Central acetylcholinesterase inhibition improves hemodynamic counterregulation to severe blood loss in alcohol-intoxicated rats

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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 297: R437–R445, 2009. First published June 10, 2009; doi:10.1152/ajpregu.00170.2009.—Acute alcohol intoxication results in impaired hemodynamic counterregulation to blood loss and is associated with an attenuated hemorrhage-induced release of catecholamines and AVP. We speculated that restoration of the neuroendocrine response to hemorrhage would improve mean arterial blood pressure (MABP) recovery during acute alcohol intoxication. Previously, we demonstrated that intracerebroventricular (ICV) choline, a precursor of acetylcholine, transiently increases sympathetic nervous system (SNS) outflow but is not capable of improving neuroendocrine and hemodynamic compensation to hemorrhage in alcohol-treated rats. We hypothesized that prolongation of the observed effect via ICV neostigmine, an acetylcholinesterase inhibitor, would enhance SNS outflow, restore the neuroendocrine response, and in turn improve hemodynamic responses to hemorrhage during acute alcohol intoxication. ICV neostigmine (1 μg) increased MABP, catecholamines, and AVP within 5 min and reversed hypotension due to 40% hemorrhage and intragastric alcohol (30% wt/vol, 2.5 g/kg) administration in chronically catheterized male Sprague-Dawley rats (225–250 g body wt). Acute alcohol intoxication before 50% hemorrhage decreased basal MABP, accentuated hypotension midhemorrhage, suppressed the hemorrhage-induced release of norepinephrine and AVP, and prevented restoration of MABP to basal levels after fluid resuscitation with lactated Ringer solution. ICV neostigmine (0.5 μg) produced a sustained increase in MABP beginning at 30 min of hemorrhage that persisted throughout fluid resuscitation in control and alcohol-treated animals. ICV neostigmine enhanced epinephrine responses and restored the hemorrhage-induced release of norepinephrine and AVP in alcohol-treated rats. These results demonstrate that inhibition of acetylcholinesterase in the central nervous system enhances SNS outflow, restores the neuroendocrine response to severe blood loss, and thereby improves hemodynamic counterregulation during acute alcohol intoxication. This study provides evidence for a central (and not peripheral) role of alcohol in impairing hemodynamic stability during hemorrhagic shock.

According to the Center for Disease Control and Prevention, trauma ranked as the fifth leading cause of death in the United States, representing 4.8% of all deaths in 2005 (20). Trauma usually consists of a combined insult of tissue injury and hemorrhagic shock, the latter of which results in severe tissue hypoperfusion, which increases the risk of end-organ damage and death (9). Fluid resuscitation reduces immediate mortality following hemorrhagic shock by improving tissue perfusion; however, long-term mortality rates remain at 22% (27).

Acute alcohol intoxication increases the risk of traumatic injury (21, 36). Nearly half of all injured patients that enter emergency departments across the United States test positive for blood alcohol (6, 24, 38), with blood alcohol levels frequently >80 mg/dl, the legal limit in most states (14, 24, 37, 38). Alcohol-intoxicated trauma patients enter the emergency department more hypotensive than their sober counterparts (41); Clinical data suggest that 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 blood loss (15); therefore, the greater hypotension noted in alcohol-intoxicated trauma patients is likely to contribute to their increased morbidity and mortality.

Previously, we demonstrated that acute 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 (single dose, 3-day binge, and 15-h constant infusion, respectively) of alcohol administration (12, 25, 26, 30, 33). This impaired hemodynamic compensatory response to hemorrhage in alcohol-intoxicated rats is associated with suppression of catecholamines (epinephrine and norepinephrine) and AVP responses (30). Taken together, these observations led us to hypothesize that the inappropriate release of these vasoactive hormones during hemorrhage is the principal mechanism by which alcohol acts to impair MABP recovery following blood loss. Thus we hypothesized that restoration of the neuroendocrine response would improve hemodynamic counterregulation to hemorrhagic shock in alcohol-treated rats.

We aimed to dissect central from systemic effects of acute alcohol intoxication on the counterregulatory responses to hemorrhage. The results from recent studies demonstrated that intracerebroventricular (ICV) administration of choline, a precursor of acetylcholine, immediately stimulates sympathetic nervous system (SNS) outflow in control and alcohol-intoxicated animals, reflected in an increase in MABP and plasma epinephrine, norepinephrine, and AVP (26). However, these effects were transient and not prolonged enough to improve hemodynamic stability following hemorrhagic shock in alcohol-intoxicated animals. The objective of the present studies was to produce sustained SNS activation by central administration of neostigmine, an acetylcholinesterase inhibitor. When injected directly into the central nervous system via the right lateral ventricle (i.e., ICV), neostigmine is confined to the brain and functions by inhibiting the breakdown of acetylcholine to acetate and choline by acetylcholinesterase and, therefore,
increasing acetylcholine availability (44). Thus any effects elicited by injection of this drug reflect a centrally mediated response, which enables isolation of central from systemic mechanisms.

To test our hypothesis, we initially confirmed that ICV neostigmine enhances SNS outflow and that, in turn, the augmented catecholamine and AVP responses contribute to the pressor effects induced by central acetylcholinesterase inhibition. We then determined whether ICV neostigmine was capable of independently reversing hemorrhage- and alcohol-induced hypotension before we subjected animals to a protocol that involved both alcohol and hemorrhage. Our results showed that ICV neostigmine produced an immediate activation of SNS outflow reflected in increased MABP, heart rate, and plasma catecholamines, AVP, and glucose. In addition, we provide strong evidence that the pressor response elicited by ICV neostigmine in normotensive and alcohol-intoxicated hypotensive animals is due to enhanced catecholaminergic and vasopressinergic activity. Taken together, these data suggest that central acetylcholinesterase inhibition restores the neuroendocrine response and improves hemodynamic compensatory responses to blood loss in alcohol-intoxicated rats. Moreover, these data provide evidence that the alcohol-induced impairment of hemodynamic counterregulation to hemorrhagic shock is elicited through attenuation of centrally mediated SNS activation.

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. During a 1-wk acclimation period, specific pathogen-free, adult male Sprague-Dawley rats (225–275 g body wt) were caged in pairs, allowed standard rat chow (2018 Teklad Global 18% Protein Rodent Diet, Harlan) and water ad libitum, and housed in a controlled-temperature (22°C) and controlled-illumination (12:12-h light-dark cycle) environment.

Surgical Procedures

ICV cannula placement. Animals were anesthetized with ketamine and xylazine (90 and 9 mg/kg, respectively) for stereotaxic implantation of guide cannulas into the right lateral ventricle, as previously described by our laboratory (25). After cannula placement, animals were returned to individual cages and allowed 7–9 days to recover from surgery, with food and water provided ad libitum. Cannula placement and patency were tested on the day of vascular and gastric catheter placement and patency were tested on the day of vascular and gastric catheter placement, with food and water provided ad libitum. Cannula placement and patency were tested.

Vascular and gastric catheter placement. Using an aseptic surgical procedure previously described by our laboratory (33), we placed sterile catheters into the left carotid artery and right jugular vein by ICV administration of 5 µl of angiotensin II (20 ng; Sigma, St. Louis, MO), which induced an immediate thirst response. Only animals that exhibited angiotensin II-induced polydipsia, reflecting accurate placement of the cannula in the lateral ventricle, were used in these studies.

Fluid Resuscitation

At the end of the 60-min hemorrhage period, an intravenous bolus of warmed (37°C) lactated Ringer solution equal to 40% of the total blood volume removed was returned to the animals, followed by a constant infusion of lactated Ringer solution of twice the blood volume removed. In total, 2.4 times the blood volume removed was returned to the animals in the form of lactated Ringer solution. MABP was monitored throughout the fluid resuscitation period. Blood (1.5 ml) was removed from hemorrhaged and time-matched sham animals at pre- and posthemorrhage time points (0 and 60 min, respectively).

Experimental Protocols

All animals were subjected to surgical procedures (ICV cannula and/or vascular and gastric catheter placement) and allowed 1–3 days to recover before initiation of the experimental protocol. In addition, animals were conscious and unrestrained throughout all the following experimental protocols.

Study 1: ICV neostigmine modulates SNS activation. To establish an optimal dose of neostigmine, we used 0.1, 0.2, 0.3, 1.2, and 3 µg of neostigmine (n = 5–12 animals per group) to construct a dose-response curve. For ICV neostigmine methyl sulfate (Sigma) administration, an injection cannula (25-gauge, 11.5-mm stainless steel tubing) was connected to a Hamilton microsyringe (10 µl) with polyethylene tubing (25–30 cm) and inserted through a guide cannula previously fixed into the skull. Neostigmine (5 µl) was infused slowly over an 8- to 10-s period into normotensive animals.

To determine whether ICV neostigmine (1 µg) increases SNS activity, we injected normotensive animals (n = 3 animals per group) with neostigmine and monitored MABP for 60 min. Other outcome measures, including heart rate and plasma epinephrine, norepinephrine, AVP, glucose, and insulin, were recorded 5 and 10 min after neostigmine injection.

Study 2: pressor response of ICV neostigmine is elicited through peripheral catecholamines and/or AVP. To determine the contribution of increased vasoactive hormone release to the pressor response of ICV neostigmine, prazosin hydrochloride, an α₁-adrenergic receptor antagonist (0.5 mg/kg; Sigma), [β-mercaptopo-
RESULTS

ICV Neostigmine Modulates SNS Activation

The dose response to ICV neostigmine (0.1, 0.2, 0.3, 1, 2, and 3 μg) was established, and 1 μg was selected on the basis of its ability to produce an immediate (within 5–10 min) increase (13 ± 3%, P < 0.001) in MABP in normotensive animals that was sustained for 60 min. The peak MABP response occurred at 10 min (20 ± 4%, P < 0.001). In

Catecholamine measurements. Blood samples were quantified for circulating epinephrine and norepinephrine levels by a high-performance liquid chromatography system consisting of a chromatographic analyzer with a catecholamine column and an electrochemical detector (Bioanalytic Systems, West Lafayette, IN), as previously described by our laboratory (25). The interassay coefficient of variability for catecholamines was 25%.

AVP measurements. AVP levels were determined in extracted plasma samples by a commercially available human-, rat-, mouse-, and ovine-specific radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA), as previously described by our laboratory (30). Briefly, plasma samples were acidified using 1% trifluoroacetic acid (buffer A) in water. C-18 Sep columns (Waters, Milford, MA) were pretreated with 1 ml of 60% acetonitrile + 40% buffer A (buffer B) followed by 3 ml of 3X buffer A. The supernatant was then loaded into the pretreated column and washed with 3 ml of 2X buffer A and then 3 ml of 1X buffer B. Finally, the eluant was evaporated to dryness using the Speed Vac Concentrator and Condensation Trap (Sarant, Farmingdale, NY) with Duo-Seal Vacuum pump (Sargeart-Welch Scientific, Skokie, IL), and the residue was reconstituted using radioimmunoassay buffer provided with the kit. Levels were determined using the manufacturer’s instructions. The radioimmunoassay reliable detection range was 10–1,280 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.

Plasma insulin measurements. Insulin levels were determined in 100 μl of nonextracted plasma samples using a commercially available rat-specific radioimmunoassay (Linco Research, St. Charles, MO). The radioimmunoassay reliable detection range was 0.1–10 ng/ml of insulin and had 100% specificity for rat insulin.

Statistical Analysis

Values are means ± SE. Statistical analysis of MABP and hormones was accomplished using one- or two-way ANOVA with or without repeated measures. Statistical analysis of survival curves was accomplished using the Gehan-Breslow test. All pair-wise multiple comparisons were completed with the Holm-Sidak method. Statistical significance was set at P < 0.05.

RESULTS

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addition, this dose of ICV neostigmine produced an immediate (within 5 min) increase in heart rate (14 ± 4%, P < 0.001), plasma glucose (37 ± 7%, P = 0.007), and circulating levels of epinephrine (399 ± 100%, P = 0.008), norepinephrine (91 ± 26%, P = 0.020), and AVP (378 ± 89%, P = 0.015) but did not significantly affect plasma levels of insulin (Table 1). Intravenously administered neostigmine (1 μg) was incapable of producing a pressor response in normotensive animals, confirming that these observed effects were centrally mediated (data not shown).

**Pressor Response to ICV Neostigmine Is Elicited Through Peripheral Catecholamine and/or AVP Release**

Basal MABP in normotensive animals pretreated with prazosin alone or prazosin + [β-mercapto-β-cyclopentamethylenepropionyl1, O-meth-Tyr2, Arg8]-vasopressin was reduced within 5 min, whereas intravenous [β-mercapto-β-cyclopentamethylenepropionyl1, O-meth-Tyr2, Arg8]-vasopressin did not alter basal MABP in normotensive animals (Table 2). Prazosin and [β-mercapto-β-b-cyclopentamethylenepropionyl1, O-meth-Tyr2, Arg8]-vasopressin blunted the pressor response to ICV neostigmine in the first 10 min after injection. Prazosin + [β-mercapto-β-b-cyclopentamethylenepropionyl1, O-meth-Tyr2, Arg8]-vasopressin abolished the pressor effect of ICV neostigmine (Fig. 2). MABP in control groups (IV saline + ICV water, IV prazosin + ICV water, and IV V1a antagonist + ICV water) remained unchanged throughout the time course; however, in the IV V1a antagonist + ICV water group, MABP decreased at 30 min after ICV water injection.

**Impact of ICV Neostigmine on MABP During Acute Alcohol Intoxication**

Acute alcohol intoxication lowered basal MABP by 12 ± 4% (P < 0.001) 30 min after administration. ICV neostigmine completely reversed the drop in MABP caused by acute alcohol intoxication (Fig. 3). ICV water did not alter alcohol-induced hypotension.

**Impact of ICV Neostigmine on MABP Response to Hemorrhage**

Removal of 40% of the estimated circulating blood volume produced a 33 ± 9% (P < 0.001) drop in MABP (Fig. 4). ICV neostigmine injected immediately after blood loss (10 min) was capable of reversing hemorrhage-induced hypotension without fluid resuscitation. MABP remained elevated at 120 min after hemorrhage. ICV water did not affect MABP in hypotensive animals. Survival was significantly improved in neostigmine-treated animals (100% vs. 25% in water controls, P = 0.027) 1 wk after hemorrhage, despite the absence of fluid resuscitation.

**Effects of ICV Neostigmine on Hemodynamic Counterregulation to Hemorrhagic Shock in Alcohol-Intoxicated Animals**

Removal of 40% of the estimated circulating blood volume decreased MABP to 60 ± 6 and 51 ± 5 mmHg within 10 min

**Table 1. Measures of SNS activity after central acetylcholinesterase inhibition via ICV neostigmine administration**

<table>
<thead>
<tr>
<th>ICV Neostigmine (1 μg)</th>
<th>Basal</th>
<th>5 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td>125 ± 4</td>
<td>142 ± 4*</td>
<td>150 ± 5*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>374 ± 18</td>
<td>426 ± 16*</td>
<td>364 ± 18</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>204 ± 29</td>
<td>615 ± 145</td>
<td>1,019 ± 205*</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>233 ± 48</td>
<td>445 ± 60*</td>
<td>243 ± 30</td>
</tr>
<tr>
<td>AVP, pg/ml</td>
<td>7.64 ± 1.0</td>
<td>36.6 ± 6.8*</td>
<td>47.0 ± 9.1*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>127 ± 6.2</td>
<td>174 ± 8.3*</td>
<td>214 ± 2.6*</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.03 ± 0.2</td>
<td>0.82 ± 0.2</td>
<td>0.93 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 normotensive rats. MABP, mean arterial blood pressure. *Significantly different from basal (P < 0.05, by 1-way ANOVA with repeated measures).

**Table 2. Effect of antagonist pretreatment on basal MABP**

<table>
<thead>
<tr>
<th>Basal Pretreatment</th>
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<tbody>
<tr>
<td>Prazosin (0.5 mg/kg)</td>
<td>115 ± 4</td>
</tr>
<tr>
<td>V1a receptor antagonist (10 μg/kg)</td>
<td>124 ± 3</td>
</tr>
<tr>
<td>Prazosin + V1a receptor antagonist</td>
<td>138 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8–12 normotensive rats at baseline. MABP was measured 5 min after administration of intravenous α1-adrenoceptor antagonist (prazosin) and/or intravenous V1a receptor antagonist ([β-mercapto-β-b-cyclopentamethylenepropionyl1, O-meth-Tyr2, Arg8]-vasopressin). Absolute basal values of MABP data in Fig. 2 are shown. *Significantly different from basal (P < 0.05, by 1-way ANOVA with repeated measures).
in control and alcohol-treated animals, respectively (Fig. 5). At midhemorrhage (21–36 min after initiation of blood removal), alcohol-treated animals (alcohol/water group) were more (29 ± 2%,
\( P < 0.001 \)) hypotensive than dextrose controls (dextrose/water group), although approximately the same amount of blood was removed from both groups at that time. Fluid resuscitation did not restore MABP to basal levels in dextrose controls (17 ± 5% from baseline, \( P < 0.01 \)) or alcohol-treated animals (20 ± 5% from baseline, \( P = 0.01 \)).

ICV neostigmine administered at 10 min into hemorrhage reversed hypotension and returned MABP to basal levels after fluid resuscitation in dextrose controls (dextrose/neostigmine group) and alcohol-treated animals (alcohol/neostigmine group). Nevertheless, alcohol-treated animals remained more hypotensive than dextrose controls (\( P = 0.040 \)) at 120 min. MABP remained constant in dextrose controls and alcohol-treated sham animals. ICV neostigmine increased MABP by 13% in all sham animals (see above).

Mortality at 48 h after hemorrhage was 29% and 33% in dextrose controls and alcohol-treated animals, respectively. Survival with ICV neostigmine was 100% in dextrose controls and alcohol-treated animals at 48 h after hemorrhage. At 1 wk after hemorrhage, survival with ICV neostigmine was 83% in dextrose controls and alcohol-treated animals, compared with 71% in the dextrose/water group and 67% in the alcohol/water group.

**Effects of ICV Neostigmine on the Neuroendocrine Response to Hemorrhage During Acute Alcohol Intoxication**

Hemorrhagic shock produced marked 542 ± 156% (\( P = 0.007 \)) and 522 ± 195% (\( P = 0.022 \)) increases in circulating levels of epinephrine at the end of hemorrhage (60 min) in dextrose controls and alcohol-treated animals, respectively (Fig. 6). ICV neostigmine did not alter posthemorrhage epinephrine levels in dextrose controls but further enhanced the hemorrhage-induced increase in alcohol-treated animals (1,193 ± 300%, \( P = 0.004 \)).

In dextrose controls, hemorrhagic shock produced a significant 123 ± 41% increase (\( P = 0.023 \)) in circulating levels of norepinephrine at the end of hemorrhage (60 min), which was prevented by alcohol (Fig. 6). ICV neostigmine did not alter the hemorrhage-induced increase in plasma norepinephrine in dextrose controls but restored the hemorrhage-induced increase in alcohol-treated animals.

Hemorrhagic shock increased circulating levels of AVP by 125 ± 58% (\( P = 0.064 \)), which was prevented by alcohol (Fig. 6). ICV neostigmine did not alter posthemorrhage plasma AVP in dextrose controls but restored the hemorrhage-induced increase in alcohol-treated animals.

**Fig. 3.** Effects of ICV neostigmine on MABP in alcohol-treated rats. ICV neostigmine (1 µg) reversed alcohol-induced hypotension (\( n = 4–5 \)). Values (means ± SE) were analyzed using 2-way ANOVA with repeated measures. *Significantly different from pre-alcohol; †significantly different from water (\( P < 0.05 \)).

**Fig. 4.** Effects of ICV neostigmine on MABP after moderate blood loss. ICV neostigmine (1 µg) administered 10 min after 40% blood loss (no fluid resuscitation) reverses hemorrhage-associated hypotension (\( n = 4–6 \)). Values (means ± SE) were analyzed using 2-way ANOVA with repeated measures. *Significantly different from 0 min; †significantly different from water (\( P < 0.05 \)).

**Fig. 5.** Impact of ICV neostigmine on hemodynamic response to hemorrhage during acute alcohol intoxication. Effects of neostigmine (0.5 µg) or sterile water (5 µl) on MABP in dextrose controls and alcohol-treated animals subjected to 50% hemorrhage and fluid resuscitation (\( n = 7–9 \)). Values (means ± SE) were analyzed using 2-way ANOVA with repeated measures. †Significantly different from dextrose/water; ‡significantly different from alcohol/water; @significantly different from 0 min (\( P < 0.05 \)).
The results presented here are the first to demonstrate that inhibition of acetylcholinesterase in the central nervous system is capable of improving hemodynamic compensation and outcome from hemorrhagic shock in alcohol-intoxicated rats. These findings indicate that the improved counterregulation occurs through enhanced release of vasoactive hormones and that central nervous system nicotinic receptors contribute to the anticholinesterase-induced pressor effect. Overall, our results demonstrate that increasing central acetylcholine availability enhances sympathetic neurotransmission in alcohol-intoxicated animals after hemorrhage. Moreover, they strongly suggest that alcohol exerts its detrimental effects on hemodynamic counterregulation to severe blood loss through central mechanisms.

Initially, we speculated that central and peripheral mechanisms contribute to impairment of hemodynamic counterregulation to blood loss during acute alcohol intoxication. Mechanisms that may explain this alcohol-induced phenomenon include a decreased circulating blood volume before hemorrhage, an impaired vascular responsiveness to vasoactive substances, and/or a blunted neuroendocrine response to hemorrhage. Preliminary findings suggest that blood volume is not markedly altered in our model of alcohol intoxication (unpublished observations). In addition, recent studies from our laboratory provide strong evidence that vascular responsiveness is not significantly altered in alcohol-intoxicated animals (28). However, our previous studies consistently demonstrated that the impaired hemodynamic counterregulation to hemorrhage in alcohol-intoxicated animals is associated with suppression of catecholamine (epinephrine and norepinephrine) and AVP responses to severe hemorrhage (MABP maintained at 40 mmHg for 60 min) (30). Taken together, these observations led us to hypothesize that the inappropriate release of vasoactive hormones during hemorrhage is the principal mechanism by which alcohol acts to impair MABP recovery after blood loss. MABP is centrally regulated in the short term through baroreceptor-mediated physiological response to hemorrhage (32) and cholinergic mechanisms (3). Both pathways are thought to affect SNS neurotransmission. The baroreceptor-mediated physiological response to hemorrhage is the result of a decrease in perfusion pressure during blood loss, causing baroreceptors in the aortic arch and carotid sinus to decrease their nerve firing rate. In turn, this results in enhanced sympathoexcitatory mechanisms through increased neurotransmission of cholinergic neurons in the rostral ventrolateral medulla (5, 32). Cholinergic pathways are independently involved in the central control of MABP through SNS activation as well (3, 18, 34, 35). The activation of sympathetic responses plays a central role in orchestrating hemodynamic counterregulation to blood loss, increasing systemic vascular resistance and cardiac output, which ultimately contributes to restoration of MABP (7). SNS activation produces an increase in the release of epinephrine and norepinephrine from the adrenal medulla and additional increases in circulating levels of norepinephrine from sympathetic postganglionic noradrenergic nerve terminals (8, 43). Baroreceptor signaling and cholinergic pathways contribute to direct increases in the release of AVP from magnocellular neurons of the supraoptic nucleus and paraventricular nucleus (32, 35). AVP contributes to maintenance of hemodynamic homeostasis by increasing systemic vascular resistance and cardiac output, which ultimately contributes to restoration of MABP (7).

![Graphs showing effects of neostigmine on plasma epinephrine, norepinephrine, and AVP in dextrose controls and alcohol-treated animals subjected to 50% hemorrhage and fluid resuscitation.](http://ajpregu.physiology.org/)

**DISCUSSION**

The results presented here are the first to demonstrate that inhibition of acetylcholinesterase in the central nervous system is capable of improving hemodynamic compensation and outcome from hemorrhagic shock in alcohol-intoxicated rats. These findings indicate that the improved counterregulation
of these hormones, resulting in impaired hemodynamic counterregulatory responses to hemorrhagic shock.

In the present studies, we chose to pharmacologically enhance SNS activation with neostigmine, an acetylcholinesterase inhibitor of the carbamate family of cholinomimetic agents that does not cross the blood-brain barrier (4). Acetylcholinesterase inhibitors have been demonstrated to reverse the hypotension associated with hemorrhagic shock and improve survival in rats (2, 13, 39, 42). Our results verify reports in the literature (11, 16, 17, 22, 23) that demonstrate the ability of neostigmine to immediately activate the SNS, indicated by increases in MABP, sympathoadrenal activation, and a decrease in insulin, when injected directly into the central nervous system (Table 1). We determined that 1 μg of neostigmine ICV was sufficient to increase MABP ≥20 mmHg. A dose of 0.5 μg was the minimally effective dose that was able to increase MABP by a similar degree. On the basis of those observations and because of the observed side effects (e.g., shaking and salivation) that occurred once the drug was administered ICV at the higher dose, the dose of neostigmine was reduced from 1 to 0.5 μg in the final study. An increase in MABP, heart rate, plasma catecholamines, AVP, and plasma glucose and a decrease in plasma insulin were interpreted as an indication of activation of SNS activity. SNS activation inhibits insulin release from the pancreatic β-cell, whereas parasympathetic nervous system activation stimulates insulin release, particularly during hyperglycemia (1). Thus we measured insulin levels as an additional confirmation that central neostigmine administration, as well as systemic physostigmine injection, results in activation of the SNS. The same dose of neostigmine was administered intravenously to rule out the possibility that leakage of ICV administered drug would contribute to the measured effects. This dose of neostigmine administered systemically did not affect any of the parameters. In addition, we have confirmed that the pressor response produced by ICV neostigmine is partially mediated through enhanced catecholamine and AVP release (Fig. 2) by blocking α-adrenergic and AVP V1 receptors with prazosin and [β-mercapto-β,β-cyclopentamethylene propionyl]1, O-me-Tyr2,Arg8]-vasopressin, respectively. The doses for the receptor antagonists used in these studies were based on reports in the literature indicating that they are effective in inhibiting pressor responses of ICV U-46619, a thromboxane A2 analog (45).

Previously, we demonstrated that a model of severe hemorrhagic shock, which involves removal of ~55–65% of estimated blood volume to maintain a target MABP of ~40 mmHg for 60 min, results in a blunted release of epinephrine, norepinephrine, and AVP in alcohol-intoxicated animals (30). In the present study, we used a more moderate hemorrhage model, where only 50% of blood was removed from dextrose controls and alcohol-treated animals. Because this produces a greater hypotension in alcohol-treated animals, a greater vasopressor release was expected. However, the data show that, despite the greater hypotension seen in acute alcohol intoxication, alcohol-intoxicated animals had a similar hemorrhage-induced rise in epinephrine and AVP levels and an attenuated rise in norepinephrine levels. Thus, although the overall levels would not appear to be markedly blunted, the relative attenuated response to a greater hypotensive insult in alcohol-intoxicated animals confirmed our previous findings. Furthermore, ICV neostigmine was effective in enhancing and restoring the hemorrhage-induced increase in catecholamines and AVP, respectively, and this improved hemodynamic counterregulation in alcohol-treated animals (Fig. 6). Taken together, these observations lend strong support for our hypothesis implicating attenuated neuroendocrine activation as a central mechanism responsible for impaired hemodynamic stability in alcohol-intoxicated hemorrhaged animals.

It is important to note that the model of alcohol administration used in these studies 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 (31). According to the National Survey of Drug Use and Health, binge drinking has been shown to increase the risk of traumatic injury and other alcohol-related deaths in an otherwise healthy population (10, 40). Therefore, the alcohol model and blood alcohol levels achieved in these studies (192 ± 21 mg/dl) are clinically relevant.

The present studies allowed us to determine the effects of acute alcohol intoxication on outcome to severe hemorrhage. Because the ability to compensate hemodynamically after blood loss correlates with improved tissue perfusion and better outcome, MABP is an appropriate outcome measure. However, this may be thought of as a limitation of the study, because heart rate and cardiac output could provide a more complete indication of tissue perfusion. It may be critical to make these measurements, inasmuch as ICV neostigmine leads to an increase in MABP, which we speculate is due to increased SNS activity, which could potentially lead to compromised blood flow to organs.

Future studies are warranted to determine the effect of central acetylcholinesterase inhibition on blood flow to specific organ beds after hemorrhage with use of more direct measures.

ICV administration of drugs allows direct administration into the central nervous system, so that the effects of the drugs on the central nervous system can be observed. However, one limitation is the lack of neuroanatomic specificity of the effects. Additional studies are warranted to identify the exact location of action by administration of the drugs into specific brain regions and/or ablation of certain brain regions.

**Perspectives and Significance**

Results from these studies and those reported in the literature indicate that ICV neostigmine enhances central cholinergic activity, activates the SNS, and improves catecholaminergic and vasopressinergic responses to hemorrhagic shock in alcohol-intoxicated rats. Restoration of the neuroendocrine response through ICV neostigmine reverses hypotension during and after hemorrhagic shock in alcohol-intoxicated animals. These results suggest that the inappropriate neuroendocrine response during hemorrhagic shock is a central mechanism involved in hemodynamic instability in alcohol-intoxicated animals. Moreover, they provide preclinical evidence for a novel approach to management of the alcohol-intoxicated traumatic injury patient that may be advantageous over present management and care. An approach to reduce the volume of resuscitation fluid necessary to restore MABP and tissue perfusion after severe hemor-
rhage would likely result in improved outcomes and decreased cost of management of the trauma/hemorrhage patient. Central administration of pharmacotherapies during fluid resuscitation in the clinical setting is not a practical approach to treat a trauma/hemorrhage patient. Therefore, studies using intravenous injection of a centrally acting acetylcholinesterase inhibitor are warranted to translate our findings to a clinically relevant scenario. Additional investigation of the impact of these drugs on tissue and organ injury, as well as their impact on metabolic and inflammatory responses to hemorrhage, is also warranted.

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