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Hemodynamic responses elicited by systemic injections of isotonic and hypertonic saline in hemorrhaged rats

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ABSTRACT

Purpose: The objectives of this study were (i) to characterize the hemodynamic responses caused by controlled hemorrhage (HEM) in pentobarbital-anesthetized rats, and (ii) to determine the responses elicited by systemic bolus injections of isotonic saline (0.15 M) or hypertonic saline (3 M) given 5 min after completion of HEM.
Results: Controlled HEM (4.3 ± 0.2 ml/rat at 1.5 ml/min) resulted in a pronounced and sustained fall in mean arterial blood pressure (MAP) to about 40 mm Hg. The fall in MAP was associated with a reduction in hindquarter vascular resistance (HQR) but no changes in renal (RR) or mesenteric (MR) vascular resistances. Systemic injections of isotonic saline (96–212 μmol/kg i.v., in 250–550 μl) did not produce immediate responses but promoted the recovery of MAP to levels below pre-HEM values. Systemic injections of hypertonic saline (750–3000 μmol/kg, i.v., in 250–550 μl) produced immediate and pronounced falls in MAP, RR, MR and especially HQR of 30–120 s in duration. However, hypertonic saline prompted a full recovery of MAP, HQR and RR to pre-HEM levels and an increase in MR to levels above pre-HEM values.
Conclusions: This study demonstrates that (i) HEM induced a pronounced fall in MAP which likely involved a fall in cardiac output and HQR, (ii) isotonic saline did not fully normalize MAP, and (iii) hypertonic saline produced dramatic initial responses, and promoted normalization of MAP probably by restoring blood volume and cardiac output through sequestration of fluid from intracellular compartments.

Introduction

Moderate to severe hemorrhage (HEM) in humans and animals results in a fall in cardiac output (CO) and mean arterial blood pressure (MAP) but an increase in total peripheral resistance (TPR), which limits the fall in MAP (Baue et al., 1967, 1991; Boyd and Mansberger, 1968; Brooks, 1935; Drucker et al., 1981; Jarhult, 1973; Mittman et al., 1976; Pirkle and Gann, 1976; Traveso et al., 1987; Wade et al., 1997). The falls in CO and MAP during HEM are partially reversed by the movement of fluid and protein from the interstitium into capillaries (Boyd and Mansberger, 1968; Drucker et al., 1981; Guyton, 1965; Pirkle and Gann, 1976; Starling, 1896). The movement of fluid and proteins is initiated by a fall in capillary hydrostatic pressure, which promotes a rapid shift of interstitial fluid into the capillaries and a less rapid shift of interstitial albumin into the plasma, which helps to support plasma oncotic pressure (Drucker et al., 1981). Intracellular fluid drawn down an osmotic gradient facilitates restitution of plasma volume after HEM by replenishing interstitial fluid volume. The increase in interstitial volume and pressure provides the driving force for transcapillary movement of fluid and albumin into plasma (Drucker et al., 1981). The driving force responsible for movement of fluid into the interstitial space and transcapillary movement of albumin, is regulated by circulating factors and especially glucose derived from the splanchnic circulation. The release of these factors is triggered by HEM-induced increases in circulating hormones levels (Drucker et al., 1981; Friedman et al., 1982; Stone et al., 1977).

The major objective of providing fluid therapy to subjects with hemorrhagic shock is to restore circulating volume and thereby support tissue perfusion (Drucker et al., 1981; Dubick et al., 2013; Falk et al., 1983; Thongrong et al., 2013). Fluids such as physiological salt solutions, Ringer's lactate, hydroxyethyl starch, albumin and dextrans have been employed in clinical and experimental settings (Dubick et al., 2013; Falk et al., 1983; Moss and Gould, 1988; Shires et al., 1995). Administration of small volumes of hypertonic saline (H-saline) to subjects in hemorrhagic shock restores MAP by increasing circulating volume (Baue et al., 1991; Coimbra et al., 1997; De Felippe and Timoner, 1980; Dontigny, 1992; Nakayama et al., 1984; Shackford et al., 1998; Traveso et al., 1987), most likely due to the movement of intracellular fluid

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into the vascular space (Drucker et al., 1981). The use of reduced volume H-saline therapy has the advantage of reducing the potential development of third space fluid sequestration (such as cerebral edema in patients with head injury), which may occur with large volume therapy (Drucker et al., 1981). In addition, there are several reports that H-saline directly increased cardiac contractility in patients with moderate to marked HEM (Ogino et al., 1998). Several studies have also examined the effects of mild to severe HEM on MAP, CO and TPR in experimental subjects (Drucker et al., 1981). It should be noted however that in recent trials, low volume H-saline resuscitation did not improve outcomes in either hypovolemic shock or traumatic head injury patients (Dubick et al., 2013), thus calling into question the benefit of such therapy for these broadly defined patient populations. A more detailed understanding of the underlying pharmacology could aid in defining a patient population that might be better served by such therapy.

There is considerable information as to effects of small volumes of isotonic saline (I-saline) and H-saline on MAP, CO and TPR in low CO-induced hypotension during HEM (Barbosa et al., 1990, 1992; Brooks, 1935; Hannon et al., 1989; Maningas et al., 1986; Nakayama et al., 1984; Smith et al., 1985; Traveso et al., 1987). However, although the changes in systemic vascular resistances during HEM in animals have received attention (see Liu et al., 2003; Whalen et al., 2007), nothing is known about the changes in systemic resistances elicited by administration of I-saline or H-saline in these rats. Such vital data would help us to understand how vascular beds subserving different physiological roles respond to HEM and to H-saline. Moreover, the rat is an ideal species to perform pharmacological studies designed to develop therapeutic strategies to treat hemorrhagic shock and other conditions associated with severe hypotension.

The first aim of this study was to determine the changes in MAP and systemic vascular resistances resulting from mild HEM in pentobarbital-anesthetized rats, in which the falls in MAP during HEM are comparable to those of conscious rats (Soucy et al., 1995a,b). The second aim was to determine the changes in MAP and vascular resistances elicited by bolus injections of I- and H-saline in these HEM rats. The changes in hindquarter (HQR), renal (RR) and mesenteric (MR) vascular resistances elicited by mild HEM were examined because of the key roles these vascular beds play in the circulatory adjustments to hemodynamic challenges, and because endocrine cells in the kidneys and mesentery release factors known to directly affect fluid movement across capillary walls and overall body fluid homeostasis (see Drucker et al., 1981).

Methods and materials

Rats and surgical procedures

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. The protocols were approved by the University of Iowa Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (250–300 g) from Harlan (Madison, WI) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A catheter was placed into a femoral vein to administer drugs. Supplemental doses of pentobarbital (5 mg/kg, i.v.) were given as necessary to maintain anesthesia. A catheter was inserted into a femoral artery to measure pulsatile arterial blood pressure (PP) and MAP, and to determine heart rate (HR). A midline laparotomy was then performed and miniature pulsed Doppler flow probes were placed on (i) the superior mesenteric artery to measure mesenteric blood flow velocities (MF) and to determine MR, (ii) a renal artery to measure renal blood flow velocities (RF) and RR, and (iii) on the descending aorta (below the level of the kidneys) to measure hindquarter blood flow velocities (HQF) and to determine HQR (Whalen et al., 1999, 2000). Vascular resistances were determined by dividing MAP by blood flow velocity. The body temperature of each rat was maintained at 37 °C via a thermostatically-controlled heating pad.

Experimental protocols

The arterial catheter was connected to a Beckman Dynograph-coupled pressure transducer to measure PP and MAP. HR was determined from PP by a cardiotachometer. The wire leads from the flow probes were connected to a Doppler flowmeter (Department of Bioengineering, University of Iowa) to continuously record blood flow velocities. The rats were allowed 15–20 min to stabilize before commencement of the HEM. Protocol 1 — In each group, blood was withdrawn to obtain a MAP value of about 40 mm Hg. In the first group of rats (n = 5), blood was withdrawn (4.3 ± 0.2 ml/rat at 1.5 ml/min) and parameters were monitored for 20 min after completion of HEM. Protocol 2 — In the second group (n = 5), blood was withdrawn (5.9 ± 0.5 ml/rat at 1.5 ml/min) and after 5 min, 100, 200 and 400 μl injections of H-saline (17.5% NaCl, 3 M) were given 3–5 min apart (at which time the responses had subsided or reached plateau values). The doses of NaCl (including the extra 150 μl volumes of isotonic NaCl used to flush the H-saline into the rats) were 750, 1500 and 3000 μmol/kg, i.v. Resting parameters were monitored for 20 min after completion of HEM. Protocol 3 — In the third group (n = 5), blood was withdrawn (6.2 ± 0.5 ml/rat at 1.5 ml/min) and after 5 min, i.v. injections (250, 350 and 550 μl) of I-saline (0.9% NaCl, 154 mM) were given 3–5 min apart. The doses of NaCl were 96, 135 and 212 μmol/kg, i.v., respectively. Resting parameters were monitored for 20 min after completion of HEM.

Statistical analyses

The data are expressed as the mean ± SEM. The data were tested and found to be normally distributed (BMDP Statistical Package, Statistical Solutions, Boston, MA). The data were then analyzed by one-way or repeated-measures analysis of variance (ANOVA) using the above statistical package, followed by Student's modified t test with Bonferroni corrections for multiple comparisons between means using the modified error mean square term from the ANOVA (Whalen et al., 1999, 2000). A value of $P < 0.05$ was taken to denote statistical difference.

Results

Hemodynamic responses produced by HEM

Resting hemodynamic parameters recorded prior to beginning the HEM protocol in the three groups are summarized in Table 1. As can be seen, there were no between-group differences in these parameters. A typical example of the responses during HEM (4.2 ml) in a rat, which did not receive subsequent injections of I- or H-saline, is shown in Fig. 1. HR, MAP and blood flow velocities began to fall about half way through

Table 1
Resting hemodynamic parameters prior to hemorrhage.

Parameter	Treatment group		
	Control	I-saline	H-saline
HR, beats/min	332 ± 16	292 ± 17	306 ± 18
MAP, mm Hg	123 ± 4	115 ± 4	116 ± 4
HQR, mm Hg/kHz	59 ± 12	76 ± 15	72 ± 18
RR, mm Hg/kHz	17 ± 2	16 ± 2	15 ± 3
MR, mm Hg/kHz	18 ± 2	17 ± 2	15 ± 2

The data are presented as mean ± SEM. The group designated "Control" did not receive injections of either hypertonic or isotonic saline after hemorrhage. The group designated I-saline received injections of isotonic saline after hemorrhage. The group designated H-saline received injections of hypertonic saline after hemorrhage. HR = heart rate. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. RR = renal vascular resistance. MR = mesenteric vascular resistance. Note that there were no between-group differences for any parameter ($P > 0.05$, for all comparisons).