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The role of the vagus nerve in hypertonic resuscitation of hemorrhagic shocked dogs

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Abstract

Previous studies have suggested a critical role for the vagi during the hypertonic resuscitation of hemorrhagic shocked dogs. Vagal blockade prevented the full hemodynamic and metabolic recovery and increased mortality. This interpretation, however, was challenged on the grounds that the blockade also abolished critical compensatory mechanisms and therefore the animals would die regardless of treatment. To test this hypothesis, 29 dogs were bled (46.0 ± 6.2 ml/kg, enough to reduce the mean arterial pressure to 40 mmHg) and held hypotensive for 45 min. After 40 min, vagal activity was blocked in a reversible manner (0ºC/15 min) and animals were resuscitated with 7.5% NaCl (4 ml/kg), 0.9% NaCl (32 ml/kg), or the total volume of shed blood. In the vagal blocked isotonic saline group, 9 of 9 dogs, and in the vagal blocked replaced blood group, 11 of 11 dogs survived, with full hemodynamic and metabolic recovery. However, in the hypertonic vagal blocked group, 8 of 9 dogs died within 96 h. Survival of shocked dogs which received hypertonic saline solution was dependent on vagal integrity, while animals which received isotonic solution or blood did not need this neural component. Therefore, we conclude that hypertonic resuscitation is dependent on a neural component and not only on the transient plasma volume expansion or direct effects of hyperosmolarity on vascular reactivity or changes in myocardial contraction observed immediately after the beginning of infusion.

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- Hypertonic sodium chloride
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- Plasma volume

Introduction

In 1980, we demonstrated that the circulatory effects of severe blood loss (40-50 ml/kg) in dogs could be successfully reversed to virtually normal function and indefinite survival by a single intravenous injection of a small volume (4-5 ml/kg) of 7.5% NaCl (hypertonic resuscitation) (1). Later, we presented evidence that the first passage of hyperosmotic blood through the pulmonary circulation and the integrity of vagal nerves are both essential for the full hemodynamic/metabolic response required for indefinite survival (2).

Our initial findings were confirmed in a canine model of selective lung denervation (3), and therefore reinforced the importance

of these neural pathways for the hypertonic resuscitation of hemorrhagic shocked dogs (3).

The existence of this proposed neural component, however, remains controversial. Other investigators challenged our hypothesis on the basis of the fact that animals with denervated lungs did not show any differences in the recovery induced by hypertonic saline after hemorrhagic shock, excluding the vagal component as the factor responsible for the hemodynamic effect of hypertonic saline (4).

In order to accommodate these new experimental findings, an alternative explanation was raised for the failure of hypertonic resuscitation observed in animals with vagal blockade submitted to hemorrhagic shock. Presumably, the definitive disruption of vagal-dependent mechanisms elicited to compensate hemorrhage could prevent the animal's recovery no matter the treatment delivered (5).

We hypothesize that a neural pathway participates directly in the action of hypertonic saline on the recovery from hemorrhagic shock. We acknowledge that this neural pathway does not consist only of a single vagal reflex, but rather includes an integrated response with afferent and efferent responses coming from several tissues and organs.

The present study was designed to determine if the vagal nerve participates as part of the neural component of the response to hypertonic saline infusion. In contrast to other protocols (1,2,6), in the present study recovery from hemorrhagic shock was investigated in animals submitted to only temporary bilateral cervical vagal blockade. Three distinct protocols of hemorrhagic shock resuscitation were used: 1) hypertonic solution (HS group, 4 ml/kg 7.5% NaCl), 2) isotonic solution (IS group, 32 ml/kg 0.9% NaCl), or 3) total shed blood replacement (blood group). In all these groups, vagal reflex was blocked by cooling the vagus nerve for 15 min, during the whole resuscitation procedure. The response of the vagal blocked animals to the resuscitation treatment provided information about the presence of the neural component in the action of hypertonic saline.

Material and Methods

Animal preparations

Experiments were performed on 29 mongrel male dogs fed standard dog chow and water *ad libitum* for at least one week in the divisional kennel. Food was removed 16 h and water 1 h prior to anesthesia (pentobarbital sodium, 25 mg/kg, supplemented with 50-75 mg whenever necessary). A tracheal cannula was inserted, but animals were allowed to breathe spontaneously throughout the experiments.

Polyethylene cannulas were inserted into the abdominal aorta via the left femoral artery for measuring arterial blood pressure and into the left femoral vein for administration of heparin (400 U/kg), supplementary anesthetic, and treatment (blood, IS or HS NaCl solutions). Aortic blood pressure was measured with strain gauge transducers (model 1280-C, Hewlett Packard, Palo Alto, CA, USA). A continuous record of blood pressure and heart rate was obtained on an 8-channel polygraph (Hewlett Packard model 1280-C).

A 7-Fr Swan Ganz catheter connected to a cardiac output computer (American Edwards Cardiac 9520 A, Santa Ana, CA, USA) was inserted through a dissected left external jugular vein, and its tip was placed in a pulmonary artery branch under radioscopic monitoring for thermal dilution determination of the cardiac output.

Animals were allowed a 90-min resting period between the end of the acute surgical procedure and the start of bleeding which was preceded by a single intravenous injection of heparin, 500 IU/kg. After this period

the animals were randomly assigned to the different resuscitation procedures.

Vagal blockade

Animals received the resuscitation procedures during bilateral cooling of the cervical vagal trunks to 0ºC. The nerve trunks were cooled by means of a water-circulation cooling device (Quimis, São Paulo, SP, Brazil). Animals from all three groups were submitted to nerve cooling. Core temperature of the nerve trunks was monitored by means of a copper-constant thermocouple. The nerves were cooled for 15 min, during infusion of the solutions, and were then reheated to 37ºC over a period of 15 min.

Experimental measurements

Arterial blood samples taken before bleeding (-45 time), immediately before resuscitation (zero time), and every 30 min thereafter (30, 60, 90, 120 time points) were analyzed for blood gases (pO_2 and pCO_2), bicarbonate and hematocrit using a 288 Blood Gas System (Ciba-Corning, East Walpole, MA, USA). Plasma protein was determined by the Biuret method at the same times and standard serum (Merck Brasil, Jacarepaguá, RJ, Brazil) was used as the standard.

Calculated parameters

1) The total blood volume (BV) was determined as BV = BW*90, where BW stands for body weight in kilograms.

2) The initial plasma volume, PV0, was determined as $PV0 = BV*(1-0.01*Ht_0)$, where Ht_0 is the initial hematocrit.

3) After the end of bleeding (shock period) and before the start of treatment, the volume of blood removed (RB) was measured and the shock plasma volume (PV_S) was determined as $PV_S = (BV - RB)*(1-0.01*Ht_S)*(P_0/P_S)$, where Ht_s is the hematocrit of shock and P_0 and P_S are initial and shock plasma protein, respectively.

4) After the isotonic and hypertonic treatment, plasma volume was determined as $PV_S = (BV - RB)*(1-0.01*Ht_X)*(P_0/P_X)$, where Ht_X and P_X respectively are the hematocrit and plasma protein measured at any given time (30, 60, 90 or 120 min after infusion).

5) After reinfusion of the removed blood, plasma volume was determined as $PV_S = BV*(1-0.01*Ht_X)*(P_0/P_X)$.

Experimental protocol

Figure 1 summarizes the main experimental protocol. 1) Hemorrhage was induced at a rate adjusted to reduce mean arterial pressure to 40 mmHg in 15 min (-45 to -30 min). 2) This pressure level was maintained for 30 min by controlled bleeding or reinfusion (-30 to 0 min). 3) Both cervical vagal trunks were cooled (≅ 0ºC) for 15 min (-25 to 10 min) and then reheated to 37ºC. 4) At the end of the bleeding period and after 5 min of vagal blockade (zero time), the animals were resuscitated with 4 ml/kg 7.5% NaCl (HS group, N = 9), 32 ml/kg 0.9% NaCl (IS group, N = 9) or the total shed blood (blood group, N = 11). 5) Animals were observed in the laboratory for more 120 min. 6) Animals were observed in the Kennel for 96 h.

The study was approved by our institutional committee for care and use of laboratory animals.

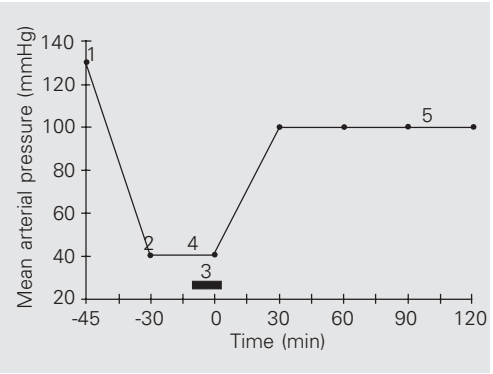

Figure 1. Experimental protocol: *1*, dogs bled to 40 mmHg (15 min); *2*, maintenance at 40 mmHg (30 min); *3*, cooling the vagi during infusion (≅ 0º - 15 min); *4*, resuscitation: HS (4 ml/kg 7.5% NaCl), IS (32 ml/kg 0.9%NaCl), blood (bled volume reinfusion); *5*, period of observation in the laboratory (120 min).

Statistical analysis

Data are reported as means ± SEM. Statistical analysis included one-way analysis of variance and Student-Newman-Keuls test for comparison of the three groups, with the level of significance set at $P < 0.05$.

Results

The experimental protocol is summarized in Figure 1. The effects of the bleeding protocol confirmed previous results (1,2). There were no statistically significant differences among animals for any of parameters up to the onset of treatment, so that the population may be considered homogeneous.

The body weight (kg) and blood volume removed (ml/kg) were: 13.6 ± 1.3 and 46.1 ± 6.2 for the HS group (N = 9), 14.9 ± 0.9 and 46.5 ± 4.2 for the IS group (N = 9), and 13.5 ± 1.6 and 45.4 ± 6.4 for the blood group (N = 11).

As expected, the three groups of animals displayed increases in plasma volume, cardiac output, and mean arterial pressure levels after the resuscitative procedures. The

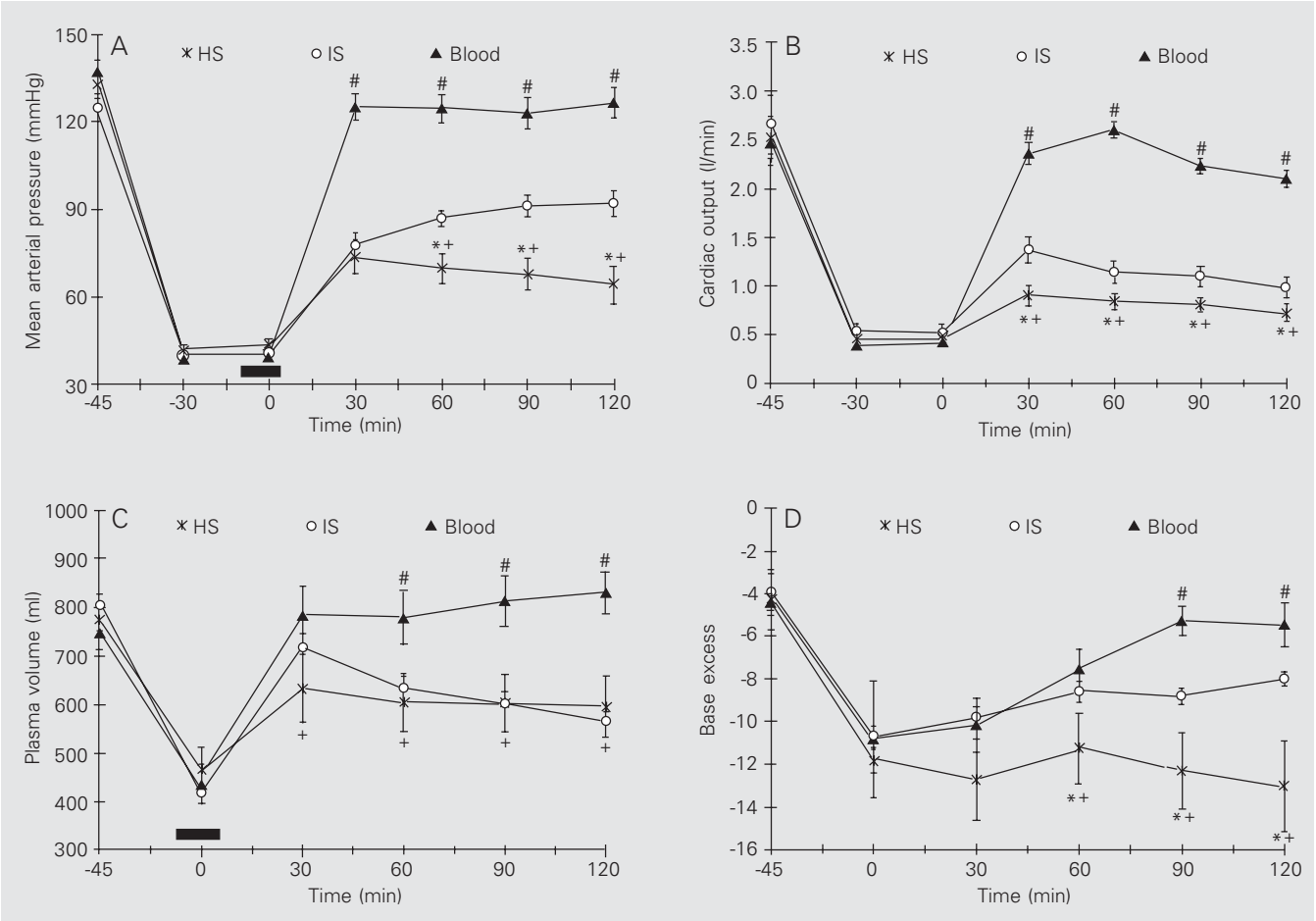

Figure 2. Effect of transitory reversible vagal blockade on the cardiovascular parameters during resuscitation of severely hemorrhagic shocked dogs with blood volume reinfusion (blood), isotonic saline reinfusion (IS), and hypertonic saline reinfusion (HS). The horizontal bar indicates when the reversible thermal vagal blockade was applied. Data are reported as means ± SEM for 9 dogs in each group. *A*, Mean arterial pressure, *$P < 0.05$, HS group *vs* IS group; $^{+}P < 0.05$, HS group *vs* blood group; $^{\#}P < 0.05$, blood group *vs* IS group. *B*, Cardiac output, *$P < 0.05$, HS group *vs* IS group; $^{+}P < 0.05$, HS group *vs* blood group; $^{\#}P < 0.05$, blood group *vs* IS group. *C*, Plasma volume, $^{+}P < 0.05$, HS group *vs* blood group; $^{\#}P < 0.05$, blood group *vs* IS group. *D*, Base excess, *$P < 0.05$, HS group *vs* IS group; $^{+}P < 0.05$, HS group *vs* blood group; $^{\#}P < 0.05$, blood group *vs* IS group. Statistical analysis: one-way ANOVA and Student-Newman-Keuls test.+

main differences observed after these procedures are summarized below.

Mean arterial pressure

During the 120-min period of observation after the resuscitation treatment, the blood group displayed higher mean arterial pressure levels when compared to HS or IS group levels ($P < 0.05$). At 60, 90 and 120 min after treatment, the IS group presented increasing mean arterial pressure values that differed statistically from the decreasing values of HS group (Figure 2A).

Cardiac output

During the 120-min period of observation after the resuscitative treatment, the blood group displayed a significantly higher cardiac output compared to the HS or IS group. There was no significant difference in cardiac output between groups that received IS or HS (Figure 2B).

Plasma volume

The HS and IS groups displayed very similar plasma volume increments that did not differ significantly during the 120-min period of post-treatment observation. However, the two groups did differ significantly from the large increase in plasma volume presented by the blood group at 90 and 120 min after treatment (Figure 2C). The plasma protein and hematocrit used for plasma volume calculations are given in Table 1.

Base excess

After the resuscitative procedure, the blood and IS groups presented a trend toward metabolic normality and base excess reached statistically different values at 90 and 120 min post-treatment. The two groups differed from the HS group and also from each other (Figure 2D).

Survival rates

The 96-h survival rates for HS, IS and blood groups were 1/9, 9/9 and 11/11. The survival rate observed for the HS group in this study was very different from previous results obtained by our group, 100% (1,2) and 92% (6) in studies in which the vagal reflex was not abolished by cooling of the nerve.

Discussion

The use of hypertonic solutions for the treatment of severe blood loss has been justified by their plasma expanding effect (7). The increase in circulating blood volume obviously benefits severely hypovolemic subjects. However, the overt and indisputable characteristics of these mechanical alterations may conceal or shift attention from the participation of other concomitant effects. In fact, several reports on *iv* administration of hypertonic solutions have suggested the participation of peripheral neuroreflexes (2,3,8-19). Several different laboratories have identified the cervical vagi as important elements of cardiovascular reflexes triggered in the cardiopulmonary area by increases in plasma osmolarity (2,3,9-14,19). Previous studies by our group have demonstrated a critical role for the vagi during the hypertonic resuscitation of hemorrhagic

Table 1. Plasma protein and hematocrit of bled animals submitted to various types of resuscitation.

Time	Plasma protein (g/dl)			Hematocrit (%)		
	HS	IS	Blood	HS	IS	Blood
-45 min	7.0 ± 0.6	6.8 ± 0.8	6.9 ± 0.9	36 ± 3	39 ± 5	38 ± 6
Zero time	5.6 ± 0.4	5.6 ± 0.6	5.5 ± 0.8	39 ± 7	45 ± 6	39 ± 4
30 min	4.6 ± 0.6	4.1 ± 0.9	6.4 ± 0.7	33 ± 7	32 ± 7	40 ± 3
60 min	4.7 ± 0.5	4.5 ± 0.9	6.5 ± 0.8	33 ± 6	34 ± 6	39 ± 6
90 min	4.7 ± 0.5	4.6 ± 0.9	6.3 ± 0.8	33 ± 6	36 ± 6	39 ± 6
120 min	4.8 ± 0.5	4.8 ± 0.9	6.2 ± 0.8	33 ± 7	37 ± 6	38 ± 6

Data are reported as means ± SEM. Blood = bled volume reinfusion group; HS = hypertonic resuscitation group; IS = isotonic resuscitation group.

shocked dogs (2,3). Transient vagal blockades prevented the full recovery and worsened the survival outcome.

However, the participation of a neural component in the hypertonic resuscitation of hemorrhagic shocked dogs continues to be a matter of controversy. This concept was challenged on the grounds that vagal blockades abolish restorative mechanisms responsible for the maintenance of the frail circulatory balance observed during hemorrhagic shock (5), and therefore no matter the treatment given the animals would finally succumb and die. The present study has readdressed this issue by determining the effect of a transitory reversible bilateral cervical vagal blockade on the recovery of hemorrhagic shocked dogs submitted to three different procedures of resuscitation: 1) hypertonic solution, 2) isotonic solution, or 3) total shed blood replacement.

In contrast with previous studies from our laboratory in which the vagal nerve was not physically manipulated, in the present study vagal blockade worsened the survival rate of bled dogs treated with hypertonic saline solution (0% in previous studies *vs* 89% after vagal blockade in the present study), as well as hemodynamic and metabolic parameters (1,2,6,18-20). In animals treated with isotonic saline solutions, transitory reversible vagal blockade did not modify the mortality rate which was near zero and similar to previous results obtained without blockade.

The data presented here indicate that transitory reversible vagal blockade during the infusion of the resuscitative treatments was not responsible for a critical disruption of the cardiovascular system regulation since the animals treated with isotonic or shed blood replacement recovered completely. The recovery of these animals treated with isotonic saline or blood can then be ascribed only to a volume-dependent mechanism. The assumption of a major interference with homeostatic mechanisms does not hold for the groups submitted to more traditional treatments and therefore should not be considered as the sole explanation for the poor outcome observed in the group treated with hypertonic solution. On the other hand, based on the present results, it is reasonable to propose that hypertonic resuscitation is partially dependent on a neural component and not only on the transient plasma volume expansion that takes place immediately after the beginning of infusion.

The hypothesis that the action of hypertonic saline in reversing hemorrhagic shock depends on a neural component is based on strong experimental evidence. From the demonstration of the role of lung innervation in triggering the response to hypertonic saline solutions (2-4,12,14,18,19,21) to the hemodynamic responses obtained when hypertonic saline solution is infused directly into the central nervous system (10,16,22), these data show that several neural pathways participate in the response to hypertonic saline infusion.

The present study is part of a search for the individual pathways that participate in this integrated net of events that culminate in the hemodynamic and metabolic effects of hypertonic saline solution resuscitation. However, further studies are needed to clarify the importance of this neural component.

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