of $L$-phenylephrine hydrochloride, SNP (Sigma Chemical, Ontario, Canada), and SNAP (World Precision Instruments, Sarasota, FL) were prepared in water at concentrations of 10, $1.68 \times 10^{-2}$, and $4.5 \times 10^{-3}$ M, respectively. The light-sensitive and rapid degradative properties of these vasoactive agents (in particular reference to SNP and SNAP, respectively) demanded that each solution be prepared immediately before use and be shielded from light. Appropriate dilutions of all stocks were obtained using HEPES-PSS.

Protocol. For each vessel, a cumulative dose-response curve to phenylephrine ($1 \times 10^{-8}$–$1 \times 10^{-3}$ M) was generated. After a 30-min stabilization period, during which time the organ bath was changed with fresh buffer (HEPES-PSS), every 10 min, the $EC_{50}$ to phenylephrine was used to preconstrict the vessel for subsequent determination of vasorelaxant responses to SNP ($1 \times 10^{-12}$–$1 \times 10^{-4}$ M) or SNAP ($1 \times 10^{-10}$–$3 \times 10^{-4}$ M). Both hilar arteries and veins returned to their respective resting tension after the 30-min HEPES-PSS washout period. The vasoconstrictor response to phenylephrine by both hilar arteries and veins was repeatable (as determined by the similar tension generated with the dose required to obtain 80% of maximal response [$EC_{80}$] to phenylephrine before and immediately after assessing the vasorelaxant responses). In addition, the $EC_{50}$ values for phenylephrine for both hilar arteries ($4 \times 10^{-7} \pm 9 \times 10^{-6}$ M SE) and veins ($3 \times 10^{-7} \pm 7 \times 10^{-8}$ M SE) were comparable ($P > 0.05$). Furthermore, the calculated $EC_{80}$ dose to phenylephrine produced a measured tension (hilar artery: $2.06 \pm 0.15$ mN/mm, $n = 5$; hilar vein: $0.38 \pm 0.02$ mN/mm, $n = 5$) that was comparable with the tension extrapolated from the cumulative dose-response curves to phenylephrine (hilar artery: $2.05 \pm 0.19$ mN/mm, $n = 5$; hilar vein: $0.38 \pm 0.03$ mN/mm, $n = 5$) ($P > 0.05$). Vessels preconstricted to $EC_{50}$ with phenylephrine were treated with either SNP or SNAP for 4-min periods at each cumulative dose. Preliminary experiments confirmed that the maximal vasorelaxant response at each dose was achieved by this time.

Statistical analysis. The significance of changes in tension at each of the cumulative doses for SNP and SNAP was analyzed by a one-way repeated-measures ANOVA, followed by the Student-Newman-Keuls method to identify the individual points of significance. Where data were not normally distributed, a repeated-measures ANOVA on ranks was used. The significance of differences between arteries and veins at particular doses was analyzed by a two-way repeated-measures ANOVA. The significance of differences in maximal vasorelaxation to SNP and SNAP in arteries and veins was determined by a one-way ANOVA. Significance was accepted at $P < 0.05$.

RESULTS

Experiment A: Effect of SNAP on MAP, Splenic Blood Flow, Fluid Extravasation, Hematocrit, and Splenic Tissue Weight

Close arterial infusion of SNAP (0.3 $\mu g\cdot 10^{-1}\cdot$ min$^{-1}$) into the splenic artery caused a significant fall in MAP (baseline: $95.6 \pm 5.3$ mmHg vs. SNAP: $91.9 \pm 4.4$ mmHg, $n = 10$; $P < 0.05$). Baseline splenic arterial inflow was significantly higher than splenic venous outflow (Fig. 3A). After infusion of SNAP, splenic arterial blood flow tended to increase and splenic venous blood flow tended to decrease, despite the fall in MAP (Fig. 3A). This resulted in a significant increase in the difference between splenic inflow and outflow (fluid extravasation) compared with baseline (time = 0) ($P < 0.05$) (Fig. 3B). There was no such change in the saline-infused control animals (data not shown). The average A-V difference over the 15-min SNAP infusion period was $\sim 1.1$ ml/min, which relates
to a total volume of fluid extravasation of 16.5 ml over 15 min. There was a SNAP-induced increase in splenic venous hematocrit (baseline: 40.8 ± 0.48%, n = 4 vs. SNAP infused: 43.0 ± 0.58%, n = 4) compared with arterial hematocrit (baseline: 39.5 ± 0.29%, n = 4 vs. SNAP infused: 39.8 ± 0.25%, n = 4) (P < 0.05), but there was no change in wet splenic tissue weight (SNAP infused: 1.0 ± 0.01 g, n = 4 vs. saline infused: 0.99 ± 0.03 g, n = 3) (P > 0.05) i.e., there was a SNAP-induced increase in loss of erythrocyte-free fluid within the splenic circulation.

**Experiment B: Effect of SNAP on Splenic Pc**

Infusion of SNAP (0.1 μg·10 ml⁻¹·min⁻¹) into the splenic artery caused a significant increase in intra-splenic Pc (saline: 10.9 ± 0.2 mmHg, n = 3 vs. SNAP: 12.2 ± 0.2 mmHg, n = 3) (P < 0.05).

**Experiment C: Effect of SNAP on Vasoreactivity of Isolated Splenic Resistance Arteries and Veins**

The passive-active tension characteristics for each individual vessel were determined (Fig. 2), and the appropriate wall tension (arterial or venous) was set accordingly (Laplace’s law). Constriction curves to phenylephrine were then constructed for each vessel so as to determine the preconstrictive dose (80% maximal constriction) to be used when testing the vasorelaxant agents. There was a significant concentration-dependent increase in tension to phenylephrine (1 × 10⁻⁸ to 1 × 10⁻³ M) in both hilar arteries (n = 9) and veins (n = 11) (P < 0.05). There was no significant difference in the phenylephrine EC₅₀ values between hilar arteries (4.86 ± 0.9 × 10⁻⁶ M) and veins (6.44 ± 1.2 × 10⁻⁶ M) (P < 0.05); i.e., although the individual preconstrictive EC₅₀ doses used for vessels varied slightly, the mean EC₅₀ dose of phenylephrine applied to arteries was not significantly different from that used for veins.

There was a concentration-dependent decrease in tension (vasorelaxation) in phenylephrine-preconstricted vessels in response to both SNP (Fig. 4) and SNAP (Fig. 5). However, the maximum vasorelaxation to SNP was significantly greater in arteries (96 ± 2.3%, n = 9) than veins (26 ± 1.9%, n = 10) (P < 0.05) (Figs. 4 and 6) i.e., SNP was fully able to counteract the constrictive activity of phenylephrine in arteries, but...
and SNAP (difference between the maximal vasorelaxation to SNP (Fig. 6). By contrast, in veins, there was no significant greater than to SNAP (50 ± 3.5%, n = 9) was significantly greater than to SNAP (50 ± 3.5%, n = 11) (P < 0.05) (Fig. 6). By contrast, in veins, there was no significant difference between the maximal vasorelaxation to SNP and SNAP (P > 0.05) (Fig. 6).

DISCUSSION

This study sought to investigate the influence of NO on intrasplenic fluid efflux. We proposed that there would be a differential vasorelaxant response to SNP between hilar arteries and veins such that intrasplenic Pcv would rise and intrasplenic fluid extravasation would increase. The results validated our hypothesis. In response to intrasplenic SNAP, there was a significant and sustained increase in the A-V flow differential (Fig. 3B) and an increase in intrasplenic Pcv; i.e., NO caused an increase in intrasplenic Pcv accompanied by an increase in intrasplenic fluid extravasation. There was a SNAP-induced increase in splenic venous hematocrit compared with arterial hematocrit (baseline: 39.5 ± 0.29%, n = 4 vs. SNAP infused: 39.8 ± 0.25%, n = 11) (P < 0.05), but there was no change in wet splenic tissue weight (SNAP infused: 1.0 ± 0.01 g, n = 4 vs. saline infused: 0.99 ± 0.03 g, n = 3) (P > 0.05); i.e., SNAP increased the loss of erythrocyte-free fluid from the splenic circulation into the systemic lymphatic system. The response of the isolated splenic resistance vessels to the NO donors indicated that the maximal vasorelaxation of hilar veins was less than that of hilar arteries and that the hilar arteries were relatively more responsive to NO-induced vasorelaxation (Figs. 4, 5, and 6). It may be calculated that, at a splenic blood flow of 2.5 ml/min in the intact anesthetized rat (Fig. 3) and a SNAP infusion rate of 0.3 µg·10 µL⁻¹·min⁻¹, the splenic arterial plasma concentration of SNAP was ~0.6 × 10⁻⁶ M. At this concentration, the SNAP cumulative dose-response curves for isolated hilar arteries and veins diverge and reveal a significant difference in vasoreactivity to SNP (Fig. 5). SNAP also has greater efficacy (maximal vasorelaxation) on hilar arteries than veins. Thus, at the doses used for the in vivo studies, NO does cause a greater vasorelaxation of the splenic resistance arteries than of the veins.

We have previously described how the spleen is the site of efflux of protein-rich fluid from the intravascular compartment into the lymphatic system (9, 21, 22). In the rat, at least 25% of fluid volume flowing into the spleen is removed from the circulating blood (9). Because blood flow to the spleen is high (~8 ml/min in the conscious rat), the rate of fluid extravasation is thus considerable (9). This is not altogether surprising given that it is by this route that activated T lymphocytes are disseminated throughout the body (24, 34). However, we have speculated that under normal physiological conditions, in addition to its immunologic function, intrasplenic efflux of isoncotic fluid may play a role in regulating intravascular volume (9).

The differential response of splenic resistance arteries and veins to NO-induced vasorelaxation may be due to a number of reasons. There may be variations between splenic resistance arteries and veins in their reliance on NO as a vasodilator system (10). Such issues have been suggested to explain the divergent responses (both quantitative and qualitative) of different vessels within a single vascular bed to a common stimulus, specifically in regard to NO-induced vasorelaxation (4, 10). Vascular studies have typically reported that NO donors cause greater vasorelaxation of veins than arteries (30). Our results show the opposite, which may indicate that the response of the splenic vasculature is distinct and unique from that of the general circulation. In whole animal experiments, NO-mediated inhibition of sympathetic vasoconstrictor tone (29) may be an additional mechanism that would affect splenic resistance arteries more than veins, because splenic arteries are preferentially innervated by sympathetic nerves (7). Further possible reasons for
the differential responses between splenic resistance arteries and veins to NO-induced vasorelaxation may reside in differences in the rate of NO generation at the cell surface of venous vascular smooth muscle cells (VSMCs) vs. arterial VSMCs, differences in cellular entry mechanisms for NO into VSMCs, and variation in sensitivity of different soluble guanylate cyclases in venous VSMCs vs. arterial VSMCs.

The Starling equilibrium describes the factors controlling movement of fluid across all vascular beds, including those within the spleen. Pre- and postcapillary changes in vascular tone largely determine net $P_C$ (20). Through a mechanism resembling that found in the renal microvasculature (where differential vasoreactivity of afferent and efferent glomerular arteries to vasoactive agents causes changes in glomerular filtration pressure) (20), the differential response between splenic resistance arteries and veins to vasoactive factors would thus alter intrasplenic $P_C$ and fluid extravasation. Unlike most other vascular beds, the discontinuous endothelium of the splenic vasculature (37) is freely permeable to plasma proteins. This enables loss of fluid that is isoncotic to plasma (22), thereby directly altering plasma volume. The extravasated fluid cannot be stored within the spleen, because the rat spleen is noncompliant and cannot acutely increase intrasplenic volume (9, 22). During the 15 min of SNAP infusion (experiment A), it may be calculated that $\sim 16.5$ ml of fluid were translocated from the intravascular space into the lymphatic system. In conscious rats, the SNAP-induced elevation in fluid shift would have been even larger than this, given our finding that anesthesia and surgery reduce intrasplenic fluid efflux (9). NO also inhibits the pumping activity of lymphatic vessels and increases their capacity; i.e., NO facilitates retention of fluid within the lymphatic system (26, 39). Ultimately, it is the balance between the loss of fluid to the lymphatic system and its return to the circulation that determines blood volume (19).

It would have been preferable to measure lymph flow directly rather than estimating it from the difference between splenic arterial inflow and venous outflow. Unfortunately, we have not been able to do this in the rat, given that there are several branches of the splenic lymph duct that cannot be placed together in the blood flow probe without damaging them. However, we do ensure vascular isolation of the spleen. Therefore, by the law of mass action, the difference between the volume of blood going into the spleen and the volume coming out must represent the fluid volume leaving the circulation. Because wet splenic tissue weight does not change (saline-infused controls: $0.99 \pm 0.03$ g vs. SNAP-infused rats: $1.0 \pm 0.01$ g), and because the volume of translocated fluid ($\sim 16.5$ ml) is many times larger than the total volume capacity of the spleen, the extravasated fluid cannot be accommodated within the parenchyma of the spleen. Therefore, the A-V differential must represent lymphatic drainage. The reported accuracy of the transonic flow probes is $\pm 2\%$ (Transonic Systems), which relates to a detectable difference in A-V blood flows of $\sim 0.05$ ml/min. Hence, given such confidence in our reported values for splenic blood flow, the SNAP-induced increase in splenic A-V difference (baseline: $0.8 \pm 0.4$ ml/min vs. peak rise during SNAP infusion: $1.3 \pm 0.4$ ml/min) must be viewed with significance. In addition, the SNAP-induced hemoconcentration of blood flowing from the splenic circulation lends further credence to our proposal that NO increases intrasplenic fluid efflux.

**Perspectives**

Physiological fluid concentrations of NO are difficult to quantify, given that this vasoactive agent has a very short half-life and normally acts in a paracrine manner (16). Despite such difficulties in measuring the quantity of NO directly in the vasculature, measurements of the metabolites of NO in the plasma have indicated increased biosynthesis in sepsis (23). To our knowledge, intrasplenic plasma levels of NO have not been measured during endotoxemia. However, there is indirect evidence to suggest that intrasplenic NO biosynthesis rises after exposure to bacterial lipopolysaccharide (11, 18). It must be noted, however, that strictly adhering to dose-response characteristics within a concentration range may not be an appropriate indicator of the amount of NO biologically available to cause vasorelaxation (3). Nevertheless, we would argue that in constructing our dose-response curves, the vessels were exposed to physiological and pharmacological concentrations of NO. Ultimately, the differential vasoreactivity of splenic resistance arteries and veins to the NO donor agents does imply a mechanism for endotoxemia-induced fluid extravasation from the splenic circulation.

Despite their biochemical differences (31), SNP and SNAP are both termed nitrovasodilators. These are vasorelaxants that act via the formation of NO and its short half-life and normally acts in a paracrine manner (8, 35). This redox versatility has impact on NO-induced vasorelaxation and, although controversial (5), it is suggested that guanylate cyclase activation has preference for the NO$^-$ redox form (15). Because SNP releases less NO redox form than SNAP (15), a greater vasorelaxant response might be expected in vessels exposed to SNAP than SNP. However, we did not find this; there was significantly greater vasorelaxation with SNP than SNAP on splenic resistance arteries, but there was no significant difference in maximal vasorelaxation to either nitrovasodilator.
on veins (Fig. 6). The basis for these responses remains unclear. The consensus opinion is that there is a number of different mechanisms via which NO may cause vasorelaxation (33), with NO-induced activation of guanylate cyclase being a prominent mechanism, although a mechanism that is independent of NO-induced activation of guanylate cyclase has been reported (1). To our knowledge, there are no studies concerning the possible mechanisms of NO-induced vasorelaxation involving splenic resistance vessels. Altogether, the aforementioned evidence suggests that the actions of the NO donors, SNAP, and SNP may be due not only to differential degrees of NO-induced guanylate cyclase activation, but possibly to different noguanylate cyclase mechanisms of NO-induced vasorelaxation within the arterial and venous sides of the splenic circulation.

In conclusion, we have previously reported a significant role for the spleen in the early changes in plasma volume during endotoxemia. We proposed that intrasplenic fluid extravasation of isosmotic fluid from the plasma into the systemic lymphatic system is responsible for the early sepsis-induced hypovolemia. During endotoxemia, NO biosynthesis increases. The results presented in this paper suggest that, under these circumstances, differential vasoreactivity of the splenic resistance arteries and veins to NO induces an elevation in intrasplenic Pc and increases fluid extravasation to the systemic lymphatic system.

The authors thank S. Jerat and Dr. S. T. Davidge for technical assistance and advice.

This study was supported by a research grant from the Medical Research Council of Canada.

REFERENCES


