The spleen is required for 5-HT1A receptor agonist-mediated increases in mean circulatory filling pressure during hemorrhagic shock in the rat

Ruslan Tiniakov and Karie Scrogin
Department of Pharmacology and Experimental Therapeutics, Loyola University
Chicago, Stritch School of Medicine, Maywood, Illinois

Running Head: 5-HT1A-agonist increases MCFP during shock

Address Correspondence to:

Karie Scrogin, Ph.D.
Associate Professor
Department of Pharmacology and Experimental Therapeutics
Loyola University Chicago, Stritch School of Medicine
2160 S. First Ave.
Maywood, IL  60153
Tel: 708-216-5652
FAX:  708-216-6596
e-mail:  kscrogi@lumc.edu
Abstract

The 5-HT$_{1A}$-receptor agonist, 8-OH-DPAT, increases whole body venous tone (mean circulatory filling pressure; MCFP), and attenuates metabolic acidosis in a rat model of unresuscitated hemorrhagic shock. To determine if improved acid-base balance was associated with sympathetic activation and venous constriction, MCFP, sympathetic activity (SA) and blood gases were compared in hemorrhaged rats following administration of 8-OH-DPAT, the arterial vasoconstrictor arginine vasopressin (AVP), or saline. To further determine if protection of acid-base balance was dependent on splenic contraction and blood mobilization, central venous pressure (CVP), MCFP, and blood gases were determined during hemorrhage and subsequent 8-OH-DPAT-administration in rats subjected to real or sham-splenectomy. Subjects were hemorrhaged to an arterial pressure of 50 mmHg for 25 min and subsequently treated with 8-OH-DPAT (30 nmol/kg, iv), AVP titrated to match the pressor effect of 8-OH-DPAT (~2 ng/min, iv), or infusion of normal saline. 8-OH-DPAT increased MAP, CVP, MCFP, SA, and decreased lactate accumulation. Arginine vasopressin did not affect CVP or SA, but raised MCFP slightly to a level intermediate between 8-OH-DPAT- and saline-treated rats. Infusion of AVP also produced a modest protection against metabolic acidosis. Splenectomy prevented the rise in CVP, MCFP and protection against metabolic acidosis produced by 8-OH-DPAT, but had no effect on the immediate pressor response to the drug. Together, the data indicate that 8-OH-DPAT produces a pattern of cardiovascular responses consistent with a sympathetic-mediated venoconstriction that is, in part, responsible for the drug's beneficial effect on acid-base balance. Moreover, blood mobilization stimulated by the spleen is required for the beneficial effects of 8-OH-DPAT.
Acknowledgements:

This study was supported by the National Institutes of Health grants HL072354 and HL076162.
Introduction

Progressive blood loss results in a complex series of autonomic responses that help to maintain perfusion pressure in the face of reduced blood volume. Such responses include an initial increase in sympathetic drive to the heart and selected vascular beds that results in an overall increase in total peripheral resistance. However, after loss of significant blood volume (~20-30%), sympathetic activity rapidly declines and cardiac vagal activation ensues resulting in a neurogenic syncope-like response characterized by bradycardia, decreased peripheral resistance and hypotension (3). Whether blood loss persists or is terminated, the syncopal response slowly reverses resulting in a gradual recovery of heart rate, arterial blood pressure and peripheral vascular resistance (40). This recovery is mediated by increased sympathetic activity as well as release of vasoactive hormones that lead to a state of compensated shock (42). However, if bleeding persists without intervention, cardiovascular decompensation ensues which can progress to irreversible cardiovascular collapse (44).

We have shown that systemic administration of the 5-HT\textsubscript{1A}-receptor agonist, 8-OH-DPAT, rapidly raises sympathetic nerve activity and blood pressure in unanesthetized rats subjected to acute blood withdrawal sufficient to initiate a syncope-like response (~15% of estimated blood volume) (29). In subsequent studies, we found that 8-OH-DPAT produced elevations in blood pressure, cardiac output, renal conductance and mean circulatory filling pressure (MCFP; an index of whole body venous tone) when given to rats subjected to blood withdrawal sufficient to produce hypovolemic shock, i.e. ~50% of estimated blood volume (40,41). The beneficial effects of drug administration persisted long after the normal half-life of the drug. Ganglionic
blockade was shown to prevent the early increase in MCFP, but affected only the delayed pressor effect of 8-OH-DPAT. Together, the data suggest that 8-OH-DPAT treatment triggers sympathetic-dependent venoconstriction to enhance venous return and peripheral perfusion. However, it is not known if a persistent increase in sympathetic activity accounts for the ability of 8-OH-DPAT to maintain the prolonged elevation in MCFP observed in this more severe hemorrhage model.

The pharmacological mechanisms that underlie the cardiovascular effects of 8-OH-DPAT are complex. Our previous work suggests that 8-OH-DPAT stimulates a rapid and transient increase in arterial tone, probably by direct activation of vascular $\alpha_1$-adrenergic receptors. In addition, the drug likely produces sympathetic-mediated activation of $\alpha_2$ adrenergic receptors that leads to constriction of the venous vasculature and mobilization of unstressed blood volume to promote a progressive and sustained increase in effective blood volume and increased perfusion of peripheral tissue (41).

Blood mobilization during hemorrhage is mediated primarily by venoconstriction within the vascular beds of the splanchnic organs including the spleen (33). Splenectomy has been shown to reduce tolerance for blood loss in rodents (11,15,32). Sympathetic-mediated contraction of the rat spleen is highly dependent upon $\alpha_2$-adrenergic receptors (20). Likewise, sympathetic-dependent increases in whole body venous tone are predominately mediated by $\alpha_2$-adrenergic receptors in rats (30). Together, the data suggest that sympathetic-dependent $\alpha_2$-adrenergic receptor-mediated mobilization of blood stores, including stores from spleen, account, at least in part, for improved cardiac output and blood gases observed with 8-OH-DPAT administration in hypovolemic shock.
However, it is not known if the spleen contributes to increased mobilization of unstressed volume in hemorrhaged rats treated with 8-OH-DPAT.

The first part of this study sought to determine whether 8-OH-DPAT produces a persistent elevation in sympathetic drive among rats in hypovolemic shock that parallels and therefore, might account for the drug's prolonged effect on MCFP. We further sought to provide additional evidence that 8-OH-DPAT raises MCFP through venoconstriction by comparing its effects with that of the arterial vasoconstrictor peptide, arginine-vasopressin (AVP) which itself has little venoconstrictor capacity. Finally, we tested the hypothesis that the pressor effect of 8-OH-DPAT, and its ability to raise MCFP and improve metabolic acidosis in rats during hypovolemic shock are dependent on the spleen.

METHODS

Animals

Male Sprague-Dawley rats weighing 300-325 g were purchased from Harlan (Madison, WI). The animals were housed within the institution's animal care facility, maintained at 23°C, with a 12 hour light/dark cycle and access to food and water ad libitum. The rats were acclimated to the housing facility for at least 1 week prior to surgery. All experiments were reviewed and approved by the institutional Animal Care and Use Committee and were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council as adopted and promulgated by the US National Institutes of Health (revised 1996).
Surgery

Protocol 1

One week prior to the experiment, rats were anesthetized with ketamine + xylazine (100 mg/kg + 7 mg/kg, im) and given the anti-sialogogue glycopyrrolate (0.5 mg/kg, sc). The jugular vein was exposed through a mid-line ventral cervical incision in the skin. A silastic balloon-tipped catheter (Vesta, Inc., Franklin, WI) was inserted into the jugular vein and advanced until the tip of the balloon was situated in the right atrium. The catheter was secured in place with silk 3-0 suture, after which the free end was tunneled subcutaneously to exit at the nape of the rat's neck. Rats were given ampicillin (150 mg/kg, sc) following surgery and again 24 and 48 hours later. The balloon was inflated with 200 μL of saline once daily prior to the experiment in order to prevent fibrin and leukocyte accumulation around the catheter tip.

One day prior to the experiment, animals were anesthetized with sodium pentobarbital (65 mg/kg, ip) for implantation of bilateral femoral arterial catheters (PE-50 heat-welded to PE-10) and a unilateral femoral venous catheter to enable simultaneous arterial blood withdrawal and arterial blood pressure recording as well as intravenous (iv) drug administration. A second femoral venous catheter made of silastic tubing (0.037” O.D.) was advanced to the thoracic vena cava for measurement of central venous pressure (CVP). All catheters were tunneled under the skin, externalized at the nape of the neck and secured in place with 3-0 silk suture. All catheters were filled with heparinized saline (75 U/mL) to preserve patency. A renal sympathetic nerve recording electrode was implanted through a left flank retroperitoneal incision as described previously (36). Briefly, a portion of renal sympathetic nerve emanating from the
aortico-renal ganglion was isolated and placed on a bipolar stainless steel recording electrode and embedded in Kwik-Sil silicone elastomer (World Precision Instruments, Sarasota, FL). The electrode leads were tunneled subcutaneously and externalized at the nape of the neck. The rats were given ampicillin (150 mg/kg, sc), and allowed to recover overnight in their home cage.

Protocol 2

Male Sprague-Dawley rats weighing between 325-360 g were anesthetized with ketamine (100 mg/kg, im) and xylazine (7 mg/kg, im) and subjected to sham- or real splenectomy at least 18 days prior to the experiment. Splenectomy was performed through a midline abdominal incision. The spleen was gently elevated from the abdominal cavity, after which, splenic blood and lymphatic vessels were ligated with 4-0 silk suture. The spleen was then excised and weighed. The abdominal muscle layer was closed with 3-0 chromic gut suture. The skin incision was closed with surgical staples. After splenectomy, all rats were given isotonic saline (3 times the spleen weight, sc). Rats subjected to sham splenectomy underwent a similar laparotomy. In this case, the spleen was pulled from the abdominal cavity, exposed to room air for 2 minutes, and returned to its normal position. Sham-splenectomized rats were given 1 ml of isotonic saline, sc. After surgery, all animals were given ampicillin (150 mg/kg/day, sc) for 5 days. Rats were allowed to recover for 2 weeks. Four days prior to the experiment, the same animals were anesthetized with sodium pentobarbital (60 mg/kg, ip) and instrumented with vascular catheters as described above. During the same surgery, an inflatable balloon-tipped catheter was inserted into the right atrium to enable
determination of MCFP. Rats were given ampicillin (150 mg/kg, sc) 1, 24 and 48 hrs after surgery. All vascular catheters were flushed daily with 150 μL of heparinized saline. The right atrial balloon was inflated for a few seconds with 200 μL of saline daily to prevent fibrin accumulation.

Data Acquisition

Mean arterial pressure (MAP), heart rate (HR), CVP and renal sympathetic nerve activity (RSNA) were recorded continuously using a PowerLab/4SP data acquisition system (ADInstruments Inc., Colorado Springs, CO) and a Macintosh PowerBook G4 computer equipped with Chart 5.2.1 software. Mean arterial pressure and CVP were measured using Transpac IV disposable transducers (Abbott Labs, North Chicago, IL) interfaced to the PowerLab through bridge amplifiers (ADInstruments Inc.). Blood pressure was recorded at 400 Hz. Heart rate was calculated using peak-to-peak detection of the pulse pressure wave. The sympathetic nerve signal was sampled at 4,000 Hz, filtered (1-1,000 Hz) and amplified (10-20,000×) with a Bio Amplifier (ADInstruments Inc.). The full-wave neurogram was rectified and integrated over 20 ms bins. To determine the amount of background noise the ganglionic blocker, hexamethonium chloride (30 mg/kg, iv), was administered at the end of experiment. The resulting average voltage was subtracted from the integrated nerve activity to determine RSNA.

Mean circulatory filling pressure was measured using methods described by Yamamoto et al. (43). In short, the right atrial balloon was rapidly inflated with 300-350 μl of saline to produce a brief (5-7 sec) circulatory arrest. During the procedure, MAP falls and stabilizes at a nadir (final arterial pressure, FAP) while CVP rises and stabilizes
at nearly the same level (venous plateau pressure, VPP). Mean circulatory filling
pressure is then calculated using the following equation:

\[ \text{MCFP} = \text{VPP} + \frac{1}{60}(\text{FAP} - \text{VPP}) \]

The \( \frac{1}{60} \) factor accounts for the difference in compliance between the arterial and venous
vasculature in the rat. Measurements of VPP and FAP were taken once hemodynamic
equilibrium was reached, usually, within 5 seconds of circulatory arrest, prior to
activation of sympathetic reflexes.

**Blood Gas Determination**

Arterial and venous blood samples (150 μl) were withdrawn through the
indwelling catheters for determination of blood gases using an i-STAT 1 blood gas
analyzer (i-STAT Corporation, East Windsor, NJ).

**Experimental protocols**

Protocol 1: Effects of 8-OH-DPAT and AVP on MCFP sympathetic activity during
hemorrhagic shock

Rats were randomly assigned to 3 experimental groups on the day of experiment.
Unanesthetized, unrestrained rats were connected to the recording instruments and
allowed to habituate for at least 30 min at normal room temperature (22-23 °C).
Hemorrhage was initiated by withdrawal of arterial blood for 6 min at 3.2 ml/kg/min. The
withdrawal rate was subsequently reduced to 0.53 ml/kg/min for an additional 4 minutes.
Additional withdrawal of small volumes (50-150 μL) of blood was performed as needed to maintain MAP at 50 mmHg for an additional 15 min. Blood withdrawal was discontinued 25 min after the initiation of hemorrhage, after which rats were given either an injection of 8-OH-DPAT (30 nmol/kg, or 9.84 μg/kg/150 μL, iv), or continuous infusion of isotonic saline (33 μL/kg/min, iv). The third group was given arginine-vasopressin (AVP) at a rate titrated to match the pressor effect of 8-OH-DPAT (~ 2 ng/kg/min, iv) for 35 min. Mean circulatory filling pressure was measured 10 min prior to, as well as 20, 30, 40, 50 and 60 min after initiation of hemorrhage. Blood gas measurements were taken at baseline (15 min prior to hemorrhage), as well as 25 and 60 min after initiation of blood withdrawal. At the end of experiment, all animals were given an injection of hexamethonium chloride (30 mg/kg, iv). Renal sympathetic nerve activity was recorded for an additional 2 min for determination of background noise. The rats were subsequently euthanized with sodium pentobarbital (200 mg/kg, iv).

Protocol 2: Effect of splenectomy on cardiovascular responses to 8-OH-DPAT

On the day of experiment, unanesthetized, unrestrained rats previously subjected to sham- or real splenectomy were connected to the recording instruments while resting in their home cage. They were allowed to habituate for at least 30 min prior to the experiment. Baseline measurements of MCFP were taken 10 min prior to blood withdrawal. Hemorrhage was initiated as described in protocol 1. At the end of the 25-minute hemorrhage period, rats were given either isotonic saline (200 μL, iv) or 8-OH-DPAT (30 nmol/kg, or 9.84 μg/kg, in 200 μL of saline, iv). Repeated measurements of MCFP were taken every 10 min beginning 20 min after initiation of hemorrhage until the
end of the experiment (60 min), after which the rats were euthanized with sodium pentobarbital (200 mg/kg, iv). Spleens of the sham-operated rats were immediately removed and weighed.

Data Analysis

Arterial blood pressure, HR and RSNA were averaged over 30-sec intervals for analysis. The average RSNA was normalized to baseline activity (% baseline) recorded for 10 min prior to the beginning of blood withdrawal. In the first study, data collected during blood withdrawal were pooled across all groups and analyzed using one-way analyses of variance (ANOVA) with repeated measures followed by Dunnett’s post-hoc tests for determination of differences from baseline. Two-way ANOVA with repeated measures were used to compare the effects of saline, 8-OH-DPAT and AVP on MAP, HR, and RSNA as well as blood gases, hematocrit and plasma protein concentration from the time of drug intervention to the end of the experiment, 60 min after the start of hemorrhage. Significant interactions between treatment and time were followed by a Dunn’s (Bonferroni) post-hoc test to compare between groups at each time point. In the second study, data collected during blood withdrawal were pooled into sham-operated and splenectomized groups, and the effect of splenectomy on MAP, HR and MCFP during hemorrhage (from time 0 to 25 min after initiation of blood withdrawal) was analyzed using 2-way ANOVA with repeated measures. Effects of 8-OH-DPAT from 25 to 60 min were assessed using 3-way ANOVA with repeated measures followed by Dunn’s (Bonferroni) post-hoc test for multiple comparisons.
RESULTS

Protocol 1: Effects of 8-OH-DPAT and AVP on sympathetic activity during hemorrhagic shock

The initial 10-min blood withdrawal produced characteristic compensatory increases in HR and RSNA followed by a rapid onset of bradycardia and sympathetic withdrawal that was paralleled by hypotension (Figure 1). Removal of variable amounts of blood was required to maintain MAP at 50 mmHg over the subsequent 15 min recording period. Total blood loss did not differ between groups (Table 1). Heart rate and RSNA began to recover prior to drug intervention. Central venous pressure fell steadily throughout the period of blood withdrawal.

Blood pressure rose slightly to ~60 mmHg in the saline-treated group within 5 min of termination of blood withdrawal and remained there for the duration of the recording period. Bolus injection of 8-OH-DPAT produced an immediate pressor response. Mean arterial pressure rose to ~80 mmHg within 5 min of injection, but continued to increase slowly over the next 15 min, eventually reaching a plateau of just under 90 mmHg. Central venous pressure also tended to increase after 8-OH-DPAT injection. Repeated measures analysis of CVP demonstrated a main effect of treatment, but the group means did not differ significantly at any one time-point. A between group comparison of mean CVP averaged over the entire post-treatment period demonstrated significantly higher CVP in 8-OH-DPAT-treated rats compared to controls (-0.78 ± 0.01 vs. -1.56 ± 0.04 mmHg; \(P<0.01\)). Infusion of AVP successfully replicated the arterial blood pressure effect of 8-OH-DPAT injection, resulting in the same large initial pressor effect followed by a slow rise and plateau by 25 min after hemorrhage termination.
Arginine vasopressin infusion had a variable effect on CVP with values fluctuating between those of saline- and 8-OH-DPAT-treated groups. Heart rate was unaffected by any treatment, but tended to decline throughout the reminder of the experiment in all groups.

8-OH-DPAT administration produced a rapid increase in RSNA that reached a maximum within the first 5 minutes of injection and then gradually waned over the duration of the post-treatment period. In contrast, neither saline- nor AVP infusion had any appreciable effect on sympathetic activity.

Inflation of the atrial balloon produced a characteristic fall in arterial pressure and an increase in CVP. As shown in Figure 2, pressure plateaus were achieved several seconds prior to reflex sympathetic activation in all groups. As such, measures of MCFP were not confounded by group differences in reflex sympathetic activation. Hemorrhage caused a significant decrease in MCFP (Figures 3). Saline infusion had virtually no effect on MCFP, while 8-OH-DPAT produced a prominent increase in MCFP that remained above that of saline-treated rats throughout the experiment. Arginine-vasopressin infusion produced a slight rise in MCFP that did not differ from that of saline-treated rats and remained below that of 8-OH-DPAT-treated animals (Figure 3). Within group analyses comparing the 20 min time point against all subsequent time points demonstrated significant effects for both 8-OH-DPAT \((P<0.01)\) and AVP \((P<0.05)\). The rise in MCFP after 8-O-DPAT treatment was significant throughout the post-treatment period. In contrast, the rise with AVP was only significant at the 60 min time point.
Progressive blood loss caused the development of primary metabolic acidosis as evidenced by decreased blood pH and reduced bicarbonate reserve (base excess) together with accumulation of lactate (Table 2). Changes in acid-base balance were paralleled by compensatory respiratory alkalosis, manifested by a substantially increased arterio-venous difference in pH, increased arterial blood oxygen saturation and decreased arterial PCO₂ (data not shown). This caused a shift on the Davenport diagram toward a primarily metabolic acidosis with a compensatory respiratory alkalosis after 25 min of hemorrhage (Figure 4). Over the next 35 min, rats treated with saline progressed to a more severe metabolic acidosis that was not sufficiently compensated by respiration. 8-OH-DPAT significantly reduced the severity of metabolic acidosis, as indicated by the lack of further pH decrease or lactate accumulation, as well as by a slight recovery of bicarbonate levels (Table 2). Infusion of AVP also provided some protection against the progression of metabolic acidosis. However, the effects of AVP were not as significant as those of 8-OH-DPAT, as evidenced by the lower venous pH in AVP-treated animals and lactate levels that did not differ significantly from saline-treated rats (Table 2). Both hematocrit and plasma protein fell significantly during the course of the experiment. However, there was no difference between groups (Table 1).

Protocol 2: Effect of splenectomy on response to 8-OH-DPAT

Splenectomy had no effect on changes in MAP or HR during the first 10 min of fixed-rate blood withdrawal (Figure 5). During the controlled hypotensive period (from 10 to 25 min after initiation of hemorrhage), CVP in sham-operated animals tended to be higher than in splenectomized animals but the difference did not reach significance. 8-
OH-DPAT produced a characteristic robust increase in arterial pressure that persisted throughout the experiment in intact rats. Splenectomized rats also demonstrated an initial pressor response to 8-OH-DPAT that was similar in amplitude to that of intact rats. However, the effect began to dissipate 25 min after drug administration. Mean arterial pressure of splenectomized animals treated with 8-OH-DPAT remained elevated above that of saline-treated splenectomized rats throughout the experiment.

Heart rate was not affected by 8-OH-DPAT injection in either group. Though highly variable, CVP tended to decline throughout the remainder of the experiment in all groups except sham-operated rats given 8-OH-DPAT. In this group, CVP rose progressively throughout the remainder of the experiment and became significantly elevated compared to splenectomized rats treated with 8-OH-DPAT and sham-operated controls given saline. Mean circulatory filling pressure decreased 6-fold following hemorrhage. 8-OH-DPAT produced a rapid increase in MCFP in sham-operated rats that was maintained for the duration of the experiment (35 min). The effect was completely abolished by splenectomy (Figure 6).

Analyses of blood gases confirmed the development of a severe metabolic acidosis 60 min after hemorrhage. The development of metabolic acidosis was not significantly influenced by prior splenectomy (Figure 7). Blood pH was somewhat more variable in the second study. Thus, the beneficial effect of 8-OH-DPAT on the progression of acidosis was not as prominent as that observed in the first experiment. However, the beneficial effect of 8-OH-DPAT on base excess in sham-operated rats remained robust. A similar trend was observed in splenectomized rats, though the effect was not significant. 8-OH-DPAT also significantly attenuated a further rise in lactate.
Prior splenectomy almost completely abolished the attenuation of lactate by 8-OH-DPAT.

Hematocrit and plasma protein concentration decreased throughout hemorrhage, and remained similar between groups (Table 4). At the end of the experiment, spleen weight was reduced in rats given 8-OH-DPAT compared to those given saline, both in absolute wet weight and when calculated as a ratio to body weight (Table 5).

**DISCUSSION**

These studies demonstrate that the 5-HT\textsubscript{1A} receptor agonist, 8-OH-DPAT, produces a dramatic rise in sympathetic drive among rats in hypovolemic shock. The sympathetic response was rapid in onset, but declined subsequently, in contrast to increases in MCFP and arterial pressure, which persisted for the duration of the experiment. These studies also demonstrate that AVP infusion produces a modest increase in MCFP among rats in hypovolemic shock. These studies are also the first to demonstrate that an intact spleen is necessary for the rise in MCFP and improved blood gases produced by 5-HT\textsubscript{1A} receptor agonist administration in rats subjected to hypovolemic shock.

In a prior study, we found that ganglionic blockade prevented the rise in MCFP generated by 8-OH-DPAT. Ganglinoic blockade also attenuated the pressor effect of the drug, particularly the prolonged pressor effect that continued to develop 15 min after drug administration (41). These findings, coupled with the immediate robust sympathoexcitatory effect of 8-OH-DPAT observed here are consistent with the view that sympathetic activation accounts for the early elevation in MCFP, and likely some of
the early pressor response observed with the drug. These findings also suggest that the early sympathoexcitatory effect of 8-OH-DPAT may mobilize sufficient unstressed blood volume to facilitate the initial rise in MCFP, and that the waning, though still elevated sympathetic activity is sufficient to maintain MCFP and arterial pressure throughout the recovery period.

In our previous study, blockade of α₂-adrenergic receptors with the hydrophilic antagonist, L-659,066, suppressed 8-OH-DPAT-induced MCFP and blood pressure responses in a manner very similar to ganglionic blockade, suggesting that α₂-adrenergic receptors in the vasculature mediate a significant portion of sympathetic response to 8-OH-DPAT. However, in a prior study, we found that epinephrine infusion had no effect on MCFP and a somewhat detrimental effect on tissue perfusion in our shock model despite the well characterized agonist effect of the hormone at α-adrenergic receptors (6). Epinephrine also has significant agonist activity at β-adrenergic receptors, and β-adrenergic receptor activation can produce splenic- and venodilation (1). Therefore, a concurrent activation of venous β-adrenergic receptors may have counteracted the vеноconstrictor effects of epinephrine and prevented extensive blood mobilization by α₂-adrenergic receptor activation. Epinephrine also stimulates lactate production through activation of β₂-adrenergic receptors and can worsen acid-base balance even in the absence of reduced tissue perfusion (23). Together these findings suggest that neural release of norepinephrine probably contributes to 8-OH-DPAT-induced vеноconstriction and attenuation of metabolic acidosis to a greater extent than does increased circulating epinephrine. At the same time, sympathetic activation of epinephrine release could conceivably have contributed to stimulation of the high density β-adrenergic receptor
population found in the terminal splanchnic venous vasculature (e.g., the portal vein) and thereby reduced resistance to blood mobilization from splanchnic blood stores during norepinephrine release (5).

We hypothesized that the venoconstrictor capacity of 8-OH-DPAT mediated the improved tissue perfusion observed with drug administration (40). Therefore, we reasoned that AVP would have little beneficial effect on blood gases given that it has only a limited constrictor effect on veins (2,39). Instead, AVP infusion slightly raised MCFP following hemorrhage and had a similar limited, but overall beneficial effect on blood gases. The mechanism by which AVP increased MCFP was not apparent. Vasopressin infusion conceivably could have had a modest effect on MCFP by activating sympathetic drive to veins. However, in the present study, AVP had virtually no effect on sympathetic activity or HR. Moreover, there is little evidence for a sympathoexcitatory effect of AVP at sites accessible from the systemic circulation. Nevertheless, AVP has recently gained attention as one of the few interventions that can successfully improve perfusion pressure in vasodilatory or decompensating hemorrhagic shock (16). The mechanism of action is not well understood but may result from inactivation of K+ currents that normally develop in vascular smooth muscle cells during metabolic acidosis (18). Reversal of these K+ currents have been suggested as a means by which vascular sensitivity to catecholamines is re-established by AVP infusion in decompensated shock (25). In the present study, drug intervention was begun prior to decompensation. Indeed, the vasopressor response to 8-OH-DPAT was still quite pronounced 25 min after the start of hemorrhage. Nevertheless, metabolic acidosis was established at this point. Thus, it is likely that some degree of vascular insensitivity
developed by the time drug intervention was employed. In fact, the pressor effect of 8-
OH-DPAT observed in the current study appeared to be attenuated (~30 vs. ~ 55 mm Hg) compared to responses in rats subjected to a smaller volume (~15% blood volume) hemorrhage in our prior studies (28,34). As such, AVP may have raised MCFP by augmenting the sensitivity of the venous vasculature to vasoconstrictors present at the time of drug intervention.

In normovolemic animals, veins contain up to 70 % of total blood volume and serve as a blood reservoir due to their large capacitance (9,33). Splanchnic veins, liver and spleen are important blood storage depots in some, but not all, mammalian species (10,32). Splanchnic vasoconstriction and splenic contraction provide potent mechanisms that promote mobilization of blood volume during vigorous physical exercise or blood loss (14,17,22,33). Splenic contraction and blood mobilization play an important role in homeostasis during blood loss in dogs and horses. In contrast, the human and rat spleen were believed to have little impact on circulating blood volume because of their relatively small size and weak contractile capacity (8,32). Nevertheless, detrimental hemodynamic effects of splenectomy have been observed during compensation from hemorrhage in anesthetized rats (11,15). In the present study, splenectomy had no effect on either the hypotensive or bradycardic response to the initial fixed-rate blood withdrawal. Moreover, the blood volume loss required to induce and maintain hypotension at 50 mmHg over the remaining 15 minutes did not differ between groups. These observations suggest that the spleen has a negligible role in maintaining arterial pressure during the initial compensatory response to blood loss in the unanesthetized rat. This may be due to a delayed sympathetic engagement of the spleen in our model of hemorrhagic shock.
Indeed, in the present study, sympathetic activity remained depressed throughout most of the hypotensive period and only began to recover between 20 and 25 min after the start of hemorrhage.

The splanchnic vasculature, including that of the spleen, houses the majority of unstressed blood volume in the rat. Assessment of the effects of hemorrhage and 8-OH-DPAT on sympathetic-mediated mobilization of unstressed volume might be best studied using splanchnic, rather than the renal sympathetic nerve recordings as performed here. However, measurements of splanchnic sympathetic activity in the conscious rat are complicated by the proximity of the accessible splanchnic nerves to the diaphragm. Thus the recordings often contain significant diaphragmatic EMG noise. In a limited number of experiments in conscious animals, we assessed splanchnic nerve activity during expiration when phrenic activity was silent (data not shown). Our evidence indicates that the activity of splanchnic and renal sympathetic nerves parallel one another during hemorrhage and subsequent 8-OH-DPAT administration and subsequent recovery. Thus, we believe that renal sympathetic activity serves as a reasonable index of the pattern of sympathetic drive to the splanchnic vasculature.

By the end of the experiment, spleen weight was reduced by 40% compared to spleens taken during splenectomy surgery suggesting that approximately 0.5 ml of blood was autotransfused from the spleen during the course of the experiment. The further decrease in splenic weight among hemorrhaged rats given 8-OH-DPAT represents an additional 0.2 ml of blood mobilization. Even if splenic hematocrit were to reach 97%, as has been reported in humans, the addition of 0.2 – 0.5 ml of high hematocrit blood into the systemic circulation would cause only a very modest change in hemoglobin and total
O₂-carrying capacity (38). Thus, autotransfusion of blood from the spleen alone would be unlikely to produce the significant hemodynamic and metabolic improvements observed after 8-OH-DPAT administration in intact animals. Moreover, in the rat most of the blood reserve resides in the extrasplenic area of splanchnic circulation primarily in splanchnic veins and liver (26,33). Redistribution of blood from extrasplenic regions likely contributed to the hemodynamic effects of 8-OH-DPAT in the current study as splenectomized-rats given drug continued to maintain elevated pressure throughout the experiment. Such redistribution must have been mediated primarily by arterial constriction and probably reduced flow to the gut since pressure remained elevated after 8-OH-DPAT in the absence of any change in MCFP. Such findings suggest that the spleen may be an important determinant in the pattern of sympathetic-mediated vasoconstriction of the venous and arterial vasculature.

Previous work by others has demonstrated an important role for splenic baroreceptors in the reflex control of cardiovascular hemodynamics. Splenic denervation produces a progressive decline of arterial pressure in rats and reduces reflex increases in cardiac output during hypoxia in anesthetized dogs (7,21). Increased splenic venous pressure in rats stimulates splenic afferent nerve activity resulting in reflex increases in renal sympathetic efferent activity, renin release and reduced renal blood flow (7,12,24). Similarly, stimulation of splenic baroreceptors produces increases in cardiac and renal sympathetic efferent activity that are paralleled by increased cardiac contractility, HR and blood pressure in dogs (13). Together, the literature indicates that splenic denervation and thus splenectomy can disable important reflex mechanisms that control cardiovascular homeostasis.
The possibility that a spleen-dependent mechanism contributed to the slow mobilization of blood stores is supported by the rather late onset of the blood pressure decline observed in splenectomized-rats treated with vehicle. Similarly, splenectomized-rats treated with 8-OH-DPAT showed a late decline in MAP that only reached significance 30 min after termination of hemorrhage. In contrast, the immediate pressor effect of 8-OH-DPAT was unaffected by splenectomy, a finding that confirms that the rapid pressor effect of the drug is due to a direct activation of vascular adrenergic receptors rather than to rapid mobilization of blood stores.

Whether 8-OH-DPAT-dependent increases in sympathetic activity contribute to increased splenic venous pressure and reflex afferent activity remains to be determined. Evidence that 8-OH-DPAT increases splenic constriction (i.e., reduces spleen volume) during increased total body venous tone suggests that splenic venous pressure may increase following drug administration. Evidence that activation of the spleno-renal reflex reduces renal blood flow in normovolemic animals suggests that splenic afferent activity reflexively increases sympathetic-mediated arterial resistance. In contrast, our previous work has shown that 8-OH-DPAT increases renal arterial conductance and does not influence overall peripheral resistance in our shock model. Nevertheless, it is plausible that the cardiovascular effects of splenic reflex activation is primarily mediated by vasoconstriction during early hypovolemia when existing arterial resistance is already elevated by vasoconstrictor hormones and sympathetic drive to the venous vasculature remains low. At this point, mobilization of the unstressed blood volume by vasoconstriction may have a greater impact on perfusion pressure than redistribution of arterial blood flow. Thus, splenic reflexes may contribute to the relatively slow
mobilization of blood stores from the splanchnic region to mediate the prolonged increase in both MCFP (through increased effective blood volume), and arterial pressure (through increased cardiac output). At present, the effect of splenoreflex activation on venous tone is not known. Moreover, it is not known whether an increase in sympathetic drive to extrasplenic veins parallels the increase in renal sympathetic activity observed here.

The receptor population that mediates the sympathoexcitatory effect of 8-OH-DPAT in hemorrhaged animals is not known. Previously we showed that low dose 8-OH-DPAT (1 μg) produced a robust and rapid sympathoexcitatory effect in hemorrhaged-rats when administered into the cisterna magna, while the same dose of drug had no effect when given systemically (34). 8-OH-DPAT readily crosses the blood brain barrier and has significant agonist activity at 5-HT1A and 5-HT7 serotonergic receptors (4,19). Our earlier work also showed that the selective 5-HT1A receptor agonist, WAY100635, dose dependently reversed the ability of centrally administered 8-OH-DPAT to maintain blood pressure during hemorrhage (35). In recent work, WAY100635 was unable to affect behavioral responses attributed to the selective 5-HT7 agonist AS 19 (31). Together, the available data favor the probability that 8-OH-DPAT acts through 5-HT1A receptors in the central nervous system to mediate its sympathoexcitatory effects. The precise region where 8-OH-DPAT acts to mediate these effects remains to be determined.

PERSPECTIVES

Together with our previous studies, results from the current work indicate that a 5-HT1A receptor agonist-mediated stimulation of sympathetic activity produces a very
different and most likely superior hemodynamic response following blood loss than does infusion of direct vasopressor agents if administered prior to decompensation in animals with an intact spleen (40,41). During decompensation the vasculature becomes unresponsive to further adrenergic stimulation (44). Given that sympathetic neurotransmission is necessary to produce the beneficial effect of 8-OH-DPAT, it is doubtful that 5-HT₁A receptor agonists would be effective when administered following decompensation, particularly when unstressed volume has been depleted. It is tempting to speculate that 5-HT₁A receptor agonists might provide a beneficial hemodynamic pattern following decompensation if administered in conjunction with AVP and volume resuscitation. However, it should be noted that in normovolemic animals, 8-OH-DPAT and other 5-HT₁A agonists produce only a transient sympathoexcitatory effect that is quickly followed by a sustained sympathoinhibition (27,29,37). These findings suggest that the sympathoexcitatory effect of 5-HT₁A receptor agonists observed during hypovolemia is due either to augmentation of some sympathoexcitatory effect-, or disruption of a sympathoinhibitory effect that develops specifically during hypovolemia. As such, 5-HT₁A receptor agonists may not provide a significant beneficial cardiovascular effect after full volume resuscitation. Instead, such drugs might be better used to enhance hemodynamic responses when given prior to volume resuscitation, or when given after limited volume resuscitation. In fact, limited volume resuscitation has been shown to reduce reperfusion injury and increase survival in animal models of uncontrolled hemorrhage (37). The results herein indicate that the efficacy of 5-HT₁A receptor agonists in such models will likely depend on the integrity of the spleen and possibly its afferent projections.


7. **Deng Y and Kaufman S.** Splenorenal reflex regulation of arterial pressure.  


40. **Tiniakov R, Osei-Owusu P and Scrogin KE**. The 5-hydroxytryptamine1A receptor agonist, (+)-8-hydroxy-2-(di-n-propylamino)-tetralin, increases cardiac


Figure Captions

Figure 1. Mean arterial pressure (MAP), heart rate (HR), change in renal sympathetic nerve activity (RSNA), and central venous pressure (CVP) during hemorrhage (gray box) and subsequent treatment with 8-OH-DPAT, saline, or AVP. Data are group means ± SEM. ** P<0.01, 8-OH-DPAT vs. Saline; ^^ P<0.01, AVP vs. Saline; ## P<0.01, 8-OH-DPAT vs. AVP; Gray and white symbols represent within group difference of P<0.05, and P<0.01 respectively vs. baseline.

Figure 2. Representative recordings of typical arterial pressure (AP), CVP, RSNA and integrated RSNA responses during balloon inflation before (A), and 15 min after hemorrhage termination and start of treatment in rats given saline (B), 8-OH-DPAT (C) or AVP (D). Horizontal bar indicates the duration of full balloon inflation. Gray shaded area indicates segment of recording from which measurements were taken for the determination of MCFP.

Figure 3. Mean circulatory filling pressure (MCFP) at baseline (-10 min), during hemorrhage (20 min) and during treatment with saline, 8-OH-DPAT or AVP. Data are group means ± SEM. ** P<0.01, 8-OH-DPAT vs. saline; ## P<0.01, 8-OH-DPAT vs. AVP; ^^ P<0.01, within group vs. 8-OH-DPAT at 20 min, †P<0.05, within group vs. AVP at 20 min.
**Figure 4.** Davenport diagram demonstrating temporal changes in acid-base balance of arterial (A) and venous blood (B) of rats subjected to hemorrhage and subsequently treated with saline, 8-OH-DPAT or AVP. Data are group means ± SEM.

**Figure 5.** Mean arterial pressure, HR and CVP in splenectomized (SplX) and sham-operated (Sham) rats during hemorrhage (gray box) and subsequent treatment with saline or 8-OH-DPAT. Data are group means ± SEM. ** P<0.01, Sham-8-OH-DPAT vs. Sham-Saline; ## P<0.01, SplX-8-OH-DPAT vs. SplX-Saline; † † P<0.05, 0.01, Sham-8-OH-DPAT vs. SplX-8-OH-DPAT.

**Figure 6.** Mean circulatory filling pressure at baseline (-10 min), during hemorrhage (20 min) and after treatment with saline or 8-OH-DPAT in splenectomized (SplX) and sham-operated rats (Sham). Data are group means ± SEM. ** P<0.01, Sham-8-OH-DPAT vs. Sham-Saline; ‡ ‡ P<0.01, Sham-8-OH-DPAT vs. SplX-8-OH-DPAT.

**Figure 7.** Davenport diagram demonstrating temporal changes in acid-base balance in arterial (A) and venous blood (B) of splenectomized- (SplX) and sham-operated rats (Sham) subjected to hemorrhage and subsequent treatment with saline or 8-OH-DPAT. Data are group means ± SEM.
Table 1. Total blood loss and temporal changes in hematocrit and plasma protein concentration over the course of the protocol 1.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Total Blood Loss (ml/kg)</th>
<th>Hematocrit (%) BL</th>
<th>Hematocrit (%) 60 min</th>
<th>Plasma Protein (g/dL) BL</th>
<th>Plasma Protein (g/dL) 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (12)</td>
<td>40.2 ± 0.9</td>
<td>36 ± 1</td>
<td>27 ± 1**</td>
<td>5.5 ± 0.2</td>
<td>4.5 ± 0.1**</td>
</tr>
<tr>
<td>8-OH-DPAT (13)</td>
<td>39.1 ± 0.7</td>
<td>37 ± 1</td>
<td>28 ± 1**</td>
<td>5.5 ± 0.1</td>
<td>4.6 ± 0.1**</td>
</tr>
<tr>
<td>AVP (12)</td>
<td>40.2 ± 0.5</td>
<td>38 ± 1</td>
<td>26 ± 1**</td>
<td>5.4 ± 0.2</td>
<td>4.3 ± 0.1**</td>
</tr>
</tbody>
</table>

Baseline (BL), 60 min after the start of hemorrhage (60 min). Data are group means ± SEM. Group n are shown in parentheses. ** P<0.01 vs. BL.
Table 2. Effects of hemorrhage and subsequent treatment with saline, 8-OH-DPAT or AVP on acid-base balance

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TIME (min)</th>
<th>TIME (min)</th>
<th>pH arterial</th>
<th>pH venous</th>
<th>PO₂ (mmHg) arterial</th>
<th>PO₂ (mmHg) venous</th>
<th>BE (mmol/L) arterial</th>
<th>BE (mmol/L) venous</th>
<th>Lactate (mmol/L) venous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (pooled)</td>
<td>25</td>
<td>60</td>
<td>7.50 ± 0.01</td>
<td>7.48 ± 0.01</td>
<td>77.0 ± 1.8</td>
<td>36.7 ± 1.4</td>
<td>8.2 ± 0.5</td>
<td>9.4 ± 0.5</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Saline (9)</td>
<td>25</td>
<td>60</td>
<td>7.47 ± 0.01</td>
<td>7.35 ± 0.02</td>
<td>97.1 ± 2.5</td>
<td>27.6 ± 2.4</td>
<td>-5.7 ± 1.1</td>
<td>-3.8 ± 1.0</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td>8-OH-DPAT (9)</td>
<td>25</td>
<td>60</td>
<td>7.45 ± 0.02</td>
<td>7.35 ± 0.02</td>
<td>97.2 ± 2.4</td>
<td>26.1 ± 1.7</td>
<td>-5.4 ± 1.4</td>
<td>-2.0 ± 1.2</td>
<td>9.0 ± 0.8</td>
</tr>
<tr>
<td>AVP (8)</td>
<td>25</td>
<td>60</td>
<td>7.44 ± 0.01</td>
<td>7.32 ± 0.03</td>
<td>101.4 ± 3.5</td>
<td>24.6 ± 2.1</td>
<td>-4.9 ± 1.1</td>
<td>-3.8 ± 1.5</td>
<td>9.1 ± 1.0</td>
</tr>
</tbody>
</table>

Baseline values are means pooled across all groups and are shown for comparison only. Values are group means ± SEM 25 and 60 min after start of hemorrhage. Group n are shown in parentheses. *,**, P<0.05, 0.01 vs. 25 min within group; #,## P<0.05, 0.01 vs. Saline; † P<0.05 vs. AVP.
Table 3. Effects of the hemorrhage and subsequent treatment with saline or 8-OH-DPAT on acid-base balance in splenectomized (Splx) and sham-operated rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TIME (min)</th>
<th>pH arterial</th>
<th>pH venous</th>
<th>PO₂ arterial</th>
<th>PO₂ venous</th>
<th>BE (mmol/L) arterial</th>
<th>BE (mmol/L) venous</th>
<th>Lactate (mmol/L) venous</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline (Sham)</strong></td>
<td></td>
<td>7.49 ± 0.01</td>
<td>7.47 ± 0.01</td>
<td>80.7 ± 1.2</td>
<td>37.1 ± 1.1</td>
<td>6.4 ± 0.6</td>
<td>8.9 ± 0.6</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Sham – Saline (6-8)</td>
<td>25</td>
<td>7.43 ± 0.01</td>
<td>7.26 ± 0.04</td>
<td>103.8 ± 2.1</td>
<td>20.6 ± 1.7</td>
<td>-8.9 ± 1.6</td>
<td>-6.8 ± 1.9</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.33 ± 0.04</td>
<td>7.18 ± 0.06</td>
<td>116.4 ± 3.5</td>
<td>22.2 ± 2.4</td>
<td>-14.7 ± 2.5</td>
<td>-12.4 ± 2.6</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>Sham – 8-OH-DPAT (8-9)</td>
<td>25</td>
<td>7.44 ± 0.01</td>
<td>7.36 ± 0.02</td>
<td>106.6 ± 9.7</td>
<td>27.9 ± 1.3</td>
<td>-6.1 ± 1.1</td>
<td>-2.8 ± 1.2</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.37 ± 0.06</td>
<td>7.32 ± 0.07</td>
<td>107.0 ± 5.5</td>
<td>31.1 ± 1.6</td>
<td>-6.5 ± 3.2**</td>
<td>-3.9 ± 3.3*</td>
<td>7.6 ± 1.7**#</td>
</tr>
<tr>
<td><strong>Baseline (SplX)</strong></td>
<td></td>
<td>7.49 ± 0.01</td>
<td>7.47 ± 0.01</td>
<td>82.9 ± 1.4</td>
<td>38.0 ± 1.1</td>
<td>6.5 ± 0.5</td>
<td>8.6 ± 0.5</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>SplX – Saline (7-8)</td>
<td>25</td>
<td>7.44 ± 0.01</td>
<td>7.30 ± 0.03</td>
<td>101.3 ± 3.3</td>
<td>22.0 ± 1.7</td>
<td>-10.1 ± 1.5</td>
<td>-7.1 ± 1.5</td>
<td>10.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.28 ± 0.07</td>
<td>7.15 ± 0.08</td>
<td>116.0 ± 4.6</td>
<td>32.7 ± 11.0</td>
<td>-15.4 ± 2.8</td>
<td>-14.4 ± 3.3</td>
<td>14.0 ± 1.6</td>
</tr>
<tr>
<td>SplX – 8-OH-DPAT (8-9)</td>
<td>25</td>
<td>7.43 ± 0.01</td>
<td>7.32 ± 0.03</td>
<td>102.6 ± 3.3</td>
<td>27.4 ± 2.5</td>
<td>-8.4 ± 1.2</td>
<td>-5.8 ± 1.3</td>
<td>9.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.36 ± 0.05</td>
<td>7.23 ± 0.07</td>
<td>117.6 ± 6.4</td>
<td>25.1 ± 2.3</td>
<td>-11.7 ± 3.0</td>
<td>-9.7 ± 3.1</td>
<td>11.4 ± 1.5</td>
</tr>
</tbody>
</table>
Baseline values are means pooled across all groups and are shown for comparison only. Values are group means ± SEM 25 and 60 min after start of hemorrhage. Group n are shown in parentheses. *,**, $P<0.05$, 0.01 vs. 25 min within group; #,# $P<0.05$, 0.01 vs. Saline; $^+$ $P<0.05$ vs. AVP.
Table 4. Total blood loss and changes in hematocrit and plasma protein concentration in splenectomized and sham-operated animals hemorrhaged to shock and treated with saline or 8-OH-DPAT

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Total Blood Loss (ml/kg)</th>
<th>Hematocrit (%)</th>
<th>Plasma Protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL 25 min 60 min</td>
<td></td>
<td>BL 25 min 30 min 60 min</td>
</tr>
<tr>
<td>SplX-Saline (11)</td>
<td>35.5 ± 0.5 46 ± 1</td>
<td>30 ± 1**</td>
<td>27 ± 1** 6.6 ± 0.1 4.6 ± 0.1** 4.3 ± 0.1**</td>
</tr>
<tr>
<td>SplX-8-OH-DPAT (11)</td>
<td>35.5 ± 0.7 47 ± 1</td>
<td>32 ± 1**</td>
<td>27 ± 1** 6.6 ± 0.1 4.8 ± 0.1** 4.3 ± 0.2**</td>
</tr>
<tr>
<td>Sham-Saline (11)</td>
<td>36.2 ± 0.7 46 ± 1</td>
<td>32 ± 2**</td>
<td>28 ± 1** 6.6 ± 0.2 4.7 ± 0.2** 4.4 ± 0.2**</td>
</tr>
<tr>
<td>Sham-8-OH-DPAT (11)</td>
<td>35.3 ± 0.6 48 ± 1</td>
<td>33 ± 1**</td>
<td>31 ± 1** 6.6 ± 0.2 4.8 ± 0.1** 4.6 ± 0.2**</td>
</tr>
</tbody>
</table>

BL – baseline, values at 25 and 60 min after the start of hemorrhage. Group n are shown in parentheses. ** P<0.01 vs. Baseline (BL) within group; +,++ P<0.05, 0.01 vs. 25 min within group.
Table 5. Effects of hemorrhage and 8-OH-DPAT injection on wet spleen weight and spleen-to-body weight ratio.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Body Weight (g)</th>
<th>Wet Spleen Weight (g)</th>
<th>Spleen/Body Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surgery</td>
<td>Experiment</td>
<td></td>
</tr>
<tr>
<td>Sham-Saline (11)</td>
<td>344 ± 2</td>
<td>342 ± 6</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>Sham- 8-OH-DPAT (11)</td>
<td>345 ± 9</td>
<td>350 ± 6</td>
<td>0.69 ± 0.04*</td>
</tr>
<tr>
<td>Splenectomized Rats (22)</td>
<td>348 ± 5</td>
<td>345 ± 5</td>
<td>1.16 ± 0.03</td>
</tr>
</tbody>
</table>

Spleens extracted during surgery are shown for comparison only. Group n are shown in parentheses. ** P<0.01 vs. Sham-Saline