Efficient Extra- and Intracellular Alkalinization Improves Cardiovascular Functions in Severe Lactic Acidosis Induced by Hemorrhagic Shock

Antoine Kimmoun, M.D., Nicolas Ducrocq, M.D., Nacira Sennoun, Ph.D., Khodr Issa, Ph.D., Charlène Strub, L.T., Jean-Marie Escanyé, Ph.D., Sébastien Leclerc, Ph.D., Bruno Levy, M.D., Ph.D.

ABSTRACT

Background: Lactic acidosis is associated with cardiovascular failure. Buffering with sodium bicarbonate is proposed in severe lactic acidosis. Bicarbonate induces carbon dioxide generation and hypocalcemia, both cardiovascular depressant factors. The authors thus investigated the cardiovascular and metabolic effects of an adapted sodium bicarbonate therapy, including prevention of carbon dioxide increase with hyperventilation and ionized calcium decrease with calcium administration.

Methods: Lactic acidosis was induced by hemorrhagic shock. Twenty animals were randomized into five groups: (1) standard resuscitation with blood retransfusion and norepinephrine (2) adapted sodium bicarbonate therapy (3) nonadapted sodium bicarbonate therapy (4) standard resuscitation plus calcium administration (5) hyperventilation. Evaluation was focused *in vivo* on extracellular pH, on intracellular pH estimated by P^{31} nuclear magnetic resonance and on myocardial contractility by conductance catheter. Aortic rings and mesenteric arteries were isolated and mounted in a myograph, after which arterial contractility was measured.

Results: All animals in the hyperventilation group died prematurely and were not included in the statistical analysis. When compared with sham rats, shock induced extracellular (median, 7.13; interquartile range, [0.10] *vs.* 7.30 [0.01]; $P = 0.0007$) and intracellular acidosis (7.26 [0.18] *vs.* 7.05 [0.13]; $P = 0.0001$), hyperlactatemia (7.30 [0.01] *vs.* 7.13 [0.10]; $P = 0.0008$), depressed myocardial elastance (2.87 [1.31] *vs.* 0.5 [0.53] mmHg/μl; $P = 0.0001$), and vascular hyporesponsiveness to vasoconstrictors. Compared with nonadapted therapy, adapted bicarbonate therapy normalized extracellular pH (7.03 [0.12] *vs.* 7.36 [0.04]; $P < 0.05$), increased intracellular pH to supraphysiological values, improved myocardial elastance (1.68 [0.41] *vs.* 0.72 [0.44] mmHg/μl; $P < 0.05$), and improved aortic and mesenteric vasoreactivity.

Conclusions: A therapeutic strategy based on alkalinization with sodium bicarbonate along with hyperventilation and calcium administration increases pH and improves cardiovascular function. **(Anesthesiology 2014; 120:926-34)**

LACTIC acidosis is one of the consequences of shock states and is associated with mortality.[1] However, it is difficult to separate the consequences of the disease associated with shock and the consequences of shock-induced lactic acidosis.

Ex vivo and organ bath studies have clearly demonstrated that severe hypoxic lactic acidosis is associated with major deleterious cardiovascular consequences.[2–4] Indeed, severe lactic acidosis (pH < 7.20) is associated with decreased catecholamine efficiency, myocardial contractile dysfunction (mainly due to decreased sensitivity of contractile proteins to Ca^{2+}), and vascular hyporesponsiveness to catecholamine, which involves K^+ adenosine triphosphate channels activation.[5–7] Several Ca^{2+} transport systems are also hindered at low pH, including sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase, the ryanodine receptor and the Na^+/Ca^{2+} exchanger.[8] Acidosis has also been shown to decrease cellular Ca^{2+} transient amplitude.[9,10] However, intracellular acidosis decreases cell metabolism and may induce a protective

What We Already Know about This Topic

- The effect of bicarbonate administration on hemodynamics and vasopressor requirements at lower pH, as well as the effect on clinical outcomes at any pH, is unknown
- This study determined the cardiovascular and metabolic effects of adapted sodium bicarbonate therapy, including prevention of carbon dioxide increase with hyperventilation and ionized calcium decrease with calcium administration, in a rat model of severe lactic acidosis induced by hemorrhagic shock

What This Article Tells Us That Is New

- A therapeutic strategy based on alkalinization with sodium bicarbonate, along with hyperventilation and calcium administration, increases pH and improves cardiovascular function

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hibernation state.[11,12] Therefore, it is unknown whether acidosis correction is beneficial under acute conditions.

Accordingly, sodium bicarbonate has been removed from the algorithm in advanced cardiac life support.[13] The Surviving Sepsis Campaign recommends against the use of sodium bicarbonate therapy for the purpose of improving hemodynamics or reducing vasopressor requirements in patients with hypoperfusion-induced lactic acidemia with pH of 7.15 or greater.[13,14]

The effect of bicarbonate administration on hemodynamics and vasopressor requirements at lower pH, as well as the effect on clinical outcomes at any pH, is unknown. However, sodium bicarbonate can induce a so-called paradoxical intracellular acidosis due to a large generation of carbon dioxide, which diffuses rapidly into the tissues, resulting in intracellular hypercapnic acidosis.[15–17] Moreover, bicarbonate decreases ionized calcium.[18] Because calcium plays a pivotal role in cellular contraction and carbon dioxide in intracellular pH regulation, both are probably involved in hemodynamic impairment associated with bicarbonate administration. Therefore, it is possible that these major side effects dampen the positive effects of sodium bicarbonate.[19] Continuous veno-venous hemofiltration using bicarbonate as a buffer has been proposed to treat metabolic acidosis. Although efficient in reversing severe metabolic acidosis in some patients, the effects of continuous veno-venous hemofiltration are progressive and may be inefficient to promptly reverse the deleterious cardiovascular effects of metabolic acidosis.[20] Boyd and Walley[19] suggested that if bicarbonate is used, consideration must be given to clear bicarbonate-induced carbon dioxide production and to correct ionized calcium if necessary. Nevertheless, the efficiency of these recommendations has not been evaluated.

We hypothesized that (1) the main putative beneficial effects of bicarbonate administration in shock with lactic acidosis are cardiovascular, (2) standard therapy including catecholamine administration should be performed before the use of bicarbonate, and (3) the prevention of carbon dioxide increase and calcium decrease through an increase in ventilation and calcium administration should enhance the beneficial effects of acidosis correction.

The aim of the current study was thus to determine the cardiovascular and metabolic effects of an adapted sodium bicarbonate therapy, including the prevention of carbon dioxide increase and ionized calcium decrease, in a resuscitated rat model of severe lactic acidosis induced by hemorrhagic shock.

Materials and Methods

Common Animal Preparation

Male Wistar rats (Charles River, Chatillon-sur-Chalaronne, L'Arbresle Cedex, France) weighing 300 to 400 g were investigated after an acclimation period of at least 1 week before experimentation. All animal experimentations were performed in accordance with institutional guidelines and protocols approved by the French Animal Care Committee in keeping with European regulations (comité d'éthique lorrain en Expérimentation Animale, Nancy, France). The procedure for the care and sacrifice of study animals was in accordance with the European Community Standards on the Care and Use of Laboratory Animals.

Anesthesia was performed with sodium pentobarbital (6 mg/100 g intraperitoneal Penthotal; Hospira, Meudon, France). Anesthesia was maintained every 30 min by intraperitoneal injection of sodium pentobarbital. After tracheotomy, animals were curarized with pancuronium bromide (0.2 mg/100 g i.v. Pavulon; MSD France, Courbevoie, France) and mechanically ventilated (tidal volume, 0.7 to 0.8 ml/100 g; ventilator frequency, 50 min^{-1}; rodent ventilator no. 683; Harvard Apparatus France, Les Ulis, France) with a fraction of inspired oxygen 100%. Ventilator frequency was adjusted to obtain an arterial P_{CO_2} between 38 and 42 mmHg. The left jugular vein was cannulated with PE-50 tubing (BD Intramedic, Le Pont de Claix, France) for infusion (1 ml h^{-1} 100 g^{-1} of body weight i.v. NaCl 0.9%; B. Braun Medical SAS, Boulogne Billancourt, France). Core body temperature was measured *via* a rectal probe and maintained at 37°C. Equal amounts of fluid were infused in all rats, and sodium intake was equivalent for all experimental groups.

Shock Model: Controlled Hemorrhagic Shock

After a 20-min stabilization period, hemorrhagic shock was induced by drawing blood in 10 min *via* an arterial catheter until reaching a mean arterial pressure (MAP) of 40 to 45 mmHg. This MAP was maintained during the remaining 50 min by redrawing blood or if necessary by transfusing the collected blood. Thereafter, an arterial blood gas was performed to ensure a pH less than 7.2 and a hyperlactatemia greater than 3 mM, which defined the time of shock.

Group Design

After establishment of shock, animals were randomized into five groups:

1. Standard resuscitation included the retransfusion of collected blood in 10 min and, if necessary, norepinephrine administration (from 1 to 6 μg kg^{-1} min^{-1}) to 90% recovery of baseline MAP (Standard group, n = 5),
2. Standard resuscitation plus administration of sodium bicarbonate 8.4% (B. Braun Medical SAS, 0.2 mmol/100 g infused over 5 min at time of shock and reinjection after 30 min) and calcium chloride 10% (Renaudin, Aïnhoa, France; 2 mg/100 g infused over 5 min at time of shock and after 30 min). The ventilator frequency was increased to 90 min^{-1} and then adjusted to obtained an arterial P_{CO_2} between 38 and 42 mmHg (Adapted group, n = 5),
3. Standard resuscitation plus administration of sodium bicarbonate (at time of shock and reinjection at 30 min) without adjustment for P_{aCO_2} or calcemia (Nonadapted group, n = 5),

4. Standard resuscitation plus calcium chloride administration (2 mg/100 g infused over 5 min at time of shock and after 30 min) without adjustment for $Paco_2$ (Calcium group, n = 5),
5. Standard resuscitation plus hyperventilation. The ventilator frequency was increased to 90 min^{-1} and then adjusted to obtained an arterial Pco_2 between 38 and 42 mmHg (Hyperventilation group, n = 5).

A sham group was also included and comprised rats undergoing surgery without hemorrhagic shock (Sham group, n = 5).

Sodium Intake

Because bicarbonate effects may be due to a volume or salt effect, NaCl 20% (B. Braun Medical SAS) diluted in glucose 5% (B. Braun Medical SAS) to obtain the same sodium load and volume as the equivalent bicarbonate solution was injected in groups 1, 4, and 5 over 5 min at time of shock and after 30 min.

Time of In Vivo *Measurements*

Hemodynamic measurements, standard biological assays (arterial blood gas, lactatemia, calcemia), pHi, and Pi/phosphocreatine were collected at baseline, at time of shock, and at 30 and 60 min after resuscitation. Experiments were continued 120 min after resuscitation for the catheter conductance study and 60 min after resuscitation for assessment of intracellular pH. All animals were sacrificed by exsanguination at the end of the *in vivo* studies.

In Vivo *Hemodynamic Measurements*

Before inducing shock, a MIKRO-Tip 2.0-French pressure–volume conductance catheter (Model SPR839; Millar Instruments, Houston, TX) was advanced into the left ventricle through the right carotid artery to measure left ventricular pressure–volume loops. Real-time volume conductance and pressure data were recorded using MPVS-300 (Millar Pressure Conductance Unit model 200; Millar Instruments Inc.). This volume measurement was based on precalibration using a series of known cylindrical volumes as per the manufacturer's instructions. Heart rate, ejection fraction, and cardiac output were computed using an analysis program (IOX 2.4.2.6*; EMKA Technologies, Paris, France) as described previously.[21] The pressure–volume relationship was assessed by compression of the inferior vena cava, which temporarily reduces preload. Index of contractility (Emax) was calculated using IOX 2.4.2.6*. In addition, MAP was measured using a pressure transducer connected to a fluid-filled catheter inserted into the left femoral artery.

Phosphorous Nuclear Magnetic Resonance Spectroscopy Protocol (See Complete Methodology in Supplemental Digital Content 1, http://links.lww.com/ALN/B15)

A second set of experiments was performed to achieve intracellular pH and energy metabolism measurements in the following groups only: Adapted, Nonadapted, and Standard.

In vivo phosphorous nuclear magnetic resonance experiments were performed on a 2.35 T 24 cm bore magnetic resonance spectroscopy system (Biospec Avance; Bruker Biospin, Wissembourg, France) as described previously.[15]

Ex Vivo *Vascular Reactivity Measurements (See Supplemental Digital Content 1, http://links.lww.com/ALN/B15, a Complete Methodology)*

After sacrifice of the animals by exsanguination at the end of conductance catheter study, the thoracic aorta and mesentery were removed and their vasoreactivity studied. Vascular reactivity of aortic and mesenteric rings was studied on a wire myograph (Danish Myo Technology, Aarhus N, Denmark) as previously described.[22]

Specific Biological Assays

Blood withdrawn at the end of the conductance catheter study was centrifuged (4,000 rpm, 15 min, 4°C) and resulting plasma aliquoted and stored at −80°C until biochemical analysis. Organs (aorta and heart) were also collected and stored at −80°C until biochemical analyses.

Cytokine Analyses

Plasma levels of interleukin (IL)-6, IL-10, and tumor necrosis factor-α were measured in duplicate with the use of rat IL-6, IL-10, and tumor necrosis factor-α enzyme-linked immunosorbent assay kits (Quantikine ELISA; R&D Systems, Chartres de Bretagne, France) according to the manufacturer's instructions. Results are expressed as picograms of measured cytokine per milliliter of plasma (pg/ml).

Measurement of Nitrite/Nitrate

NO_2^- and NO_3^- are the primary oxidized products of nitric oxide reacting with water and, therefore, total concentration of NO_2^-/NO_3^- in plasma was used as an indicator of nitric oxide production *in vivo*. In brief, the nitrate in the supernatant was first reduced to nitrite by incubation with nitrate reductase (10 U/ml) and nicotamide adenine dinucleotide phosphate (629.2 μg/ml) at room temperature for 30 min. Thereafter, total nitrite concentration in the samples was measured by Griess reaction after the addition of 100 μl of Griess reagent to 100 μl of sample in a 96-well plate with a flat, transparent bottom. The optical density at 550 nm was measured by an enzyme-linked immunosorbent assay microplate reader and normalized with optical density at 550 nm of standard saline solutions.

RNA Extraction and Quantitative Real-time–Polymerase Chain Reaction (RT-PCR)

Primers for quantitative RT-PCR were obtained from Eurogentec (Angers, France). Total RNA extraction was carried out with the RNA Plus mini kit (Qiagen, Courtaboeuf Cedex, France) according to the manufacturer's instructions. Total RNA was reverse transcribed to complementary DNA by using iScript One-Step RT-PCR Kit for Probes (Biorad, Marnes-la-Coquette, France). Complementary DNA obtained from the RT reaction was subjected to quantitative

PCR by using iTaq Fast SYBR Green Supermix With ROX (Biorad). The primer and concentrations were optimized according to manufacturer's guidelines. Expressions of inductible isoform of nitric oxide synthase of messenger RNA (mRNA) and Hif-1 mRNA were measured using iTaq Fast SYBR Green Supermix (Biorad). PCR parameters were as follows: incubation at 50°C for 2 min, incubation at 95°C for 10 min, and thereafter 40 denaturation cycles at 95°C for 15 s and annealing and extension at 60°C for 1 min. Each sample was determined in duplicate. A standard curve method was used to determine the relative mRNA levels as described in the Applied Biosystems User Bulletin: A standard curve for each gene was created using RNA isolated from the Standard group. Isolated RNA was reverse transcribed, and dilution series of complementary DNA ranging from 1 pg to 10 ng were subjected to RT-PCR. The obtained threshold cycle values were plotted against the dilution factor to create a standard curve. Relative mRNA levels in test samples were then calculated from the standard curve.

Statistical Analysis

Because of the small sample size, most of the analyses were performed with nonparametric methods. The aforementioned characteristics were compared between Sham and experimental groups at time of shock (Wilcoxon–Mann–Whitney test) and between the different groups at 60 min after shock (Kruskal–Wallis tests). Comparisons between groups were specifically done at 120 min only for the total norepinephrine intake and the specific biological assays (Kruskal–Wallis tests). Further comparisons were performed upon reaching statistical significance in the Kruskal–Wallis test with the Dunn test, a *post hoc* nonparametric test. Intergroup differences for *in vivo* myocardial function, intracellular pH, and energy metabolism measurements were tested using a two-way ANOVA (repeated time measurements and drug as independent variables). *Ex vivo* vasoreactivity was assessed in the several groups for increasing concentrations of phenylephrine (Friedman test). The nonlinear curve fitting tool of PRISM (GraphPad Software, San Diego, CA) was used to depict the results of the vasoactivity testing. The two-tailed statistical significance level was set at 0.05. All statistical analyses were performed using SAS® 9.1 system software (SAS Institute Inc., Cary, NC,).

Results

All the animals survived the experimental period with the exception of all rats included in the Hyperventilation group, which rapidly died from refractory hypotension.

Hemorrhagic shock is associated with lactic acidosis, depressed myocardial function, and vascular hyporesponsiveness to vasoconstrictors (table 1 and fig. 1 and Supplemental Digital Content 2, fig. 1, *http://links.lww.com/ALN/B16, which depicts the ex vivo vasoreactivity of aortic rings to phenylephrine).* When compared with sham animals, hemorrhagic shock was associated with hyperlactatemia, extracellular metabolic acidosis, and intracellular acidosis (7.26 [0.18] *vs.* 7.05 [0.13]; $P = 0.0001$). Neither $Paco_2$ nor ionized calcium levels significantly differed between the two groups (*table 1*).

Changes detected by conductance catheter readings confirmed that both load-dependent and load-independent parameters of left ventricular function were impaired in shock-induced animals. Cardiac output as well as Emax, a load-independent measure of systolic function, were significantly lower than in sham-treated animals (cardiac output: 55,558 [49,459] *vs.* 95,047 [8,231] µl/min, $P = 0.0488$; Emax: 0.5 [0.53] *vs.* 2.87 [1.31] mmHg/µl, $P = 0.007$; *table 1*).

Although phenylephrine induced a dose-dependent increase in tension in aortic rings and small mesenteric vessels in control rats, hemorrhagic shock conversely blunted phenylephrine-stimulated contraction ($P < 0.01$) (*fig. 1* and Supplemental Digital Content 2, fig. 1, http://links.lww.com/ALN/B16).

Adapted acidosis therapy is associated with extracellular pH normalization and intracellular pH increase (table 2 and fig. 2 and Supplemental Digital Content 2, fig. 2, *http://links.lww.com/ALN/B16, which depicts the course of high-energy phosphates [Pi/phosphocreatine] in striated muscle in Adapted, Nonadapted, and Standard groups).* When compared with the Standard group, bicarbonate therapy coupled with calcium and increased ventilation (Adapted group) increased extracellular pH to normal range and intracellular pH to supraphysiological values (*table 2* and *fig. 2*). In contrast, bicarbonate administration without ventilation adjustment (Nonadapted group) increased arterial $Paco_2$, leading to a further decrease in extracellular pH. Consequently, bicarbonate also induced a transient (30 min) paradoxical intracellular acidosis (*fig. 2*).

Calcium administration (Calcium group) did not alter extracellular and intracellular pH (not shown), whereas inorganic phosphate/phosphocreatine, an index of the bioenergy

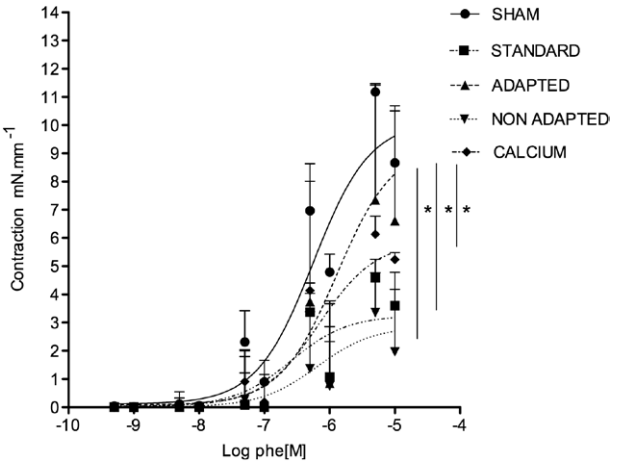

Fig. 1. *Ex vivo* vasoreactivity of first-order mesenteric arteries. X axis: Increasing concentration of phenylephrine express in logarithm of phenylephrine [M]. Y axis: contraction of the vessel in mN/mm. *Vasoreactivity is significantly improved in the Adapted group compared with the other groups ($P < 0.05$). The symbols (*point*, *squares*, and *triangles*) represent median. Upper edges of *error bars* represent 75th percentile in each group.

Table 1. Metabolic and Hemodynamic Alterations between Rats at Establishment of Shock and Sham Animals

	Establishment of Shock, N = 20 (80%)		Sham, N = 5 (20%)		
	Median	Interquartile	Median	Interquartile	*P* Value
pH	7.13	0.10	7.30	0.01	0.0007
Lactatemia (mM)	9.6	3.6	2.5	1.4	0.0008
$Paco_2$ (mmHg)	36	7	43	2	0.0824
BE (mM)	–16.0	5.5	–5.0	3.0	0.0006
Ionized calcium (mM)	1.1	0.2	1.3	0.1	0.0614
HR (min^{-1})	350	55	395	24	0.0066
MAP (mmHg)	43	3	133	9	0.0006
EF (%)	39	21	64	23	0.0043
CO (μl/min)	55,558	49,459	95,047	8,231	0.0488
Emax (mmHg/μl)	0.50	0.53	2.87	1.31	0.0007
Ea (mmHg/μl)	0.40	0.40	0.82	0.22	0.0228

No difference was observed between treated groups.

BE = base excess; CO = cardiac output; Ea = arterial elastance; EF = ejection fraction; Emax = maximal elastance; HR = heart rate; MAP = main arterial pressure; $Paco_2$ = partial pressure of carbon dioxide in blood.

supply of the cell, did not exhibit any difference between the three studied groups (Supplemental Digital Content 2, *fig. 2*, http://links.lww.com/ALN/B16).

Adapted acidosis therapy is associated with an improvement in vascular response to catecholamine (figs. 1 and *3 and Supplemental Digital Content 2, fig. 1, http://links.lww.com/ALN/B16)*. Total infused norepinephrine required to maintain a 90% baseline MAP throughout the experiment (*fig. 3*) was reduced in the Adapted group when compared with the other groups. *Ex vivo* vasoreactivity of aorta and mesenteric arteries (*fig. 1* and Supplemental Digital Content 2, fig. 1, http://links.lww.com/ALN/B16) was restored to sham levels while improving significantly in the Calcium group. Conversely, in the Nonadapted group, the maximal contractile capacity of the aorta and small mesenteric arteries further decreased.

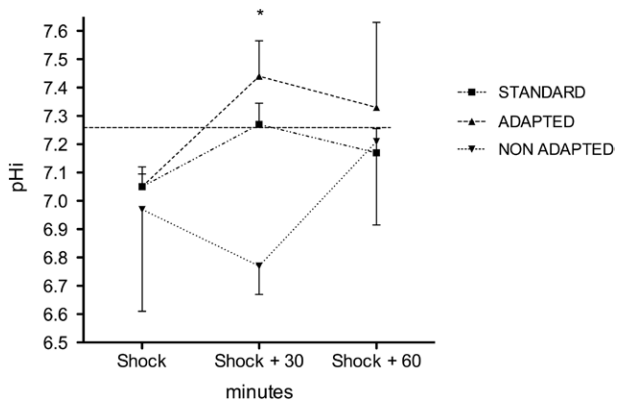

Fig. 2. Course of intracellular pH at establishment of shock, and 30 and 60 min thereafter in Adapted, Nonadapted, and Standard groups. *Dashed line*: normal intracellular pH value in Sham rats. *At 30 min, the Adapted group had a significantly higher pH compared with the two other groups ($P < 0.05$). X axis: time in minutes. Y axis: pH values. The symbols (*point*, *squares*, and *triangles*) represent median. Upper edges of *error bars* represent 75th percentile in each group.

*Adapted acidosis therapy is associated with an improvement in myocardial function (*table 2*, Supplemental Digital Content 2, fig. 3, http://links.lww.com/ALN/B16, which depicts the change [%] in Emax from establishment of shock [100%], and 30 and 60 min thereafter)*. As shown, Emax (as expressed in percentage of variation) displayed a linear increase in the Adapted group compared to the other groups.

Effect of the Different Treatment Strategies on Cytokine Expression (Supplemental Digital Content 2, figs. 4 and 5, http://links.lww.com/ALN/B16)

Hemorrhagic shock was associated with an increase in IL-6, IL-10, and tumor necrosis factor-α levels ($P < 0.05$), which was not modified with the three treatment strategies. Expression levels of inductible isoform of nitric oxide synthase mRNA increased in the aorta but not in the heart. Expression levels of Hif-1 mRNA (hypoxia-induced factor-1), modulated by hypoxia and/or nuclear factor κB under normoxic conditions, remained at a constant level between groups in both aorta and heart (Supplemental Digital Content 2, figs. 4 and 5, http://links.lww.com/ALN/B16).

Discussion

The main finding of the current study is that bicarbonate therapy with prevention of hypercapnia together with calcium administration markedly improves cardiovascular function in severe lactic acidosis associated with hemorrhagic shock. These beneficial effects are associated with a restoration of catecholamine efficiency both at the myocardial and vascular level. Our results confirm the deleterious effects of bicarbonate alone especially in demonstrating a paradoxical intracellular acidosis.

Model Characteristics

Given that experimental models of lactic acidosis have failed to show a beneficial effect of alkali therapy alone when

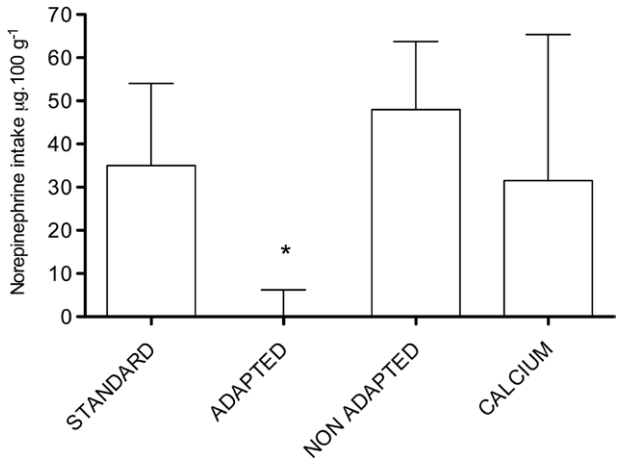

Fig. 3. Total norepinephrine intake over the resuscitation period in each group in order to maintain a 90% baseline mean arterial pressure. *Total norepinephrine dose was significantly lower in the Adapted group compared with Nonadapted and Calcium groups (P = 0.02). Upper edges of *error bars* represent 75th percentile in each group.

compared with the effects of sodium therapy, we used a more clinically relevant model of lactic acidosis induced by low cardiac output (circulatory hypoxia) and subsequently treated according to clinical standards, for example, volume expansion, ventilation, transfusion, and norepinephrine.[15,23–29] Our model displayed all the characteristics of a severe shock after a severe ischemia–reperfusion state despite aggressive conventional treatment: major lactic acidosis, vasopressor hyporesponsiveness, decreased myocardial function, and inflammatory state.[30]

Effects of Bicarbonate

First, we observed that arterial pH decreased after bicarbonate administration secondary to a marked increase in arterial P_{CO_2}. When compared with other previous experimental models using the same bicarbonate concentration, we observed a greater increase in arterial P_{CO_2}.[28,31] This is likely due to (1) the concomitant use of neuromuscular blockade, (2) a major stagnant venous hypercarbia secondary to low cardiac output and not eliminated by the lungs,[32] and (3) the severity of metabolic acidosis before bicarbonate administration. During shock, carbon dioxide production in the absence of bicarbonate administration is mainly under the dependency of a nonbicarbonate buffering system. Under severe hypoxemic conditions such as in our model, more H^+ ions are available to react with the exogenously administered bicarbonate thereby increasing carbon dioxide generation ($H^+ + HCO_3^- \leftrightarrow H_2O + CO_2$).[33,34] Kindig *et al.*[35] have demonstrated that carbon dioxide production in severe shock/systemic acidosis is accompanied by the generation of a considerable amount of carbon dioxide. In the current experiment, this carbon dioxide overproduction could not be eliminated by the lungs because our animals were paralyzed. The major consequence of this extracellular metabolic and respiratory acidosis translated in a worsened although transient extracellular and intracellular pH.[15] When examining the literature, a decreased pH after a sodium bicarbonate load was not observed in less severe models and in human clinical trials.[15,23,28,29,36] Morever, the two most relevant human studies assessing the effectiveness of bicarbonate load on hemodynamic mainly included shocked patients with only moderate acidosis. Last, the use of a neuromuscular-blocking agent was not specified.[18,37]

Table 2. Comparison of Biological and Hemodynamic Characteristics 60 min after Establishment of Shock

	Standard Group, N = 5 (25%)		Adapted Group, N = 5 (25%)		Nonadapted Group, N = 5 (25%)		Calcium Group, N = 5 (25%)		
	Median	Interquartile	Median	Interquartile	Median	Interquartile	Median	Interquartile	*P* Value
pH	7.12	0.30	7.36	0.04*,†,‡	7.03	0.12	7.03	0.14	<0.0001
Lactatemia (mM)	4.6	1.3	10.0	2.5	10.2	1.5	5.6	5.6	0.0417
$Paco_2$ (mmHg)	36	5	37	2†	62	12	36	5	0.0088
BE (mM)	–20	10	–7	4*	–19	2	–16	7	0.0334
Calcemia (mM)	1.1	0.2	1.3	0.1	1.0	0.1	1.2	0.1	0.0337
HR (min^{-1})	376	34	343	26	398	46	427	71	0.2760
MAP (mmHg)	88	54	82	23	98	39	123	43	0.2502
EF (%)	39	30	52	15	50	37	60	31	0.4233
CO (µl/min)	61,762	50,157	114×10^3	40,213	115×10^3	3,650	86,751	20,457	0.0406
ΔEmax (mmHg/µl)	0.28	0.68	1.11	0.59*,†	0.12	0.66	0.45	0.95	0.0058
Ea (mmHg/µl)	0.5	0.28	0.41	0.49	0.35	0.14	0.67	0.44	0.0321

Intervention in each group: Standard: standard resuscitation with blood retransfusion and norepinephrine; Adapted group: standard resuscitation plus administration of sodium bicarbonate and calcium chloride. Ventilator frequency was increased to obtained an arterial P_{CO_2} in range; Nonadapted group: standard resuscitation plus administration of sodium bicarbonate without adjustment for $Paco_2$ or calcemia; Calcium group: standard resuscitation plus calcium administration. Upon statistical significance in Kruskal–Wallis test, a Dunn protected *post hoc* test was performed between the Adapted group and other treated groups. None of the rats included in the Hyperventilation group survived at 60 min, hence the rats in this group are not included in the study comparisons.

* $P < 0.05$ *vs.* Standard. † $P < 0.05$ *vs.* Nonadapted. ‡ $P < 0.05$ *vs.* Calcium.

BE = base excess; CO = cardiac output; Ea = arterial elastance; EF = ejection fraction; Emax = maximal elastance; ΔEmax = evolution between establishment of shock and 60 min thereafter; HR = heart rate; MAP = main arterial pressure; $Paco_2$ = partial pressure of carbon dioxide in blood.

Second, bicarbonate administration in our experimental conditions did not lead to improvement nor deterioration of cardiac function and vascular contractile function when compared with rats treated with a standard resuscitation regimen. Interestingly, although not statistically significant, bicarbonate therapy increased cardiac output when compared with standard resuscitation. These effects are likely related to a decrease in systemic vascular resistances induced by hypercapnia and acidosis and relatively higher norepinephrine doses.[38,39]

Adapted Bicarbonate Therapy Improves Cardiovascular Function

Use of adapted bicarbonate therapy revealed that (1) arterial pH increased to normal values and (2) arterial Pco_2 did not change. Changes in extracellular pH were mimicked into muscle intracellular pH, which increased slightly above normal values. These changes in extra/intracellular pH were associated with a marked improvement in intrinsic cardiac function assessed by conductance catheter, which is the current reference technique for measuring this parameter.[40] These results further confirm the findings by Schotola *et al.*[41] in humans who demonstrated that mild metabolic acidosis (pH, 7.2) reduces cardiac contractility and significantly impairs the β-adrenergic force response in failing human myocardium. Moreover, adapted buffer therapy in the current study was associated with recovery of micro/macrovascular norepinephrine responsiveness. These results confirm previous experimental works and clinical observation whereby correction of acidosis improves vascular responsiveness to catecholamines.[42,43] In systemic circulation, acidosis induces a decrease in contraction of vascular smooth cells with subsequent vasodilatation, whereas alkalosis produces the opposite effects.[32,44]

Calcium administration improves vascular function and transiently increases myocardial performance. It is also interesting to note that calcium administration in this situation improved vascular reactivity without normalizing it and transiently increased myocardial performance. Nevertheless, these effects were always less efficient when compared with the adapted bicarbonate therapy.

Limitations and Perspectives

A first limitation is the apparent small sample size in each group. When pooling the two experiments (*in vivo* hemodynamic and pHi measurements), the same percentage of variation *versus* baseline was obtained in each group with regard to MAP, heart rate, total infused norepinephrine, and arterial blood gas. Moreover, all rats evolved in the same manner according to their group, hence conferring reliability and reproducibility to the results.

A second and main limitation of the study is that given the use of a neuromuscular-blocking agent, rats were not able to compensate the acidosis induced by the increase in Pco_2. Therefore, both the effects of shock-induced acidosis and deleterious bicarbonate effects may have been enhanced. However, the current model clearly highlights the beneficial effects of sodium bicarbonate when taking into account its potential deleterious effects, hence offering certain clinical application perspectives. Bicarbonate therapy as proposed could be useful in acute situations in which an urgent restoration of hemodynamics is needed in the expectation of specific therapeutic efficiency such as, for example, in acute cardiogenic shock with very low cardiac output before reperfusion, noncontrolled hemorrhagic shock before/during surgery, and septic shock with cardiac and vascular hyporesponsiveness.

Transposing this experimental study to clinical practice would warrant the following key points. First, before infusing bicarbonate, arterial Pco_2 should be in the lower normal ranges if possible and paralyzing agents should be avoided. Before and during bicarbonate infusion at 1 to 2 mmol/kg, ventilation should be increased and pH and $Paco_2$ monitored. The bicarbonate dose should be sufficient to significantly increase arterial pH without any increase in $Paco_2$. We also suggest administering calcium chloride (1 to 2 g) and to further monitor ionized calcium levels. This proposed bundle might prove useful, after clinical study confirmation, only in the most critical situation pending the initiation of continuous veno-venous ultrafiltration to control acid–base status.

Nevertheless, bicarbonate therapy should not be proposed in patients unable to compensate for increased carbon dioxide related to sodium bicarbonate infusion (severe acute respiratory distress syndrome) or in whom hyperventilation is contraindicated. In such cases, continuous veno-venous ultrafiltration may be the better therapeutic approach to increase pH.[45]

Conclusions

The current experimental study of severe lactic acidosis after resuscitated hemorrhagic shock in rats demonstrates that a therapeutic strategy based on systemic alkalinization with sodium bicarbonate along with hyperventilation and calcium administration increases extra/intracellular pH and improves cardiovascular function. Further studies are urgently needed to determine which patients may benefit from this strategy.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Levy: CHU Nancy, Service de Réanimation Médicale Brabois, Pole Cardiovasculaire et Réanimation Médicale, Hôpital Brabois, 54511 Vandoeuvre les Nancy, France. b.levy@chu-nancy.fr. Information on

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