EFFECTS OF CARDIAC NA^+-H^+ EXCHANGE BLOCKADE ON MYOCARDIAL CONTRACTILE FUNCTION DURING HEMORRHAGIC SHOCK

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Background: Myocardial contractile dysfunction has been described following hemorrhagic shock. While the Na^+-H^+ exchanger is well known to be a major regulator of intracellular pH, its role in hemorrhagic shock remains unclear. However, there has been intensive research investigating the myocardial protective role of several Na^+-H^+ exchangers in ischemia-reperfusion, which is similar to hemorrhagic shock as both result in extracellular acidosis.

The purpose of the present study was to examine the effects of blocking the cardiac Na^+-H^+ exchanger, using 100 μM amiloride, on myocardial contractile function after *ex vivo* perfusion of isolated rat heart following one hour of hemorrhagic shock.

Methods: Anesthetized male Sprague-Dawley rats were assigned to either hemorrhagic treated or untreated groups, or a similar time-matched control group (n=6 per group). Rats were hemorrhaged using a reservoir model. Arterial blood pressure was monitored for one hour, and maintained at a mean arterial blood pressure of 40 mm Hg, via an intra-arterial catheter inserted into the left carotid artery. Two arterial blood samples were taken, one at baseline and another at 60 minutes of hemorrhage. These blood samples were analyzed for pH and lactate levels.

Hearts were harvested and perfused either with a balanced salt solution for 60 minutes or a solution containing 100 μM amiloride. Myocardial function was determined with a balloon- tipped catheter inserted into the left ventricle via the mitral valve.

Indices of left ventricular function were measured, including left ventricular end diastolic pressure (LVEDP), left ventricular peak systolic pressure (LVPSP), coronary perfusion pressure (Pp) and left ventricular balloon volume (BV) . The left ventricular ± dP/dt, which is the left ventricular index of contractility, was calculated. Left ventricular compliance (C) was also calculated.

Results: The results showed that inhibition of the Na^+-H^+ exchanger for 5 minutes of *ex vivo* perfusion of the isolated hearts following hemorrhagic shock, improved myocardial contractile function as compared to the hemorrhage untreated hearts. The results also showed that hemorrhagic shock decreased myocardial contractile function as compared to controls.

Conclusion: Blocking the Na^+-H^+ exchanger for a short period on *ex vivo* perfusion of isolated hearts has a protective effect on myocardial contractile function. Further research is needed to investigate the exact mechanism of protection using more specific Na^+-H^+ exchanger inhibitors.

Key Words: Hemorrhage, rat, isolated heart, contractility, amiloride, calcium, sodium, lactate, pH, Langendorff

Introduction

HEMORRHAGIC SHOCK HAS RESULTED in myocardial contractile dysfunction (McDonald, Milligan *et al.* 1975; Shoemaker, Pietzman *et al.* 1996; Gloviczki 2002), both in vivo (McDonald, Milligan *et al.* 1975; McDonough, Giaimo *et al.* 1999) as well as in the ex vivo isolated perfused heart The exact mechanism of the myocardial contractile dysfunction following hemorrhagic shock is not well known. One explanation is that it may be related to the extracellular acidosis produced by hemorrhagic shock (Davis and Shackford 1991; Abramson, Scalea *et al.* 1993; Wu, Zhang *et al.* 1993; Turvey and Allen 1994; Bannon, O'Neil *et al.* 1995; Manikis, Jankowski *et al.* 1995; Modelska, Matthay *et al.* 1997; Alfaro, Pesquero *et al.* 1999; Baron, Sinert *et al.* 1999; Burris, Rhee *et al.* 1999; Luchette, Robinson *et al.* 1999).

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The role of the Na^+-H^+ exchanger in affecting myocardial contractile function following hemorrhagic shock has not been investigated. However, intensive research has been done on the role of the Na^+-H^+ exchanger in ischemia reperfusion injury. The Na^+-H^+ exchanger is one of the major regulators of the intracellular pH. Several isoforms have been identified; Na^+-H^+ exchanger isoforms differ in tissue distribution, molecular structures, sensitivity to pharmacological inhibitors, and roles in cell regulation.

Na^+-H^+ exchanger -1 has been recognized for some time as the 'housekeeping' isoform as it is present in most tissues and is the primary Na^+-H^+ exchanger subtype found in mammalian cardiac cells (Fliegel and Frohlich 1993; Orlowski and Grinstein 1997; Wakabayashi, Shigekawa *et al*. 1997). The function of Na^+-H^+ exchanger -2, which is found in renal and intestinal epithelia, is still not well understood. Na^+-H^+ exchanger -3 occurs in the apical membrane of epithelia and is generally responsible for the apical entry of sodium ions in trans-epithelial Na transport. Na^+-H^+ exchanger -4 is found mainly in the stomach and kidney. Na^+-H^+ exchanger -5 is found in the brain and testis (Frohlich and Karmazyn 1997). Among these Na^+-H^+ exchanger isoforms, Na^+-H^+ exchanger -1 is the sole isoform detectable in the cardiac myocyte (Fliegel and Dyck 1995) and its function and regulation has the greatest relevance for cardiovascular physiology and pathology.

Ischemia-induced acidosis represents a major stimulus for Na^+-H^+ exchanger activation. Stimulation of the Na^+-H^+ exchanger will cause Na^+ influx and H^+ efflux with subsequent elevation of $[Ca^{2+}]$ via the Na^+- Ca^{2+} exchanger (Renlund, Gerstenblith *et al*. 1984). Rapid and excessive accumulation of $[Ca^{2+}]i$ will trigger a cascade of pathological and biochemical alterations (Nayler, Panagiotopoulos *et al*. 1988; Panagiotopoulos, Daly *et al*. 1990) and subsequent cell injury and contractile dysfunction We hypothesize that rapid resuscitation of the heart following severe hemorrhagic shock will lead to cardiac dysfunction via stimulation of the Na^+-H^+ exchanger. The mechanism may result from a large increase in the $[Ca^{2+}]i$ and $[Na^+]i$ as demonstrated in the case of ischemia-reperfusion.

Amiloride is a non-specific Na^+-H^+ exchanger blocker. That means it blocks the Na^+-H^+ exchanger isoform 1 as well as other isoforms. Other amiloride analogues have been identified which block isoform 1 specifically, which is present in the heart. The clinical significance of these specific blockers is that they act only in the heart. Previous studies have demonstrated the protective effects of blocking the Na^+-H^+ exchanger using Na^+-H^+ exchanger inhibitors such as amiloride (Karmazyn 1988) and its analogs (Scholz, Albus *et al*. 1993; Hendrikx, Mubagwa *et al*. 1994; Sack, Mohri *et al*. 1994; Yasutake, Ibuki *et al*. 1994; Klein, Pich *et al*. 1995; Shimada, Hearse *et al*. 1996; Xue, Aye *et al*. 1996). However, there is a lack of data on the protective effect of Na^+-H^+ exchanger inhibition in the case of resuscitation from shock.

The purpose of this study was to investigate the role of Na^+-H^+ exchanger in causing myocardial contractile dysfunction following hemorrhagic shock and to demonstrate the protective effects of blocking the Na^+-H^+ exchanger using amiloride on improving myocardial contractile function following hemorrhagic shock in an isolated rat heart model.

Materials and Methods

Surgical Procedure

Male Sprague-Dawley rats were injected intra-peritonealy (i.p.) with heparin sodium 2000 I.U 15 minutes prior to anesthesia. The rats were then anaesthetized using urethane 125mg/kg intra-peritoneally. The left carotid artery was cannulated using polyethylene tubing size 60, and was connected to an in-line pressure transducer for continuous blood pressure monitoring. Animals were allowed to stabilize for a period of 30 minutes. Animals were injected with heparin sodium 2000 I.U. intra-arterially. The animals were assigned randomly to either an experimental or time-matched control group (n = 6 per group).

Hemorrhage

Rats were hemorrhaged using a reservoir (a 10 mL syringe) was connected to the arterial (carotid artery) by three way stopcocks. Opening the stopcock and aspirating gently and gradually with the syringe induces hemorrhage. Blood was aspirated at a rate of 1 mL/min. Blood was continuously withdrawn or reinfused to the animal to maintain a mean arterial pressure of approximately 40 mmHg. The same surgical procedure was performed for the sham hemorrhage

group except rats were not hemorrhaged. This group served as the time-matched control group to compare the data from the hemorrhaged group.

After one hour data collection, hearts were perfused *ex vivo* with KHB containing amiloride 100 μM/L for 5 minutes, then shifted to KHB without amiloride for 55 minutes in the Langendorff system. Two arterial blood samples were collected at 0 and 60 minutes of hemorrhage. The hearts were harvested and perfused using the Langendorff system with KHB solution containing amiloride 100 μM/L for 5 minutes, then perfused with KHB solution for 55 minutes.

Harvest and Ex vivo Perfusion

During the agonal period of hemorrhage and in time-matched controls, a sub-xiphoid, transverse incision was made in the abdomen and extended superiorly along both mid-axillary lines. The diaphragm was carefully transected along its ventral margin and the ventral rib cage was lifted revealing the beating heart. Hearts were then excised quickly and placed into iced physiologic saline to arrest the heart. Hearts were attached by the aorta to the proper size cannula in the Langendorff system. The hearts were perfused with non-circulating Krebs-Henseleit- Bicarbonate (KHB) buffer consisting of the following (in mM): sodium chloride, 118; calcium chloride, 1.25; potassium chloride, 4.7; sodium bicarbonate, 21; magnesium sulphate, 1.2; glucose, 11; potassium biphosphate, 1.2; and EDTA, 0.5. An apical stab incision was made in the left ventricle using a #15 scalpel blade. A saline-filled cellophane balloon-tipped catheter was placed into the left ventricle (LV) via the mitral valve and was used to measure LV pressure and balloon volume. LV and perfusion pressures were measured using transducers placed at the levels of the heart and aorta. Hearts were stimulated electrically at 300 beats per minute using an electrical stimulator (6020 Stimulator from Harvard Apparatus). Perfusion pressure was maintained at 50 mm Hg. LVEDP was maintained at 5 mm Hg. Perfusate temperature was maintained at 37°C. The perfusate was gased with a mixture of 95% O_2 + 5% CO_2 at a pH of 7.4 for the duration of the experiment.

Arterial Blood Samples

Two arterial blood samples (1ml) were collected from controls and hemorrhage rats after 30 minutes of cannulization (immediately before hemorrhage in the hemorrhage group hemorrhage) and before harvesting the hearts. Blood was analyzed for lactate and pH.

Hemodynamic and Cardiodynamic Measurements

Left ventricular end diastolic pressure (LVEDP) and the left ventricular peak systolic pressure (LVPSP) were measured continuously. Coronary vascular resistance (CVR) was calculated as coronary perfusion pressure (CPP) divided by the coronary flow (Q), ($CVR = \Delta CPP / \Delta Q$). Since coronary flow was maintained at a constant, any change in perfusion pressure was directly proportional to changes in coronary vascular resistance.

Statistical Analysis

All data were initially analyzed with Bartlett's test for homogeneity. Data found not to be homogeneous were transformed and reanalyzed. Data were analyzed with multivariate analysis of variance (MANOVA). Means were analyzed using Duncan's test and were considered significant when yielding a P value less than or equal to 0.05. Data are expressed as means ± SEM.

Results

Figure 1 shows the changes in the left ventricular peak systolic pressure during the one-hour experimental protocol. Left ventricular peak systolic pressure was significantly decreased in the hemorrhage group compared to the sham hemorrhage group and remained depressed throughout the experimental protocol ($P<0.05$). The peak systolic pressure was significantly higher in the amiloride treated hemorrhage group as compared to the hemorrhage group ($P<0.05$). Figure 2 shows the changes in left ventricular end diastolic pressure, which was significantly higher in the hemorrhage group compared to the sham hemorrhage group ($P<0.05$). Figure 2 also shows a significant decrease in the left ventricular end diastolic pressure in the hemorrhage-treated group compared to the hemorrhage group ($P<0.05$). Figure 3 shows changes in the left ventricular generated pressure, which was significantly low in the hemorrhage group compared to the sham hemorrhage group, while the hemorrhage-treated group showed a significant increase in the left ventricular generated pressure compared to the

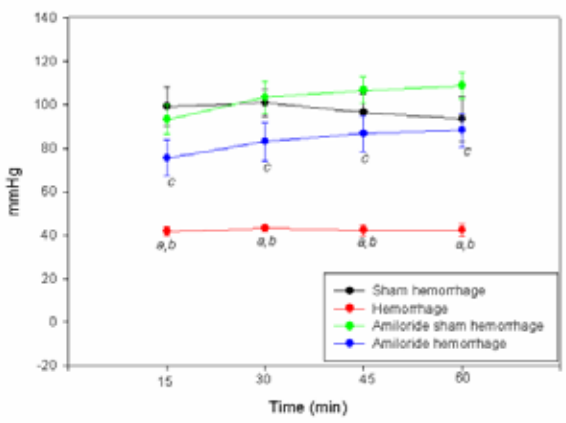

Figure 1. Left ventricular peak systolic pressure of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour ex vivo perfusion (n=6).
a = p < 0.05 compared to amiloride hemorrhage
b = p < 0.05 compared to sham hemorrhage
c = p < 0.05 compared to amiloride sham hemorrhage

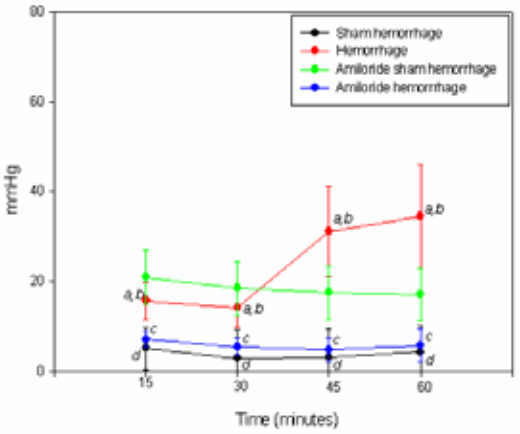

Figure 2. Left ventricular end-diastolic pressure of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour of ex vivo perfusion (n=6).
a = p < 0.05 compared to amiloride hemorrhage
b = p < 0.05 compared to sham hemorrhage
c = p < 0.05 compared to amiloride sham hemorrhage
d = p < 0.05 compared to amiloride sham

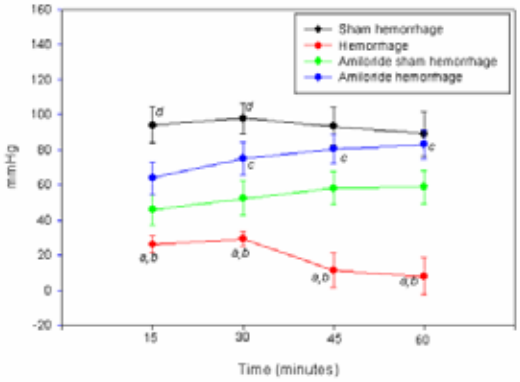

Figure 3. Left ventricular generated pressure of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour ex vivo perfusion (n=6).
a = p < 0.05 compared to amiloride hemorrhage
b = p < 0.05 compared to sham hemorrhage
c = p < 0.05 compared to amiloride sham hemorrhage
d = p < 0.05 compared to amiloride sham hemorrhage

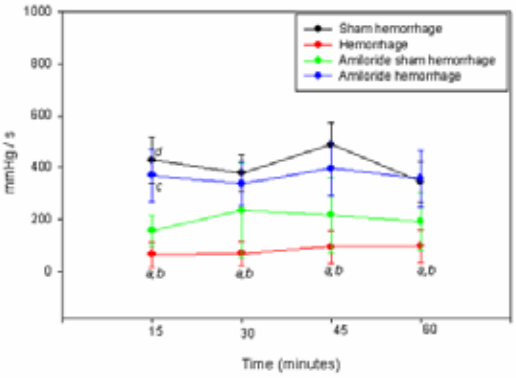

Figure 4. Left ventricular +dP/dt of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour ex vivo perfusion (n=6).
a = p < 0.05 compared to amiloride hemorrhage
b = p < 0.05 compared to sham hemorrhage
c = p < 0.05 compared to amiloride sham hemorrhage
d = p < 0.05 compared to amiloride sham hemorrhage

hemorrhage group. Figure 2 shows the changes in left ventricular end diastolic pressure, which was significantly higher in the hemorrhage group compared to the sham hemorrhage group (P<0.05). Left ventricular maximum + dP/dt (Figure 4) was significantly lower in the hemorrhage group as

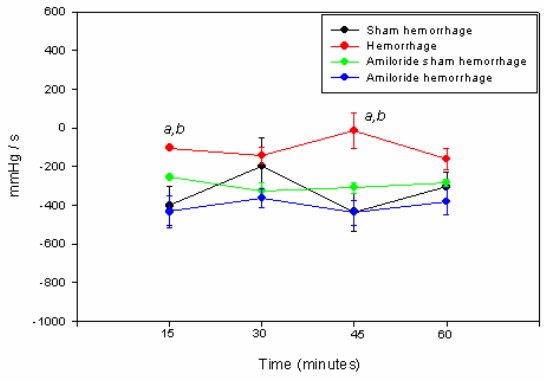

Figure 5. Left ventricular –dP/dt of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour ex vivo perfusion (n=6).
a = p < 0.05 compared to amiloride sham hemorrhage
b = p < 0.05 compared to sham hemorrhage

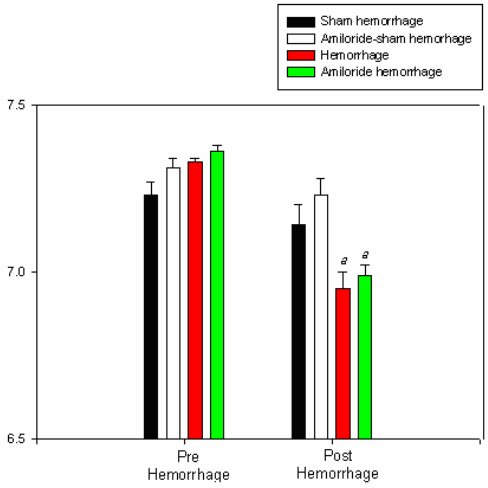

Figure 6. Arterial pH in two blood samples: pre-hemorrhage and post-hemorrhage in the four experimental groups
a = p < 0.05 compared to pre-hemorrhage

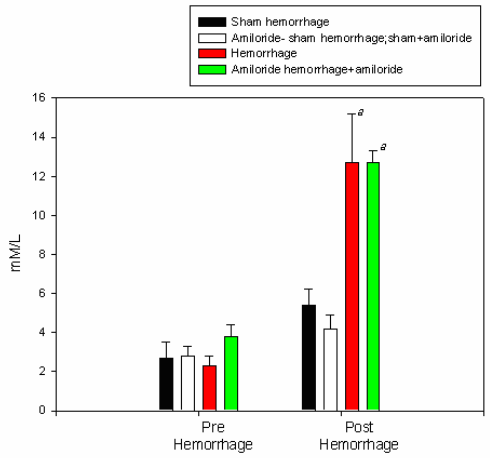

Figure 7. Arterial lactate in two blood samples: pre-hemorrhage and post-hemorrhage in the four experimental groups.
a = p < 0.05 compared to pre-hemorrhage

compared to the sham hemorrhage group and significantly higher in the hemorrhage-treated compared to the hemorrhage group. Figure 2 shows the changes in left ventricular end diastolic pressure, which were significantly higher in the hemorrhage group compared to the sham hemorrhage group (P<0.05). Maximum - dP/dt was significantly higher in the hemorrhage group than the sham hemorrhage group at the 15 and 45 minutes of perfusion as shown in figure 5 (P<0.05), while – dP/dt was significantly lower in the hemorrhage treated compared to the hemorrhage group (P<0.05).

Figure 6 shows arterial pH in two blood samples: pre-hemorrhage and post-Hemorrhage in the four experimental groups. In the sham hemorrhage and amiloride sham hemorrhage groups there were no differences in the pH levels between the pre- and post-hemorrhage groups (P>0.05). In the hemorrhage and amiloride hemorrhage groups, pH was significantly reduced during the post-hemorrhage time period (P<0.05). The reductions in pH in these two groups were not significantly different from one another (P>0.05). Figure 7 shows arterial lactate in two blood samples: pre-hemorrhage and post-hemorrhage in the four experimental groups. In the sham hemorrhage and amiloride sham hemorrhage groups there were no differences in the lactate concentrations between the pre- and post-hemorrhage groups (P>0.05). In the hemorrhage and amiloride hemorrhage groups there were significant increases in lactate between the

Table 1. *Baseline data from hemorrhage and control groups treated and untreated with 100 μM amiloride. (Total blood volume = calculated, based upon 9% of rat weight)*

	Hemorrhage	Amiloride Hemorrhage	Sham Hemorrhage	Amiloride Sham-hemorrhage
Rat weight (gm)	468.33 ±25.87	393.33 ± 8.82	377.17 ± 10.1	434.17 ± 23.47
Heart weight (gm)	1.15 ± 0.06	1.28 ± 0.02	1.38 ± 0.07	1.39 ± 0.12
Total hemorrhage volume (ml)	11.0 ± 1.06	8.08 ± 0.74	-	-
Total hemorrhage time (min)	64.93 ± 7.12	58.71 ± 7.75	59.30 ±7.76	60.48 ± 13.97
Hemorrhage %	25.95 ± 1.32	22.85 ±1.99	-	-
Basal MABP (mmHg)	139.03 ± 7.77	126.40 ± 6.86	131.11 ± 8.19	135.99 ± 7.04
Total blood volume (ml)	41.98 ± 2.42	35.40 ± 0.79	33.94 ± 0.90	39.08 ± 2.11

Table 2 *One hour perfusion of the isolated hearts from hemorrhage group.*

	15 Minutes	30 Minutes	45 Minutes	60 Minutes	
Coronary Flow (ml/min)	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	
Coronary Perfusion Pressure (mmHg)	50.00 ± 1.3	51.67 ± 2.11	59.17 ± 6.51	88.17 ± 7.85	(a)
Total Balloon Volume (ml)	0.29 ± 0.05	0.29 ± 0.05	0.29 ± 0.05	0.29 ± 0.05	
Coronary Vascular Resistance (mmHg/ml/min)	5.13 ± 0.14	5.67 ± 0.3	6.92 ± 0.56	7.54 ± 0.64	(b)

a = $p < 0.05$ compared to within group 15 minute time period
b = $p < 0.05$ compared to within group 15 minute time period

pre- and post-hemorrhage groups ($P<0.05$). However, the increase in lactate in these groups was not different from one another ($P>0.05$).

Table 1 shows the baseline data from the hemorrhage group. Rat weight was 468.33 ± 25.87 gm, heart weight was 1.15 ± 0.06 gm, total hemorrhage time was 64.93 ± 7.12 minutes, total hemorrhage volume was 11.0 ± 1.06 mL, that is 25.95 ± 1.32 %, total blood volume was calculated to be 41.98 ± 2.42 ml and basal mean arterial blood pressure was 139.03 ± 7.77 mmHg.

Hemodynamic data, including coronary flow, coronary perfusion pressure, total balloon volume and coronary vascular resistance, remained unchanged throughout the experimental protocol (tables not shown) except for the hemorrhage group (Table 2) where the coronary perfusion pressure significantly increased at 60 minutes (88.17 ± 7.85) compared to 15 minutes values (50 ± 1.3). Also, the coronary vascular resistance showed a significant increase at 60 minutes (7.54 ± 0.64 mmHg/mL/min) as compared to 15 minutes (5.13 ± 0.14 mmHg/mL/min).

Discussion

Data from the present study showed reperfusion of the hemorrhagic shocked hearts on the Langendorff following one hour of hemorrhagic (MABP= 40 mmHg), which resulted in myocardial contractile dysfunction and impaired recovery of function, as demonstrated by:

1) a decrease in left ventricular generated pressure in hemorrhage group as compared to controls,
2) a decrease in max ± dP/dt,
3) an increase in left ventricular end diastolic pressure,
4) a decrease in left ventricular compliance,

This study is the first to our knowledge to show that blocking the cardiac sarcolemmal Na^+-H^+ exchanger with amiloride, a non-specific Na^+-H^+ exchanger blocker, during the first few minutes of resuscitation from hemorrhagic shock, improved the cardiac contractile function of the isolated perfused hearts. Our finding is in agreement with other reports (McDonald, Milligan *et al*. 1975; Shoemaker, Pietzman *et al*. 1996; Gloviczki 2002) that showed that hemorrhagic shock resulted in cardiac contractile dysfunction.

In 1975, McDonald *et al* (McDonald, Milligan *et al*. 1975) studied the effects of hemorrhagic shock on myocardial contractility *in vivo* in dogs. They concluded that it may be the onset of resuscitation, and the duration and severity of hemorrhagic shock that affect the recovery of myocardial contractility. Previous studies showed that hemorrhagic shock decreased myocardial contractile function, both *in vivo* (McDonald, Milligan *et al*. 1975; McDonough, Giaimo *et al*. 1999) as well as *in vitro* isolated perfused hearts (Horton 1989; Toropov and Dolgikh 2000). Raymond (Raymond 2002) showed that agonal hemorrhagic shock (MABP=35 mmHg) resulted in reversible myocardial depression in ex vivo perfused rat hearts.

The exact mechanism of the cardiac dysfunction following hemorrhagic shock is not well known. One explanation is that it may be related to the extracellular acidosis produced by the hemorrhagic shock (Luchette, Robinson *et al*. 1999). Hemorrhagic shock results in a decrease in tissue perfusion and oxygen delivery. When oxygen delivery falls below a critical level, intracellular metabolism shifts to anaerobic glycolysis with consequent lactate production. Data from the present study showed that hemorrhagic shock resulted in extracellular acidosis, as shown by the decrease in the arterial pH in the hemorrhage group as well as an increase in the arterial lactate levels.

Data from our present study showed evidence of increased $[Ca^{2+}]_i$ during hemorrhagic shock, which was associated with an increase in the left ventricular end diastolic pressure (Figure 2), a decrease in left ventricular compliance (Table 2) and increased – dP/dt. The compliance was calculated by dividing the change in balloon volume (BV) by the change in left ventricular end diastolic pressure (LVEDP), ($C= \Delta V/\Delta P$). The balloon volume in the hemorrhage group is decreased and LVEDP was increased, indicating that the stiffness of the heart is increased in the hemorrhage group. In other words, the compliance is decreased in our hemorrhagic shock model. Our data is also in agreement with other studies. In 1983, Alyono *et al* (Alyano *et al*. 1983) showed a significant decrease in left ventricular compliance in intact dogs that undergo 2 hours of hemorrhage (mean arterial pressure 40 mm Hg), followed by reinfusion of the shed blood. Diebel *et al* in 1993 (Diebel 1993) also reported a decrease in the right ventricular compliance in an anaesthetized swine model after hemorrhagic shock. This decrease in compliance was caused by an increase in intracellular calcium.

Studies of models of hemorrhagic shock have shown increased $[Ca^{2+}]_i$. Other studies showed evidence of the increase in $[Ca^{2+}]_i$ including: LVEDP, -dP/dt, LV balloon volume and decreased LV compliance on *ex vivo* reperfusion following hemorrhagic shock. These data may be related to activation of the Na^+-H^+ exchanger during reperfusion as blocking of the Na^+-H^+ exchanger abolished these changes. This raises a possibility of involvement of Na^+-H^+ exchanger in mediating myocardial dysfunction following hemorrhagic shock

However, there is a lack of data on the role of the Na^+-H^+ exchanger during reperfusion following hemorrhagic shock. Our present study focused on investigating the role of the Na^+-H^+ exchanger in mediating myocardial contractile dysfunction following *ex vivo* reperfusion of hearts after hemorrhagic shock. The mechanism of myocardial contractile dysfunction after *ex vivo* reperfusion of the hearts following one hour of hemorrhagic shock is unclear, and there is considerable lack of data on the role of Na^+-H^+ exchanger in mediating this dysfunction. However, there has been intensive study of the role of the Na^+-H^+ exchanger in mediating the so-called reperfusion injury following ischemic conditions (Grinwald 1982; Karmazyn 1988). It is generally accepted that when hearts are exposed to ischemic conditions for prolonged periods, recovery is incomplete and can be

associated with greater injury than that seen in the initial ischemic episodes (Grinwald 1982). The mechanisms underlying reperfusion injury are not known with certainty, although they likely involve defective Ca^{2+} handling by the cardiac cell leading to $[Ca^{2+}]_i$ overload (Koomen, Schevers *et al.* 1983).

Undoubtedly, the most widely studied [models] in terms of the role of the Na^+-H^+ exchanger in cardiac pathology pertain to its role in the ischemic and reperfused myocardium. During ischemia extracellular pH drops, intracellular pH drops and protons accumulate. The Na^+-H^+ exchanger causes removal of the intracellular protons and results in accumulation of $[Na^+]_i$. The increased levels of $[Na^+]_i$ reportedly stimulate the Na^+-Ca^{2+} exchanger to extrude Na^+ in exchange for Ca^{2+}. Excess Ca^{2+} therefore accumulates. Reperfusion of the ischemic myocardium would likely result in rapid restoration of the extracellular acidosis that would eventually cause rapid and excessive stimulation of the Na^+-H^+ exchanger leading to a large increase in the intracellular Na^+ (Regan, Broisman *et al.* 1980; Grinwald 1982). The $[Na^+]_I$ dependent accumulation of $[Ca^{2+}]_i$ would be further compounded by the fact that Na^+-K^+ ATPase activity is likely inhibited by ischemia (Karmazyn 1988), which would increase $[Na^+]_I$ with a subsequent elevation in $[Ca^{2+}]_i$ via the Na^+-Ca^{2+}exchange mechanism. The large increase in the $[Na^+]_i$ will cause an abnormally large $[Ca^{2+}]_i$ via the Na^+-Ca^{2+} exchanger (Renlund, Gerstenblith *et al.* 1984).

The first study demonstrating protection (Karmazyn 1988) showed that amiloride, the Na^+-H^+ exchanger inhibitor, enhanced ventricular recovery from reperfused ischemic isolated rat hearts (figures 1.10., 1.11., 1.12.). Another study has shown that the protective effect of amiloride is associated with a reduced incidence of arrhythmias and preservation of ultrastructural integrity (Duan and Karmazyn 1992). Early studies have shown that this protection is associated with diminished tissue contents of Na^+ and Ca^{2+}, in support of a close association between the Na^+-H^+ exchanger and Na^+-Ca^{2+} exchange activity (Tani and Neely 1989).

More recent studies have used more specific and potent benzoylguanidine Na^+-H^+ exchanger inhibitors, such as the HOE compounds, to demonstrate protection and implicate Na^+-H^+ exchanger involvement. For example, the first such agent, HOE 694, has been shown to exert protective effects in terms of virtually all parameters studied both under *in vitro* conditions and in animals subjected to coronary artery occlusion and reperfusion (Scholz, Albus *et al.* 1993; Hendrikx, Mubagwa *et al.* 1994; Sack, Mohri *et al.* 1994; Yasutake, Ibuki *et al.* 1994; Klein, Pich *et al.* 1995; Shimada, Hearse *et al.* 1996; Xue, Aye *et al.* 1996) . The GUARDIAN (Guard During Ischemia Against Necrosis) study, was the first clinical evaluation of Na^+-H^+ exchanger inhibition. It is a Phase II/Phase III double-blind, randomized placebo-controlled study of >11 500 patients, assessed by different doses of cariporide in individuals with acute coronary syndromes. The study failed to demonstrate an overall significant attenuation (10%) of the two primary events, mortality and incidence of myocardial infarction; however, favorable effects among the three major subgroups were observed in those patients receiving the highest dose (120 mg every 8 hours) of the drug, including a significant event rate reduction in high-risk patients undergoing coronary artery bypass surgery. These results are therefore encouraging and overall support the concept that Na^+-H^+ exchanger inhibition represents a safe, therapeutic approach for cardioprotection that undoubtedly deserves future attention.

The present study showed that inhibition of the Na^+-H^+ exchanger using amiloride for 5 minutes during *ex vivo* reperfusion from hemorrhagic shock improved myocardial contractile function. Moreover, evidence of lower $[Ca^{2+}]_i$ (lower LVEDP, -dP/dt and increased compliance). This may suggest involvement of the Na^+-H^+ exchanger, in part or in total, in causing the myocardial contractile dysfunction during the *ex vivo* reperfusion of hearts following hemorrhagic shock, since this dysfunction was improved by inhibiting the exchanger for short period on reperfusion.

Data from the present study may be of clinical beneficial as it may show a sort of myocardial protection against rapid resuscitation following prolonged severe hemorrhagic shock that has been associated with multiple organ failure (Ba, Wang *et al.* 2000) and high mortality rates(Burris, Rhee *et al.* 1999). Further intensive research needs to be done, using more specific Na^+-H^+ exchanger blockers, to measure the $[Na^+]_i$ and $[Ca^{2+}]_i$ as well as pH_i to confirm the involvement of Na^+-H^+ exchanger. Also, further studies are needed to show the relation between the beneficial effects of the Na^+-H^+ exchanger inhibition and the depth and duration of hemorrhagic shock.

In summary, our study is the first to show that blocking the Na^+-H^+ exchanger using amiloride, a non specific Na^+-H^+ exchanger inhibitor, during the first 5 minutes of *ex vivo* reperfusion on the Langendorff System, following one hour of hemorrhagic shock, improved recovery of left ventricular contractile function. The exact mechanism of protection of Na^+-H^+ exchanger blockers on resuscitation of hemorrhagic shock is not clear. One possible explanation is that it could be due to prevention of the excessive stimulation of the Na^+-H^+ exchanger on rapid reperfusion, which resulted in an abnormally large increase in $[Na^+]_i$ and $[Ca^{2+}]_i$. However, further research is required to clarify the exact mechanism of protection.

Conclusion

The results of the present study showed beneficial effects of blocking the cardiac Na^+-H^+ exchanger, using amiloride, on myocardial contractile function in the *ex vivo* perfused rat heart following hemorrhagic shock. On resuscitation, the Na^+-H^+ exchanger is excessively stimulated to recover from intracellular acidosis, allowing extrusion of protons at the expense of a large increase in $[Na^+]_i$. The rise in $[Na]_i$ resulted in a large increase in $[Ca^{2+}]_I$ through stimulation of the Na^+-Ca^{2+} exchanger. Blocking the cardiac Na^+-H^+ exchanger for a short time during *ex vivo* reperfusion following hemorrhagic shock may allow the cell to balance the increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ without potentially causing injury to the cell. Although the precise mechanism(s) underlying the beneficial effects of amiloride in the present study remains to be determined, it may be related to amiloride's well-known ability to inhibit the Na^+-H^+ exchange mechanism. These results are in agreement with those who have suggested such a protective effect of blocking the cardiac Na^+-H^+ exchanger following ischemia-reperfusion injury. Hemorrhagic shock results in accumulation of lactate and intracellular acidification. Intracellular acidification may be the initial stimulus for a cascade of events contributing to intracellular calcium overload. Thus the observed effects of improving myocardial contractile function may be related to preventing these potential changes in the $[Ca^{2+}]_i$. Further research is required to determine the mechanisms involved, particularly with more specific Na^+-H^+ exchange blockers, and for measurements of pH_i , $[Na^+]_i$ and $[Ca^{2+}]_i$.

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