Role of NHE1 in the Cellular Dysfunction of Acute Metabolic Acidosis

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\textbf{Key Words}
Metabolic acidosis · NHE1 · Cardiac function · Lactic acidosis · Ketoacidosis

\textbf{Abstract}
Background: Metabolic acidosis is associated with impaired cellular function. This has been attributed to the accompanying reduction in intracellular and interstitial pH of the myocardium. Recent studies suggest that activation of the cellular Na\textsuperscript{+}-H\textsuperscript{+} exchanger NHE1 might contribute to myocardial dysfunction. This review examines the experimental evidence which supports the role of NHE1 in the genesis of acidosis-induced cellular dysfunction, the benefits of its inhibition, and the type of acidosis that might benefit from therapy. Summary: Information was obtained by searching MEDLINE for articles published between 1969 and 2013 using the terms: NHE1, metabolic acidosis, lactic acidosis, ischemia-reperfusion, shock, resuscitation, high anion gap acidosis, and non-gap acidosis. Each article was also reviewed for additional suitable references. Nineteen manuscripts published between 2002 and 2013 assessed the impact of inhibition of NHE1 on cellular function. They revealed that NHE1 is activated with metabolic acidosis associated with hypoxia, hypoperfusion, hemorrhagic shock, and sepsis. This was associated with a rise in cellular sodium and calcium and cardiac dysfunction including reduced contractility and a predisposition to cardiac arrhythmias. Inhibition of NHE1 with specific inhibitors improved cardiac function, reduced blood and tissue levels of proinflammatory cytokines, and decreased mortality. Key Message: These results suggest that use of inhibitors of NHE1 might be worthwhile in the treatment of some types of acute metabolic acidosis, specifically the lactic acidosis associated with hypoxia, hemorrhagic shock, and cardiac arrest. Its potential role in the treatment of other forms of acute metabolic acidosis remains to be determined.

\textbf{Introduction}

The pH of the intracellular compartments (pH\textsubscript{i}) is normally maintained between 6.9 and 7.2, the higher pH values being found in cells with high rates of proton trans...
port such as those of the kidney and stomach [1]. Maintenance of a normal pH is important, because the function of several critical proteins and enzymes involved in metabolism, RNA and DNA synthesis, and other essential processes is impaired by a decrease in pH. Indeed, in vitro and in vivo studies have revealed cellular dysfunction associated with a reduction in pH [2, 3]. Also, a reduction in systemic pH and presumably pH is associated with a poor clinical outcome [2].

The pH of the interstitial fluid is normally slightly higher than that of the intracellular compartment averaging 7.3. Maintenance of a stable interstitial pH is also important for optimal cellular function: binding of catecholamines and insulin to their cognate receptors in various tissues is pH dependent [4], and a reduction in interstitial pH reduces binding and cellular responsiveness to each of these hormones. A decrease in interstitial pH also affects the activity of certain channels or receptors, such as the acid-sensing ion channel in the central nervous system and the proton-gated G-protein-coupled receptors, and transient receptor potential vanilloid 1 in various tissues. Opening of these channels increased the influx of calcium and sodium and contributed to cellular dysfunction and injury [5, 6].

Several disorders are associated with acute metabolic acidosis and have varying clinical outcomes. At present, elimination of the triggering condition is the only effective therapy.

A component of the abnormalities in cellular function and the presence of a poor clinical outcome have been attributed to the acidic acid-base milieu. Therefore, an additional therapy has been amelioration of the intracellular acidosis and acidemia by various means, including administration of base. This nonselective therapy has been applied to all forms of acute metabolic acidosis with varying success [2, 7]. Indeed, administration of base, at least in the form of sodium bicarbonate, has failed to improve cellular function or clinical outcomes in patients with either ketoacidosis or lactic acidosis [3]. These findings support an unmet need for targeted therapy.

Recent studies have identified a role for activation of the ubiquitous Na⁺-H⁺ exchanger NHE1 in the myocardium, and possible other tissues, in the cellular dysfunction of certain types of acute metabolic acidosis, specifically hypoxic lactic acidosis [8, 9]. These data suggest that the use of a NHE1 inhibitor might be beneficial in the treatment of this type of acute lactic acidosis.

The present review summarizes the mechanisms of cellular injury resulting from activation of NHE1 and their potential role in various types of acute metabolic acidosis as derived from animal studies. It highlights the impact of selective inhibition of this transporter on hemodynamic parameters and clinical outcomes in acute lactic acidosis. This evidence from animal studies supports a potential role for inhibition of NHE1 in the treatment of acute hypoxic lactic acidosis. Whether this treatment would be beneficial in other forms of acute metabolic acidosis remains to be determined.

**Role of NHE1 in Cellular Injury with Acidosis**

The possibility that activation of the Na⁺-H⁺ exchanger NHE1 contributed to myocardial injury with metabolic acidosis was based on studies of ischemia-reperfusion of the myocardium [10]. During ischemia, an intense lactic acidosis ensues, causing a reduction in pH. The decrease in pH activates Na-dependent acid/base transporters including NHE1, a Na⁺-HCO₃⁻ cotransporter, and Na⁺-independent transporters including an H⁺-ATPase and an H⁺-lactate symporter (primarily activated with lactic acidosis) in an attempt to return cellular pH to its baseline [11, 12]. As a consequence of activation of the Na⁺-dependent transporters, influx of sodium occurs. Ischemia constrains normal efflux of sodium from the cell because of decreased Na⁺-K⁺ ATPase activity resulting from reduced ATP production. Accumulation of sodium in the cell either slows the activity of the Na⁺-Ca²⁺ exchanger or reverses its action, both events leading to an increase in cellular calcium [9]. These events are depicted in figure 1. Examination of the contribution of each process to proton efflux revealed that NHE1 accounted for the majority of proton efflux by myocardial cells in response to acidification of the cell [10, 11]. Therefore, the impact of activation of NHE1 alone is highlighted.

When blood flow to tissues is restored, washout of protons further accelerates the rate of Na⁺-H⁺ exchange at least transiently (as well as sodium and calcium accumulation) as the proton gradient is decreased [13]. Both the increase in cellular sodium and calcium cause cellular dysfunction with reduced myocardial contractility and increased arrhythmias. Based on this schema, factors crucial to maximal activation of NHE1 and therefore the greatest accumulation of calcium included the severity of the reduction in pH, the suppression of sodium extrusion by Na⁺-K⁺-ATPase, and the pH gradient between the cell and interstitial fluid. To the extent that various forms of acute metabolic acidosis have different effects on each of these factors, the value of inhibition of NHE1 in the treatment of acute metabolic acidosis might vary. Of interest,
although inhibition of NHE1 will reduce cellular Na\(^+\) and Ca\(^{2+}\), it could also impair the regulation of pH\(_i\). It appears, based on the data obtained in vitro and in vivo, that even were this to happen, it would not prevent improvement in cardiac function resulting from administration of the NHE1 inhibitor.

**Hypoxic Lactic Acidosis**

Lactic acidosis associated with shock or severe tissue hypoxia produces a reduction in pH\(_i\) and interstitial pH\(_i\); pH\(_i\) falling to values as low as 6.4 [14]. The low pH\(_i\) stimulates the activity of sodium-dependent transporters including NHE1 and a monocarboxylic proton transporter which transports both lactate and hydrogen in an attempt to return pH\(_i\) to baseline. A byproduct of stimulation of NHE1 is accumulation of sodium within the cells. Normally, much of the sodium would be ejected by the ubiquitous Na\(^+\)-K\(^+\)-ATPase. However, because hypoxia reduces the generation of ATP necessary for increased Na\(^+\)-K\(^+\)-ATPase activity, efflux of sodium from the cell is slowed favoring sodium accumulation. As noted, accumulation of sodium slows or reverses the Na\(^+\)-Ca\(^{2+}\) exchanger, increasing cellular calcium. Thus, during the period of tissue hypoxia, conditions are such to favor large increments in cellular sodium and calcium. If reperfusion of the affected tissues then ensues, washout of prostols will reduce the gradient against which NHE1 transports protons increasing the activity of the transporter and further, at least transiently, increasing cellular sodium and calcium. Therefore, ischemia with and without reperfusion is the prototypical situation in which NHE1 might play an important role in cellular injury and dysfunction. This is illustrated by the results of 19 studies using different models characterized by tissue hypoxia and lactic acidosis published in the last 11 years. Models used included hypovolemic shock and volume resuscitation [8, 15–19], hypoperfusion accompanied by lactic acid infusion [20], asphyxia with severe hypoxia [21], and severe trauma [17]. These studies have demonstrated that inhibition of NHE1 improves cardiac and neurological function, reduces blood and/or tissue concentrations of proinflammatory cytokines, and often strikingly reduces mortality [22, 23].

**Cardiac Arrest**

Both hypobicarbonatemia and hypercarbia are characteristic of the acid-base milieu observed with cardiac arrest [14]. In this regard, it is distinctly different than the classic lactic acidosis commonly observed with shock or trauma. The rise in carbon dioxide concentrations in tissues can be particularly large with values reaching 150 mm Hg. As a consequence, administration of base such as sodium bicarbonate is not effective in raising the pH level of myocardial tissues since it tends to cause generation of carbon dioxide. The value of so-called carbon dioxide-consuming bases such as THAM and Carbicarb for myocardial pH is variable [3]. Some studies of carbicarb in dogs showed that it raised the pH level of the myocardial cell [24]. Its effect on myocardial carbon dioxide concentration is not clear. Be that as it may, elegant studies of cardiac arrest induced by ventricular fibrillation revealed improvement in cardiac function during fibrillation [25] and significant attenuation of postresuscitation ventricular arrhythmias including prevention of recurrent episodes of ventricular fibrillation with cariporide and other NHE1 inhibitors in both pig and rat models (table 1) [26–32].

![Fig. 1. Schema depicting the mechanisms linking a reduction of pH\(_i\) to cellular injury and dysfunction. Since NHE1 is responsible for the majority of proton efflux by the cell, NHE1 is highlighted. A reduction in pH\(_i\) occurring with acute metabolic acidosis activates acid-base transporters, including NHE1 in an attempt to return pH\(_i\) to baseline. The activation of NHE1 promotes the entry of Na into the cell. Particularly, in the presence of hypoxia which constrains the efflux of sodium out of the cell, intracellular sodium rises (Na\(_i\)). This increment in sodium will either slow the sodium calcium exchanger (Na\(^+\)-Ca\(^{2+}\)) or reverse its action (Ca\(^{2+}\)-Na\(^+\)), both events leading to an increase in cellular calcium (Ca\(_i\)), cellular injury, and dysfunction.](image-url)
Table 1. Effect of treatment with NHE1 inhibitors on cardiac function, inflammatory response, and outcome in different experimental models of acute metabolic acidosis

<table>
<thead>
<tr>
<th>Model</th>
<th>Animals</th>
<th>Effect on cardiac and/or neurologic function</th>
<th>mortality</th>
<th>proinflammatory cytokines</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypovolemic shock</td>
<td>pigs</td>
<td>increase in cardiac output with resuscitation</td>
<td>NA</td>
<td>NA</td>
<td>[8]</td>
</tr>
<tr>
<td>Severe hypovolemic shock</td>
<td>pigs</td>
<td>increase in cardiac output with resuscitation</td>
<td>decreased mortality</td>
<td>NA</td>
<td>[15]</td>
</tr>
<tr>
<td>Severe hypovolemic shock</td>
<td>pigs</td>
<td>increase in blood flow to vital organs; improvement in neurological outcome</td>
<td>decreased mortality</td>
<td>decreased blood proinflammatory cytokines</td>
<td>[16]</td>
</tr>
<tr>
<td>Severe trauma</td>
<td>rats</td>
<td>improved hemodynamics</td>
<td>decreased mortality</td>
<td>decreased blood proinflammatory cytokines</td>
<td>[17]</td>
</tr>
<tr>
<td>Severe trauma</td>
<td>pigs</td>
<td>increased cardiac output; improvement in cardiac function</td>
<td>NA</td>
<td>decreased tissue proinflammatory cytokines</td>
<td>[18, 19]</td>
</tr>
<tr>
<td>Lactic acidosis produced by lactic acid infusion and hypotension</td>
<td>pigs</td>
<td>increased cardiac output; improvement in cardiac function</td>
<td>decreased mortality</td>
<td>decreased blood proinflammatory cytokines</td>
<td>[20]</td>
</tr>
<tr>
<td>Asphyxia-induced hypoxic acidosis</td>
<td>pigs</td>
<td>increased cardiac output; improvement in cardiac function</td>
<td>NA</td>
<td>decreased blood proinflammatory cytokines</td>
<td>[21]</td>
</tr>
<tr>
<td>Severe perinatal asphyxia</td>
<td>pigs</td>
<td>cerebral protection</td>
<td>NA</td>
<td>NA</td>
<td>[22]</td>
</tr>
<tr>
<td>Neonatal asphyxia</td>
<td>rats</td>
<td>decreased seizures in neonates</td>
<td>NA</td>
<td>NA</td>
<td>[23]</td>
</tr>
<tr>
<td>Sepsis</td>
<td>rats</td>
<td>improvement in cardiac function</td>
<td>NA</td>
<td>NA</td>
<td>[37]</td>
</tr>
<tr>
<td>Ventricular fibrillation-induced cardiac arrest</td>
<td>pigs</td>
<td>increased cardiac output; improvement in cardiac function</td>
<td>NA</td>
<td>NA</td>
<td>[25, 26]</td>
</tr>
<tr>
<td>Ventricular fibrillation-induced cardiac arrest</td>
<td>pigs</td>
<td>reduced ventricular arrhythmias after resuscitation</td>
<td>NA</td>
<td>NA</td>
<td>[26]</td>
</tr>
<tr>
<td>Ventricular fibrillation-induced cardiac arrest</td>
<td>pigs</td>
<td>improvement in cardiac function; reduced ventricular arrhythmias after resuscitation</td>
<td>NA</td>
<td>NA</td>
<td>[27]</td>
</tr>
<tr>
<td>Ventricular fibrillation-induced cardiac arrest</td>
<td>rats</td>
<td>improvement in cardiac function</td>
<td>NA</td>
<td>NA</td>
<td>[29]</td>
</tr>
<tr>
<td>Ventricular fibrillation-induced cardiac arrest</td>
<td>rats</td>
<td>increased cardiac output; decreased intracellular Na⁺ and Ca²⁺</td>
<td>NA</td>
<td>NA</td>
<td>[30]</td>
</tr>
<tr>
<td>Ventricular fibrillation-induced cardiac arrest</td>
<td>pigs</td>
<td>increased cardiac output; improvement in neurological outcome</td>
<td>no difference</td>
<td>NA</td>
<td>[31]</td>
</tr>
<tr>
<td>Deep hypothermia-induced circulatory arrest</td>
<td>pigs</td>
<td>improvement in neurological outcome</td>
<td>NA</td>
<td>NA</td>
<td>[32]</td>
</tr>
</tbody>
</table>

NA = Not available.

**Diabetic Ketoacidosis**

Diabetic ketoacidosis, along with lactic acidosis, is one of the most common causes of severe metabolic acidosis [33]. Treatment is restricted to administration of insulin and fluids. Base treatment has not been shown to accelerate the rate of recovery or reduce complications [7]. Indeed, administration of sodium bicarbonate has been linked to important complications including cerebral edema [34]. Since this disorder is often characterized by significant volume losses, it is likely that local or diffuse hypoxia could be present. A potential role of activation of NHE1 in major complications such as cerebral edema is worth pursuing and the benefits of inhibition of NHE1 investigated.

**Lactic Acidosis with Sepsis**

Lactic acidosis occurring with sepsis can be due to tissue hypoxia analogous to the situation with tissue ischemia. However, several investigators have shown that lactic acidosis in animals and humans can result from

NHE1 in Acute Metabolic Acidosis
marked stimulation of the Na⁺-K⁺-ATPase by circulating catecholamines in the absence of tissue hypoxia [35, 36]. In this case, although activation of NHE1 might be prominent, rapid efflux of sodium from the cells due to high activity of NHE1 might lessen the rise in cellular sodium and calcium. In a single study in which metabolic acidosis was induced by producing sepsis in rats, inhibition of NHE1 was associated with decreased cellular sodium and calcium and improved cardiac function [37]. Whether tissue hypoxia was present in this model was not examined. Comparison of the benefits of inhibition of NHE1 in sepsis, both with and without tissue hypoxia, will be useful in further describing the benefits of this therapy.

**Non-Gap Metabolic Acidosis**

Non-gap acidosis (hyperchloremic acidosis) is increasingly common in hospitalized patients. In this setting, it is associated with administration of large quantities of sodium chloride. There remains controversy about whether this represents a type of dilution acidosis. Tissue perfusion can be impaired or intact depending on the success of the volume resuscitation. Diarrhea is also associated with a non-gap acidosis, and this cause of acidosis is common in Third World countries where cholera is frequent. Should the diarrhea be severe, volume depletion and impaired tissue perfusion could be present.

Given the diverse mechanisms leading to a non-gap acidosis, it is not obvious, as to the potential role of NHE1 in cellular dysfunction with this type of metabolic acidosis. It is likely that activation of NHE1 will occur with all causes on non-gap acidosis since a decrease in pHᵢ should be present. However, the increment in cellular sodium and calcium and the response to NHE1 inhibition is likely to depend in part on whether the Na⁺-K⁺-ATPase is stimulated. The value of inhibition of NHE1 in the treatment of non-gap metabolic acidosis remains to be determined.

**Impact of Coadministration of Base with NHE1**

Continual proton transport into the interstitial space by NHE1 slows the activity of the transporter as a proton gradient develops. As noted, administration of base is often a component of treatment of acute metabolic acidosis. Should this therapy raise the pH level of the interstitial space, it will lessen the pH gradient and encourage acceleration of the NHE1 activity. In support of this possibility, perfusate of an isolated heart subject to ischemia-reperfusion with an acidic solution during the reperfusion period to minimize so-called washout of protons was more protective than more rapid restoration of extracellular pH by administration of an alkaline solution [13]. Thus, theoretically the impact of administered base on NHE1 activity is likely to depend on its ability to raise the pH level of the intracellular and interstitial compartments. For example, administration of sodium bicarbonate particularly in the face of impaired tissue perfusion might lower pHᵢ and interstitial pH as CO₂ generated by buffering is trapped in tissues. The impact on NHE1 activity will depend on the composite effects of these changes. On the other hand, the impact of base such as THAM which can penetrate cells and raise both pHᵢ and extracellular pH depends on the rapidity and magnitude of increments in both parameters.

Recent studies of asphyxia-induced lactic acidosis compared the hemodynamic response in piglets with asphyxia treated with vehicle, NHE1 inhibitor alone, sodium bicarbonate alone, or sodium bicarbonate and NHE1 inhibition [38]. A marked fall in cardiac contractility was observed with acidosis. It was unimproved with vehicle or administration of sodium bicarbonate despite a rapid rise in systemic pH in the latter group. Administration of the selective inhibitor sabiporide with sodium bicarbonate resulted in a marked improvement in contractility. These studies support the importance of NHE1 inhibition in preserving cardiac function during acidosis both with and without improvement in systemic pH. Whether this effect will be additive to the actions of buffers that are capable or raising pHᵢ and extracellular pH remains a question of importance.

**Clinical Studies in Humans**

Large-scale randomized controlled clinical studies have only been performed examining the impact of NHE1 inhibition on cardiac ischemia both during ischemia and after reperfusion. Of note, most cardioprotection was achieved when the NHE1 inhibitor was given during or prior to onset of ischemia, and not when given during reflow [39]. These results would suggest that the NHE1 inhibitor might be most effective when given early in the course of lactic acidosis. However, this remains to be determined.
Potential Complications of NHE1 Inhibition

In addition to effectiveness, the safety of the drug is important in its potential value. Most of the NHE1 inhibitors used in clinical trials were not associated with serious side effects. However, in the Expedition study [40], a higher mortality related to thrombotic strokes was found in patients receiving high-dose cariporide intravenously. This complication was not found in other studies where this agent or other NHE1 inhibitors were administered, and some have attributed this complication to overdosage with this agent. Be that as it may, delineation of the mechanism of this effect and the risk of its occurrence will be important before the introduction of NHE1 inhibition to clinical use in treatment of acute metabolic acidosis.

Conclusions and Future Directions

Metabolic acidosis, particularly when it is severe, causes significant hemodynamic dysfunction and contributes to increased mortality [2, 3]. The recognition that increased activity of the Na⁺–H⁺ exchanger NHE1 might contribute to myocardial depression, activation of proinflammatory cytokines, and increased mortality with certain acute metabolic acidoses could represent an important contribution to our understanding of the pathophysiological events that transpire with this acid-base disorder. More importantly, they could lay the groundwork for the addition of targeted treatments to the usual measures utilized in the care of patients with acute metabolic acidosis. However, several important questions remain to be answered. The overwhelming majority of studies in animals have been done with models of tissue hypoxia, theoretically producing lactic acidosis. Whether any benefit will accrue when used with other types of metabolic acidosis is not clear. Also, the timing of drug administration related to the phase of metabolic acidosis needs to be defined. Finally, the safety of the drug under the circumstances of acute metabolic acidosis will have to be established.

Therapy of metabolic acidosis other than targeting the underlying cause of the acidosis has been confined to base administration. This nonspecific therapy has not been successful. Further delineation of the role of NHE1 in abnormalities of hemodynamic function and clinical outcome of diverse causes of metabolic acidosis will be valuable in establishing its place, if any, in the treatment of patients with acute metabolic acidosis.

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Disclosure Statement

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