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, isotonc 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 to determine the sympathetic effects of intravenous (IV) hypertonic or isotonc saline resuscitation after hemorrhage, five sheep were used for examining the same effects after bilateral intracarotid (IC) infusion of hypertonic saline, three sheep were hemorrhaged and not resuscitated (time controls), and a further six sheep were used to investigate cardiovascular changes of hemorrhagic resuscitation with Gelofusine or hypertonic saline, with or without preinfusion of the β-blocker propranolol. In all sets of experiments, the animals served as their own controls, i.e., in a randomized fashion, and at separate occasions they received all treatments specific for those experiments. Each experiment was separated by at least 3 days and was only performed if the cardiovascular variables and sympathetic nerve activity (SNA) were found to be normal. Sheep were fed a diet of oaten chaff (800 g/day), and water was offered ad libitum.

Surgical procedures. Before experiments, all sheep underwent sterile surgery. Anesthesia was induced with sodium thiopental (15 mg/kg iv), and, after endotracheal intubation, anesthesia was maintained with 1.5–2.0% isoflurane-O2. 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 (CZ1745SPC, 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.

Cardiac 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 during both experiments in seven animals for CSNA and six animals for RSNA. Hemorrhage was performed by manual withdrawal of blood (1 ml·kg$^{-1}$·min$^{-1}$) from a jugular vein for 20 min. At 5 min after the hemorrhage was stopped, an IV resuscitation was started, consisting of either 0.2 ml·kg$^{-1}$·min$^{-1}$ of 2.4 mosmol/kg NaCl (total sodium load 4.8 mosmol/kg) or 1.2 ml·kg$^{-1}$·min$^{-1}$ isotonic NaCl (total sodium load 3.6 mosmol/kg). The infusion was discontinued after 10 min, and the shed blood retransfused after an additional 60 min. The volume of isotonic saline was chosen to match the plasma volume expansion caused by hypertonic saline, as determined by changes in plasma protein concentration. Venous blood samples were taken for analyzes of plasma sodium, chloride, and protein concentration before hemorrhage, after hemorrhage, following resuscitation, and 30 and 60 min thereafter. In addition, experiments were completed on an additional three sheep in which no resuscitation was performed after hemorrhage.

The cerebral component of hypertonic saline resuscitation. A bilateral IC infusion of hypertonic saline was performed in five conscious sheep to investigate if the sympathetic effects were due to a direct cerebral effect of the resuscitation. Recordings of nerve activity with satisfactory quality were obtained in four animals for CSNA and RSNA. Five minutes after a hypotensive hemorrhage, hemorrhage, as determined by changes in plasma protein concentration.

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.

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 $22.7 \pm 2.9$ spikes/s and CSNA $22.5 \pm 4.8$ 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. 2E); 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/)

**Fig. 1.** 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></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...
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...
NaCl (7, 32). 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).

The cardiac effects of hypertonic saline we observed are similar to those from experiments performed in conscious individuals that demonstrated positive inotropic effects of hypertonic resuscitation (8, 27, 28). In contrast, studies investigating the direct cardiac effects of hypertonicity in vitro (25) and in isolated heart preparations (34), as well as in anesthetized animals and humans (5, 10, 13, 22, 38), found no or negative effects on cardiac contractility and/or output. It has been demonstrated that anesthesia induces greater and longer lasting reductions in CSNA than RSNA in cats (17), and we have found that, in sheep, isoflurane anesthesia abolishes CSNA, but does not inhibit RSNA (data not shown). Thus the absence of a positive inotropic effect of hypertonicity in these studies is likely to be related to impaired sympathetic control of the heart, either by anesthesia or denervation. This effect of anesthesia is emphasized by the finding that the beneficial cardiovascular effects of intracerebroventricular hypertonic saline during hemorrhage were abolished by anesthesia (10).

In addition to increasing CSNA, and thus CO, hypertonic 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
progressive fall in renal blood flow, and, with hemorrhage volumes of >14 ml/kg, renal function deteriorates, as shown by large falls in glomerular filtration rate and urine output (29). Resuscitation with hypertonic saline restores renal function (29), and the inhibition of RSNA caused by this treatment may well contribute to this beneficial effect. The heterogeneous control of CSNA and RSNA was also demonstrated in the present study by the lower burst incidence of CSNA than RSNA in the resting state, in agreement with previous findings (24). This low resting level of CSNA provides scope for large increases in activity when increases in CO are required, as during hemorrhage or exercise.

The findings from this and previous studies indicate that the cardiovascular effects of hypertonic saline resuscitation are at least partly dependent on increases in cerebral sodium concentration (9, 10). Based on previous studies, the IV (9) and the IC (unpublished) hypertonic saline infusions would have increased sodium concentration in the brain by a similar amount to the increases in plasma (~10 mmol/l). Currently, the cerebral pathways that lead to the simulation of CSNA and inhibition of RSNA are not well defined (18). In normovolemic animals, increases in cerebral sodium concentration stimulate CSNA and inhibit RSNA, and these effects are inhibited by intracerebroventricular infusion of the AT₁-antagonist losartan (19, 37). The inhibition of RSNA relies on the integrity of the lamina terminalis (20) and the paraventricular nucleus of the hypothalamus (3, 11). These findings suggest that the hypernatremia during hypertonic saline resuscitation is detected by osmotic or sodium sensors in the lamina terminalis, which, via an angiotensinergic link possibly to the paraventricular nucleus of the hypothalamus, leads to stimulation of CSNA and inhibition of RSNA, for which there must be an inhibitory synapse in the pathway (18). Activation of such pathways by hypertonic saline in hemorrhage 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 caulatal 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 Euler’s vag 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


