Splenic denervation worsens lipopolysaccharide-induced hypotension, hemoconcentration, and hypovolemia

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Andrew, Peter S., and Susan Kaufman. Splenic denervation worsens lipopolysaccharide-induced hypotension, hemoconcentration, and hypovolemia. Am J Physiol Regulatory Integrative Comp Physiol 280: R1564–R1572, 2001.—During lipopolysaccharide (LPS)-induced endotoxemia, increased intrasplenic fluid efflux contributes to a reduction in plasma volume. We hypothesized that splenic sympathetic nerve activity (SSNA), which increases during endotoxemia, limits intrasplenic fluid efflux. We reasoned that splenic denervation would exaggerate LPS-induced intrasplenic fluid efflux and worsen the hypotension, hemoconcentration, and hypovolemia. A non-lethal dose of LPS (150 μg·kg⁻¹·h⁻¹ for 18 h) was infused into conscious male rats bearing transit time flow probes on the splenic artery and vein. Fluid efflux was estimated from the difference in splenic arterial inflow and venous outflow (A-V). LPS significantly increased the (A-V) flow differential (fluid efflux) in intact rats (saline −0.01 ± 0.02 ml/min, n = 8 vs. LPS +0.21 ± 0.06 ml/min, n = 8); this was exaggerated in splenic denervated rats (saline −0.03 ± 0.01 ml/min, n = 7 vs. LPS +0.41 ± 0.08 ml/min, n = 8). Splenic denervation also exacerbated the LPS-induced hypotension, hemoconcentration, and hypovolemia (peak fall in mean arterial pressure: denervated 19 ± 3 mmHg, n = 10 vs. intact 12 ± 1 mmHg, n = 8; peak rise in hematocrit: denervated 6.7 ± 0.3%, n = 8 vs. intact 5.6 ± 0.3%, n = 8; decrease in plasma volume at 90-min post-LPS infusion; denervated 1.05 ± 0.15 ml/100 g body wt, n = 7 vs. intact 0.54 ± 0.08 ml/100 g body wt, n = 8). The exaggerated LPS-induced hypovolemia associated with splenic denervation was mirrored in the rise in plasma renin activity (90 min post-LPS: denervated 11.5 ± 0.8 ng·ml⁻¹·h⁻¹, n = 9 vs. intact 6.6 ± 0.7 ng·ml⁻¹·h⁻¹, n = 8). These results are consistent with our proposal that SSNA normally limits LPS-induced intrasplenic fluid efflux.

spleen; plasma volume

EXTRAVASATION OF PROTEIN-RICH fluid (isooncotic to plasma) out of the splenic circulation causes a reduction in plasma volume (9, 21, 22), i.e., the spleen plays a significant role in the regulation of fluid movement between the intravascular and extravascular fluid compartments. We have proposed that the driving force for this fluid efflux is increased intrasplenic microvascular pressure (Pc) caused by differential vasoconstrictor tone of pre- vs. postcapillary splenic resistance vessels (3, 36). Because the rat spleen has no storage capacity and is noncontractile (32), fluid that extravasates from the splenic circulation is not retained within the parenchyma of the spleen but drains into the systemic lymphatic system (9, 22). We have reported that blood flow through the spleen is as high as 8 ml/min in the conscious rat and that under euvolemic conditions, ~25% of fluid flowing into the spleen is removed from the circulating blood (9). We have also previously reported that lipopolysaccharide (LPS) increases intrasplenic fluid efflux in anesthetized rats (3). This route of fluid extravasation probably contributes significantly to LPS-induced cardiovascular collapse, because splenectomy completely abolishes the early changes in blood pressure, hematocrit, and blood volume (3).

The splenic nerve consists predominantly of sympathetic vasoconstrictor fibers, directed primarily to the splenic arterioles rather than venules (1, 4, 30). The tone of these resistance vessels depends on the balance between splenic sympathetic vasoconstrictor nerve activity and the opposing vasodilatory actions of local and circulating vasoactive agents (7). Because the splenic sympathetic nerves predominantly target the precapillary vessels, increased splenic sympathetic nerve activity (SSNA) would thus be anticipated to increase precapillary resistance, lower intrasplenic Pc, and limit fluid extravasation from the splenic circulation.

Bacterial endotoxin increases SSNA (24). However, endotoxin also initiates the release of endogenous cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) (6, 40), both of which promote the release of vasoactive factors that contribute to the hemodynamic alterations characteristic of endotoxemia (6, 29). We proposed that removal of SSNA (by splenic denervation) would leave the vasodilatory actions of local and circulating vasoactive factors unopposed (33, 34), which would allow Pc to rise, and would exaggerate LPS-induced intrasplenic fluid efflux.
This study sought to investigate the influence of the splenic nerves on intrasplenic fluid efflux and hemodynamic parameters during a nonlethal low-dose infusion of LPS (150 μg·kg⁻¹·h⁻¹ for 18 h) in conscious male rats. To this end, the following measurements were made: intrasplenic fluid efflux [estimated from the difference between splenic arterial inflow and venous outflow (A-V)], mean arterial pressure (MAP), heart rate, hematocrit, plasma volume, and plasma renin activity (PRA). Wet splenic tissue weight was measured on completion of LPS infusion to verify the absence of splenic storage of the extravasated isoncotic fluid. Total catecholamine content of splenic tissue was measured to verify splenic denervation. Plasma TNF-α concentration was measured to assess whether splenic denervation had a significant effect on the early LPS-induced increase in plasma TNF-α levels. We hypothesized that the LPS-induced increase in intrasplenic fluid efflux, hypotension, hemococoncentration, and hypovolemia would be exacerbated by splenic denervation.

**METHODS**

The experiments described in this paper were examined by the local Animal Welfare Committee, University of Alberta and were 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).

**Animals and housing.** Male Long-Evans rats were obtained from Eastern Canada (Charles River, St. Foy, Quebec, Canada). They were held in the University Animal Facility until they attained the requisite weight range (450–600 g), were exposed to light of a 12:12-h cycle, in a humidity- and temperature-controlled environment, and were maintained on a 0.3% sodium diet and water ad libitum. Postsurgical housing was for at least a week (or until the animal regained its presurgical body weight).

**Surgery.** Anesthesia was induced with pentobarbital sodium (62 mg/kg body wt ip), followed by penicillin (0.1 ml im; Ethacillin Rogas/STB, London, Ontario, Canada) and atropine (0.1 ml, 0.4 mg/ml sc). Buprenorphine (0.01 mg/kg sc) was given after the completion of surgery. Throughout the surgical procedures, the rats were maintained on a Delaphase isothermic heating pad (Braintree Scientific) that maintained body temperature at 37°C. Isotonic saline (4 ml/h iv) was infused into each animal throughout the surgery. Animals were allowed 1 wk to recover from surgery and to regain their preoperative body weight.

**Cannulations.** A nonocclusive cannula (0.51-mm inside diameter (ID) × 0.94-mm outside diameter (OD); Silastic) was implanted into the inferior vena cava for blood samples. A smaller, nonocclusive cannula (0.28-mm ID × 0.61-mm OD; Silastic) was placed caudal to this for injection of Evans blue dye (Baker Chemical, Phillipnburg, NJ). The jugular vein was cannulated (0.51-mm ID × 0.94-mm OD; exactly 80 mm in length; Silastic) for infusion of LPS or isotonic saline, via a subcutaneous osmotic minipump (Alza, Palo Alto, CA).

**Denervation of the spleen.** The splenic nerve was first visualized under a microscope, and the splenic bundle (which consists of the splenic artery, splenic vein, and splenic nerve) was carefully cleared from the surrounding pancreatic and connective tissue. At the cleared section of the splenic bundle, the splenic nerve was detached from the splenic vessels, and a 2-mm section of nerve was cut away and discarded. The severed ends of the splenic nerve were painted with liquefied 5% phenol-alcohol solution (Fisher Scientific, Edmonton, Alberta, Canada).

**Implantation of MAP recording device.** A pressure transducer (PA-C40, Data Sciences International) was implanted in the abdominal aorta (midway between the branch of the left renal artery and the bifurcation to the femoral arteries) as previously described (3). MAP was continuously recorded using the PhysioTel Telemetry System (Data Sciences International).

**Implantation of blood flow probes.** The use and calibration of these probes have been previously described (9). Flow probes (1RB series; Transonic Systems, Ithaca, NY) were placed around the splenic artery and vein, and the probe leads were sutured securely to the body wall. Splenic blood flows were continuously recorded online using a flowmeter (Transonic Systems) plus Windaq software (Windaq, DATAQ Instruments, Akron, Ohio). Blood samples were rapidly transferred to heparinized microhematocrit tubes. They were centrifuged and read immediately following collection.

**Measurement of hematocrit.** Blood samples (50 μl) were taken from the central venous catheter into heparinized microhematocrit tubes. They were centrifuged and read immediately following collection.

**Measurement of plasma volume.** Plasma volume was determined by use of the Evans blue dye dilution method (22). In short, an initial blood sample was taken (0.25 ml), followed by the addition of Evans blue dye (0.3 ml, 0.5 g/100 ml in sterile isotonic saline) via the smaller indwelling venous cannula. The line was flushed with 0.2 ml saline. At 10, 20, 30, 40, and 60 min postinfusion of Evans blue, blood samples (0.15 ml) were taken from the larger venous cannula. The blood was replaced with the same volume of saline. Blood samples were rapidly transferred to heparinized Fisherbrand Caraway tubes (Fisher Scientific) and centrifuged. Hematocrit was measured and the plasma separated from the red blood cells. Plasma samples (50 μl) were diluted in 950 μl saline, and absorbance was measured at 605 μm on a Spectrophotometer (LKB Biochrom, model 4049, Cambridge, England). Recordings were compared with standards obtained by adding 0, 1, and 2 μl of the 0.5% Evans blue solution to 50 μl of the initial plasma sample plus 950 μl saline. Plasma volume was calculated by extrapolation back to time zero.

**Measurement of PRA.** PRA was determined in blood samples collected at time intervals of basal (−24 h) and then at 90 min, 8 h, and 18 h postinfusion of either saline or LPS. The PRA was measured using an ANG I radioimmunoassay kit (New England Nuclear, Boston, MA). The subsequent reports on the interference and sensitivity of this assay are based on data provided in the NEN ANG I radioimmunoassay kit handbook. The method reports an absence of nonspecific interference in the assay by plasma constituents. The sensitivity of the method, defined as the mass equivalent to twice the standard deviation of the zero binding, is ~40 pg/ml.

**LPS infusion.** LPS (150 μg·kg⁻¹·h⁻¹), derived from *Escherichia coli* (serotype 055:B5), was supplied by Sigma Chemicals as a lyophilized powder, chromatographically purified by gel filtration, with a protein content of <1%. At 1 wk after surgery, to implant the indwelling cannulas, an osmotic minipump (Alza) was implanted under isoflurane anesthesia (Abbott Labs, Montreal, Canada) and connected to the jugular vein cannula. The length of the jugular cannula (80 mm) gave a “lead-in” time of exactly 2 h at the delivery rate of 8 μl/h, i.e., *time zero* was 2 h after implantation of the pump, at which time the rat was conscious and fully recovered from the brief period of anesthesia. Infusion of LPS continued over...
the entire 18-h experimental period, and with a mean body weight per rat of 530 ± 7.07 g, the total dose of LPS over the entire 18-h infusion period was 0.07 mg.

Measurement of total catecholamine content of splenic tissue after denervation. The total catecholamine content of intact and denervated splenic tissue (postinfusion of either saline or LPS) was measured using a catecholamine 3H radioenzymatic assay system (Amersham Pharmacia Biotech, Baie d’Urfe, Quebec, Canada). The subsequent report on the sensitivity of this assay is taken from data provided in the catecholamine 3H radioenzymatic assay system handbook. The expected sensitivity of this assay for each individual catecholamine (noradrenaline, adrenaline, and dopamine) is from 2.5 pg for noradrenaline and adrenaline to 15–20 pg for dopamine per 50-μl volume of plasma analyzed. All our plasma samples were analyzed in a single assay.

Measurement of wet splenic tissue weight. After the 18-h infusion of saline or LPS, the rats were decapitated and the spleen was removed. The wet weight of the spleen was measured.

Measurement of TNF-α in plasma. The TNF-α concentration was determined in plasma samples collected at time intervals of basal (−24 h), 90 min, and 3 h postinfusion of either saline or LPS. The TNF-α concentration of plasma was measured using a TNF-α rat ELISA system (Amersham Pharmacia Biotech). The subsequent reports on the specificity and sensitivity of this assay are taken from data provided in the handbook that accompanied the assay (Amersham Pharmacia Biotech). The ELISA is specific for measurement of natural and recombinant rat TNF-α; it does not cross-react with rat IL-1α or IL-1β. The reported sensitivity of this ELISA in plasma was reported as <10 pg/ml. All our plasma samples were analyzed in a single assay.

Measurement of heart rate. The heart rate (beats/min) was calculated by analysis of the MAP data using Windaq software (DATAQ Instruments). Heart rate was determined by counting the number of spikes in the MAP recordings from data collected for a 5-min period before each timed blood sample collection; at 0, 20, 40, 60, and 90 min, and 3 h post-LPS or saline infusion.

Experimental groups. There were four investigative groups: intact rats infused with isotonic saline, intact rats infused with LPS, splenic denervated rats infused with isotonic saline, and splenic denervated rats infused with LPS.

Protocol for splenic blood flow. The start of the experimental measurements (time 0) was defined as the commencement of LPS (or saline) infusion into the jugular vein. Splenic arterial and venous blood flows were continuously recorded and later analyzed (Windaq, DATAQ Instruments). Splenic blood flows were derived from data collected for a 10-min period before each timed blood sample collection; at 0, 20, 40, 60, and 90 min, and 3, 8, and 18 h post-LPS infusion into the jugular vein.

Protocol for MAP. Basal (−24 h) measurements of MAP were determined on the day before the start of the experiment. The start of the experimental measurements (time 0) was defined as the commencement of LPS (or saline) infusion into the jugular vein. MAP was continuously recorded and later analyzed (Windaq, DATAQ Instruments). MAP was derived from data collected for a 10-min period before each timed blood sampling.

Protocol for hematocrit. Basal (−24 h) measurements of hematocrit were determined on the day before the start of the experiment. Serial measurements of hematocrit were derived from blood samples taken at 0, 20, 40, 60, and 90 min, and 3, 8, and 18 h post-LPS/LPS infusion into the jugular vein.

Protocol for plasma volume. Basal (−24 h) plasma volume was determined on the day before the start of the experiment and then the following day at 90 min postsaline/LPS infusion into the jugular vein.

Protocol for TNF-α ELISA. Serial measurements of plasma TNF-α concentration were derived from blood samples taken at 0 min, 90 min, and 3 h postsaline/LPS infusion into the jugular vein.

Protocol for PRA. Blood samples for determination of PRA were drawn 24 h before the onset of saline/LPS infusion and at 90 min, 8 h, and 18 h postsaline/LPS infusion into the jugular vein.

Statistical analysis. The significance of changes in splenic arterial and venous blood flows, splenic A-V difference, MAP, heart rate, percent hematocrit, plasma volume, plasma TNF-α concentration, and PRA across time was analyzed by a two-way, repeated-measures ANOVA, followed by the Student-Newman-Keuls test to identify the individual points of significance. If the data were not normally distributed, a repeated-measures ANOVA on ranks was used, followed by Dunn’s method to identify individual points of significance. Plasma volume was analyzed using an unpaired t-test to assess the absolute decrease in plasma volume between intact and denervated LPS-infused groups. Total catecholamine content was analyzed using an unpaired t-test. Differences in the splenic wet weight were analyzed using an unpaired t-test. Significance was accepted at P < 0.05.

RESULTS

There were no significant differences between the intact and denervated groups in the resting (time = 0) splenic arterial or venous blood flows or in the resting (time = 0) A-V flow differential (fluid efflux) (Fig. 1).

![Fig. 1. Splenic arterial (A) and venous (B) blood flows in denervated (○, n = 10) and intact (●, n = 8) conscious rats during lipopolysaccharide (LPS) infusion (150 μg·kg⁻¹·h⁻¹) and fluid efflux (●; arterial inflow and venous outflow (A-V) difference) in denervated (○, n = 8) and intact (●, n = 8) groups. Vertical bars delineate standard error of the mean. *Significant difference between LPS-infused groups and their respective saline-infused control groups (data not shown) and between the LPS-infused groups and their respective preinfusion (0 min) basal values, P < 0.05. §Significant difference between intact and denervated LPS-infused groups, P < 0.05.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00482.2017)
(\(P > 0.05\)). Nor were there any changes in splenic blood flows or A-V flow differential over the course of 18 h in the respective saline-infused control groups (data not shown) (\(P > 0.05\)). After infusion of LPS, splenic arterial blood flow increased significantly in both intact and denervated groups with regard to both basal values and their respective saline-infused control group (Fig. 1A) (\(\$P < 0.05\)). The LPS-induced increase in splenic arterial blood flow was significantly greater in the denervated group than in the intact group at 60 min, 90 min, and 3 h postinfusion (Fig. 1A) (\(\$P < 0.05\)). There was no significant difference in venous blood flow between denervated and intact groups infused with LPS (Fig. 1B) (\(P > 0.05\)). The A-V flow differential significantly increased in response to LPS in both the denervated and intact groups, both with regard to basal values and their respective saline-infused control group (Fig. 1C) (\(\$P < 0.05\)). The LPS-induced rise in intrasplenic fluid efflux was significantly greater in the splenic denervated than in the intact group at 60 min, 90 min, 3 h, and 8 h postinfusion (Fig. 1C) (\(\$P < 0.05\)). The mean increase in intrasplenic fluid efflux over the 18-h LPS infusion period was significantly greater in the denervated (+ 0.41 ± 0.08 ml/min, \(n = 7\)) than in the intact (+ 0.21 ± 0.06 ml/min, \(n = 8\)) group (\(P < 0.05\)).

There was no significant difference in basal (−24 h) heart rate between the intact and denervated groups (Fig. 3D) (\(P > 0.05\)), nor were there any significant changes in heart rate over the course of 18 h in the respective saline-infused controls (data not shown) (\(P > 0.05\)). After 90 min of LPS infusion, heart rate increased in both the intact and denervated groups compared with their respective basal values and saline-infused controls (Fig. 3D) (\(\*P < 0.05\)). However, the increase in heart rate was significantly greater in the denervated group compared with the intact group at 90 min, 3 h, and 8 h post-LPS infusion (\(\*P < 0.05\)).

Basal (−24 h) plasma volume was significantly greater in the splenic denervated group than in the intact group (Fig. 4) (\(\$P < 0.05\)). After 90 min of LPS infusion, there was a significant fall in plasma volume

\[ MAP = \text{mean arterial pressure} \]

\[ Hct = \text{hematocrit} \]

\[ A-V \text{ flow differential} = \text{arterial flow} - \text{venous flow} \]

\[ \text{Intrasplenic fluid efflux} = \text{increase in fluid out of spleen} \]

\[ \text{Heart rate} = \text{number of heartbeats per minute} \]

\[ \text{Plasma volume} = \text{volume of plasma in the blood} \]

\[ \text{Basal values} = \text{values before any treatment} \]

\[ \text{LPS} = \text{lipopolysaccharide} \]

\[ \$P < 0.05, \*P < 0.05 \] indicate statistically significant differences.
from respective basal control values in both intact ($^P < 0.05$) and denervated ($^P < 0.05$) groups (Fig. 4). There was a significant difference in plasma volume between the intact and denervated groups at 90 min post-LPS infusion (Fig. 4) ($^P < 0.05$). Moreover, the decrease in plasma volume from basal to 90 min post-LPS infusion was significantly greater in the denervated group (1.08 ± 0.15 ml/100 g body wt, $n = 7$) than in the intact group (0.54 ± 0.08 ml/100 g body wt, $n = 8$) ($P < 0.05$).

Basal (−24 h) PRA tended to be higher in the denervated saline-infused control group (7.5 ± 0.5 ng ANG 1·ml⁻¹·h⁻¹, $n = 7$) than in the intact saline-infused control group (4.7 ± 0.3 ng ANG 1·ml⁻¹·h⁻¹, $n = 7$) ($P = 0.323$). There was no significant change in PRA over the 18-h period of saline infusion in intact or denervated saline-infused controls (data not shown) ($P > 0.05$). LPS caused a significant time-dependent increase in PRA in both intact ($^P < 0.05$) and denervated ($^P < 0.05$) groups (Fig. 5). Moreover, the increase in PRA was significantly greater in the denervated group than in the intact group at 90 min, 8 h, and 18 h post-LPS infusion (Fig. 5) ($^P < 0.05$). The intra-assay and interassay variability of the PRA measurements was 6 and 11%, respectively.

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Fig. 3. Splenic (A-V) difference (A), MAP (B), Hct (C), and heart rate (D) during intravenous infusion of LPS (150 μg·kg⁻¹·h⁻¹) in intact (●) and splenic denervated (○) conscious rats. Vertical bars delineate standard error of the mean. *Significant difference between denervated and intact groups, $P < 0.05$. §Significant difference between LPS-infused groups (intact and denervated) and their respective basal values and saline-infused groups, $P < 0.05$.

Fig. 4. Plasma volume at basal (−24 h) and 90 min postinfusion of LPS (150 μg·kg⁻¹·h⁻¹) in intact (closed bars, $n = 8$) and denervated (open bars, $n = 7$) conscious rats. Vertical bars delineate standard error of the mean. †Significant difference in plasma volume in intact rats at basal vs. 90 min post-LPS infusion, $P < 0.05$. §Significant difference in plasma volume in denervated rats at basal vs. 90 min post-LPS infusion, $P < 0.05$. *Significant difference in basal plasma volume between intact and denervated groups, $P < 0.05$. §Significant difference in plasma volume at 90 min post-LPS infusion between intact and denervated LPS-infused groups, $P < 0.05$. 

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Total catecholamine content of splenic tissue at 18 h postsaline/LPS infusion was significantly less in denervated (31.6 ± 7.6 ng/ml, n = 10) than in intact (222.2 ± 34.1 ng/ml, n = 10) groups (P < 0.05). The intra-assay variability of the catecholamine measurements was 2%.

Basal (−24 h) plasma TNF-α concentration in the denervated and intact groups was below the detection limit of the assay. There was no detectable change in plasma TNF-α concentration in either intact or denervated control groups at 90 min and 3 h postsaline infusion. LPS caused a significant increase in plasma TNF-α concentration in both intact (90 min: 2,244 ± 654 pg/ml; 3 h: 1,606 ± 113 pg/ml, n = 4) and denervated (90 min: 2,231 ± 532 pg/ml; 3 h: 2,006 ± 577 pg/ml, n = 4) groups compared with their respective basal value and saline-infused controls (P < 0.05). Plasma TNF-α concentrations were not significantly different between the denervated and intact groups at 90 min or 3 h post-LPS infusion (P > 0.05). The intra-assay variability of the plasma TNF-α measurements was 5%.

There were no significant differences in the wet weight of splenic tissue at 18 h postinfusion among intact saline-infused (1.27 ± 0.111 g, n = 13), intact LPS-infused (1.28 ± 0.07 g, n = 10), denervated saline-infused (1.27 ± 0.07 g, n = 11), and denervated LPS-infused (1.26 ± 0.05 g, n = 13) groups (P > 0.05).

**DISCUSSION**

The spleen participates in the regulation of blood volume by controlling the efflux of protein-rich fluid from the intrasplenic circulation into the systemic lymphatic system (9, 21, 22). We recently reported that during low-dose intravenous infusion of LPS (150 μg·kg⁻¹·h⁻¹) in anesthetized rats, intrasplenic fluid efflux increases (3). We suggested that because splenectomy abolishes LPS-induced hypotension, hemo-concentration, and hypovolemia, efflux of isooncotic fluid from the splenic circulation probably contributes significantly to endotoxemic cardiovascular dysregulation (3). This current study sought to investigate the influence of the splenic nerves on LPS-induced changes in intrasplenic fluid efflux and hemodynamics in conscious rats. Splenic denervation (verified by comparison of the total catecholamine content of intact and denervated splenic tissue) removes sympathetic vasoconstrictor tone, predominantly over precapillary splenic resistance vessels. This has two effects. First, splenic denervation impairs the contribution of the splenic vasculature to the reflex increase in total peripheral resistance in response to the LPS-induced decrease in MAP (Fig. 3B). Second, denervation prevents the LPS-induced increase in sympathetic tone from limiting the rise in intrasplenic P C (which drives intrasplenic fluid efflux) (24, 36). Consequently, splenic denervation had been expected to exaggerate intrasplenic fluid efflux and thus worsen the hypotension, hemoconcentration, and hypovolemia. This is exactly what we found.

We have previously shown that under euvolemic conditions in conscious rats, as much as 25% of fluid flowing into the spleen is removed from the circulating blood (9). The current data are consistent with this finding (splenic A-V flow differential of ∼0.9 ml/min at time = 0, Fig. 1C). In intact rats, nonlethal low-dose infusion of LPS caused a significant increase in splenic A-V flow differential (Fig. 1C), indicating enhanced intrasplenic fluid efflux into extravascular spaces. This was exacerbated in splenic denervated rats. The LPS-induced increase in intrasplenic fluid efflux was associated with hypotension (Fig. 2A), hemoconcentration (Fig. 2B), tachycardia (Fig. 3D), and hypovolemia (Fig. 4). Specifically, the early LPS-induced hypotension at 20, 40, 60, and 90 min, and 3 h (Fig. 3B) was consistent with an increase in splenic A-V flow differential (Fig. 3A) in both intact and denervated rats. These LPS-induced hemodynamic perturbations were exacerbated in the splenic denervated rats. The transient recovery of MAP in LPS-infused denervated rats (at 90 min) was correlated with a pronounced increase in heart rate (Fig. 3, B and D), suggesting that the exaggerated hypotension may be driving the enhanced tachycardia in denervated rats during the early stages of endotoxemia.

The time course of differences in intrasplenic fluid efflux and decreases in MAP, between intact and denervated rats infused with LPS, do not suggest a simple causal relationship; the more pronounced fall in MAP in the denervated rats (at 20 and 40 min post-LPS infusion) preceded the difference in intrasplenic fluid efflux (Fig. 3, A and B). Loss of the vasoconstrictor effect of an LPS-induced increase in SSNA over the splenic vascular bed (and its contribution to total peripheral resistance) may be the reason for this exaggerated decrease in MAP in denervated rats at 20 and 40 min post-LPS infusion. At the time of significant increases in hematocrit and intrasplenic fluid efflux (90 min post-LPS infusion) (Fig. 3, A and C), plasma...
volume in the denervated rats was significantly less than in the intact rats (Fig. 4). This may be due to the exaggerated intrasplenic fluid efflux that occurred in the preceding time period in the denervated rats, i.e., at 60 to 90 min post-LPS infusion (Fig. 3A).

There was a significant time-dependent increase in PRA in intact and denervated LPS-infused groups (Fig. 5). The exaggerated increase in PRA in the denervated rats is consistent with the greater fall in plasma volume. Although, on the basis of this evidence, an exaggerated intrasplenic fluid efflux may not account entirely for the changes in MAP following LPS infusion; our results do provide evidence that enhanced fluid extravasation from the splenic circulation may contribute to the exaggerated LPS-induced hypotension, hemoconcentration, and hypovolemia in the early hours of endotoxemia in denervated rats. Our results thus support the proposal that SSNA limits intrasplenic fluid efflux during endotoxemia.

The rat spleen is noncompliant and cannot acutely store blood volume (32). This was confirmed by our findings that there were no significant differences in splenic tissue wet weight between groups, despite significant differences in the A-V flow differential. The spleen has a discontinuous vascular endothelium (37). Thus given an elevation in intrasplenic P_c, it is possible for protein-rich fluid to pass unhindered from the intravascular compartment into extravascular spaces (9, 22, 36). This fluid drains into the systemic lymphatic system (21) and, if not returned to the intravascular compartment, contributes to a fall in plasma volume. Ultimately, it is the balance between this loss of fluid to the systemic lymphatic system and its return to the circulation that determines blood volume (19).

We estimated lymphatic flow from the splenic circulation by measuring the difference in splenic A-V (Fig. 1C). Much as we would have liked to directly measure lymphatic flow from the spleen, this is not technically feasible in the rat. Because the weight of the spleen did not change despite significant changes in splenic A-V flow differential (Fig. 1C), and because the volume of extravasate was many times the total volume capacity of the rat spleen, this fluid must have been transferred to another site, namely the systemic lymphatic system. The reported accuracy of the transonic flow probes is ±2% (Transonic Systems), which relates to a detectable difference in A-V blood flows of ~0.05 ml/min. Hence, given such confidence in our reported values for splenic blood flow, the mean increase in splenic A-V difference between intact control −0.01 ± 0.02 ml/min vs. LPS +0.21 ± 0.06 ml/min and denervated control −0.03 ± 0.01 ml/min vs. LPS +0.41 ± 0.08 ml/min) rats must be viewed with significance. These large increases in intrasplenic fluid extravasation would cause an enormous loss of plasma during the 18-h period of LPS infusion were it not for the fact that most of this fluid would be returned to the vascular system from the lymphatic system (19). However, the capacity of the lymphatic system is increased by such agents as atrial natriuretic factor (31), the circulating levels of which increase in endotoxic shock (2). Thus the enhanced storage capacity of the systemic lymphatic system during endotoxemia may enable a greater volume of extravasated fluid to be held within this compartment without return to the vascular system. The fact that plasma volume was less in denervated than intact rats at 90 min post-LPS infusion (Fig. 4) is thus probably a combination of enhanced intrasplenic fluid efflux plus increased capacity of the lymphatic system to retain this extravasated fluid.

There is ample evidence for the existence of sympathetic vasoconstrictor fibers within the splenic nerve (1, 4, 30). However, it is also well established that there are also [despite one report to the contrary (30)] sensory afferent fibers (10, 23, 27, 41). These sensory afferents have been proposed to form part of a neural reflex pathway between the spleen and kidney, termed a spinal splenorenal reflex (27, 41). The sensory afferent traffic from the spleen inhibits renal sympathetic nerve activity (RSNA), thus limiting PRA. Removal of this inhibition (by splenic denervation) would consequently elevate RSNA and thus cause a rise in plasma volume over the long term (i.e., over the 1-wk period of recovery after surgery). Our findings lend credence to this proposal, given that splenic denervated rats tended to have an increased basal PRA (Fig. 5) plus an expanded basal plasma volume (Fig. 4) compared with intact rats. In interpreting our findings, it is important to distinguish between short- (minutes to hours) and long-term (days) regulation of plasma volume. The latter is more likely to involve renal control of extracellular fluid volume. It is an alteration in these mechanisms that would be responsible for increasing basal plasma volume in the splenic denervated rats (Fig. 4). The rise in basal PRA in denervated rats may be the primary driving force behind the elevated basal plasma volume observed in this current study.

Our study found an increase in splenic arterial blood flow following intravenous infusion of LPS (Fig. 1A); this has also been reported elsewhere (34). The mechanism for the increase in splenic blood flow has been proposed to be the LPS-induced production of the vasoactive cytokine IL-1, because the IL-1 receptor antagonist abolishes the response (34). As well as directly affecting the vascular tone of splenic resistance arteries, the splenic sympathetic nerve has been suggested to mediate central modulation of immune cell function within the spleen (17). Activation of immune cells elevates their production of vasoactive cytokines (16, 20, 26, 28, 35). Thus sympathetic nerve activity can modulate the secretion of cytokines from LPS-exposed immune cells (25), thereby possibly altering the production of vasoactive agents. Hence, there is the possibility that surgical denervation of the spleen removes the inhibitory action of sympathetic nerves on the production of vasoactive cytokines by immune cells residing in the spleen. This alteration in production of vasoactive cytokines could potentially have contributed to the exaggerated LPS-induced hemodynamic changes found in the splenic denervated rats. However, our measurements of plasma TNF-α concentration do not support this proposal. TNF-α is one of the first primary
cytokines released during sepsis (6), and it has been implicated in the endotoxemic-induced hemodynamic alterations (14, 15, 29, 40). In response to LPS, plasma TNF-α concentration increased equally in the denervated and intact rats, i.e., splenic denervation did not significantly attenuate the early LPS-induced increase in plasma TNF-α levels (P > 0.05). Thus altered production of vasoactive cytokines cannot account for the LPS-induced hemodynamic differences we have observed between intact and denervated rats.

Nonlethal low-dose infusion of LPS over a long period, as used in the present study, has been reported elsewhere to cause hemodynamic changes similar to those we observed (3, 13). Our use of a model characterized by morbidity rather than mortality enables us to identify the changes that occur during the initial stages of endotoxemia. By contrast, those studies that use much larger bolus doses of LPS induce increases in systemic permeability characteristic of established endotoxemic shock (38, 39). To our knowledge, there are no studies concerning the effects of splenic denervation on the cardiovascular response to endotoxin in the rat or any other species. However, there are studies reporting that splenectomy (which we propose removes the pathway for intrasplenic fluid efflux from the intravascular compartment into the systemic lymphatic system) attenuates LPS-induced hemodynamic changes in both rats (3) and dogs (11, 12). Consequently, we suggest that the significance of intrasplenic fluid efflux in the regulation of plasma volume during endotoxemia is not peculiar to the rat.

Perspectives

This study confirms the importance of the splenic nerves in influencing plasma volume homeostasis during endotoxemia. Splenic denervation enhances intrasplenic fluid efflux and worsens hypotension, hemoconcentration, and hypovolemia during LPS-induced endotoxemia. We propose that in intact animals, SSNA concentration, and hypovolemia during LPS-induced splenic fluid efflux and worsens hypotension, hemococoncentration in influencing plasma volume homeostasis during endotoxemia.

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