Published in final edited form as:

Shock. 2009 October ; 32(4): 410–415. doi:10.1097/SHK.0b013e31819e2d13.

Transient central cholinergic activation enhances sympathetic nervous system activity but does not improve hemorrhage-induced hypotension in alcohol-intoxicated rodents

Keisa W. Mathis and Patricia E. Molina

LSUHSC Department of Physiology and Alcohol and Drug Abuse Center of Excellence, New Orleans, LA 70112-1393

Abstract

Morbidity and mortality following traumatic injury and hemorrhagic shock is exacerbated in the alcohol-intoxicated individual. The level of hypotension at the time of admittance into the emergency department is a critical indicator of outcome from injury. Previously we have demonstrated that acute alcohol intoxication (AAI) decreases basal mean arterial blood pressure (MABP), exaggerates hypotension throughout hemorrhagic shock (HS), and attenuates the pressor response to fluid resuscitation (FR) in male rodents. This AAI-induced impaired hemodynamic counter-regulation to blood loss is associated with dampened neuroendocrine activation [i.e., epinephrine (EPI), norepinephrine (NE), and arginine vasopressin (AVP) release]. We hypothesize that the blunted neuroendocrine response is the principal mechanism involved in hemodynamic instability during and following HS in AAI. The present study investigates whether enhancing central cholinergic activity via intracerebroventricular (ICV) choline, a precursor of acetylcholine, would restore the neuroendocrine response, and as a result, improve hemodynamic compensation following HS. Chronically-catheterized, conscious, male Sprague-Dawley rats (225–250g) received a primed 15-h alcohol infusion (30% w/v; total ~8g/kg) prior to ICV choline (150μg) injection and were subsequently subjected to fixed-volume HS (50%) and FR with lactated Ringers (2× volume removed). There were a total of eight experimental groups (n=5–12 rats per group): alcohol-treated not hemorrhaged (alcohol/sham), dextrose-treated not hemorrhaged (dextrose/sham), alcohol-treated hemorrhaged (alcohol/hemorrhage), and dextrose-treated hemorrhaged (dextrose/hemorrhage), with ICV choline or water injection. ICV choline immediately increased basal MABP in both control (16%) and AAI animals (12%), but did not alter MABP following HS in either group. ICV choline increased basal plasma EPI (196%), NE (96%), and AVP (145%), and enhanced the HS-induced rise in EPI and AVP, without altering NE responses to HS, in control animals. AAI blunted choline-induced neuroendocrine activation and prevented the HS-induced rise in NE, without affecting post-HS EPI and AVP levels. ICV choline administration to AAI animals enhanced the HS-induced rise in EPI without affecting post-HS NE or AVP. These results indicate that ICV choline produced immediate neuroendocrine activation and elevation in MABP that was not sustained sufficiently to improve hemodynamic counter-regulation in alcohol-treated animals.

Keywords

choline; alcohol; hemorrhage; sympathetic nervous system; vasopressin; catecholamines; rats

INTRODUCTION

In 2000, traumatic injury ranked as the 5th leading cause of death in the United States (1). Traumatic injury is more frequent in alcohol-intoxicated individuals than in the non-intoxicated population (2,3,4,5). In fact, nearly half of injured victims that enter emergency departments across the United States test positive for alcohol (6,7,8), with blood alcohol levels (BAL) frequently exceeding 80 mg/dL, the legal limit in most states (7,8,9,10). Several studies have reported that alcohol-intoxicated injured patients are more susceptible to acute medical complications such as pneumonia and other nosocomial infections and have a greater need for intensive care, higher occurrence of permanent disability, and increased risks of mortality during their hospital stay (11,12). Factors that may lead to greater complications in this patient population include severity of the injury, genetic makeup of the individual, history of alcohol consumption, and blood alcohol concentrations at the time of injury (7,13).

Clinical studies have reported that alcohol-intoxicated injured victims present with decreased systolic blood pressure at the time of entry into the emergency department (14). Because mean arterial blood pressure (MABP) at the time of admittance into the emergency department is one of the most critical indicators of a patient's outcome and survival from traumatic injury and the associated hemorrhagic shock (15), the inability to reverse hypotension likely contributes to greater morbidity and mortality in alcohol-intoxicated victims.

The pathophysiological mechanisms involved in the response to trauma and hemorrhage during acute alcohol intoxication have been extensively researched by our laboratory. We have previously demonstrated that alcohol intoxication, produced by intragastric administration of alcohol, decreases baseline MABP, accentuates hypotension throughout hemorrhage, and blunts the pressor response to fluid resuscitation, regardless of the dose (1.75, 5, and 8 g/kg) and frequency of alcohol administration (single dose, three-day binge, and 15-h constant infusion, respectively) (16,17,18). This lack of hemodynamic compensation to blood loss is associated with attenuated catecholamine and arginine vasopressin (AVP) release in alcohol-intoxicated animals following severe hemorrhage where MABP is maintained at 40 mmHg for 60 minutes (19). We hypothesize that this inappropriate neuroendocrine response is the principal mechanism underlying the hemodynamic instability observed in alcohol-treated animals following blood loss. It is possible that alcohol, in addition to impairing the neuroendocrine response, could be producing a decrease in circulating blood volume, and/or reducing vascular reactivity to circulating hormones and neurotransmitters. Thus, we speculate that alcohol exerts its effects not only in peripheral tissues, but centrally as well. In the studies presented here, we aimed to restore the neuroendocrine response to hemorrhage by centrally enhancing cholinergic activation to test the hypothesis that pharmacological interventions aimed at

enhancing neuroendocrine activation will improve hemodynamic counter-regulation following hemorrhagic shock in alcohol-intoxicated animals. We predicted that stimulation of central cholinergic neurons, an approach known to activate the sympathetic nervous system (SNS) (20), would improve hemodynamic counter-regulatory responses and recover MABP in alcohol-intoxicated, hemorrhaged rodents. We chose to administer choline centrally to enable us to dissect central from peripheral mechanisms involved in alcohol-induced impairment of the hemodynamic counter-regulatory responses to HS. Our results showed an immediate stimulation of SNS outflow following ICV choline administration as indicated by the increases in MABP, catecholamines, and AVP. However, these effects were transient and not sustained enough to improve hemodynamic stability following hemorrhagic shock in alcohol-intoxicated animals.

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 (225–250g) arrived to the institution and were allowed one week to acclimate to their surroundings. During this time, they were caged in pairs, fed standard rat chow (Purina, St. Louis, MO) and water *ad libitum*, and housed in a controlled-temperature (22 °C) and controlled-illumination (12-h light/dark cycle) environment.

Experimental Design

The animals were subjected to all surgical procedures (ICV cannula and vascular and gastric catheter placement) 8–12 days prior to the experimental protocol. Alcohol administration was initiated the evening prior to hemorrhagic shock and fluid resuscitation. Details of the experimental design are outlined below (also see Figure 1).

Intracerebroventricular cannula placement—Animals were anesthetized with ketamine/xylazine (90 mg/kg and 9 mg/kg, respectively) in order to stereotactically implant guide cannulas into the right lateral ventricle. Briefly, a burr hole was drilled 1.3 mm lateral to bregma and a 22-gauge stainless steel guide cannula inserted through the hole to a depth of 3.5 mm below the skull surface. All stereotaxic coordinates were selected based on an atlas of the rat's brain. The guide cannula was cemented with dental acrylic to stainless steel support screws placed in the skull. A 30-gauge stylet (dummy cannula) was inserted into the guide to maintain patency. Animals were returned to individual cages, allowed 7–9 days to recover from surgery, and provided with food and water *ad libitum*. Cannula placement and patency were tested on the day of vascular and gastric surgery using 5 µL of angiotensin II (20 ng; Sigma, St. Louis, MO), which induced an immediate thirst response.

Vascular and gastric catheter placement—Sterile catheters were placed into the left carotid artery and right jugular vein, as well as the antrum of the stomach, using aseptic surgical procedures as previously described by our laboratory (16–18). The catheters were routed subcutaneously through a trocar and exteriorized at the nape of the neck. Following

this surgical procedure, the animals were returned to their individual cages and allowed to recover for 2–3 days, again with food and water provided *ad libitum*.

Alcohol administration—All experimental procedures were conducted in conscious and unrestrained animals. On the day prior to hemorrhage at approximately 4:00 pm, animals were administered 30% ethyl alcohol over 15 h, as previously described (16). Briefly, animals received an alcohol bolus (1.75 g/kg) followed by a 15-h constant infusion of alcohol (~300 mg/kg/h). The next morning, animals received a final bolus of alcohol (1.75 g/kg). The total dose received over the alcohol administration period was approximately 8 g/kg, achieving BALs of 174 mg/dL. Time-matched control animals received isocaloric/ isovolumic dextrose (13.9 g/kg).

This mode of alcohol administration reflects a human binge drinking episode, described as the consumption of five or more drinks over a period of time sufficient to elevate blood alcohol levels above intoxicating levels (21). Binge drinking is frequent in the alcohol-consuming population (22) and has been repeatedly shown to increase the risk of traumatic injury in an otherwise healthy population (23).

Drug administration—Choline (150 µg; Sigma, St. Louis, MO) was administered 30 min after the completion of alcohol infusion and approximately 5 min prior to initiating hemorrhage (Figure 1). Time-matched controls received equal volumes of vehicle (H₂O, 5 µL) ICV. For ICV drug administration, an injection cannula (25-gauge, 11.5 mm stainless steel tubing) was inserted through the guide cannula. The injection cannula was connected to a Hamilton microsyringe (10 µL) with polyethylene tubing (25–30 cm). Choline was then infused slowly over an 8–10-second period. The injection volume was monitored by observing the movement of an air bubble placed in the polyethylene tubing. The dose of choline used in this study was chosen based on reports in the literature that demonstrated that the pressor response induced by ICV choline is dose-dependent and that the highest documented dose (150 µg) immediately increases MABP, catecholamines, and AVP (20,24). The MABP response to 150 µg choline is sustained for approximately 15 minutes as shown by our preliminary studies as well as studies in the literature (36). Only animals that had a positive response to angiotensin II-induced polydipsia, reflecting accurate placement of the cannula in the lateral ventricle, were used in these studies.

Hemorrhagic Shock and Fluid Resuscitation—Conscious, unrestrained animals were subjected to fixed-volume hemorrhage as previously described by our laboratory (17). Briefly, half the circulating blood volume (calculated by 6% of animals body weight in grams) was removed over 60 min, with the bulk of the blood (40% of circulating blood volume) removed within the first 10 minutes and the remaining blood removed at T = 30 and 60 minutes of hemorrhage. Animals were not heparinized at any time during the experimental protocol. Arterial blood samples were collected pre- and post-hemorrhage into a heparinized syringe. At the end of the 60-min hemorrhage period, an intravenous bolus of warmed (37°C) lactated Ringers equal to 40% of the total blood volume removed was returned, followed by a constant infusion of lactated Ringers of two times the blood volume removed. In total, 2.4 times the blood volume removed was returned to the animal in the form of lactated Ringers. MABP and heart rate were monitored throughout the hemorrhage

and fluid resuscitation period using Powerlab (Powerlab, AD Instruments, Colorado Springs, CO). Blood (1.5 mL) was removed from time-matched sham animals at 0 and 60 minutes for pre- and post-hemorrhage hormone analysis, whereas the blood sample taken at 120 minutes was terminal and collected for future analysis. There were a total of eight experimental groups: alcohol-treated not hemorrhaged (alcohol/sham), dextrose-treated not hemorrhaged (dextrose/sham), alcohol-treated hemorrhaged (alcohol/hemorrhage), and dextrose-treated hemorrhaged (dextrose/hemorrhage), with ICV choline or water injection. At the end of fluid resuscitation, the animals were euthanized by an intravenous injection of sodium pentobarbital (125 mg/kg) followed by exsanguination.

Blood Sample Analysis

Arterial blood samples were collected in chilled heparinized syringes and aliquots were placed in tubes containing catecholamine preservative (9% ethylenediaminetetraacetic acid, 6% glutathione and dH₂O at a pH of 6.0–7.4) at 20 μ L/mL of blood. Blood samples were centrifuged for 15 min at 10000 rpm for plasma separation. Blood alcohol levels were measured using an amperometric oxygen electrode (Analox Instruments Limited., London, England).

Catecholamine measurements—High-performance liquid chromatography was used to quantify circulating epinephrine and norepinephrine levels. Plasma samples were spiked with 30 μ L of 3,4-dihydroxybenzylamine, the internal standard, absorbed into a small quantity of aluminum oxide (alumina) and separated from the alumina by eluting solution (Bioanalytic Systems, West Lafayette, IN). The samples were quantified for circulating epinephrine and norepinephrine levels using a high-performance liquid chromatography system consisting of a chromatographic analyzer with a catecholamine column and an electrochemical detector (Bioanalytic Systems, West Lafayette, IN). The interassay coefficient of variability for catecholamines was 11.8%.

Arginine vasopressin measurements—Plasma AVP levels were determined using a commercially available human, rat, mouse and ovine-specific radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA). Briefly, plasma samples were acidified using 1% trifluoroacetic acid in water. The supernatant was then loaded into a pre-treated C-18 SEP-Column (Waters Corporation, Milford, MA) and washed with 60% acetonitrile in 1% trifluoroacetic acid. After several washes, the eluant was evaporated and the powder was reconstituted using radioimmunoassay buffer. The radioimmunoassay reliable detection range was 10–1280 pg/mL of AVP and had 100% specificity for [Arg8]-vasopressin (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH₂), the form of vasopressin found in most mammals.

Statistical Analysis

All data are presented as mean $\pm$ standard error of the mean (SEM) with the number of animals used per group indicated in the figure legend. Statistical analysis of MABP, catecholamines, and arginine vasopressin was accomplished using two-way analysis of variance (ANOVA) with or without repeated measures (indicated in figure legend). All pair-wise multiple comparisons were completed with the Holm-Sidak method. Statistical significance was set at $p < 0.05$.

RESULTS

ICV choline immediately increases mean arterial blood pressure

ICV choline increased MABP in both dextrose controls (16%; $p < 0.001$) and alcohol-treated animals (12%; $p < 0.001$; Figure 2) within five minutes of injection. ICV water did not alter MABP in either group of animals. Dextrose administration did not alter basal MABP; however, alcohol administration decreased basal MABP by 10% ($p < 0.001$).

ICV choline does not improve hemodynamic counter-regulation to hemorrhagic shock during acute alcohol intoxication

MABP in dextrose-treated animals dropped approximately 49% below basal levels, following removal of 40% of the estimated circulating blood volume, averaging 58 ± 6 mmHg within 15 min of the hemorrhage protocol (Figure 3). Alcohol exacerbated the hypotension (29%; $p = 0.009$) within 15 min of hemorrhage with MABP averaging 38 ± 4 mmHg. ICV choline ameliorated hypotension in alcohol-treated animals within the first 15 min of hemorrhage. However, alcohol-treated animals were more hypotensive than time-matched dextrose controls throughout the remainder of the observation period (an average of 52 ± 1 and 72 ± 2 mmHg, respectively; Figure 4). MABP in alcohol-treated animals (87 ± 6 mmHg) remained lower than dextrose controls (104 ± 9 mmHg) at completion of the resuscitation period ($p = 0.098$). Despite the apparent favorable pressor response to ICV choline during early hemorrhage, a significant improvement in MABP following hemorrhage was not observed in either control (102 ± 8 mmHg) or alcohol-treated animals (83 ± 3 mmHg), with alcohol-treated animals significantly lower ($p = 0.008$).

MABP at 60 and 120 minutes were similar to basal levels in dextrose- and alcohol-treated sham animals with or without ICV choline injection.

ICV choline immediately enhances sympathetic nervous system activity but does not completely restore neuroendocrine response to hemorrhagic shock during alcohol intoxication

ICV choline increased basal (or pre HS) plasma epinephrine (196%; $p = 0.002$), norepinephrine (96%; $p < 0.001$), and AVP (145%; $p = 0.012$) in dextrose-treated controls within five minutes of injection (Figure 5; pre-HS panel). Alcohol administration alone did not alter the prevailing basal hormone levels. However, alcohol prevented the choline-induced increases in plasma epinephrine, norepinephrine, and AVP.

Hemorrhagic shock produced a marked 422% ($p = 0.048$) and 479% ($p = 0.004$) increase in circulating levels of epinephrine at T=60 minutes in dextrose controls and alcohol-treated animals, respectively (Figure 5; post HS panel). ICV choline further enhanced post-HS plasma epinephrine levels in both dextrose- (106%; $p = 0.008$) and alcohol-treated (76%; $p = 0.004$) animals.

Hemorrhagic shock produced a significant 165% increase ($p = 0.004$) in circulating levels of norepinephrine at T=60 minutes in dextrose-treated animals, which was prevented by

alcohol (Figure 5; post HS panel). ICV choline did not alter post-HS plasma norepinephrine in either the control or alcohol-treated groups.

Hemorrhagic shock increased circulating levels of AVP by 92% ($p = \text{NS}$) and 87% ($p = 0.003$) in dextrose-treated controls and alcohol-treated animals, respectively, at $T=60$ minutes (Figure 5; post HS panel). ICV choline further enhanced post-HS plasma AVP levels (69%; $p = 0.011$) in dextrose controls. The choline-induced enhancement in AVP release following hemorrhage was prevented by alcohol.

DISCUSSION

Our results demonstrate that ICV choline produced an immediate activation of the sympathetic nervous system as evidenced by the increase in MABP and the rise in plasma epinephrine, norepinephrine, and AVP within five minutes of administration in dextrose controls. Acute alcohol intoxication lowered basal MABP and prevented choline-induced neuroendocrine activation, but did not alter the pressor response of central choline. ICV choline produced few to no improvements in hemodynamic and neuroendocrine counter-regulation in alcohol-intoxicated animals following hemorrhagic shock. We speculate that the inability of ICV choline to reverse hemorrhage-induced hypotension and to produce a sustained increase in vasoactive hormone release throughout the duration of hemorrhage in alcohol-treated animals is due to transient enhancement of descending SNS outflow that was extinguished prior to the end of the HS protocol.

In the emergency room setting, hypotension reversal in hemorrhaging patients is currently achieved by fluid resuscitation with colloids, crystalloids, and other plasma expanders (25,26). Treatment of the injured and hemorrhaged alcohol-intoxicated victim is more complicated because alcohol further exacerbates hypotension, as well as disrupts metabolic parameters and mental status (10). Thus, identification of effective pharmacological management to enhance restoration of MABP following trauma and hemorrhage would be an important addition to the current armamentarium used for the management of the intoxicated trauma victim in the acute setting.

Previous studies from our laboratory have demonstrated that acute alcohol intoxication decreases basal MABP, exaggerates hypotension during hemorrhagic shock, and attenuates the pressor response to fluid resuscitation in rats (16–18). Others have reported similar effects of alcohol on hemodynamic stability and compensation during and following hemorrhagic shock in guinea pigs (27,28) and swine (29). We have demonstrated that the impaired hemodynamic counter-regulatory response to hemorrhage in rats is associated with a blunted hemorrhage-induced increase in circulating epinephrine, norepinephrine, and arginine vasopressin (19). This led to the hypothesis that restoring the neuroendocrine response in alcohol-treated rodents would prove beneficial in normalizing MABP following blood loss.

Several approaches could have been utilized to enhance sympathetic nervous system outflow including subjecting the animals to physical or psychological stressors or administering sympathetic agonists. However, we were primarily interested in isolating central effects of

alcohol from systemic effects. The results of our studies, and those of others, provide strong evidence that central choline administration produces immediate sympathetic nervous system activation. ICV choline was proven in the literature to be effective in increasing central acetylcholine levels in a study by Buccafusco (30) in which he injected ICV [^3H]-choline and observed an immediate increase in [^3H]-acetylcholine in brain regions such as the cerebral cortex, medulla oblongata, and the hypothalamus. Our preliminary studies are compatible with published studies (24,31,32,33) that show ICV choline increases MABP in both control and alcohol-intoxicated animals. While ICV injection of acetylcholine has also been shown to increase MABP (34), we predict that the actions of ICV acetylcholine would have been even shorter lived than those observed with choline administration. Moreover, we demonstrated that ICV choline was effective in producing an increase in plasma epinephrine, norepinephrine, and vasopressin levels under baseline conditions in control animals. In additional studies we have observed that ICV choline administration to conscious naïve rats increased heart rate and plasma glucose levels while decreasing plasma insulin concentrations within five minutes of injection (unpublished data). Therefore we chose ICV choline, based on the aforementioned data suggesting the timing and magnitude of sympathetic activation following ICV choline administration.

The exact brain regions affected by ICV choline have not yet been the focus of our laboratory. Reports in the literature suggest that the observed effects are mediated via central nicotinic receptors (35). In addition, the brain renin-angiotensin system may be involved in the pressor response elicited by ICV choline because ICV pretreatment with captopril, an angiotensin converting enzyme inhibitor, has been shown to inhibit the pressor response induced by ICV choline (36).

As previously observed, fifteen-hour infusion of alcohol produced a significant decrease in MABP. One could speculate that this initial drop in MABP prior to hemorrhagic shock contributes to the overall impaired hemodynamic counter-regulation in alcohol-intoxicated animals. Our preliminary studies showed that choline quickly restored MABP reversing the alcohol-induced hypotension (Figure 2). Because alcohol blunted the choline-induced increases in basal epinephrine, norepinephrine, and AVP (Figure 5), we speculate that the immediate (within 5 min) choline-induced increase in MABP in alcohol-treated animals is not due to the increased circulating concentrations of vasoconstrictors measured in this study, but possibly through increased sympathetic nerve activity leading to increased heart rate and cardiac output.

Previously we have demonstrated that fixed-pressure HS, which involves removal of approximately 55–65% blood volume to maintain a target MABP of approximately 40 mmHg for 60 minutes, results in increase release of vasopressors, namely epinephrine, norepinephrine, and AVP in control animals with (19). Using that model of hemorrhage, we have demonstrated that the rise in these vasoactive hormones and mediators is attenuated by alcohol-treated animals, which we speculate is the principal underlying mechanism for impaired hemodynamic counter-regulation following HS. Because a smaller blood volume is removed from alcohol-intoxicated animals to achieve fixed pressure hemorrhage ongoing studies have used a fixed-volume model of HS. This involves removal of 50% of the circulating blood volume in both control and alcohol-treated animals. Because this produces

a greater hypotension in alcohol-treated animals, a greater vasoactive mediator release was expected. In contrast, our results show that alcohol-intoxicated animals had a similar HS-induced rise in epinephrine and AVP levels and an attenuated rise in norepinephrine levels; all in the context of a greater hypotensive response to a given blood loss.

The inability of choline to improve hemodynamic counter-regulation following HS in alcohol-intoxicated animals is speculated to be due to its acute and transient action. The literature and our preliminary studies suggested the pressor response of ICV choline is of 15–20 min duration (37). Thus if the initial greater hypotension produced in alcohol-intoxicated animals following blood loss were a critical determinant of outcome, one would have expected to see a greater efficacy in ICV choline's effects. In addition, the enhancement in plasma epinephrine and AVP levels in dextrose controls, but not of norepinephrine release post-HS suggests a possible mechanism why MABP was not restored following HS and fluid resuscitation. Additional studies are warranted to determine which vasoactive substance is most important in producing improved blood pressure recovery during resuscitation.

In summary, our results demonstrate that ICV choline is capable of immediately activating the SNS (increasing MABP, plasma epinephrine and norepinephrine) and increasing AVP release. However, due to its transient effect ICV choline did not improve MABP at the end of HS and fluid resuscitation in control or alcohol-treated rodents. Future studies from our laboratory will investigate whether prolonging central cholinergic activity will improve hemodynamic counter-regulatory responses to hemorrhagic shock in the alcohol-intoxicated host.

Acknowledgments

The authors would like to thank Patrick Greiffenstein, MD for his outstanding surgical expertise, and Jean Carnal and Curtis Vande Stouwe for their excellent technical assistance. The authors would also like to extend a special thanks to the staff of the animal care facility at Pennington Biomedical Research for all their support following Hurricane Katrina. This research was supported by the ONR N00014-97-1-0248, DOD PR-054196, and NIAAA-AA7577.

References

1. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004; 291(10):1238–1245. [PubMed: 15010446]
2. Rehm J, Room R, Graham K, Monteiro M, Gmel G, Sempos CT. The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease: An overview. *Addiction*. 2003; 98:1209–1228. [PubMed: 12930209]
3. Li G, Keyl PM, Smith GS, Baker SP. Alcohol and injury severity: Reappraisal of the continuing controversy. *Journal of Trauma*. 2004; 56(5):1029–1032. [PubMed: 15179242]
4. Cherpitel CJ. Alcohol consumption and injury in the general population: From a natural sample. *Drug Alcohol Dependence*. 1994; 34(3):217–24. [PubMed: 8033759]
5. Borges G, Cherpitel C, Orozco R, Bond J, Ye Y, Macdonald S, Rehm J, Poznyak V. Multicentre study of acute alcohol use and non-fatal injuries: Data from the WHO collaborative study on alcohol and injuries. *Bulletin of the World Health Organization*. 2006; 84(6):453–460. [PubMed: 16799729]
6. Rivara FP, Jurkovich GJ, Gurney JG, Seguin D, Fligner CL, Ries R, Raisys VA, Copass M. The magnitude of acute and chronic alcohol abuse in trauma patients. *Archives of Surgery*. 1993; 128(8):903–905. [PubMed: 8343063]

7. Madan AK, Yu K, Beech DJ. Alcohol and drug use in victims of life-threatening trauma. *Journal of Trauma*. 1999; 47(3):568–571. [PubMed: 10498317]
8. Cherpitel CJ, Bond J, Ye Y, Borges G, Macdonald S, Stockwell T, Giesbrecht N, Cremonese M. Alcohol-related injury in the ER: A cross-national meta-analysis from the Emergency Room Collaborative Alcohol Analysis Project (ERCAAP). *Journal of Studies of Alcohol*. 2003; 64(5): 641–649.
9. Reyna TM, Hollis HW, Hulsebus RC. Alcohol-related trauma. *Annals of Surgery*. 1984; 201:194–197. [PubMed: 3970599]
10. Hadfield RJH, Mercer M, Parr MJA. Alcohol and drug abuse in trauma. *Resuscitation*. 2001; 48:25–36. [PubMed: 11162880]
11. Jurkovich GJ, Rivara FP, Gurney JG, Fligner C, Ries R, Mueller BA, Copass M. The effect of acute alcohol intoxication and chronic alcohol abuse on outcome from trauma. *The Journal of the American Medical Association*. 1993; 270(1):51–56.
12. Heckbert SR, Vedder NB, Hoffman W, Winn RK, Hudson LD, Jurkovich GJ, Compass MK, Harlan JM, Rice CL, Maier RV. Outcome after hemorrhagic shock in trauma patients. *Journal of Trauma*. 1998; 45(3):545–549. [PubMed: 9751548]
13. Schwacha MG, Holland LT, Chaudry IH, Messina JL. Genetic variability in the immune-inflammatory response after major burn injury. *Shock*. 2005; 23(2):123–128. [PubMed: 15665726]
14. Shih H, Hu S, Yang C, Ko J, Wu J, Lee C. Alcohol intoxication increases morbidity in drivers involved in motor vehicle accidents. *American Journal of Emergency Medicine*. 2003; 21:91–94. [PubMed: 12671806]
15. Hill DA, Abraham KJ, West RH. Factors affecting outcome in resuscitation of severely injured patients. *Australia and New Zealand Journal of Surgery*. 1993; 63(8):604–609.
16. Phelan H, Stahls P, Hunt J, Bagby GJ, Molina PE. Impact of alcohol intoxication on hemodynamic, metabolic and cytokine responses to hemorrhagic shock. *Journal of Trauma*. 2002; 52:675–682. [PubMed: 11956381]
17. Mathis KW, Zambell K, Olubadewo JO, Molina PE. Altered hemodynamic counter-regulation to hemorrhage by acute moderate alcohol intoxication. *Shock*. 2006; 26(1):55–61. [PubMed: 16783199]
18. Greiffenstein P, Mathis KW, Vande Stouwe C, Molina PE. Alcohol binge before trauma/hemorrhage impairs integrity of host defense mechanisms during recovery. *Alcoholism: Clinical and Experimental Research*. 2007; 31(4):1–12.
19. Molina PE, Zambell KL, Norenberg K, Eason J, Phelan H, Zhang P, Vande Stouwe C, Carnal JW, Porreta C. Consequences of alcohol induced early dysregulation of responses to trauma/hemorrhage. *Alcohol*. 2004; 33:217–227. [PubMed: 15596090]
20. Ulus IH, Arslan BY, Savci V, Kiran BK. Restoration of blood pressure by choline treatment in rats made hypotensive by haemorrhage. *Br J Pharmacol*. 1995; 116(2):1911–1917. [PubMed: 8528579]
21. Naimi TS, Brewer RD, Mokdad A, Denny C, Serdula MK, Marks JS. Binge drinking among US adults. *Journal of the American Medical Association*. 2003; 289(1):70–75.
22. Johnston, LD.; O'Malley, PM.; Bachman, JC. NIH Publication No. 03-5376. Bethesda, MD: National Institute of Drug Abuse; 2003b. Monitoring the future—national survey results on drug use, 1975–2002: College students and young adults ages 19–40.
23. Gmel G, Bissery A, Gammeter R, Givel J, Calmes J, Yersin B, Daeppen J. Alcohol-attributable injuries in admissions to a Swiss emergency room—an analysis of the link between volume of drinking, drinking patterns, and preattendance drinking. *Alcoholism: Clinical and Experimental Research*. 2006; 30(3):501–509.
24. Savci V, Goktalay G, Ulus IH. Intracerebroventricular choline increases plasma vasopressin and augments plasma vasopressin response to osmotic stimulation and hemorrhage. *Brain Research*. 2002; 942:58–70. [PubMed: 12031853]
25. Rizoli SB. Crystalloids and colloids in trauma resuscitation: a brief overview of the current debate. *Journal of Trauma*. 2003; 54:S82–S88. [PubMed: 12768108]
26. Sapsford W. Should the 'c' in 'abcde' be altered to reflect the trend towards hypotensive resuscitation? *Scandinavian Journal of Surgery*. 2008; 97:4–11. [PubMed: 18450202]

27. Horton JW. Cardiac contractile effect on ethanolism and hemorrhagic shock. *American Journal of Physiology: Heart and Circulatory Physiology*. 1992; 31:1096–1103.
28. McDonough KH, Giaimo ME, Miller HI. Low-dose ethanol alters the cardiovascular, metabolic, and respiratory compensation for severe blood loss. *Journal of Trauma*. 2002; 53(3):541–548. [PubMed: 12352494]
29. Bottoms GD, Fessler JF, Johnson M, Coatney M, Voorhees W. Effects of acute alcohol intake on tolerance to hypotension. *Alcoholism: Clinical and Experimental Research*. 1990; 14(5):776–780.
30. Buccafusco JJ. Kinetics of [^3H]choline and [^3H]acetylcholine metabolism in several regions of rat brain following intracerebroventricular injection of [^3H]choline: Effects of haloperidol. *Biochemical Pharmacology*. 1982; 31(8):1599–1605. [PubMed: 7092951]
31. Arslan BY, Ulus IH, Savci V, Kiran BK. Effects of intracerebroventricular injected choline on cardiovascular functions and sympathoadrenal activity. *J Cardiovasc Pharmacol*. 1991; 17(5):814–821. [PubMed: 1713998]
32. Savci V, Ulus IH. Central choline reverses hypotension caused by alpha-adrenoceptor or ganglion blockade in rats: the role of vasopressin. *Eur J Pharmacol*. 1996; 311(2–3):153–161. [PubMed: 8891595]
33. Gurun MS, Savci V, Ulus IH. Intracerebroventricular choline reverses hypotension induced by acute chemical sympathectomy. *J Auton Pharmacol*. 1997; 17(3):155–163. [PubMed: 9278775]
34. Peres-Polon VL, Correa FM. Pressor effects of acetylcholine injected into the lateral septal area of conscious rats. *Neuropharmacology*. 1994; 33(12):1537–1544. [PubMed: 7760976]
35. Sarter M, Parikh V. Choline transporters, cholinergic transmission and cognition. *Nature Reviews Neuroscience*. 2005; 6:48–56.
36. Isbil-Buyukcoskun N, Gulec G, Ozluk K, Ulus IH. Central injection of captopril inhibits the blood pressure response to intracerebroventricular choline. *Brazilian Journal of Medical and Biological Research*. 34:815–820. 200. [PubMed: 11378673]
37. Ulus IH, Millington WR, Buyukuysal RL, Kiran BK. Choline as an agonist: determination of its agonistic potency on cholinergic receptors. *Biochem Pharmacol*. 1988; 37(14):2747–2755. [PubMed: 3395355]

Figure 1. Experimental design

The experiment was conducted in the order indicated. Crosses denote alcohol bolus (1.75 g/kg). Details are outlined in *Materials and Methods*.

Figure 2. ICV choline increased basal MABP within five minutes

Mean arterial blood pressure (MABP; mmHg) in dextrose- and alcohol-treated animals ($n = 5-12$) injected with intracerebroventricular (ICV) choline or water. Values are means $\pm$ SEM, $p^* < 0.05$ vs. pre-dextrose or alcohol infusion, $p+ < 0.05$ vs. post-infusion. Statistical analysis was completed using two-way ANOVA with repeated measures.

Figure 3. ICV choline improved hemorrhagic shock-induced hypotension in alcohol-treated animals within first 15 minutes

Percent change in mean arterial blood pressure (MABP) in dextrose-treated (black bars) and alcohol-treated (white bars) animals ($n = 5-12$) within 15 min of hemorrhagic shock. Values are means $\pm$ SEM, $p^* < 0.05$ vs. time-matched, dextrose-treated animals. Statistical analysis was completed using two-way ANOVA.

Figure 4. ICV choline does not reverse hypotension in alcohol-treated animals following hemorrhagic shock and fluid resuscitation

Mean arterial blood pressure (MABP; mmHg) in dextrose- and alcohol-treated ($n = 5-12$) throughout fixed-volume hemorrhagic shock and fluid resuscitation. Dextrose-treated animals injected with ICV water (dextrose/water) are represented with black circles, alcohol/water with black triangles, dextrose/choline with white circles, and alcohol/choline with white triangles. Values are means $\pm$ SEM, $p^* < 0.05$ vs. dextrose/water and $p+ < 0.05$ vs. dextrose/choline. Statistical analysis was completed using two-way ANOVA with repeated measures.

Figure 5. ICV choline produced transient increase in circulating hormones

Circulating levels of epinephrine, norepinephrine, and arginine vasopressin (AVP) in pg/mL pre- and post-hemorrhagic shock (HS; T=60 minutes) in dextrose- and alcohol-treated animals (n = 5–12) injected with either intracerebroventricular (ICV) choline or water. Dextrose-treated animals injected with ICV water (dextrose/water) are represented with solid white bars, alcohol/water with diagonal bars, dextrose/choline with cross-hatch bars, and alcohol/choline with solid black bars. Values are means $\pm$ SEM, $p^* < 0.05$ vs. pre-HS time point, $p^+ < 0.05$ vs. dextrose/water, $p^@ < 0.05$ vs. dextrose/choline, and $p^\# < 0.05$ vs. alcohol/water. Statistical analysis was completed using two-way ANOVA with repeated measures. (NOTE: The hemorrhage-induced increase in AVP levels was not significant, possibly due to a single outlier in the dextrose/water group.)