Published in final edited form as:

J Trauma Acute Care Surg. 2013 January ; 74(1): 196–202. doi:10.1097/TA.0b013e31826fc747.

Hypertonic saline resuscitation enhances blood pressure recovery and decreases organ injury following hemorrhage in acute alcohol intoxicated rodents

Jesse K. Sulzer, PhD, Annie M. Whitaker, BS, and Patricia E. Molina, MD, PhD

LSUHSC Department of Physiology and Alcohol and Drug Abuse Center of Excellence New Orleans, LA

Abstract

Background—Acute alcohol intoxication (AAI) impairs the hemodynamic and arginine vasopressin (AVP) counter-regulation to hemorrhagic shock (HS) and lactated Ringer's (LR) fluid resuscitation (FR). The mechanism of AAI-induced suppression of AVP release in response to HS involves accentuated nitric oxide (NO) inhibitory tone. In contrast, AAI does not prevent AVP response to increased osmolarity produced by hypertonic saline (HTS) infusion. We hypothesized that FR with HTS during AAI would enhance AVP release by decreasing PVN NO inhibitory tone subsequently improving mean arterial blood pressure (MABP) and organ perfusion.

Methods—Male Sprague Dawley rats received a 15h alcohol infusion (2.5g/kg + 0.3 g/kg/h) or dextrose (DEX) prior to HS (40mmHg × 60 min) and FR with HTS (7.5%; 4ml/kg) or LR (2.4 × blood volume removed). Organ blood flow was determined and brains collected for NO content at 2h post-FR.

Results—HTS improved MABP recovery in AAI (109 vs 80mmHg) and DEX (114 vs 83mmHg) animals compared to LR. This was associated with higher (>60%) circulating AVP levels at 2h post-FR than those detected in LR animals in both groups. Neither AAI alone nor HS in DEX animals resuscitated with LR altered organ blood flow. In AAI animals, HS and FR with LR reduced blood flow to liver (72%), small intestine (65%), and large intestine (67%) compared to shams. FR with HTS improved liver (3-fold) and small intestine (2-fold) blood flow compared to LR in AAI-HS animals. The enhanced MABP response to HTS was prevented by pretreatment with a systemic AVP V1_a receptor antagonist. HTS decreased PVN NO content in both groups 2h post-FR.

Conclusions—These results suggest that FR with HTS in AAI results in removal of central NO inhibition of AVP, restoring AVP levels and improving MABP and organ perfusion in AAI-HS.

Address correspondence to: Patricia E. Molina, MD, PhD, LSUHSC Department of Physiology, 1901 Perdido Street, New Orleans, LA 70112-1393, Phone: 504-568-6171, Fax: 504-568-6158, pmolin@lsuhsc.edu.

Jesse Sulzer PhD: jsulze@lsuhsc.edu

Annie Whitaker BS: awhita@lsuhsc.edu

Patricia Molina MD, PhD: pmolin@lsuhsc.edu

The authors declare no conflicts of interest.

Author Contributions

JKS participated in literature search, study design, data collection, data analysis, data interpretation, writing, and production of figures. AMW participated in study design, data collection and writing. PEM participated in study design, data interpretation and writing.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

BACKGROUND

Traumatic injury and subsequent hemorrhagic shock (HS) is a leading cause of death (1). These rates are further increased in alcohol-intoxicated individuals with approximately 40% of trauma victims having positive blood alcohol concentrations (2). Complicating care for the intoxicated patient are alterations in initial assessments including decreased mean arterial blood pressure (MABP) (3-6).

Acute alcohol intoxication (AAI) dysregulates the response to HS including impaired hemodynamic stability during HS and FR (7). Impaired MABP recovery is associated with attenuated circulating levels of the counter-regulatory hormones arginine vasopressin (AVP), epinephrine, and norepinephrine (8, 9). Recently, we demonstrated that enhanced nitric oxide (NO) inhibitory tone in the hypothalamic paraventricular nucleus (PVN) contributes to the attenuated AVP levels following HS during AAI (10). A key additional finding was that AAI did not prevent the AVP response to osmotic stimulation with hypertonic saline (HTS), and furthermore, that this was associated with decreased PVN NO content. Thus, we predicted FR with HTS would reduce PVN NO content, and increase circulating levels of AVP following HS in AAI.

While clinical trials have failed to demonstrate benefit on 28-day survival, preclinical studies demonstrate that HTS improves survival, MABP recovery and tissue perfusion, and decreases organ injury (11-14). The potent vasopressor, AVP, is a critical mediator of the hemodynamic effects of HTS (15). AVP is elevated following HTS administration in normotensive animals and during shock states, and AVP V1_a receptor inhibition prevents the pressor response to HTS during endotoxic shock (16). Given that the response to osmotic stimulation of AVP release is not inhibited by AAI, we hypothesized that HTS could provide a physiologic approach to restore the normal AVP response and improve MABP recovery in the intoxicated host.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center and were performed in accordance with the guidelines of the National Institutes of Health. Specific pathogen-free, adult male Sprague-Dawley rats (250-275g) arrived to the institution and were housed in a controlled environment with a 12-h light/dark cycle and standard rat chow (Purina, St. Louis, MO) and water *ad libitum* for one week prior to surgical procedures.

Surgical Procedures

Animals were anesthetized with ketamine/xylazine (90 and 9mg/kg) and implanted with sterile vascular (carotid and jugular) and gastric catheters (PE50) using aseptic surgical technique as previously described (17). Animals were allowed 1-3 days to recover, were housed individually and provided food and water *ad libitum*.

Alcohol administration

On the evening prior to HS, conscious and unrestrained animals received an intra-gastric bolus of 30% alcohol (2.5 g/kg) followed by a 15-hour constant infusion of alcohol (300 mg/kg/hour) achieving plasma alcohol levels of 238 ± 10 mg/dl. This model mimics an episode of binge drinking, which has been shown to increase the risk of traumatic injury (18, 19). Time-matched controls received isocaloric/isovolumic dextrose (12.2g/kg).

Hemorrhagic Shock

Conscious and unrestrained animals were randomized to sham or HS and subjected to fixed-pressure HS as previously described (n=8-12/group as indicated in figure legends) (7). Briefly, blood was removed through the carotid catheter to reduce and maintain MABP between 40-50 mmHg during the 60-min HS period. MABP was monitored throughout the HS and resuscitation periods (Powerlab, AD Instruments, Colorado Springs, CO).

Fluid Resuscitation

Animals were randomly assigned to resuscitation with warmed HTS (7.5%; 4ml/kg over 10min) or LR (bolus of 40% total blood removed followed by 2x total blood removed over 60min). To determine the contribution of AVP to MABP recovery, a subset of animals received the V1_a antagonist [b-mercapto-b, b-cyclpentamethylenepropionyl¹, O-me-Tyr², Arg⁸]-vasopressin, (10ug/kg in ~0.25ml normal saline; Sigma Aldrich, St. Louis, MO) intravenously 5 min prior to FR. This dose was previously shown to inhibit the pressor effects of AVP following central neostigmine administration (9).

Organ Blood Flow

Organ blood flow was determined 2-hours post-FR by injection of 15µm fluorescent microspheres (Invitrogen, Carlsbad, CA). This time point was based on studies demonstrating differences in regional flow between resuscitation strategies at this time (20). Animals were anesthetized with ketamine/xylazine and a sterile catheter (PE50) placed in the left femoral artery. Microspheres suspended in 10% dextran and 0.01% Tween80 at a concentration of 1,000,000/ml were injected (~250,000/injection) into the carotid catheter over 20s. A blood sample was withdrawn from the femoral artery catheter with a pump (Harvard Apparatus, Holliston, MA) beginning 20s before microsphere infusion and continuing for 60s at a rate of 0.7ml/min. Saline was infused at 0.7ml/min through the carotid catheter immediately after microsphere infusion to replace blood lost. Five minutes after the microsphere injection, animals were euthanized by sodium pentobarbital overdose (125 mg/kg), organs harvested, weighed, and digested in ~8 ml of ethanolic KOH at 80°C. The microspheres were collected by centrifugation (2000xg for 10min) and washed twice prior to extraction of the fluorescent dye with 2-ethoxyethanolamine. The reference blood sample was processed using a similar approach as that used for tissues. Fluorescence intensity was measured on a plate reader (Tecan 2000) at an excitation of 540nm and emission of 560nm. Organ blood flow was calculated as:

$$\text{Organ blood flow (ml/min/100g tissue)} = (f_i/f_{\text{ref}}) \times 0.7\text{ml/min}$$

where f_i is the fluorescence of the tissue sample, and f_{ref} is the fluorescence of the reference blood sample.

Blood Sample Analysis

Blood was collected from the carotid artery catheter into chilled syringes washed with Aprotinin/Heparin. Plasma alcohol concentrations were determined using an Analox GM7 analyzer based on the oxidation of ethanol. Circulating alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined with a colorimetric reagent kit (Stanbio, Boerne, TX). BUN and creatinine were determined with colorimetric assay kits (BioAssay Systems, Hayward, California). Plasma AVP concentrations were measured using a rat-specific enzyme-linked immunoassay kit according to manufacturer's instructions (Phoenix Pharmaceuticals, Burlingame, CA).

PVN Nitrate/nitrite

The PVN was isolated and flash frozen as previously described (10). Tissue was homogenized on ice in PBS (100 μ l/mg tissue), centrifuged (10,000 rpm $\times$ 10min at 4°C) and the supernatant filtered using a 30kDa molecular weight cut off filter (Millipore, MA). Nitrate/nitrite was measured in the filtered supernatant using a colorimetric assay kit (Cayman Chemical, Ann Harbor, MI).

Myeloperoxidase (MPO) activity

Lung and small intestine (gut) were homogenized in 20mM phosphate/0.1mM EDTA, pH 7.4 and MPO content determined as previously described (7). MPO activity was expressed as the amount of enzyme necessary to produce a change in absorbance of 1.0/minute/gram of tissue.

Statistical Analysis

All data are presented as mean $\pm$ SEM with the number of animals indicated in the figure legends. In general, statistical comparisons were accomplished by one-way repeated measures analysis of variance (ANOVA), followed by Newman-Keul's test using Sigma Plot 11.0 statistical software. Statistical significance was accepted at $p < 0.05$.

RESULTS

HTS improved MABP recovery during AAI

Basal MABP was lower in AAI animals compared to dextrose-treated (113 ± 2 vs 121 ± 5 mmHg; $p = \text{NS}$). Similar MABP was achieved in all experimental groups during HS. FR with LR increased MABP in dextrose- and alcohol-treated HS animals (average of 36 ± 6 and 28 ± 4 mmHg above end-HS) (Figure 1). HTS significantly enhanced MABP compared to LR for the first 18 minutes in dextrose- and AAI-HS animals. Average MABP during resuscitation with HTS was higher in dextrose and AAI animals (average of 40 ± 5 and 52 ± 8 mmHg above end-HS).

Improved MABP recovery with HTS is mediated by AVP

Baseline AVP values were similar among groups (Figure 2). Circulating AVP levels were increased ~ 4 -fold at the end of HS. AVP levels were lower in AAI-HS (~ 3 -fold increase). AVP values decreased following LR in dextrose-treated animals to values similar to the AAI LR-treated animals (~ 2 -fold above baseline). Following HTS, AVP values remained at post-HS values in dextrose-treated and increased to ~ 4 -fold above baseline in AAI-HS animals. Antagonism of the $V1_a$ receptor prevented the HTS-induced rise in MABP in both dextrose- and AAI-treated animals without altering MABP recovery in LR-treated animals (Figure 1).

HTS decreased PVN NO content

PVN NO content was not altered by AAI alone in sham animals (Figure 3). HS and resuscitation with LR resulted in a significant increase in PVN NO content at 2-hours post-FR in both dextrose (33 ± 11 vs 1.8 ± 0.6 μ moles/g tissue) and AAI-animals (48 ± 10 vs 1.1 ± 0.2 μ moles/g tissue). PVN NO content was markedly reduced following HTS in dextrose (5 ± 1.8 vs 33 ± 11 μ moles/g tissue) and AAI (5 ± 1 vs 48 ± 10 μ moles/g tissue) as compared to LR.

HTS improved tissue perfusion in AAI-HS

Organ blood flow was not altered by AAI alone or by HS and FR with LR in dextrose-treated animals (Figure 4). In contrast, AAI-HS animals had reduced blood flow to the liver

(72% vs. sham; $p<0.05$), kidney (63% vs. sham; $p=NS$), small intestine (65% vs. sham; $p<0.05$), and large intestine (67% vs. sham; $p<0.05$). HTS increased blood flow to the liver (3-fold vs. LR; $p<0.05$), kidney (~3-fold vs. LR; $p=NS$), small intestine (2-fold vs. LR; $p<0.05$), and large intestine (1.8 fold vs. LR; $p=NS$) in AAI-HS animals. In contrast, HTS resuscitation in dextrose-HS animals decreased large intestine blood flow (43% vs. sham; $p<0.05$) without altering blood flow to other organs.

HTS improved markers of liver injury

Circulating levels of ALT were not altered by AAI alone or by HS in dextrose-treated animals regardless of FR (Table, Supplemental Digital Content 2). In contrast, ALT was significantly increased in AAI-HS animals (~4-fold above time-matched AAI-sham). The rise in ALT in AAI animals was prevented by HTS resuscitation. Circulating levels of AST were not significantly altered by AAI, HS, or AAI-HS (Table, SDC 2). Plasma creatinine levels were significantly elevated in both dextrose- and AAI-HS animals following LR and this was not altered by HTS (Table, SDC 2). BUN was not altered by HS in dextrose- or AAI-treated animals regardless of FR (Table, SDC 2).

HTS decreased organ neutrophil influx

AAI alone did not alter MPO content in lung or gut (Figure 5). HS did not alter MPO content in dextrose-treated animals. In contrast, lung (75% vs. dextrose-sham; $p<0.05$) and gut (73% vs. dextrose-sham; $p<0.05$) MPO content were significantly increased in AAI-HS animals resuscitated with LR. HTS resuscitation did not alter MPO content in dextrose-HS animals, but significantly reduced MPO content in the gut and attenuated the rise in lung MPO in AAI-HS animals.

DISCUSSION

The present study examined the impact of HTS resuscitation on AVP levels and hemodynamic recovery following HS during AAI. Previously, we demonstrated that AAI selectively disrupts signaling mechanisms controlling AVP release in response to HS without impairing the AVP response to HTS. Moreover, PVN NO inhibitory tone contributes to the AAI-mediated disruption of AVP release during HS (10). Because the response to HTS remained intact during AAI, we hypothesized that FR with HTS would overcome the NO inhibitory tone resulting from AAI, enhance AVP release, and improve MABP recovery and organ perfusion following HS. Indeed, this study showed that HTS enhanced AVP levels in both dextrose- and AAI animals. Additionally, the immediate pressor response to HTS was mediated by the AVP V_1 receptor. These results demonstrate that HTS enhanced MABP through elevated AVP, which we speculate resulted from decreased PVN NO inhibitory tone. In addition, restoration these effects were associated with improved MABP recovery and enhanced end organ perfusion in the AAI host.

AVP is a critical hormone contributing to the recovery of MABP during HS. Pharmacologic inhibition of the AVP V_1 receptor exacerbates hypotension during HS and resuscitation (21). Because AAI impairs the HS-induced rise in AVP, strategies aimed at enhancing AVP were predicted to improve MABP recovery. This hypothesis was supported by studies demonstrating benefits of exogenous AVP administration in MABP recovery following HS (22). While these studies suggest an important role for AVP in response to HS and indicate therapeutic potential for exogenous administration, pressor use can lead to complications including impaired organ perfusion (23, 24). Thus, we sought a physiologic approach to stimulate endogenous AVP release following HS during AAI. Our results support previous reports that the pressor response to HTS is mediated mainly by AVP (15). The role of AVP

in MABP recovery following HTS was confirmed using a V_{1a} AVP receptor antagonist which abolished the initial pressor effects of HTS.

A potential mechanism of the improved AVP response following HTS resuscitation is decreased PVN NO inhibitory tone. We previously demonstrated that the attenuated AVP response to HS during AAI results from enhanced NO inhibitory tone in the PVN (10). Furthermore, HTS decreased PVN NO content in normotensive and euvoletic AAI animals. Thus, we examined if HTS would decrease PVN NO content and improve circulating AVP levels following hemorrhage. In the present study, HTS decreased PVN NO content, suggesting a role for removal of NO inhibitory tone in the improved AVP response.

An important potential mechanism in addition to osmoreceptor- and baroreceptor-mediated release of AVP is modulation by angiotensin II (25, 26) Central AT_1 receptor antagonism prevents the increase in AVP following HTS administration (27). Angiotensin II enhances presynaptic inhibition of GABA and NO release; thus providing a potential mechanism by which HTS may reduce NO inhibitory tone (28). The impact of AAI on the central renin-angiotensin system as with other central signaling pathways potentially involved with the response to HTS is currently unknown and warrants future study.

Enhanced MABP recovery does not always directly improve tissue perfusion. HS causes a selective redistribution of blood flow from the splanchnic circulation to vital organs such as the heart and brain (29). In animal models, standardized LR resuscitation partially restores tissue perfusion (20) and vasopressor use produces varied effects (24, 30). In contrast, HTS improves tissue perfusion (31). Hyperosmolality induces pre-capillary dilation, and thus may improve tissue perfusion directly or prevent damage due to excess vasopressor stimulation (31). Direct effects of HTS on the microvasculature may also contribute to decreased organ injury. HTS reduces endothelial swelling increasing arteriole diameters and blood flow (32). Our results demonstrated that HTS restored hepatic blood flow and attenuated the fall in gut perfusion suggesting that the enhanced MABP recovery with HTS is not at the expense of end-organ perfusion.

Additional microvascular benefits of HTS resuscitation have been reported (33) Hemorrhage results in neutrophil influx into various organs (34). Exacerbation of this response is implicated in organ damage (35). AAI increases neutrophil content following injury or HS via enhanced chemokines, increased adhesion molecule expression, and delayed apoptosis (36, 37). In contrast, HTS is associated with lower adhesion molecule expression (33). In the present study, HTS reduced neutrophil influx and subsequent organ damage during AAI as evidenced by the attenuated rise in gut and lung MPO content. This may have conferred protection through decreasing reactive oxygen species and frequency of leukocyte plugging. (38). We speculate that prevention of neutrophil influx may decrease development of organ injury during recovery as reflected by the reduction in circulating ALT, a marker of liver damage. Thus, the decreased organ injury was likely the result of the HTS modulation of multiple pathways including but not limited to improved AVP release, organ perfusion, and neutrophil recruitment and infiltration.

Despite the promising findings presented here and other pre-clinical studies, clinical trials with HTS have failed to demonstrate benefit over traditional crystalloid resuscitation (11). Nevertheless, although 28-day mortality was not improved, HTS does limit the incidence of negative secondary outcomes such as need for ventilation and renal failure (11) and confers, immunomodulatory benefits (39). One could speculate that while in the non-intoxicated trauma victim, current resuscitation measures may already be optimized, the impaired hemodynamic and neuroendocrine responses of AAI hemorrhaged victims may be benefited by HTS.

This study examined a single administration of a standard dose of HTS, administered immediately following controlled HS. We have previously reported the critical time-dependent effects of fluid resuscitation of AAI-HS animals.(40). An additional consideration, given the robust pressor response to HTS is the presence of active bleeding at the time of FR. Moreover, the possible benefit of repeated administration of small volumes of HTS is quite likely as we have observed in preliminary studies. Therefore, situational considerations as well as optimal dosing and timing of administration warrant future study.

In conclusion, HTS during AAI improved hemodynamic counter-regulation by enhancing AVP release, and this was associated with improved organ perfusion and decreased organ injury. Future studies are warranted to examine the mechanism by which HTS reduces the AAI-induced increase in neutrophil influx, which may have implications for the reduction of organ injury. To this end, the use of HTS as an immunomodulator independent of its traditional role in resuscitation may yield a new strategy for ameliorating organ injury and dysfunction during recovery from traumatic injury and HS, particularly during AAI.

Acknowledgments

The authors thank Drs. Jerry Breslin, John Hunt, Jay Kolls, Daniel Kapusta and Kathleen McDonough for their thoughtful insight and discussions.

Funding: DOD-PR-054196, LA BoR-110350057A, NIAAA-AA7577, NIAAA-F30AA019587-01A1, AMA Foundation

References

1. Heron M. Deaths: leading causes for 2007. *Natl Vital Stat Rep.* 2011; 59:1–95. [PubMed: 21950210]
2. Cherpitel CJ. Alcohol and casualties: a comparison of emergency room and coroner data. *Alcohol Alcohol.* 1994; 29:211–218. [PubMed: 8080603]
3. Jurkovich GJ, Rivara FP, Gurney JG, et al. The effect of acute alcohol intoxication and chronic alcohol abuse on outcome from trauma. *JAMA.* 1993; 270:51–56. [PubMed: 8510296]
4. Jurkovich GJ, Rivara FP, Gurney JG, et al. Effects of alcohol intoxication on the initial assessment of trauma patients. *Ann Emerg Med.* 1992; 21:704–708. [PubMed: 1590611]
5. Koval KJ, Cooley M, Cantu RV, et al. The effects of alcohol on in-hospital mortality in drivers admitted after motor vehicle accidents. *Bull NYU Hosp Jt Dis.* 2008; 66:27–34. [PubMed: 18333825]
6. Shih HC, Hu SC, Yang CC, et al. Alcohol intoxication increases morbidity in drivers involved in motor vehicle accidents. *Am J Emerg Med.* 2003; 21:91–94. [PubMed: 12671806]
7. Molina PE, Zambell KL, Norenberg K, et al. Consequences of alcohol-induced early dysregulation of responses to trauma/hemorrhage. *Alcohol.* 2004; 33:217–227. [PubMed: 15596090]
8. Mathis KW, Sulzer J, Molina PE. Systemic administration of a centrally acting acetylcholinesterase inhibitor improves outcome from hemorrhagic shock during acute alcohol intoxication. *Shock.* 2010; 34:162–168. [PubMed: 20023599]
9. Mathis KW, Molina PE. Central acetylcholinesterase inhibition improves hemodynamic counterregulation to severe blood loss in alcohol-intoxicated rats. *Am J Physiol Regul Integr Comp Physiol.* 2009; 297:R437–445. [PubMed: 19515985]
10. Whitaker AM, Sulzer JK, Molina PE. Augmented central nitric oxide production inhibits vasopressin release during hemorrhage in acute alcohol intoxicated rodents. *Am J Physiol Regul Integr Comp Physiol.* 2011
11. Mattox KL, Maningas PA, Moore EE, et al. Prehospital hypertonic saline/dextran infusion for post-traumatic hypotension. The U.S.A. Multicenter Trial. *Ann Surg.* 1991; 213:482–491.

12. Matsuoka T, Hildreth J, Wisner DH. Liver injury as a model of uncontrolled hemorrhagic shock: resuscitation with different hypertonic regimens. *J Trauma*. 1995; 39:674–680. [PubMed: 7473953]
13. Us MH, Ozkan S, Oral L, et al. Comparison of the effects of hypertonic saline and crystalloid infusions on haemodynamic parameters during haemorrhagic shock in dogs. *J Int Med Res*. 2001; 29:508–515. [PubMed: 11803735]
14. Homma H, Deitch EA, Feketeova E, et al. Small volume resuscitation with hypertonic saline is more effective in ameliorating trauma-hemorrhagic shock-induced lung injury, neutrophil activation and red blood cell dysfunction than pancreatic protease inhibition. *J Trauma*. 2005; 59:266–272. [PubMed: 16294064]
15. Batista MB, Bravin AC, Lopes LM, et al. Pressor response to fluid resuscitation in endotoxic shock: involvement of vasopressin. *Crit Care Med*. 2009; 37:2968–2972. [PubMed: 19770747]
16. Giusti-Paiva A, Martinez MR, Bispo-da-Silva LB, et al. Vasopressin mediates the pressor effect of hypertonic saline solution in endotoxic shock. *Shock*. 2007; 27:416–421. [PubMed: 17414425]
17. Phelan H, Stahls P, Hunt J, et al. Impact of alcohol intoxication on hemodynamic, metabolic, and cytokine responses to hemorrhagic shock. *J Trauma*. 2002; 52:675–682. [PubMed: 11956381]
18. Gmel G, Bissery A, Gammeter R, et al. Alcohol-attributable injuries in admissions to a swiss emergency room—an analysis of the link between volume of drinking, drinking patterns, and preattendance drinking. *Alcohol Clin Exp Res*. 2006; 30:501–509. [PubMed: 16499491]
19. Savola O, Niemela O, Hillbom M. Alcohol intake and the pattern of trauma in young adults and working aged people admitted after trauma. *Alcohol Alcohol*. 2005; 40:269–273. [PubMed: 15870091]
20. Yang S, Zou LY, Bounelis P, et al. Glucosamine administration during resuscitation improves organ function after trauma hemorrhage. *Shock*. 2006; 25:600–607. [PubMed: 16721268]
21. Fujisawa Y, Miyatake A, Hayashida Y, et al. Role of vasopressin on cardiovascular changes during hemorrhage in conscious rats. *Am J Physiol*. 1994; 267:H1713–1718. [PubMed: 7977803]
22. Voelckel WG, Convertino VA, Lurie KG, et al. Vasopressin for hemorrhagic shock management: revisiting the potential value in civilian and combat casualty care. *J Trauma*. 2010; 69(Suppl 1):S69–74. [PubMed: 20622623]
23. Muller S, How OJ, Hermansen SE, et al. Vasopressin impairs brain, heart and kidney perfusion: an experimental study in pigs after transient myocardial ischemia. *Crit Care*. 2008; 12:R20. [PubMed: 18291025]
24. Matejovic M, Trager K, De Backer D. Noradrenaline and the kidney: foe, friend, or both? *Intensive Care Med*. 2005; 31:1476–1478. [PubMed: 16088409]
25. Mouw D, Bonjour JP, Malvin RL, et al. Central action of angiotensin in stimulating ADH release. *Am J Physiol*. 1971; 220:239–242. [PubMed: 4321203]
26. Ramsay DJ, Keil LC, Sharpe MC, et al. Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion. *Am J Physiol*. 1978; 234:R66–71. [PubMed: 203199]
27. Velasco IT, Rocha-e-Silva M. Hypertonic saline resuscitation is prevented by intracerebroventricular saralasin but not by captopril. *Braz J Med Biol Res*. 1989; 22:237–239. [PubMed: 2676039]
28. Li DP, Chen SR, Pan HL. Angiotensin II stimulates spinally projecting paraventricular neurons through presynaptic disinhibition. *J Neurosci*. 2003; 23:5041–5049. [PubMed: 12832527]
29. Wang P, Ba ZF, Burkhardt J, et al. Trauma-hemorrhage and resuscitation in the mouse: effects on cardiac output and organ blood flow. *Am J Physiol*. 1993; 264:H1166–1173. [PubMed: 8476095]
30. Dudkiewicz M, Proctor KG. Tissue oxygenation during management of cerebral perfusion pressure with phenylephrine or vasopressin. *Crit Care Med*. 2008; 36:2641–2650. [PubMed: 18679110]
31. Zakaria el R, Tsakadze NL, Garrison RN. Hypertonic saline resuscitation improves intestinal microcirculation in a rat model of hemorrhagic shock. *Surgery*. 2006; 140:579–587. discussion 587–578. [PubMed: 17011905]
32. Corso CO, Okamoto S, Leiderer R, et al. Resuscitation with hypertonic saline dextran reduces endothelial cell swelling and improves hepatic microvascular perfusion and function after hemorrhagic shock. *J Surg Res*. 1998; 80:210–220. [PubMed: 9878316]

33. Alam HB, Sun L, Ruff P, et al. E- and P-selectin expression depends on the resuscitation fluid used in hemorrhaged rats. *J Surg Res.* 2000; 94:145–152. [PubMed: 11104654]
34. Zakaria el R, Campbell JE, Peyton JC, et al. Postresuscitation tissue neutrophil infiltration is time-dependent and organ-specific. *J Surg Res.* 2007; 143:119–125. [PubMed: 17950080]
35. Peitzman AB, Billiar TR, Harbrecht BG, et al. Hemorrhagic shock. *Curr Probl Surg.* 1995; 32:925–1002. [PubMed: 7587344]
36. Ono M, Yu B, Hardison EG, et al. Increased susceptibility to liver injury after hemorrhagic shock in rats chronically fed ethanol: role of nuclear factor-kappa B, interleukin-6, and granulocyte colony-stimulating factor. *Shock.* 2004; 21:519–525. [PubMed: 15167680]
37. Akhtar S, Li X, Kovacs EJ, et al. Interleukin-18 delays neutrophil apoptosis following alcohol intoxication and burn injury. *Mol Med.* 2011; 17:88–94. [PubMed: 20844839]
38. Mazzoni MC, Borgstrom P, Intaglietta M, et al. Capillary narrowing in hemorrhagic shock is rectified by hyperosmotic saline-dextran reinfusion. *Circ Shock.* 1990; 31:407–418. [PubMed: 1697790]
39. Angle N, Cabello-Passini R, Hoyt DB, et al. Hypertonic saline infusion: can it regulate human neutrophil function? *Shock.* 2000; 14:503–508. [PubMed: 11092681]
40. Sulzer JK, Molina PE. Delayed resuscitation with physostigmine increases end organ damage in alcohol intoxicated rats. *Shock.* 2011; 35:74–79. [PubMed: 20577152]

Figure 1.

Impact of hypertonic saline (HTS) resuscitation on mean arterial blood pressure (MABP) recovery following hemorrhage. Values are mean $\pm$ SEM increase in MABP from end HS values over time (minute) during fluid resuscitation. $n=5-8$ /group. a: lactated Ringer's (LR) vs 60 min; b: HTS vs 60 min; c: LR + V1 receptor antagonist vs 60 min; d: HTS + V1 receptor antagonist vs 60 min; * all groups vs HTS + V1 antagonist; # HTS vs LR

Figure 2.

Circulating levels of arginine vasopressin (AVP) (pg/ml) during hemorrhage and resuscitation with lactated Ringer's (LR) or hypertonic saline (HTS). Values are mean $\pm$ SEM of plasma concentrations. n=5-8/group. * p<0.05 vs baseline # p<0.05 vs LR

Figure 3.

Nitrate/nitrite ($\mu\text{mol/mg tissue}$) concentrations in the periventricular nucleus (PVN) 2h post-resuscitation with lactated Ringer's (LR) or hypertonic saline (HTS). n=5-8/group. * $p < 0.05$ vs baseline # $p < 0.05$ vs LR

Figure 4. Organ blood flow (ml/min/100g tissue) 2h post-resuscitation with lactated Ringer's (LR) or hypertonic saline (HTS). Values are mean $\pm$ SEM. n=3-4/group. * p<0.05 vs time-matched sham # p<0.05 vs LR

Figure 5.

Myeloperoxidase (MPO) activity in A. lung and B. gut 2h post-resuscitation with lactated Ringer's (LR) or hypertonic saline (HTS). Values are mean $\pm$ SEM. n=5-8/group. * p<0.05 vs time-matched sham # p<0.05 vs LR