

Critical role of bicarbonate and bicarbonate transporters in cardiac function

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Author contributions: Shull GE conceived and wrote the review sections of the manuscript; Wang HS wrote the sections dealing with Figures 2 and 3; Wang HS and Chen Y performed the experiments in Figures 2 and 3; and Vairamani K generated the data in Table 1 and Figure 1.

Supported by National Institutes of Health Grants HL061974 to Shull GE; and ES017263 to Wang HS

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Received: January 1, 2014 Revised: March 6, 2014

Accepted: May 16, 2014

Published online: August 26, 2014

Abstract

Bicarbonate is one of the major anions in mammalian tissues and extracellular fluids. Along with accompanying H^+ , HCO_3^- is generated from CO_2 and H_2O , either spontaneously or *via* the catalytic activity of carbonic anhydrase. It serves as a component of the major buffer system, thereby playing a critical role in pH homeostasis. Bicarbonate can also be utilized by a variety of ion transporters, often working in coupled systems, to transport other ions and organic substrates across cell membranes. The functions of HCO_3^- and HCO_3^- -transporters in epithelial tissues have been studied extensively, but their functions in heart are less well understood. Here we review studies of the identities and physiological functions of Cl^-/HCO_3^- exchangers and Na^+/HCO_3^- cotransporters of the *SLC4A* and *SLC26A* families in heart. We also present RNA Seq analysis of their cardiac

mRNA expression levels. These studies indicate that *slc4a3* (AE3) is the major Cl^-/HCO_3^- exchanger and plays a protective role in heart failure, and that *Slc4a4* (NBCe1) is the major Na^+/HCO_3^- cotransporter and affects action potential duration. In addition, previous studies show that HCO_3^- has a positive inotropic effect in the perfused heart that is largely independent of effects on intracellular Ca^{2+} . The importance of HCO_3^- in the regulation of contractility is supported by experiments showing that isolated cardiomyocytes exhibit sharply enhanced contractility, with no change in Ca^{2+} transients, when switched from Hepes-buffered to HCO_3^- -buffered solutions. These studies demonstrate that HCO_3^- and HCO_3^- -handling proteins play important roles in the regulation of cardiac function.

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Key words: SLC4; SLC26; Slc26a6; AE1; AE2; NBCn1

Core tip: Bicarbonate is one of the major anions in mammalian tissues and fluids. It plays a critical role in pH homeostasis and is utilized by various transporters to transport other ions and organic substrates across cell membranes. Here we review studies of the physiological functions of Cl^-/HCO_3^- exchangers and Na^+/HCO_3^- cotransporters in heart, present RNA Seq analysis of their cardiac mRNA expression levels, and show that bicarbonate is required for optimal contractility in isolated cardiac myocytes. These studies demonstrate that HCO_3^- and HCO_3^- handling proteins are abundant in heart and play important roles in the regulation of cardiac function.

Wang HS, Chen Y, Vairamani K, Shull GE. Critical role of bicarbonate and bicarbonate transporters in cardiac function. *World J Biol Chem* 2014; 5(3): 334-345 Available from: URL: <http://www.wjgnet.com/1949-8454/full/v5/i3/334.htm> DOI: <http://dx.doi.org/10.4331/wjbc.v5.i3.334>

INTRODUCTION

In mammalian tissues, bicarbonate/CO₂ is the major extrinsic buffer system of both extracellular and intracellular fluids. HCO₃⁻/CO₂ are likely to play a particularly important role in cardiac muscle^[1,2], which relies almost entirely on oxidative metabolism and continuously converts large quantities of O₂ to CO₂. *In vivo*, HCO₃⁻ is usually formed by carbonic anhydrase-mediated hydration of CO₂^[2,3], in a reaction that also generates H⁺ (CO₂ + H₂O → H⁺ + HCO₃⁻). As might be expected, cardiac myocytes express a variety of ion transporters that mediate extrusion of H⁺ and either extrusion or uptake of HCO₃⁻. This allows fine control of intracellular pH (pH_i) and coupling of H⁺ and HCO₃⁻ transport to the transport of other ions, thereby affecting not only pH_i, but also cell volume and both cellular and systemic ion homeostasis^[4-6].

H⁺ and HCO₃⁻ are, in effect, transient ions^[2] that can be used to transport other ions and organic substrates across cell membranes, both directly and by secondary active transport. These ion transporters include Cl⁻/HCO₃⁻ exchangers, Na⁺/HCO₃⁻ cotransporters (NBCs), and Na⁺/H⁺ exchangers (NHEs). The functions of the various acid-base transporters have been studied most extensively in epithelial tissues; however, they exhibit a surprising abundance and diversity in cardiac tissues. In this paper we review studies describing the identities, membrane locations, and functions of the major HCO₃⁻ transporters in heart. In addition, we report relative mRNA expression levels in mouse heart for members of the *SLC4A* and *SLC26A* anion transporter families^[7-9], which include all of the known Cl⁻/HCO₃⁻ exchangers and Na⁺/HCO₃⁻ cotransporters. Finally, we discuss previous studies of the effects of HCO₃⁻ on the isolated heart^[10] and correlate those results with new data using isolated cardiac myocytes. The available evidence shows that a diverse group of transporters are responsible for movements of HCO₃⁻ into and out of the heart and demonstrate that the presence of HCO₃⁻ has a major stimulatory effect on contractility that is, at least in part, independent of changes in intracellular Ca²⁺.

IDENTIFICATION AND LOCATIONS OF CARDIAC HCO₃⁻ TRANSPORTERS

Cloning and hybridization analyses have led to the identification of three Cl⁻/HCO₃⁻ exchangers of the *SLC4A* family in heart. These anion exchangers are termed AE1, AE2, and AE3 (Anion Exchanger 1, 2, and 3; gene symbols, *Slc4a1-3*), and one Cl⁻/HCO₃⁻ exchanger of the *SLC26A* family, termed Slc26a6 or PAT1 (putative anion transporter 1). Among the known Na⁺/HCO₃⁻ cotransporters (NBCs), which are members of the *SLC4A* family, the electrogenic NBCe1 and electroneutral NBCn1 (gene symbols, *Slc4a4* and *Slc4a7*) have been identified in heart. Excellent reviews of the *SLC4A* and *SLC26A*^[7-9,11,12] families of transporters have been published recently and provide detailed information about specific

isoforms, including their ion transport specificities and their physiological functions in various tissues.

AE1 is the band 3 Cl⁻/HCO₃⁻ exchanger that is expressed most prominently in red blood cells^[13] and also includes a kidney variant^[14] derived from an alternative promoter in intron 4 of the erythrocyte transcription unit^[15]. Erythrocyte AE1 plays major roles in maintaining the stability of the cytoskeleton^[16] and in gas exchange^[17]. Cardiac AE1 mRNA identified in rat heart is smaller than that of the erythroid and kidney variants^[18]. It encodes a truncated protein based on immunoblot analyses, although the exact identity of the cardiac AE1 protein in rat heart has not been determined^[19]. Immunofluorescence studies of rat heart suggest that truncated cardiac AE1 protein is restricted to intercalated discs^[20]. Expression of AE1 is sharply reduced in adult mouse heart compared with its levels in fetal heart^[21], consistent with the RNA Seq data discussed below. AE2 is expressed at low levels in heart^[18,21,22], and AE2a, one of 4 variants derived from the use of alternative promoters^[23], was the only variant detected^[22]. The membrane location of AE2 in heart has not been determined. AE3 mRNAs are expressed at very high levels in heart^[18,24] and encode both a full-length variant (AE3fl) that is expressed in brain and other tissues and a much more abundant cardiac variant^[25-27]. The cardiac AE3 (AE3c) mRNA is derived from an alternative promoter located in intron 6 of the longer transcription unit and has a unique 73-amino acid sequence that replaces the first 270 amino acids of AE3fl^[25]. In fetal mouse heart, AE3fl is the predominant form; however, in adult heart, AE3fl is largely restricted to the atria, while AE3c is the predominant form in ventricles^[27]. In cardiac myocytes, AE3 protein has been localized to t-tubules and the sarcolemma, with apparent foci of expression at costameres^[28].

The *SLC26A* family transports a broad range of anions, including sulfate, chloride, iodide, bicarbonate, oxalate, and formate, and some isoforms can function as anion channels^[9]. The first member of this family shown to function as a Cl⁻/HCO₃⁻ exchanger was *Slc26a3*^[29]; however, it is primarily an epithelial transporter and is expressed at only low levels in adult heart^[21]. Slc26a6 can mediate Cl⁻/HCO₃⁻ exchange^[30-32], which appears to be its major function in apical membranes of the intestine^[33]. In the renal proximal tubule, however, Slc26a6 functions primarily as a Cl⁻/formate and Cl⁻/oxalate exchanger^[34,35]. Slc26a6 also mediates Cl⁻/OH⁻ exchange and has been proposed to serve both as a Cl⁻/HCO₃⁻ exchanger and as a Cl⁻/OH⁻ exchanger in heart^[21,36]. Slc26a6 protein has been localized to the t-tubules and sarcolemma^[28].

Prior to the molecular cloning of Na⁺/HCO₃⁻ cotransporter (NBC) isoforms, both electroneutral^[37,38] and electrogenic^[39,40] Na⁺/HCO₃⁻ cotransport activities had been identified in cardiac muscle. NBCe1 (gene symbol, *Slc4a4*), the first NBC to be cloned^[41,42], is electrogenic. In kidney, NBCe1 mediates outward transport of 1 Na⁺ and 3 HCO₃⁻ across the basolateral membrane of proximal tubule epithelial cells^[41,42]. In most other tissues, including

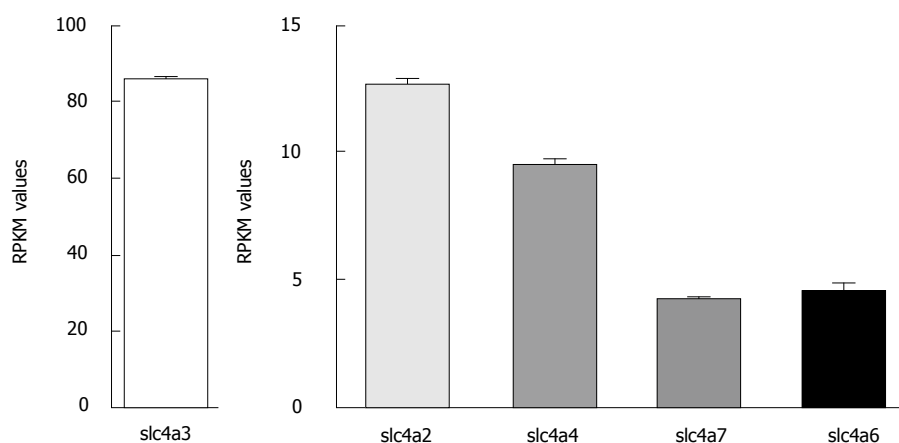


Figure 1 Relative expression levels of the major $\text{Cl}^-/\text{HCO}_3^-$ exchangers and $\text{Na}^+/\text{HCO}_3^-$ cotransporters in mouse heart. RPKM values \pm SE as determined by RNA Seq analysis (see Table 1 legend) are shown for the most abundant known HCO_3^- transporters in wild-type FVB/N mouse hearts ($n = 4$). Note the difference in scale for AE3 and the other transporters.

heart, regulation *via* phosphorylation results in a stoichiometry of 1:2^[43]. Cloning of the cardiac form of NBCe1 revealed that it has a different N-terminus than the kidney variant^[44], which is derived from an alternative promoter and first exon. NBCn1 is electroneutral and transports Na^+ and HCO_3^- in a 1:1 ratio. It was cloned from rat smooth muscle^[45] and skeletal muscle^[46] and shown to be expressed in heart. A study using an antibody to the N-terminal sequence of an NBCn1 variant indicated that expression in heart was restricted to endothelial and smooth muscle cells^[47]. However, NBCn1 transcripts undergo extensive alternative splicing, including a relatively cardiac-specific exon^[48], and the use of alternative promoters that yield alternative N-termini^[49]. Both NBCe1 and NBCn1 have been shown by Western blots to be expressed in cardiac myocytes^[50]. Immunolocalization studies demonstrated that both isoforms are localized to t-tubules, lateral sarcolemma, and intercalated discs^[50].

EXPRESSION LEVELS OF HCO_3^- TRANSPORTERS IN HEART

As discussed above, the major HCO_3^- transporters in the mammalian heart include both $\text{Cl}^-/\text{HCO}_3^-$ exchangers and $\text{Na}^+/\text{HCO}_3^-$ cotransporters of the *SLC4A* gene family and at least one $\text{Cl}^-/\text{HCO}_3^-$ exchanger of the *SLC26A* family. Gene expression data for hearts of wild-type FVBN mice were determined by RNA Seq analysis^[51], a powerful method for determining the expression levels of all known mRNAs in a tissue of interest^[52,53]. Relative mRNA expression levels for the *SLC4A* and *SLC26A* families are shown in Table 1. Transcript levels are expressed as RPKM values (reads per kilobase of exon per million mapped reads), which normalizes expression to the length of the mRNA. Graphical representations of mRNA expression levels of the HCO_3^- transporters that have been identified in heart and appear to be expressed at physiologically relevant levels are shown in Figure 1.

Among the *SLC4A* transporters, the AE3 $\text{Cl}^-/\text{HCO}_3^-$ exchanger was expressed at very high levels (RPKM =

85.7 \pm 0.6), suggesting that it serves as the major HCO_3^- efflux mechanism in mouse cardiac myocytes. AE2 (RPKM = 12.57 \pm 0.28) was expressed at lower levels than AE3, but its levels of expression were still greater than some of the other transporters. Although NBC4 (*Slc4a5* or NBCe2) was reported in human heart^[54], it is not expressed at significant levels in mouse heart or in rat heart^[55]. Of the two major $\text{Na}^+/\text{HCO}_3^-$ cotransporters in mouse heart, NBCe1 (RPKM = 9.45 \pm 0.25) was more abundant than NBCn1 (RPKM = 4.24 \pm 0.05). By comparison, RPKM values for the NHE1 Na^+/H^+ exchanger^[51], recognized as the major Na^+ -dependent acid extruder in heart, were 9.10 \pm 0.20.

Among the *SLC26A* transporters, Slc26a2, Slc26a6, and Slc26a10 were expressed most abundantly. However, among these three *SLC26A* transporters, the only known HCO_3^- transporter is Slc26a6 (RPKM = 4.56 \pm 0.26). As discussed above, it has been shown to function as a $\text{Cl}^-/\text{HCO}_3^-$ exchanger, but it also mediates Cl^-/OH^- , $\text{Cl}^-/\text{formate}$, and $\text{Cl}^-/\text{oxalate}$ exchange^[9]. Slc26a2 is a sulfate transporter and also transports Cl^- and oxalate^[9]. The ion specificity of Slc26a10 has not been determined^[9], but its high level of expression (RPKM = 31.66 \pm 2.55) suggests that it plays an important role in mouse heart.

Some of the HCO_3^- transporters expressed at low levels (*e.g.*, the Slc4a8 Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchanger and Slc26a3 $\text{Cl}^-/\text{HCO}_3^-$ exchanger) could still play important roles in heart, particularly if they were restricted to specialized regions of the heart or were expressed primarily in earlier stages of development or in cell-types other than cardiac myocytes. For example, Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchange, which could be due to the activity of Slc4a8^[56], has been identified in both chicken embryonic cardiomyocytes^[57] and in vascular endothelial cells^[58].

PHYSIOLOGICAL FUNCTIONS OF CARDIAC HCO_3^- TRANSPORTERS

Cl⁻/HCO₃⁻ Exchangers

Because of the high Cl^- concentrations of extracellular

Table 1 Relative mRNA levels for the *Slc4a* and *Slc26a* anion transporters in mouse heart

<i>SLC4A</i> family			<i>SLC26A</i> family		
Gene symbol	Transporter name and major function(s); alternate names	Average \pm SE	Gene symbol	Transporter name and major function(s); alternate names	Average \pm SE
<i>Slc4a1</i>	AE1 Cl ⁻ /HCO ₃ ⁻ exchanger; Band 3	0.2 \pm 0.05	<i>Slc26a1</i>	SAT1 sulfate/anion exchanger; Slc26a1	0.34 \pm 0.07
<i>Slc4a2</i>	AE2 Cl ⁻ /HCO ₃ ⁻ exchanger	12.57 \pm 0.28	<i>Slc26a2</i>	DTDST sulfate/anion exchanger; Slc26a2	2.98 \pm 0.17
<i>Slc4a3</i>	AE3 Cl ⁻ /HCO ₃ ⁻ exchanger	85.7 \pm 0.64	<i>Slc26a3</i>	DRA Cl ⁻ /HCO ₃ ⁻ exchanger; Slc26a3	0.64 \pm 0.05
<i>Slc4a4</i>	NBCe1 Na ⁺ /HCO ₃ ⁻ cotransporter; NBC1	9.45 \pm 0.25	<i>Slc26a4</i>	Pendrin Cl ⁻ /HCO ₃ ⁻ exchanger; Slc26a4	0.02 \pm 0.01
<i>Slc4a5</i>	NBCe2 Na ⁺ /HCO ₃ ⁻ cotransporter; NBC4	0.003 \pm 0.002	<i>Slc26a6</i>	PAT1 Cl ⁻ /HCO ₃ ⁻ , Cl ⁻ /formate exchanger; Slc26a6	4.56 \pm 0.26
<i>Slc4a7</i>	NBCn1 Na ⁺ /HCO ₃ ⁻ cotransporter; NBC2; NBC3	4.24 \pm 0.05	<i>Slc26a7</i>	Slc26a7 Cl ⁻ /HCO ₃ ⁻ exchanger, Cl ⁻ channel; TAT1	0.15 \pm 0.04
<i>Slc4a8</i>	NDCBE Na ⁺ -driven Cl ⁻ /HCO ₃ ⁻ exchanger; Slc4a8	0.73 \pm 0.04	<i>Slc26a9</i>	Slc26a9 Cl ⁻ /HCO ₃ ⁻ exchanger, Cl ⁻ channel	0.01 \pm 0.01
<i>Slc4a9</i>	AE4 Cl ⁻ /HCO ₃ ⁻ exchanger	0.02 \pm 0.01	<i>Slc26a10</i>	Slc26a10, transporter function unknown	31.66 \pm 2.55
<i>Slc4a10</i>	NBCn2 Na ⁺ /HCO ₃ ⁻ cotransporter	0.02 \pm 0.003	<i>Slc26a11</i>	Slc26a11 anion exchanger, Cl ⁻ channel; KBAT	1.15 \pm 0.14

Relative mRNA expression levels were determined using RNA from 4-month-old male FVB/N mouse hearts ($n = 4$) as described previously^[51]. Values are RPKM (reads per kilobase per million mapped reads) \pm SE and are a measure of the relative abundance of specific gene transcripts^[53]. For some *Slc26a* transporters, ion transport specificities are more complex than indicated; see Alper and Sharma^[9].

fluids, electroneutral Cl⁻/HCO₃⁻ exchangers mediate outward transport of HCO₃⁻ and inward transport of Cl⁻. The direct effect of this activity is to reduce pHi, thereby contributing to pHi regulation^[59], and to enhance Cl⁻-loading, which could affect Cl⁻ currents that in turn could affect action potentials or rhythmicity^[60,61]. Also, when coupled with Na⁺-dependent acid extrusion mechanisms, Cl⁻/HCO₃⁻ exchange facilitates Na⁺-loading, which can affect contractility as discussed below and may contribute to cardiac hypertrophy^[2]. AE1, AE2, and AE3 are electroneutral, but Cl⁻/HCO₃⁻ exchangers of the *SLC26A* family are reported to support both electroneutral or electrogenic anion exchange^[9,12].

The physiological functions of AE1 in heart are unclear. An AE1 global knockout mouse has been shown to develop cardiac hypertrophy^[28]. The investigators noted, however, that the levels of AE1 in the adult heart are relatively low (confirmed by the data in Table 1) and that null mutants exhibit severe hemolytic anemia and spherocytosis. They attributed the hypertrophy to the blood defect and concluded that one of the more abundant Cl⁻/HCO₃⁻ exchangers, possibly AE3, was more likely to provide the HCO₃⁻ extrusion capability that has been proposed to balance Na⁺-dependent acid extrusion *via* transporters such as the NHE1 Na⁺/H⁺ exchanger (discussed below). AE2 is a potential candidate for this activity as it is known to operate in concert with NHE1 on basolateral membranes of colonic epithelial cells^[62]. AE2 is much less abundant than AE3 and its functions in heart have not been determined.

It has been suggested that one of the major functions of Cl⁻/HCO₃⁻ exchange in heart is to counter the alkalinizing effects of Na⁺/H⁺ exchange. This would allow increased Na⁺/H⁺ exchange activity^[2,63], which in turn would lead to increased Na⁺-loading and Ca²⁺-loading *via* reverse activity of the Na⁺/Ca²⁺ exchanger^[64]. Inhibition of Na⁺/H⁺ exchange reduces cardiac hypertrophy^[4,65] and overexpression of an activated NHE1 Na⁺/H⁺ exchang-

er induces hypertrophy and increases cytosolic Na⁺, Ca²⁺ transients, and contractility^[66]. Studies have shown that the reduction in hypertrophy in spontaneously hypertensive rats in response to angiotensin II blockade involves reductions in both Na⁺/H⁺ exchange and Cl⁻/HCO₃⁻ exchange^[67]. Intracellular pH in NHE1-overexpressing myocytes was significantly higher when they were maintained in Hepes-buffered media than in CO₂/HCO₃⁻-buffered media^[66]. This is consistent with the view^[68,69] that Cl⁻/HCO₃⁻ exchange balances the alkalinizing effects of Na⁺/H⁺ exchange, which would be expected to facilitate pHi-neutral Na⁺-loading *in vivo*.

The Cl⁻/HCO₃⁻ exchanger that has been most heavily studied in heart is AE3. Its mRNA is expressed at much higher levels in heart than those of the other HCO₃⁻ transporters (see Table 1) and it has a cardiac specific variant^[25-27], indicating that it serves a specialized function. Earlier studies showed that Cl⁻/HCO₃⁻ exchange and Na⁺/H⁺ exchange were increased in the hypertrophic heart of spontaneously hypertensive rats^[67] and that AE3fl mRNA was upregulated^[70]. Although this might suggest that AE3fl accounts for the increased anion exchange activity, the investigators cautioned against this interpretation as AE3fl is expressed at low levels in the adult rat and mouse heart^[19,25,27]. Treatment of papillary muscles with an inhibitory anti-AE3 antibody led to an increase in the slow-force response to stretch, which is dependent on Na⁺/H⁺ exchange, and caused a substantial reduction in Cl⁻/HCO₃⁻ exchange, supporting the view that AE3 is the major Cl⁻/HCO₃⁻ exchanger in cardiac muscle^[71]. Analysis of pHi in tissues treated with the anti-AE3 antibody indicated that AE3 is the major anion exchanger responsible for countering the alkalinizing effects of NHE1-mediated Na⁺/H⁺ exchange^[71]. It has been suggested that activation of AE3 and NHE1 together might contribute to hypertrophy^[72], but so far there is no direct proof of this hypothesis.

The initial studies of a gene-targeted AE3-null mouse

showed that the loss of AE3 did not impair cardiovascular performance *in vivo* under basal conditions or after β -adrenergic stimulation, and it also had no effect on ischemia-reperfusion injury using the Langendorff-perfused heart^[6]. The latter finding was surprising as there is evidence that Cl/HCO_3^- exchange mediates some of the changes in pH_i and intracellular Cl that contribute to reperfusion injury^[73]. Heart weight/body weight ratios were significantly reduced in null mutants relative to wild-type mice, consistent with the possibility that loss of AE3 activity might contribute to a reduction in hypertrophy. When AE3-null mice were crossed with an NKCC1 $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter-null mouse, which had normal contractility^[74], the double mutant mice exhibited a contractility defect *in vivo* and in isolated myocytes, and Ca^{2+} extrusion mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger was increased^[6]. NKCC1 has been shown to provide substantial Na^+ influx in chick cardiac myocytes^[75] and to affect $\text{Na}^+/\text{Ca}^{2+}$ exchange in mouse astrocytes^[76]. Therefore, it is possible that the additional loss of NKCC1 caused a reduction in Na^+ -loading in AE3/NKCC1 double mutants, with subsequent effects on contractility.

The above studies were consistent with the possibility that loss of AE3 might protect against hypertrophy; however, they also showed that its absence can impair cardiac function under certain conditions. To test whether AE3-deficiency might protect against hypertrophy, AE3-null mice were crossed with a transgenic hypertrophic cardiomyopathy mouse model^[77] carrying a Glu180Gly mutation in α -tropomyosin^[78]. The additional loss of AE3 in the Glu180Gly mutant caused no decrease in the degree of hypertrophy and led to more rapid decompensation, heart failure, and death. Cardiac performance in response to β -adrenergic stimulation was severely impaired in double mutants. The double mutants exhibited more arrhythmic events as heart rates were increased by electrical pacing to assess force-frequency responses and Ca^{2+} -handling was also impaired. It was concluded that AE3 activity is needed for better preservation of cardiac function during heart failure and that it would not be an appropriate therapeutic target for cardiac arrhythmias or hypertrophy. In a more recent study^[79] it was shown that hearts of AE3-null mice exhibit blunting of the force-frequency response when they are paced to higher heart rates *in vivo*. Phosphorylation of Akt, which plays a central role in mechanosensory signaling, was increased in paced AE3-null hearts and phosphorylation of adenosine 5'-monophosphate-activated protein kinase (AMPK) was reduced^[79]. These data suggest that the increased susceptibility of AE3-null mice to decompensation in heart failure might be due impaired rate dependent inotropy, an insufficient response to biomechanical stress, and metabolic perturbations.

The functions of Slc26a6 in heart have not yet been determined. As discussed by Alper and Sharma^[9], there is controversy about the electrogenicity of the Cl/HCO_3^- exchange activity of Slc26a6, which is the only known Cl/HCO_3^- exchanger of the *SLC26A* family expressed

at significant levels in heart^[21]. Some studies^[80,81] reported electrogenic Cl/HCO_3^- exchange for Slc26a6 with a stoichiometry of $1\text{Cl}/2\text{HCO}_3^-$ and others^[82] reported electroneutral exchange with a 1:1 ratio. In the latter study^[82], the investigators could detect electrogenic $\text{Cl}/\text{oxalate}$ transport mediated by Slc26a6 in oocytes but Cl/HCO_3^- and Cl/OH^- exchange appeared to be electroneutral. Cl/OH^- exchange in cardiac myocytes, which has been attributed to Slc26a6^[21], is electroneutral^[36], and Slc26a6-mediated $\text{Cl}/\text{formate}$ exchange is electroneutral^[83]. Regardless of whether Slc26a6-mediated Cl/HCO_3^- exchange is electrogenic, during most of the excitation-relaxation cycle, when the membrane potential is negative, it would transport HCO_3^- out of the cell. However, it is possible that reversal of electrogenic Cl/HCO_3^- exchange might occur at positive membrane potentials. The potential functions of electrogenic Slc26a6-mediated Cl/HCO_3^- exchange in heart have not been studied. Furthermore, it is not clear which of the various transport functions of Slc26a6 is the most important in heart.

$\text{Na}^+/\text{HCO}_3^-$ Cotransporters

As discussed above, there is evidence for both electroneutral and electrogenic NBC activities in cardiac myocytes^[37-40,84,85]. NBCe1 and NBCn1, along with NHE1, have been immunolocalized in rat myocytes^[50]. These three transporters are the major Na^+ -dependent alkalinizing mechanisms in cardiac myocytes. In the isolated perfused ferret heart, NBC and NHE activities contributed equally to recovery of pH_i, both after an acid load^[86] and also during reperfusion following ischemia^[87]. In the latter study, it was suggested that NBC-mediated Na^+ influx might contribute to Ca^{2+} overload and injury after reperfusion. Later studies using an inhibitory antibody showed that inhibition of NBCe1 protected against ischemia-reperfusion injury in the isolated rat heart^[88]. Similarly, in rat ventricular myocytes subjected to anoxic conditions, simultaneous inhibition of NBC and NHE1 activities prevented hypercontracture induced by Ca^{2+} -overload during reoxygenation, whereas inhibition of either activity alone was insufficient^[89]. This suggests that NBCe1, like the NHE1 Na^+/H^+ exchanger, can be a significant source of Na^+ -loading, although the magnitude of Na^+ -loading *via* NBCe1 has been estimated to be lower than that of Na^+/H^+ exchange^[64]. Also, NHE1 has cardioprotective effects that appear to be independent of effects on Na^+ and Ca^{2+} loading^[51,90].

NBCe1 is localized to t-tubules^[50], along with the L-type Ca channel (LTCC) and NCX1 $\text{Na}^+/\text{Ca}^{2+}$ exchanger^[91], whereas NHE1 is expressed at highest levels in intercalated discs^[50,92]. Thus, NBCe1 appears to be well situated to affect excitation-contraction coupling^[93], particularly since it is electrogenic. In fact, a substantial NBC-mediated HCO_3^- current has been demonstrated beginning at -50 millivolts^[84], and electrogenic NBC activity causes a shortening of the action potential duration (APD) and affects the resting membrane potential^[40,94]. By shortening the APD, NBCe1 could reduce the open time of the LTCC and

with its location in the t-tubule it could reduce intraluminal (extracellular) pH and increase pHi, both of which reduce LTCC-mediated Ca^{2+} currents^[95]. Thus, while NBCe1 activity may serve as a Na^+ -loading mechanism that could, in principle, contribute to Ca^{2+} -loading *via* reverse mode activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, its effects on the APD and on LTCC activity might counteract this tendency. NBCn1 is also in t-tubules of ventricular myocytes^[50] and because it is electroneutral it could operate throughout the excitation-contraction cycle.

Both NBCe1 and NBCn1, along with NHE1, were induced in rat heart during pressure overload hypertrophy^[55]. After an acid load, the rate of pHi recovery *via* NBC and NHE activities were increased accordingly, and NBC activity in the physiological pHi range was similar to that of NHE1^[55]. Additional experiments^[55] showed that when rats were subjected to pressure overload and then treated with losartan, an angiotensin II AT_1 receptor antagonist, both hypertrophy and the induction of NBCe1 and NBCn1 were sharply reduced. The results suggest, but do not prove, that increased NBC activities contribute to the development of hypertrophy. NBCe1 mRNA and protein were also induced in human failing hearts^[88] and in rat hearts following myocardial infarction^[96]; however, in the latter study, treatment with an angiotensin II AT_1 receptor antagonist had no effect on NBCe1 expression. The effects of angiotensin II on NBC activity in cardiac myocytes are complex as some studies report activation of NBC activity^[97,98] and others report inhibition^[99]. A more recent study showed that cardiac expression of both NBCn1 and NBCe1 were induced in spontaneously hypertensive rats in which angiotensin II plays a major role^[100]. However, NBCe1 activity was reduced due to a reduction in its protein expression in t-tubule and sarcolemmal membranes; nevertheless, total NBC activity increased due to an increase in NBCn1 activity. The authors noted that a reduction in NBCe1 activity leads to an increase in APD, which is a common occurrence during cardiac hypertrophy^[101], and that this would likely cause an increase in Ca^{2+} -influx *via* LTCC^[102]. Thus, NBCe1 activity, rather than inhibition of its activity, may be cardioprotective in some disease conditions.

EFFECTS OF HCO_3^- ON CONTRACTILITY AND Ca^{2+} IN ISOLATED HEARTS

Given the abundance and diversity of HCO_3^- transporters in heart and the fact that HCO_3^- is part of the major buffer system in biological systems, it is surprising that there has been little reported work on the specific effects of HCO_3^- on contractility and Ca^{2+} -handling. In an interesting and important study, Fülöp *et al.*^[10] analyzed Langendorff-perfused guinea pig hearts in the presence of both Krebs solution buffered with $\text{CO}_2/\text{HCO}_3^-$ and Tyrode solution buffered with HEPES^[10]. Contractility in isolated hearts was significantly greater in Krebs solution than in Tyrode solution. However, when Tyrode solution was supplemented with $\text{CO}_2/\text{HCO}_3^-$, in the continuing

presence of HEPES, contractility increased to the levels observed in Krebs solution. Changes in contractility were reversible as the buffers were switched between those containing $\text{CO}_2/\text{HCO}_3^-$ or HEPES alone. Despite increased contractility, both the amplitude and duration of the Ca^{2+} transients were lower in solutions containing $\text{CO}_2/\text{HCO}_3^-$ buffer, indicating that enhanced Ca^{2+} transients were not responsible for the enhanced contractility. Analyses of isolated trabeculae also revealed increased contractility in $\text{CO}_2/\text{HCO}_3^-$ buffer, with faster times to peak tension and shorter relaxation times^[10]. In purkinje fibers and papillary muscles, the duration of the action potential was reduced in the presence of $\text{CO}_2/\text{HCO}_3^-$ buffer^[10]. This finding is consistent with the proposed effects of NBCe1 activity on the action potential^[103].

The reduced contractility in the isolated heart and isolated tissues in response to the absence of HCO_3^- may have been due to reduced pHi or buffering power^[10]. However, in wild-type myocytes used in a study of the effects of Na^+/H^+ exchange on Ca^{2+} and contractility, pHi was the same in HEPES buffer as in buffer containing $\text{CO}_2/\text{HCO}_3^-$ ^[66]. Also, in myocytes overexpressing an activated NHE1, the absence of $\text{CO}_2/\text{HCO}_3^-$ led to an increase in pHi^[66]. These results suggest that a HCO_3^- -dependent transport mechanisms, *i.e.*, $\text{Cl}^-/\text{HCO}_3^-$ exchange, is needed to counter the alkalinizing effects of NHE1.

EFFECTS OF HCO_3^- ON CONTRACTILITY AND Ca^{2+} IN ISOLATED MYOCYTES

Although studies of cardiac myocyte mechanics and Ca^{2+} handling are commonly performed in HEPES buffered solution (Tyrode's solution), we are unaware of studies directly comparing the effects of the two buffer conditions. Therefore, we performed experiments to assess the effects of HEPES-buffered and HCO_3^- -buffered solutions on both contraction of rat ventricular myocytes and Ca^{2+} transients. The concentrations of cations were identical for the two solutions, and their osmolarities were the same.

Switching from HEPES-buffered solution to HCO_3^- -buffered solution had a bi-phasic effect on myocyte contraction, determined by measurements of cell shortening as described previously^[104]. It first resulted in transient suppression of myocyte contraction, followed by reversal of suppression, and enhancement of contraction (Figure 2A). On average, the downward suppressive phase lasted about 50 s (Figure 2B), resulting in a peak suppression of cell shortening from 7.04% in control (*i.e.*, HEPES) to 4.35% (Figure 2C). This was followed by gradual stimulation of contraction; at steady-state, contraction was significantly increased to 11.39% (Figure 2C). Switching from HEPES-buffered Tyrode's solution to an isosmotic solution that contained both HEPES and HCO_3^- produced the same bi-phasic effect and reached the same steady-state increase in contractility. Thus, the stimulation of contraction by HCO_3^- in isolated cardiac myocytes was fully reversible, as observed previously in the isolated

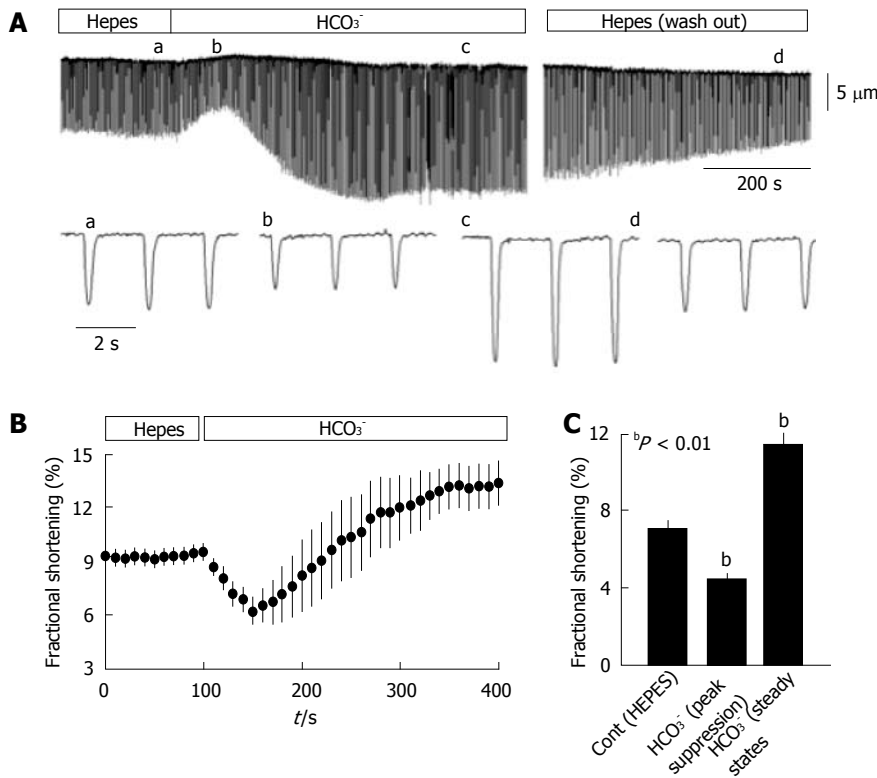


Figure 2 Isolated myocytes exhibit greater contractility in CO₂/HCO₃⁻ buffer than in HEPES buffer. Ventricular myocytes from rat hearts were enzymatically dissociated using Langendorff perfusion^[104] and myocyte mechanics were analyzed at room temperature (24 °C), with stimulation at 0.5 Hz as described previously^[107]. Myocytes were switched between HEPES-buffered Tyrode's solution (in mmol/L: NaCl 140, KCl 5.4, MgCl₂ 1, CaCl₂ 1.8, glucose 10, and Na-HEPES 5; pH = 7.4; bubbled with 100% O₂) and HCO₃⁻-buffered Krebs solution (in mmol/L: NaCl 120, NaHCO₃⁻ 25, KCl 4.2, KH₂PO₄ 1.2, MgCl₂ 1, CaCl₂ 1.8, and glucose 10; pH = 7.4 when bubbled with 95% O₂ and 5% CO₂). A: Representative contraction tracing of a myocyte bathed in HEPES buffer, then switched to HCO₃⁻ buffer, and then returned to HEPES buffer; the lower tracings show an expanded scale for the indicated (a-d) time points. The lower panels show the time course of fractional shortening (B) and average fractional shortening (C) of myocytes (n = 19) in HEPES buffer and then switched to HCO₃⁻-containing buffer. Experiments were performed using myocytes from 3 hearts and statistical analysis was conducted using a paired t-test. Values are means ± SE.

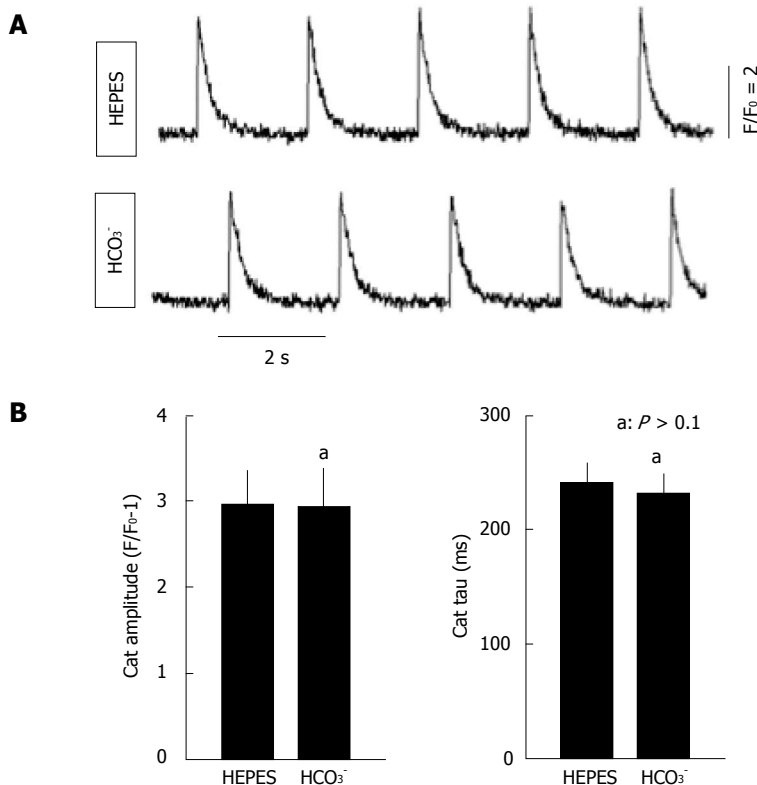


Figure 3 Ca²⁺ transient analysis in isolated rat myocytes bathed in CO₂/HCO₃⁻ buffer and in HEPES buffer. For recording of Ca²⁺ transients, isolated ventricular myocytes were loaded with fluo-4 acetoxymethyl ester (5 μmol/L, Molecular Probes, Eugene, OR) and activated with field stimulation at 0.5 Hz. Fluorescence signals were measured using a Nikon TE 2000 microscope and an InCyt Standard PM photometry system (Intracellular Imaging, Cincinnati, OH) as described previously^[104]. A: Representative Ca²⁺ transients in HEPES and HCO₃⁻-containing buffers; B: Average Ca²⁺ transient (CaT) amplitudes and tau values, a measure of the rate of decay of the Ca²⁺ transient in HEPES and HCO₃⁻-containing buffers (n = 12). The same cells were imaged in both buffers. Myocytes were from the same preparations used in Figure 2, with statistical analyses performed using a paired t-test. No significant differences were observed.

heart^[10].

In addition to affecting myocyte contractility, HCO₃⁻ also had a small but significant effect on myocyte length. Switching from HEPES to the HCO₃⁻-buffered solution transiently increased myocyte length by approximately 1% (data not shown). Such transient lengthening was inde-

pendent of myocyte contraction, and was also observed in unpaced, quiescent myocytes. This suggests the possibility that cell volume was increased by the addition of HCO₃⁻, which is reasonable given the role of Cl⁻/HCO₃⁻ exchange in cell volume regulation^[105,106].

Interestingly, the marked effect of HCO₃⁻ on myo-

cyte contraction was not accompanied by any detectable change in the Ca^{2+} transient. Figure 3A shows Ca^{2+} transients from the same myocyte in the presence of HEPES or HCO_3^- solutions. Switching from HEPES-buffered solutions to HCO_3^- -buffered solutions altered neither the amplitude nor the time constant of the Ca^{2+} transient (Figure 3B). These data using isolated myocytes correlate well with the previous studies using isolated hearts^[10]. Both sets of data show that HCO_3^- has a major effect on contractility, without any major effects on the amplitude of the Ca^{2+} transient.

CONCLUSION

The studies reviewed here show that the mammalian heart contains an abundance of HCO_3^- transporters, which include both $\text{Cl}^-/\text{HCO}_3^-$ exchangers and $\text{Na}^+/\text{HCO}_3^-$ cotransporters. Their most obvious function is regulation of pH_i, although it is possible that this is not their major function. This is particularly apparent in the case of the $\text{Cl}^-/\text{HCO}_3^-$ exchangers because at the high frequencies occurring *in vivo*, cardiac myocytes generate a substantial acid load. Thus, there would appear to be little need to maintain a robust capacity for recovery from an alkaline load. Nevertheless, it is possible that the cardiac anion exchangers regulate the pH or electrolyte concentrations of sub-sarcolemmal or t-tubule microdomains. With regard to electrolyte homeostasis, coupling of $\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{HCO}_3^-$ cotransport (or Na^+/H^+ exchange) can serve as a pH_i-neutral Na^+ - and Cl^- -loading mechanism, with Na^+ affecting Ca^{2+} -loading *via* $\text{Na}^+/\text{Ca}^{2+}$ exchange. In addition to effects on Na^+ -loading, electrogenic $\text{Na}^+/\text{HCO}_3^-$ cotransport can affect the duration of the action potential^[40,84,94] and, by affecting subsarcolemmal and t-tubular pH, it might also affect the activity of L-type Ca^{2+} channels^[95]. Finally, the available data show that the presence of $\text{CO}_2/\text{HCO}_3^-$ buffer has a major effect on contractility that cannot be readily explained by effects on Ca^{2+} -handling, thus suggesting that HCO_3^- homeostasis plays an important role in the regulation of cardiac contractility. The mechanism is not known, but it is conceivable that intracellular HCO_3^- concentrations affect myofibrillar function and that dynamic transporter-mediated HCO_3^- fluxes have a major effect on electrical and ionic properties of the myocyte. Further studies of the effects of HCO_3^- and the cardiac functions of each of the major HCO_3^- transporters will be needed to resolve these issues.

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Endocrinology 2012; **153**: 712-720 [PMID: 22166976 DOI: 10.1210/en.2011-1772]

P- Reviewer: Bies J, Laghmani K, Tatulian S **S- Editor:** Wen LL
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