Mechanisms of Increased Myocardial Contractility with Hypertonic Saline Solutions in Isolated Blood-Perfused Rabbit Hearts

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Hypertonic saline improves organ perfusion and animal survival during hemorrhagic shock because it expands plasma volume and increases tissue oxygenation. Because both decreased and increased myocardial performance have been reported with hypertonic saline, the effects of hyperosmolarity and the mechanism accounting for it were investigated in isolated blood-perfused rabbit hearts. Coronary blood flow (CBF), myocardial contractility, relaxation, and oxygen consumption were measured during administration of blood perfusates containing 140–180 mmol sodium concentrations ($[Na^+]$). In two other series of experiments, the role of Na^+-Ca^{2+} exchange in the inotropic effect of hyperosmolarity (160 mmol sodium concentration) and hypertonicity (sucrose) were also investigated. Hypertonic $[Na^+]$ induced a significant increase in contractility and relaxation, combined with a coronary vasodilation. Myocardial oxygen consumption (Mvo_2) increased at all hypertonic $[Na^+]$ without significant change in coronary venous oxygen tension (Pvo_2) and content (Cvo_2). Amiloride (0.3 mmol) inhibited the improved contractility observed with 160 mmol sodium. Similar Na^+-Ca^{2+} exchanger blockade did not inhibit the inotropic effect of sucrose. These results confirm the positive inotropic effect of hypertonic $[Na^+]$. The inhibition of this improvement by amiloride suggests that calcium influx through the sarcolemna could be the major mechanism of this effect.

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In various experimental models, resuscitation of hemorrhagic shock with hypertonic saline 7.5% has been shown to improve organ perfusion (1) and survival (2). These improvements are not mainly due to plasma volume expansion (3) but to improved venous return (4), and arteriolar vasodilation (5,6). A putative positive inotropic effect of hypertonic saline might also contribute to these beneficial effects, but is still under discussion. Hypertonic saline, unlike non-saline hypertonic perfusates, impairs myocardial contractility in experimental studies (7,8), but other reports suggest that hypertonic saline may have positive direct or indirect inotropic effects (9). To determine the direct effects of hypertonicity on myocardial performance, blood perfusates with increasing concentrations of sodium were infused into isolated rabbit hearts. The sodium concentrations studied were those previously obtained, *in vivo*, after hypertonic saline infusion in animals and humans. Because changes in intracellular sodium concentration ($[Na]_i$) by high sodium concentration (10) might affect the calcium flux through the Na^+-Ca^{2+} exchanger (11,12), myocardial performance during infusion of high sodium concentrations were compared before and after inhibition of the Na^+-Ca^{2+} exchanger by amiloride. In addition, to distinguish the effects of the sodium concentration from those induced by other changes in blood osmolarity, the effects of similar hypertonicity achieved with sucrose were also studied with or without blockade of Na^+-Ca^{2+} exchange.

Methods

Outdated packed human red cells stored at 4°C were used to prepare perfusate (13). After centrifugation, the buffy coat and plasma were discarded and the erythrocytes were washed twice with 150 mmol NaCl solution. Blood was reconstituted by mixing red blood cells in a modified Krebs-Henseleit bicarbonate buffer containing (in millimoles) NaCl 118, K^+ 5.9, free Ca^{2+} 2.5, $MgSO_4$ 0.5, $NaPO_4H_2$ 1.17, $NaHCO_3$ 28, glucose 11, lactate 0.9, serum albumine 0.5%. Reconstituted blood was filtered through a 40-μm filter (Pall Ultipor

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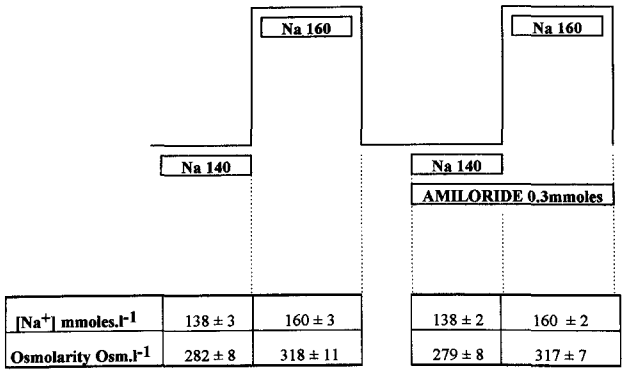
SQ 40S) to eliminate polymorphonuclear cells and avoid microaggregate formation. The blood was then oxygenated, using a membrane oxygenator with a gas mixture of oxygen 20%, carbon dioxide 5%, and nitrogen 75%. After rewarming to 37°C, electrolyte concentrations were adjusted to achieve physiologic concentrations and $NaHCO_3$ was added to obtain a standard acid-base balance.

The study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture, the recommendations of the Helsinki Declaration, and the study was approved by our institutional animal investigation committee. New Zealand albino male rabbits (2–2.5 kg) were anesthetized with ether. After thoracotomy, the heart and aortic arch were rapidly excised and immersed in cold isotonic saline solution. The pericardium was quickly removed under immersion and the aorta prepared for cannulation. The heart was mounted on an aortic cannula and aortic retrograde perfusion at a hydrostatic perfusion pressure of 80 mm Hg was begun, according to the Langendorff technique. As previously reported in detail (13), the apparatus was modified to reduce red blood cell sedimentation and filling volume of the circuit, and to enable continuous recording of coronary blood flow (CBF). The column used to set perfusion pressure was replaced by a syringe with a plunger containing mercury. The plunger was attached to a displacement transducer that controlled the coronary pump speed. Mean coronary perfusion pressure (PP) was recorded from a small catheter, located above the aortic valve. Atrial pacing maintained a constant heart rate (HR).

The apparatus was enclosed in a thermostatic chamber at 37°C. Coronary sinus drainage was collected through a small pulmonary artery catheter. A cannulated fluid-filled balloon connected to a pressure transducer by a rigid catheter, was placed in the left ventricle through a left atrial incision and inflated to maintain constant left ventricular volume. HR was recorded and the maximum positive (dP/dt_{max}) and negative (dP/dt_{min}) derivatives of left ventricular pressure were electronically derived from the left ventricular signal. Since intraventricular volume was held constant, dP/dt_{max} and dP/dt_{min} were considered as inotropic and lusitropic indices, respectively.

Arterial and coronary venous oxygen tensions (Pao_2, Pvo_2), carbon dioxide tensions and pH were measured with standard electrodes at 37°C. The arterial hemoglobin concentration and arterial and coronary venous saturations were measured with a hemoximeter. Arterial and coronary venous O_2 content (Cao_2, Cvo_2) and myocardial O_2 consumption (Mvo_2) were derived using standard formula. The sodium concentration was measured with a standard electrode and perfusate osmolarity was measured with an osmometer.



$[Na^+]$ mmoles.l^{-1}	138 ± 3	160 ± 3	138 ± 2	160 ± 2
Osmolarity Osm.l^{-1}	282 ± 8	318 ± 11	279 ± 8	317 ± 7

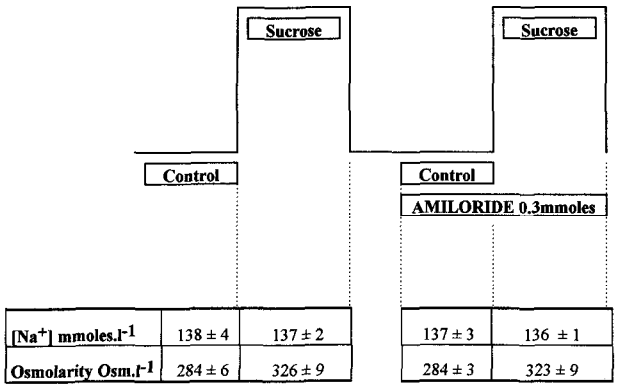
Figure 1. Experimental protocol and measured sodium concentrations and plasma osmolarity of the perfusates. The sodium concentration and plasma osmolarity were significantly higher in the 160 mmol $[Na^+]$ ($P < 0.01$). These changes were similar with or without amiloride.

After aortic cannulation, CBF and myocardial performances were allowed to stabilize to baseline value. Part of the control blood perfusate was withdrawn and 20% NaCl was added to obtain resultant perfusates concentrations of 150, 160, 170, and 180 mmol sodium. After control measurements obtained during infusion of 140 mmol sodium, hypertonic perfusates containing 150–180 mmol $[Na^+]$ were infused until stabilization of CBF and of myocardial performance indices. Before and after each infusion, arterial and coronary venous blood samples were collected. Control perfusate of 140 mmol $[Na^+]$ was then reinfused and recovery was allowed for return to baseline value. Nine hearts were used in this subset.

In 11 other rabbit hearts, after baseline measurements obtained during infusion of 140 mmol sodium, effects of 160 mmol $[Na^+]$ perfusate were determined. Then, control perfusate was infused again and 0.3 mmol amiloride, an inhibitor of the Na^+-Ca^{2+} exchanger, was added. After stabilization, the effects of 160 mmol $[Na^+]$ perfusate were studied again in the presence of amiloride (Figure 1).

To distinguish effects of the sodium concentration from those induced by variation of the plasma osmolarity, hypertonic nonsaline blood perfusate with a constant sodium concentration was infused into a third group of eight hearts. This solution was prepared by addition of sucrose to obtain a perfusate osmolarity equal to the one measured in the 160 mmol $[Na^+]$ perfusate. The same experimental protocol as with $[Na^+]$ 160 mmol perfusate was performed with and without amiloride (Figure 2).

Analysis of the changes in the variables studied with the sodium concentration were made by analysis of variance (ANOVA) for repeated measures. When a



$[Na^+]$ mmoles.l^{-1}	138 ± 4	137 ± 2	137 ± 3	136 ± 1
Osmolarity Osm.l^{-1}	284 ± 6	326 ± 9	284 ± 3	323 ± 9

Figure 2. Experimental protocol and measured sodium concentrations and plasma osmolarity of the perfusates. The sodium concentration remained unchanged whereas osmolarity increased significantly in sucrose perfusate ($P < 0.01$). This increase in plasma osmolarity was similar with or without amiloride.

significant effect of sodium concentration was found, the relationship between $[Na^+]$ and the studied variable was further analyzed by decomposition in orthogonal polynomials allowing the testing of linear and quadratic contrasts. The significance level was fixed at 5% but due to the nature of the ANOVA we used the criterion of Huynh and Feld rather than the classical F value. In addition, Helmert contrast was used on dP/dt_{max} to determine the $[Na^+]$ at which inotropy did not change anymore.

The effects of amiloride on the inotropic effect of 160 mmoles $[Na^+]$ were analyzed with two-way ANOVA for repeated measures. The two tested "within" factors were the sodium level and presence or absence of drug treatment. The interaction between the two factors allowed us to test whether the effect of changing sodium concentration was significantly affected by the drug treatment. When interaction was significant, we tested the effect of sodium using Student's t-test for matched pairs. When the interaction was not significant, the effect of the sodium was determined by the two-way ANOVA. All results are expressed as means ± SD and $P < 0.05$ was considered significant.

Results

Composition of reconstituted blood (in millimoles), pH, and blood gas values were as follows: Na, 138 ± 3.2; K^+, 5.2 ± 1.5; Cl, 100 ± 4; HCO_3^{2-}, 25 ± 3.2; glucose, 8.5 ± 1.3, Ca^{2+} 2.36 ± 2.3; pH, 7.37 ± 0.07; Pao_2, 148 ± 19 mm Hg; $Paco_2$, 38.1 ± 1.8 mm Hg. The hemoglobin and arterial oxygen content values did not change during the study (Table 1). Sodium concentrations in perfusates were (in millimoles) 138 ± 3, 148 ± 3, 159 ± 3, 169 ± 4 and 181 ± 4, respectively. Coronary PP, HR, and left ventricular end diastolic pressure (LVEDP) also remained unchanged throughout the study. CBF, dP/dt_{max}, dP/dt_{min}, and Mvo_2 returned to baseline values between each hypertonic perfusion.

Hypertonic perfusates induced nonlinear increases in dP/dt_{max} and dP/dt_{min} (Table 1). Maximum increase of dP/dt_{max} occurred when sodium concentration reached 160 mmol. Nonlinear increase in CBF was observed with infusion of increasing $[Na^+]$ (Table 1). These were associated with increased Mvo_2 without variations of Pvo_2 or Cvo_2 (Table 1).

In the second part of the study, baseline HR, PP, and LVEDP values were 130 ± 9 bpm, 75 ± 3 mm Hg, and 5 ± 4 mm Hg, respectively, and did not vary with changes in perfusates. A significant increase in dP/dt_{max} (2307 ± 483 mm Hg/s vs 2623 ± 469 mm Hg/s, $P < 0.01$) and dP/dt_{min} (1575 ± 308 mm Hg/s vs 1695 ± 327 mm Hg/s, $P < 0.01$) occurred during 160 mmol $[Na^+]$ infusion without amiloride. These increased dP/dt_{max} and dP/dt_{min} were not observed during amiloride infusion (Figure 3). The interaction between the factor "sodium level" and the factor "drug treatment" in the two-way ANOVA was significant ($P < 0.01$ and $P < 0.05$ for dP/dt_{max} and dP/dt_{min}, respectively).

In the third part of the study, sodium concentration did not vary during the study whereas plasma osmolarity was significantly higher in the sucrose perfusate ($P < 0.01$). Changes in osmolarity were similar with or without amiloride (Figure 2). Baseline values of HR, PP, and LVEDP were 125 ± 16 bpm, 76 ± 2 mm Hg, and 4 ± 2 mm Hg, respectively, and remained unchanged during the study. Perfusion of sucrose significantly increased dP/dt_{max} (1862 ± 405 mm Hg/s vs 2300 ± 571 mm Hg/s, $P < 0.01$) and dP/dt_{min} (1303 ± 321 mm Hg/s vs 1538 ± 468 mm Hg/s, $P < 0.05$). Similar increases in dP/dt_{max} (1888 ± 383 mm Hg/s vs 2334 ± 384 mm Hg/s, $P < 0.01$) and dP/dt_{min} (1369 ± 281 mm Hg/s vs 1669 ± 240 mm Hg/s, $P < 0.05$) by sucrose were observed in presence of amiloride (Figure 4).

Discussion

Both negative (7,8) and positive inotropic effects (9) have been demonstrated with hypertonic saline in various experimental models. However, direct intracoronary perfusion of hypertonic saline performed in some of these studies (8) induced marked and acute changes in plasma osmolarity and sodium concentration that greatly differed from the moderate changes that occur after intravenous infusion (14). The cardiac effects of hypertonic saline *in vivo* are influenced by changes in the autonomic nervous system (15) and direct effects of hypertonic saline on myocardial fibers might be difficult to differentiate from those mediated by variations in autonomic nervous tone. The

Table 1. Effect of Hypertonic Saline on the Myocardial Performance, Coronary Blood Flow, and Oxygen Consumption ($n = 9$)

	Na 140	Na 150	Na 160	Na 170	Na 180	Linear	Nonlinear
LVEDP (mm Hg)	9.3 ± 5.0	7.6 ± 4.9	7.8 ± 4.2	7.6 ± 4.2	8.7 ± 4.9	NS	NS
HR (bpm)	101 ± 13	104 ± 19	107 ± 22	106 ± 22	110 ± 23	NS	NS
PP (mm Hg)	83 ± 2	83 ± 2	82 ± 2	82 ± 2	82 ± 2	NS	NS
dP/dt_{max} (mm Hg/s)	1861 ± 469	2133 ± 447	2355 ± 567	2370 ± 581	2394 ± 598	$P < 0.001$	$P < 0.05$
dP/dt_{min} (mm Hg/s)	1167 ± 361	1383 ± 401	1422 ± 499	1363 ± 485	1333 ± 504	NS	$P < 0.01$
Hb (g/dL)	10.4 ± 1	10.3 ± 0.9	10.4 ± 0.9	10.3 ± 1	10.5 ± 0.8	NS	NS
Cao_2 (mL/100 mL)	13.8 ± 1.5	13.7 ± 1.2	13.8 ± 1.2	13.7 ± 1.2	13.7 ± 1	NS	NS
Pvo_2 (mm Hg)	34 ± 6	36 ± 5	34 ± 5	34 ± 6	36 ± 7	NS	NS
Cvo_2 (mL/100 mL)	10.2 ± 1.4	10.6 ± 1.1	10.4 ± 1.1	10.2 ± 1.2	10.5 ± 1.2	NS	NS
CBF ($mL/min^{-1} \cdot g^{-1}$)	1.55 ± 0.7	2.27 ± 0.8	1.98 ± 0.9	2.13 ± 0.9	2.32 ± 1	$P < 0.001$	$P < 0.05$
Mvo_2 ($mL \cdot min^{-1} \cdot 100\ g^{-1}$	5.2 ± 1.9	6.7 ± 2.5	6.2 ± 2	7 ± 2.4	7.3 ± 2.7	$P < 0.001$	NS

LVEDP = left ventricular end-diastolic pressure; HR = heart rate; PP = coronary perfusion pressure; dP/dt_{max} = maximum positive derivative of the left ventricular pressure; dP/dt_{min} = maximum negative derivative of the left ventricular pressure; Hb = hemoglobin concentration; Cao_2 = coronary arterial oxygen content; Pvo_2 = coronary venous oxygen tension; Cvo_2 = coronary venous oxygen content; CBF = coronary blood flow; Mvo_2 = myocardial oxygen consumption; NS = not significant.

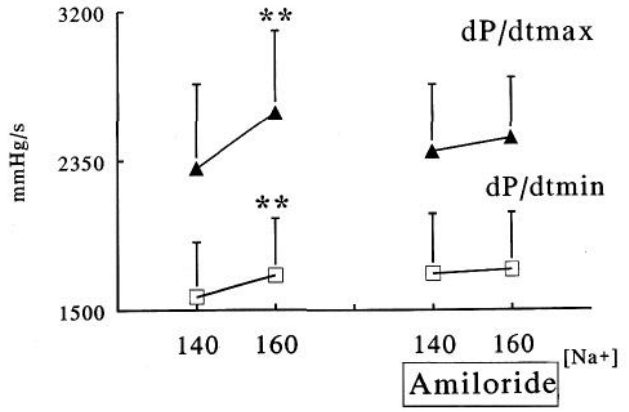


Figure 3. Effect of amiloride on the positive inotropic effect of 160 mmol sodium concentration ($n = 11$). Perfusion of 160 mmol [Na] perfusate significantly increased the myocardial performances. This improvement of myocardial performance was inhibited by amiloride (**$P < 0.01$).

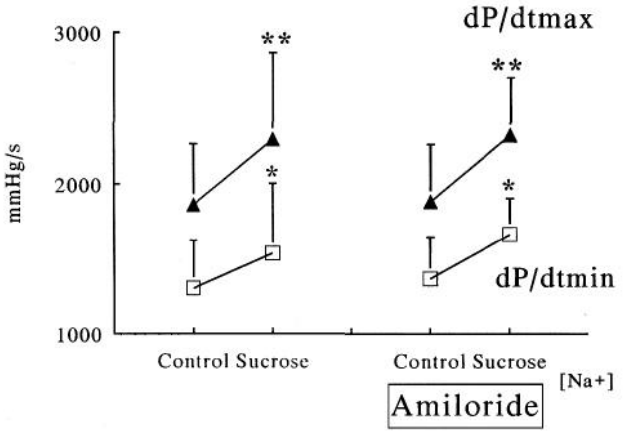


Figure 4. Effect of amiloride on the positive inotropic effect of sucrose ($n = 8$). Perfusion of sucrose perfusate significantly increased contractility (dP/dt_{max}) and relaxation (dP/dt_{min}) with or without amiloride (*$P < 0.05$; **$P < 0.01$).

isolated heart preparation allowed us to exclude these interactions.

We studied a wide range of sodium concentrations, including those previously found *in vivo* (145–155 mmol) after intravenous infusion of hypertonic saline 7.5% (14). In agreement with Kien and Kramer (9) our dose-response curve indicated increases in myocardial contractility and relaxation with this range of concentrations. In contrast, Brown et al. (7) reported impairment of myocardial function in the isolated rat heart. These conflicting findings could be species-related effects because contractile protein properties (16) and calcium metabolism of rat hearts (17) differ greatly from other mammals. In addition, differences in Pao_2 and Cao_2 in crystalloid or blood perfusates (18) which were shown to markedly affect CBF (13,18), myocardial metabolism (18), and cardiac effects of anesthetics (13), could also be involved in the cardiac depression and unchanged CBF in Brown et al.'s study (7), whereas improvement of myocardial contractility and well known vasodilatating effects of hypertonic saline (5,6) were observed in our experiments. Mvo_2 also increased with all hypernatremic solutions studied (150–180 mmol [Na^+]). Since the left ventricular preload and HR were maintained constant, this increase in Mvo_2 was due to the improvement of myocardial contractility by perfusion of higher sodium concentrations. Coronary vasodilation occurred in response to increased metabolic load, as suggested by unchanged Pvo_2 and Cvo_2.

Because changes in the sodium concentration are associated with variations in plasma osmolarity, we cannot exclude the possibility that hypertonicity may, *per se*, affect inotropy and lusitropy. Our experiments with sucrose confirm the positive inotropic effect of osmolarity lower than 400 mOsm (19) which might be due to an increased intracellular calcium concentration (20). Easier flow through arterioles and capillaries

due to decreased red cell volume and endothelial shrinkage has been suggested with hypertonic saline (3). These microcirculatory effects may affect oxygen supply to tissue by lowering local resistances to flow leading to more homogeneous local flow distribution, increasing local oxygen supply and, consequently, myocardial performance. This might partly explain the positive inotropic and vasodilatory effects of hypertonic saline.

Increased intracellular sodium ($[Na^+]_i$) by hypertonic saline (10) might be another putative mechanism to explain the inotropic effect of hypernatremic perfusion. In studies using the patch clamp method, greater $[Na^+]_i$ underneath the sarcolemmal membrane has been shown to induce calcium influx from the "reverse mode" of Na^+-Ca^{2+} exchange (11,12,21) and to stimulate calcium release from the sarcoplasmic reticulum (21). Increased $[Na^+]_i$ induced by high extracellular sodium concentration (10) could be large enough to favor the "reverse mode" of Na^+-Ca^{2+} exchange which plays a major role in contractility and is involved in the reinforcement of myocardial performance by digitalis (22). In our study, rabbit hearts were perfused with 160 mmol sodium concentration or sucrose in the presence of 0.3 mmol amiloride. This sodium concentration was chosen as a reference because it induced the maximum effect on myocardial performance in our dose-response curve. The concentration of amiloride we used has been shown to inhibit the Na^+-Ca^{2+} exchange (23) but did not markedly affect inotropy in preliminary experiments. In the present study, the effects of 160 mmol $[Na^+]$ on myocardial performance were significantly altered by amiloride but the improvement of contractility obtained by a similar sucrose-induced increase in extracellular osmolarity was not affected. Since the sodium concentration and plasma osmolarity varied to the same extent with and without amiloride, the inhibition of the positive inotropic effect of sodium was obviously related to the Na^+-Ca^{2+} exchange blockade. As suggested by Rocha e Silva et al. (24), the sodium ion itself has a key role in this improvement of myocardial contractility and mediates increased myocardial performance through a specific mechanism involving Na^+-Ca^{2+} exchange. Na^+-Ca^{2+} exchange is not involved in the sucrose-induced improvement of contractility which must therefore be due to other mechanisms, such as an increase in intracellular calcium concentration mediated by dehydration of the myocardial cells (20) or an increase in myofilament sensitivity to calcium (25).

In conclusion, hypertonic saline increased myocardial contractility and relaxation of blood-perfused isolated rabbit hearts. This improvement of myocardial performance reflects the direct effect of hypertonic saline on the myocardium and could be involved in the beneficial effects of hypertonic saline in hemorrhagic shock.

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