Hypertonic sodium resuscitation after hemorrhage improves hemodynamic function by stimulating cardiac, but not renal, sympathetic nerve activity

Robert Frithiof, Rohit Ramchandra, Sally G. Hood, and Clive N. May
Howard Florey Institute, University of Melbourne, Parkville, Victoria, Australia
Submitted 15 September 2010; accepted in final form 3 December 2010

Frithiof R, Ramchandra R, Hood SG, May CN. Hypertonic sodium resuscitation after hemorrhage improves hemodynamic function by stimulating cardiac, but not renal, sympathetic nerve activity. Am J Physiol Heart Circ Physiol 300: H685–H692, 2011. First published December 10, 2010; doi:10.1152/ajpheart.00930.2010.—Small volume hypertonic saline resuscitation can be beneficial for treating hemorrhagic shock, but the mechanism remains poorly defined. We investigated the effects of hemorrhagic resuscitation with hypertonic saline on cardiac (CSNA) and renal sympathetic nerve activity (RSNA) and the resulting cardiovascular consequences. Studies were performed on conscious sheep instrumented with cardiac (n = 7) and renal (n = 6) sympathetic nerve recording electrodes and a pulmonary artery flow probe. Hemorrhage (20 ml/kg over 20 min) caused hypotension and tachycardia followed by bradycardia, reduced cardiac output, and abolition of CSNA and RSNA. Resuscitation with intravenous hypertonic saline (1.2 mol/l at 2 ml/kg) caused rapid, dramatic increases in mean arterial pressure, heart rate, and CSNA, but had no effect on RSNA. In contrast, isotonic saline resuscitation (12 ml/kg) had a much delayed and smaller effect on CSNA, less effect on mean arterial pressure, no effect on heart rate, but stimulated RSNA, although the plasma volume expansion was similar. Intracarotid infusion of hypertonic saline (1 ml/min bilaterally, n = 5) caused similar changes to intravenous administration, indicating a cerebral component to the effects of hypertonic saline. In further experiments, contractility (maximum change in pressure over time), heart rate, and cardiac output increased significantly more with intravenous hypertonic saline (2 ml/kg) than with Gelofusine (6 ml/kg) after hemorrhage; the effects of hypertonic saline were attenuated by the β-receptor antagonist propranolol (n = 6). These results demonstrate a novel neural mechanism for the effects of hypertonic saline resuscitation, comprising cerebral stimulation of CSNA by sodium chloride to improve cardiac output by increasing cardiac contractility and rate and inhibition of RSNA.

Methods

Ethical approval. Experimental procedures were approved by the Animal Experimental Ethics Committee of the Howard Florey Institute, in accordance with the Prevention of Cruelty to Animals Act 1986, under the guidelines of the National Health and Medical Research Council of Australia’s Code of Practice for the Care and Use of Animals for Experimental Purposes, which conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23, revised 1996).

All experiments were conducted on adult Merino ewes (35–45 kg), housed in individual metabolism cages. Eight sheep were used for examining the sympathetic effects of intravenous (IV) hypertonic or isotonic saline resuscitation after hemorrhage, five sheep were used to determine the sympathetic effects of intravenous (IV) hypertonic or isotonic saline resuscitation after hemorrhage, five sheep were used to determine the sympathetic effects of intravenous (IV) hypertonic or isotonic saline resuscitation after hemorrhage, five sheep were used to determine the sympathetic effects of intravenous (IV) hypertonic or isotonic saline resuscitation after hemorrhage. For the two first sets of experiments, surgery was performed to implant recording electrodes into the cardiac and renal sympathetic nerves. Under general anesthesia, the right or left renal artery was exposed via a paracostal retroperitoneal approach. With the aid of a dissection microscope, the renal nerve was...
identified running along or parallel to the renal artery and cleared of surrounding fat. The recording electrodes consisted of tungsten wire (0.05-mm diameter), etched to a fine point, glued into the end of Teflon-coated 25-strand silver-coated copper wires (C2174SPC, Cooner, Chatsworth, CA). The exposed point of the electrode (1.5–2.0 mm in length) was inserted obliquely through the nerve sheath, ensuring that the tip was positioned in the center of the nerve. Up to five electrodes were implanted along the exposed length of nerve and fixed in place with cyanoacrylate glue. The wires were looped and exteriorized through the sutured wound. For a ground, a custom-made stainless steel plate with an attached metal loop was inserted subcutaneously close to the exit site of the electrodes, leaving the loop protruding through the skin. Cardiac sympathetic nerve recording electrodes were implanted in the thoracic cardiac nerves via a thoracotomy. Experiments were conducted on standing, conscious sheep and, to minimize any effect of surgical stress, were started on the 4th day after implantation of the electrodes.

To facilitate arterial blood pressure measurements, easy cannulation for IC infusion of hypertonic saline, and insertion of a left ventricular catheter, the sheep were anesthetized and subjected to unilateral or bilateral (for the second set of experiments) exteriorization of the carotid arteries into cervical skin loops. Special care was taken not to damage or stretch the vagus nerve. This was performed at least 2 wk before the implantation of sympathetic nerve recording electrodes. For the third set of experiments, the sheep were implanted with a transit-time flow probe (20 mm, Transonic Systems, Ithaca, NY) on the pulmonary artery, via a left-sided thoracotomy. Antibiotic (900 mg of procaine penicillin; Troy Laboratories, Sydney, NSW, Australia) was administered prophylactically for 3 days after surgery. Postsurgical analgesia was maintained with intramuscular injection of flunixin meglumine (1 mg/kg; Mavlab) at the start of surgery and then 4 and 16 h postsurgery.

In all animals, at least 1 day before experiments with the use of aseptic techniques, a Tygon cannula was inserted into a carotid artery for measurement of arterial pressure. Three polyethylene cannulas were inserted into a jugular vein to facilitate blood withdrawal and for infusions. In addition, in the sheep in which cardiac effects of sympathetic activation by hypertonic saline were investigated, a Tygon cannula was advanced under pressure guidance to the left ventricle via the left carotid artery.

**Sympathetic nerve recording.** CSNA and RSNA were recorded differentially between the pair of electrodes with the best signal-to-noise ratio. The signal was amplified (×100,000) and filtered (band-pass 300–1,000 Hz), displayed on an oscilloscope, and passed through an audio amplifier and loud speaker. SNA (5000 Hz), CO (100 Hz), and arterial and venous blood pressures (100 Hz) were recorded on computer using a CED micro 1401 interface and Spike 2 software (Cambridge Electronic Design).

Data were analyzed on a beat-to-beat basis using custom-written routines in the Spike 2 program. For each heartbeat, the program determined diastolic, systolic, and mean arterial pressure (MAP) heart period. The threshold was set just above background so that spikes from small bursts were counted. The background noise was taken as the spikes per second during the hypotensive phase of hemorrhage, when SNA was abolished, and this was subtracted from the data collected on the day. The burst incidence was calculated as the number of bursts per 100 heartbeats.

**Sympathetic and hemodynamic responses to hypertonic or isotonic saline resuscitation.** The effect of hemorrhage and subsequent resuscitation with hypertonic saline on CSNA and RSNA was investigated in eight conscious sheep. Recordings of nerve activity with satisfactory quality were obtained in four animals for CSNA and RSNA. Five minutes after a hypotensive hemorrhage, performed as above, 2.4 mosmol/kg NaCl were infused 1 ml/min for 10 min into each carotid. The shed blood was retransfused 5 min after hemorrhage and following resuscitation. Jugular venous blood samples were taken for analysis of plasma sodium and protein concentration before hemorrhage, after hemorrhage, and following resuscitation.

**Cardiac effects of sympathetic activation by hypertonic saline.** At occasions separated by at least 3 days and in a randomized fashion, six conscious sheep went through three different protocols to investigate β-receptor stimulatory effects on the heart of the increased CSNA induced by hypertonic saline. Hemorrhage was performed as described above, and 5 min thereafter a 2-min IV infusion, consisting of either 2 ml/kg of 2.4 mosmol/kg NaCl or 6 ml/kg 4% modified fluid gelatin (Gelofusine), was started. In the third protocol the hypertonic saline infusion was preceded by a bolus injection (30 mg) and a 60-min infusion (2 mg/min) of the β-receptor antagonist propranolol. The shed blood was retransfused 60 min after the resuscitation ended.

Venous blood samples were taken for analyses of plasma sodium, chloride, and protein concentration before hemorrhage, after hemorrhage, following resuscitation, and 30 and 60 min thereafter.

**Data analyses.** All statistical calculations were performed using Statistica 8 (Statsoft, Tulsa, OK), and the graphs were created with Sigma Plot 8.02 (SPSS, Chicago, IL). Data are expressed as means ± SE. Changes in plasma and cardiovascular parameters over time were analyzed using two-way repeated-measures ANOVA, including all treatment groups in each experiment. In case of a significant main effect, pairwise comparisons of treatments, as well as a one-way repeated-measures ANOVA for each treatment, were performed. A significant effect of time was interpreted as due to the resuscitation, and a significant time-treatment interaction was taken as a difference in the response between treatments. The average level of nerve activity during the different treatments was analyzed using Wilcoxon matched-pairs test. Differences in time until the first burst between treatments and nerves were evaluated with Bonferroni corrected Student’s t-tests. The significance level was set at $P \leq 0.05$.

**RESULTS**

Baseline cardiovascular, CSNA, and RSNA data are shown in Table 1.

**Sympathetic and hemodynamic responses to hemorrhage.** Hemorrhage caused progressive decreases in CO, MAP, left ventricular pressure, and total peripheral conductance (TPC), whereas heart rate (HR) initially increased (Fig. 1A). After $\sim 15$ ml/kg of blood loss, HR fell abruptly to control levels, together with a pronounced reduction in MAP, CO, and left ventricular pressure. At this time, TPC increased slightly, indicating peripheral vasodilatation.

Both CSNA and RSNA increased in response to hemorrhage, although the CSNA response was more prominent, with a twofold increase in activity when blood loss reached 14...
ml/kg (Fig. 1B). Subsequently, coinciding with the fall in HR and blood pressure, activity in both nerves decreased significantly until it was abolished at 20 ml/kg blood loss (Fig. 1, B and C). The baseline mean level of RSNA was significantly higher than the corresponding mean level of CSNA (P < 0.02), but, during hemorrhage, the activity in both nerves increased to similar absolute values (RSNA 22.7 ± 2.9 spikes/s and CSNA 22.5 ± 4.8 spikes/s after 14 ml/kg hemorrhage).

**Sympathetic and hemodynamic responses to hypertonic (1.2 mol/l) or isotonic saline resuscitation.** After hemorrhage, IV infusion over 10 min of hypertonic saline (2 ml/kg) or isotonic saline (12 ml/kg) caused comparable degrees of plasma volume expansion (Fig. 2A). As expected, only hypertonic saline increased plasma sodium concentration (Fig. 2B). Hypertonic saline infusion increased MAP more rapidly and to a higher level compared with isotonic saline (Fig. 2C). This was associated with a significant increase in HR not seen with isotonic saline (Fig. 2D).

At the end of hypotensive hemorrhage, when CSNA was abolished, resuscitation with hypertonic saline caused a rapid stimulation in CSNA to above control levels. Isotonic saline also restored CSNA, but the average activity during the infusion was significantly lower than with hypertonic resuscitation (Fig. 2E). Interestingly, the opposite effect was noted when investigating the RSNA response (Fig. 2F). Whereas RSNA was partially restored by isotonic saline, following hypertonic saline, there was only a very minor increase in RSNA. These observations were further emphasized by analysis of the latency from start of resuscitation until the occurrence of the first bursts in the respective recordings (Fig. 2G). The duration until the first occurrence of bursts during hypertonic saline infusion was significantly less for CSNA than for RSNA (Fig. 2G); indeed, renal sympathetic bursts were sometimes not seen until after the hypertonic saline infusion was finished. During isotonic saline, CSNA returned at approximately the same time as

![Fig. 1](http://ajpheart.physiology.org/) Effects of a controlled venous hemorrhage (20 ml/kg body wt over 20 min) in conscious sheep. A: changes in mean arterial pressure (MAP; n = 15), heart rate (HR; n = 15), left ventricular pressure (LVP; n = 6), cardiac output (CO; n = 6), and total peripheral conductance (TPC; n = 6). B: changes in renal sympathetic nerve activity (RSNA; n = 6) and cardiac sympathetic nerve activity (CSNA; n = 8). Values are means ± SE. C: original trace showing 8-s recordings of arterial pressure (AP), RSNA, and CSNA before and during a controlled venous hemorrhage (20 ml/kg body wt in 20 min). Basal levels are presented in Table 1.

### Table 1. Baseline values of MAP, HR, CO, LVP, dP/dt\(_{\text{max}}\), CSNA, and RSNA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>88</td>
<td>2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>85</td>
<td>2</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.5</td>
<td>0.2</td>
</tr>
<tr>
<td>LVP, mmHg</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>dP/dt(_{\text{max}}), mmHg/s</td>
<td>3,434</td>
<td>254</td>
</tr>
<tr>
<td>CSNA, spikes/s</td>
<td>11.2</td>
<td>2.4</td>
</tr>
<tr>
<td>RSNA, spikes/s</td>
<td>18.4</td>
<td>1.8</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; LVP, left ventricular pressure; dP/dt\(_{\text{max}}\), maximum change in pressure over time; CSNA, cardiac sympathetic nerve activity; RSNA, renal sympathetic nerve activity.
RSNA, but was significantly delayed compared with CSNA during hypertonic resuscitation (Fig. 2G).

The cerebral component of hypertonic saline resuscitation. To investigate the contribution of cerebral mechanisms to the effect of hypertonic saline resuscitation, we determined the responses to bilateral IC infusion at a rate chosen to have minimal systemic effects. Nevertheless, plasma volume, as well as plasma sodium concentration, increased slightly but insignificantly in both groups. However, there was a significant difference in the latency to the first bursts in the cardiac and renal sympathetic nerves between the two groups: the latency was significantly longer in the 1.2 mol/l NaCl group compared with the isotonic NaCl group (P < 0.05). The time from the start of resuscitation until the first bursts were noted in the cardiac and renal sympathetic nerves is denoted by the symbol *.

Fig. 2. Intravenous resuscitation with 2 ml/kg hypertonic (1.2 mol/l) NaCl or 12 ml/kg isotonic NaCl over 10 min, started 5 min after a 20 ml/kg controlled venous hemorrhage. A–F: effects on plasma volume expansion (n = 8; A), plasma jugular vein sodium concentration ([Na⁺]; n = 8; B), MAP (n = 8; C), HR (n = 8; D), CSNA (n = 7; E), and RSNA (n = 6; F). All animals received both treatments at different occasions and in a randomized fashion. Also presented are control experiments in three animals receiving no resuscitation. BPM, beats/min. Significant *change over time, #difference in effect over time between 1.2 mol/l NaCl and isotonic NaCl, §difference in effect over time between 1.2 mol/l NaCl and no resuscitation, and $difference in average level of nerve activity between 1.2 mol/l NaCl and isotonic NaCl during resuscitation: P < 0.05. G: time from start of resuscitation until the first bursts were noted in the cardiac and renal sympathetic nerves. *P < 0.05. Values are means ± SE.

AJP-Heart Circ Physiol • VOL 300 • FEBRUARY 2011 • www.ajpheart.org
significantly (Fig. 3, A and B), although the changes were very much less than with IV hypertonic saline (Fig. 2, A and B). IC infusion caused progressive increases in MAP and HR (Fig. 3, C and D), and, as seen with IV hypertonic resuscitation, it caused a large, significant stimulation of CSNA, but had no effect on the renal sympathetic nerves, which remained silent (Fig. 3, E and F).

Cardiac effects of sympathetic activation by hypertonic saline. In the final set of experiments, the cardiac response to the increased CSNA was assessed by evaluating the effect of β-adrenoceptor blockade with propranolol. To more accurately mimic the clinical situation, the hypertonic saline resuscitation was given rapidly over 2 min. Considering the rapid infusion rate, Gelofusine was used as the control fluid, as it is a more potent plasma volume expander than isotonic saline and thus requires less volume to be infused to replicate the change in plasma volume seen with hypertonic saline. As before, plasma volume and sodium levels were elevated by hypertonic saline, whereas Gelofusine only caused plasma volume expansion (Fig. 4, A and B). In the presence of propranolol, the hypertonic saline-induced tachycardia was prevented, and contractility was only restored to the level achieved by volume expansion per se (Fig. 4, C and D). These effects of propranolol significantly reduced the improvement in CO caused by hypertonic saline (Fig. 4E). There was, however, no significant difference in the response of MAP to the resuscitations (Fig. 4F), because hypertonic saline caused peripheral vasodilatation, as shown by a significantly increased TPC (Fig. 4G).

DISCUSSION

We found that resuscitation after hemorrhage with small-volume hypertonic saline stimulated CSNA, but inhibited RSNA. These effects were probably cerebrally mediated, as an infusion of hypertonic saline acting mainly on the brain caused similar results. In further investigations, we demonstrated that the ability of hypertonic saline to increase CO, via chronotropic and inotropic actions induced by stimulation of CSNA, depended on activation of cardiac β-receptors. These findings demonstrate the presence of a novel neural mechanism that contributes significantly to the beneficial cardiovascular effects of hypertonic saline resuscitation, in addition to its ability to cause plasma volume expansion.

Hemorrhage causes a biphasic response in SNA, HR, and vascular resistance, with an initial increase and subsequent reduction in these variables (26). The peripheral vasodilatation is coupled to a pronounced fall in blood pressure and marks the start of microcirculatory impairment, ischemia, and organ dysfunction, all hallmarks of hemorrhagic shock. Withdrawal of SNA during the hypotensive, decompensation phase of actual or simulated hemorrhage has been described to occur in renal and muscle sympathetic nerves (35), whereas activity remains in adrenal sympathetic nerves (33). This study showed that CSNA, after a significant increase, was abolished by hemorrhage.

Resuscitation with hypertonic saline after hemorrhage improves survival compared with an equal volume of isotonic...
As confirmed by the present study, MAP and CO are rapidly restored by hypertonic saline administered in a volume of 10% of shed blood volume (21), which is largely due to a rapid increase in intravascular volume. However, these effects cannot be attributed entirely due to volume expansion, because resuscitation with isotonic saline or Gelofusine, which caused the same increase in plasma volume, had less effect on MAP and CO (Figs. 2 and 4). Instead, we provide evidence that stimulation of CSNA by hypertonic saline had potent chronotropic and inotropic actions, which, together with improved preload and reduced afterload, resulted in increased CO. These data provide a mechanism by which hypertonic saline resuscitation caused peripheral vasodilatation, as shown by the increase in TPC (Fig. 4G). This has been shown to be mainly due to hyperosmolality-induced vascular smooth muscle relaxation (12), which, together with the improvement in cardiac pump function, may improve microcirculatory blood flow and attenuate ischemia. In this context, the stimulatory function of CSNA on CO is crucial to prevent augmentation of the hypotension by the peripheral vasodilatation, as has been described after rapid administration of hypertonic saline in anesthetized, but not conscious, animals (15).

One of the most striking findings of this study was the differential effect of hypertonic saline resuscitation on sympathetic outflow to the heart and kidneys. In contrast to the potent stimulation of CSNA by hypertonic saline resuscitation, there was no increase in RSNA. Indeed, compared with the stimulatory effect of isotonic saline resuscitation on RSNA, hypertonic saline, in fact, inhibited RSNA. In the setting of hemorrhage, this renal sympathoinhibition may have beneficial effects on renal function. During hemorrhage, there is a significant decrease in renal blood flow and glomerular filtration rate (18, 20), which may be partly due to reduced sympathetic vasoconstrictor tone in the renal vasculature (19). Hypertonic saline resuscitation may reduce this hypotension by decreasing renal vascular resistance and improving renal perfusion, thereby preserving renal function during hemorrhage.

Fig. 4. Intravenous resuscitation with 2 ml/kg hypertonic NaCl (1.2 mol/l), 6 ml/kg Gelofusine (6% modified fluid gelatine), or 2 ml/kg hypertonic NaCl (1.2 mol/l) preceded by treatment with propranolol (30 mg bolus followed by infusion at 2 mg/min for 60 min). A–G: effects on plasma [Na⁺] (n = 6; A) and plasma volume expansion (n = 6; B), HR (n = 6; C), maximum change in pressure over time (dP/dtmax; n = 6; D), CO (n = 6; E), MAP (n = 6; F), and TPC (n = 6; G). Resuscitation was started 5 min after a 20 ml/kg controlled venous hemorrhage and was discontinued after 120 s. The intravenous infusions were kept at constant rates of 1 ml·kg⁻¹·min⁻¹ (hypertonic NaCl) and 3 ml·kg⁻¹·min⁻¹ (Gelofusine). All animals received all treatments at different occasions and in a randomized fashion. Significant *change over time, #difference in effect over time between 1.2 mol/l NaCl and Gelofusine, and §difference in effect over time between 1.2 mol/l NaCl and 1.2 mol/l NaCl preceded by propranolol: P ≤ 0.05.
intracerebroventricular infusion of the AT1-antagonist losartan by blocking cerebral AT1 receptors attenuates the improvement in renal function during hypertonic saline resuscitation is supported by the finding that blocking cerebral AT1 receptors attenuates the improvement in systemic hemodynamics after resuscitation with hypertonic saline (9, 31). The areas of the brain thought to be involved in the initiation of the compensatory phase, such as the rostral ventrolateral medulla, the ventrolateral periaqueductal gray, and the caudal midline medulla (4, 14, 23), may also be affected by the hypertonic saline.

Besides a direct central action, hypertonic saline may also act on neural afferent pathways to modulate SNA. A possible action on the carotid bodies is suggested by the finding that sinoaortic denervation attenuated the recovery of MAP in response to hemorrhagic resuscitation with hypertonic saline (6). An intact innervation of the lung has been suggested to be essential for the full hemodynamic response to hypertonic saline (16, 39), but other studies have questioned this hypothesis (1). Furthermore, the inhibition of RSNA may, in theory, be due to activation of cardiopulmonary mechanoreceptors caused by the volume expansion, but this is unlikely, as the animals were both hypovolemic and hypotensive during a major part of the resuscitation. In conclusion, we present a novel neural mechanism by which hypertonic saline resuscitation reverses hemorrhagic shock. Our results indicate that hypertonic saline acts to selectively stimulate CSNA and inhibit RSNA, and that the former effect is crucial for the full cardiovascular response to resuscitation. Furthermore, these findings suggest that these contrasting sympathetic effects are centrally mediated. Because autonomic function is extensively modified by hemorrhage and by common pharmacological treatments used in conjunction with hemorrhage, such as anesthetic agents and opioids, our results are of importance for the accurate use of this resuscitation in hemorrhagic shock. Moreover, hypertonic saline activation of CSNA may be beneficial in other forms of shock, sepsis, traumatic brain injury, and vasovagal syncope, but further studies are necessary to elucidate this.

ACKNOWLEDGMENTS

The authors thank Tony Dornom and Alan McDonald for excellent technical assistance and David Trevaks for Spike 2 programming. Current address of R. Frithiof: Karolinska Institutet, Physiology & Pharmacology, von Eulers vagn 8, Stockholm 17177, Sweden.

GRANTS

This work was supported by the National Health and Medical Research Council of Australia (NHMRC) (Grant 509204). R. Frithiof was the recipient of a Post-doctoral Research Fellowship from the Swedish Heart and Lung Foundation, C. N. May was supported by an NHMRC Research Fellowship (566819), and R. Ramchandra was supported by a National Heart Foundation Post-doctoral Research Fellowship (PF07M3293).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

H692

SYMPATHETIC EFFECTS OF HYPERTONIC RESUSCITATION


