The electrogenic Na\(^+\)/HCO\(_3^-\) cotransport modulates resting membrane potential and action potential duration in cat ventricular myocytes

Maria C. Villa-Abrille, Martin G. Vila Petroff and Ernesto A. Aiello

Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata 1900, Argentina

Perforated whole-cell configuration of patch clamp was used to determine the contribution of the electrogenic Na\(^+\)/HCO\(_3^-\) cotransport (NBC) on the shape of the action potential in cat ventricular myocytes. Switching from Heps to HCO\(_3^-\) buffer at constant extracellular pH (pH\(_e\)) hyperpolarized resting membrane potential (RMP) by 2.67 ± 0.42 mV (n = 9, P < 0.05). The duration of action potential measured at 50% of repolarization time (APD\(_{50}\)) was 35.8 ± 6.8% shorter in the presence of HCO\(_3^-\) than in its absence (n = 9, P < 0.05). The anion blocker SITS prevented and reversed the HCO\(_3^-\)-induced hyperpolarization and shortening of APD. In addition, no HCO\(_3^-\)-induced hyperpolarization and APD shortening was observed in the absence of extracellular Na\(^+\). Quasi-steady-state currents were evoked by 8 s duration voltage-clamped ramps ranging from -130 to +30 mV. A novel component of SITS-sensitive current was observed in the presence of HCO\(_3^-\). The HCO\(_3^-\)-sensitive current reversed at -87 ± 5 mV (n = 7), a value close to the expected reversal potential of an electrogenic Na\(^+\)/HCO\(_3^-\) cotransport with a HCO\(_3^-\):Na\(^+\) stoichiometry ratio of 2:1. The above results allow us to conclude that the cardiac electrogenic Na\(^+\)/HCO\(_3^-\) cotransport has a relevant influence on RMP and APD of cat ventricular cells.

(Resubmitted 30 August 2006; accepted after revision 27 November 2006; first published online 30 November 2006)

Corresponding author: E. A. Aiello: Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, 60 y 120, La Plata 1900, Argentina. Email: aaiello@atlas.med.unlp.edu.ar

Na\(^+\)/HCO\(_3^-\) cotransport (NBC) was first described by Boron & Boulpaep (1983) in the renal proximal tubule of the salamander, with a HCO\(_3^-\)/Na\(^+\) stoichiometry of 3:1, which generates a net flux of negative charge across the cell membrane. In the heart, this mechanism was first reported to be present in sheep Purkinje fibres (Dart & Vaughan-Jones, 1992) and isolated guinea pig ventricular myocytes (Lágadic-Gossman et al. 1992) as an electroneutral transporter. However, the lack of electrogenicity of the NBC in myocardium was challenged by experiments performed in cat heart multicellular preparations (Camilón de Hurtado et al. 1995, 1996) and rat ventricular myocytes (Aiello et al. 1998) where the presence of an NBC with a HCO\(_3^-\)/Na\(^+\) stoichiometry of 2:1 was suggested. More recently, the data proved by molecular biology supported the notion that one electroneutral (NBC3 or NBCn1) (Pushkin et al. 1999; Choi et al. 2000) and two electrogenic isoforms (NBC1b or hhNBC or NBCe1-B and NBC4 or NBCe2-c) (Choi et al. 1999; Pushkin et al. 2000; Sassani et al. 2002; Virkki et al. 2002) coexist in the myocardium. A recent work described in detail the functional diversity of the electrogenic NBC in ventricular myocytes from rat, rabbit and guinea pig (Yamamoto et al. 2005). Although we have previously suggested that the electrogenic NBC contributes to the modulation of the spike-like rat action potential (AP) waveform, the participation of this transporter in the configuration of the typical prolonged cardiac AP of larger mammals is an interesting issue that remains to be studied. Thus, in this study we present evidence for the presence of an electrogenic NBC in isolated cat ventricular myocytes, and for its contribution to the modulation of resting membrane potential (RMP) and AP duration (APD).

Methods

Cell isolation

All experiments were performed in accordance with the guidelines for Animal Care of the Scientific Committee of the University of La Plata School of Medicine. Cats (body weight 3–4 kg) were anaesthetized by intraperitoneal injection of sodium pentobarbitone (35 mg (kg body weight))\(^{-1}\)). The chests were opened when plane three of phase III of anaesthesia was reached,
verified by the loss of the corneal reflex and appearance of slow deep diaphragmatic breathing. The hearts were quickly removed, mounted in a Langendorff apparatus and retrogradely perfused with Krebs-Henseleit solution (K-H) containing (mm): NaCl 123, KCl 4.69, CaCl$_2$ 1.35, NaHCO$_3$ 20, Na$_2$HPO$_4$ 1.2, MgSO$_4$ 1.2, glucose 11, pH 7.35 after gassing with 95% O$_2$–5% CO$_2$. Hearts were perfused at constant pressure for a stabilization period of 10–15 min. Single ventricular myocytes were isolated by an enzymatic dispersion technique in which hearts were perfused with nominally Ca$^{2+}$-free K-H solution for 5 min before treatment with collagenase (74.5 u ml$^{-1}$, Worthington Biochemical Corp., Lakewood, NJ, USA) in Ca$^{2+}$-free K-H solution for 45 min. The left ventricle was then removed, placed in Ca$^{2+}$-free solution, and cut into small pieces (2 × 2 mm). After a final wash, the tissues were kept in K-H at room temperature, and single myocytes were obtained by gentle trituration.

**Patch-clamp recordings**

Isolated cat ventricular myocytes were placed in a recording chamber and superfused with bath solution at a flow rate of 1.5 ml min$^{-1}$. Only rod-shaped myocytes with clear and distinct striations and an obvious marked shortening and relaxation on stimulation were used. Experiments were performed at room temperature (20–22°C), at 30°C or at 37°C.

The nystatin perforated whole-cell configuration of the patch clamp technique (Korn et al. 1991) was used for voltage- and current-clamp recordings with a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Union City, CA, USA). Patch pipettes were pulled with a PP-83 puller (Narishige, Tokyo, Japan) and fire-polished with a MF-88 Microforge (Narishige) to a final resistance of 0.5–1 MΩ when filled with a control pipette solution. Membrane voltage and whole-cell currents (sampling rate = 1 kHz; low pass filter = 1 kHz) were digitally recorded directly to hard disk via an analog-to-digital converter (Digidata 1200, Axon Instruments) interfaced with an IBM clone computer running pClamp software (Axon Instruments). A pacing rate of 0.2 Hz was applied in the current-clamp recordings. Data analysis was performed with pCLAMP (Clampfit). A Ag/AgCl wire directly in contact with the extracellular solution was used as reference electrode. Since the pipette potential was nulled in external solution, all current-clamp tracings and voltage-clamp protocols required corrections for junction potential. This was accomplished by filling 20 pipettes with standard internal solution. They were then nulled in internal solution, and the difference in potential on immersion in external solution was recorded. The measured junction potential value was consistently −10 mV, and this value was used to correct all current-clamp data and voltage-clamp protocols. There were no significant differences in the value of junction potential among all the external solutions used in the present work. For each cell, capacitative current was recorded to determine the membrane capacitance, and the currents were normalized for cell capacitance. Average cell capacitance was 126.6 ± 11.1 pF ($n = 52$).

**pH$_i$ measurements**

After enzymatic isolation, myocytes were loaded with the membrane-permeant acetoxymethyl ester form of the fluorescent H$_{1}$-sensitive indicator SNARF-1/AM. Cell suspensions (2 ml) were exposed to a final concentration of 4 μM SNARF-1/AM and 0.6% v/v DMSO. After 10 min, the myocytes were gently centrifuged for 2 min and resuspended in Hepes buffer and stored at room temperature until use. pH$_i$ and cell length were monitored on the stage of a modified inverted microscope, as previously described (Vila Petroff et al. 2000). After excitation at 530 ± 5 nm, the ratio of SNARF-1/AM emission at 590 ± 5 nm to that of 640 ± 5 nm was used as a measure of pH$_i$ according to an in vivo calibration, obtained from SNARF-1/AM-loaded myocytes exposed to solutions of varying pH values containing 140 mm KCl, 20 μM nigericin, 1 μM valinomycin, and 1 μM carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone at room temperature.

**Cell shortening**

In some experiments, cell shortening, as an index of contractility, was measured simultaneously with the perforated-patch recordings or the pH$_i$ measurements. Resting cell length and cell shortening were measured by a video-based motion detector (Crescent Electronics, UT, USA) and stored by software for an off-line analysis.

**Solutions**

The HCO$_3$$^-$/free external solution (Hepes-buffered) contained (mm): NaCl 133, KCl 5, MgSO$_4$ 1.2, MgCl$_2$ 0.8, glucose 10, CaCl$_2$ 1.35, and Hepes 10, pH 7.35 with 5 mm NaOH (total Na$^+$ 138 mm). The HCO$_3$$^-$/buffered solution contained (mm): NaCl 118, KCl 5, MgSO$_4$ 1.2, MgCl$_2$ 0.8, glucose 10, CaCl$_2$ 1.35, Hepes 10, choline-Cl 15, and NaHCO$_3$ 20. The pH was titrated to 7.35 with TrisBase after gassing with 95% O$_2$–5% CO$_2$. In some experiments the extracellular solution contained 5 mm instead of 20 mm NaHCO$_3$, and no gassing with O$_2$/CO$_2$ was assayed. This solution was prepared fresh for each experimental day, in order to minimize the CO$_2$ hydration reaction. In the Na$^+$-free experiments, the external NaCl was replaced completely with LiCl in both, Hepes- and HCO$_3$$^-$/buffered solutions (in the zero Na$^+$ HCO$_3$$^-$/buffered solution, NaHCO$_3$ was replaced with...
choline-HCO$_3^-$, LiCl was 133 mM and no choline-Cl was added). The pH of the Na$^+$-free Hepes-buffered solution was titrated to 7.35 with Tris base. NaHCO$_3$ was replaced completely with choline-HCO$_3^-$ in the HCO$_3^-$-buffered solution. The Nystatin pipette solution contained (mM): K-glucuronate 130, KCl 10, NaCl 8, MgCl$_2$ 0.5, EGTA 1, Hepes 10, and Nystatin 0.3 mg ml$^{-1}$. The pH was titrated to 7.15 with KOH. NaCl was replaced with choline-Cl in the pipette solution of the Na$^+$-free experiments. In all the cases, the estimated equilibrium potential for Cl$^-$ was $-52$ mV.

**Statistics**

Data are expressed as mean $\pm$ s.e.m. and were compared with Student's $t$ test for paired values, and repeated measures ANOVA followed by the Student-Newman-Keuls test. A value of $P < 0.05$ was considered statistically significant (two-tailed test).

**Results**

Figure 1A shows representative traces of cat ventricular APs before and after 1, 2, 5 and 10 min of replacing the extracellular Hepes-buffered solution (nominal HCO$_3^-$-free) with a CO$_2$/HCO$_3^-$-buffered solution (HCO$_3^-$ 20 mM) at constant pH$_i$. The RMP hyperpolarized rapidly by approximately 3 mV in the presence of the physiological buffer. APD shortened gradually until steady-state was reached after 5 min in HCO$_3^-$$. Single cardiac myocytes with long APDs frequently exhibit variability in APD from one beat to another, even when paced at a constant rate, possibly leading to a misinterpretation of our results. However, in the present work we did not detect substantial beat-to-beat APD variability, as shown in the time course of beat-to-beat APD, before and after HCO$_3^-$, depicted in Fig. 1B. Thus, it seems unlikely that the changes in APD detected in the presence of HCO$_3^-$ were due to beat-to-beat APD variability.

Figure 2A shows the time course of the average changes in RMP during the replacement of Hepes-buffered superfusate with HCO$_3^-$-buffered solution. On average, RMP hyperpolarized 2.67 $\pm$ 0.47 mV ($n = 9$) after 5 min in the presence of HCO$_3^-$$. Figure 2B depicts percentage average changes in APD after 50% (APD$_{50}$) and 90% (APD$_{90}$) of repolarization time induced by the presence of HCO$_3^-$ in the bath media. A significant and relevant APD$_{50}$ (~35%) and APD$_{90}$ (~25%) shortening was observed as early as 2 min after exposing the myocytes to HCO$_3^-$, remaining constant afterwards during the rest of the experiment. Although without reaching statistical significance, the effect of HCO$_3^-$ was more pronounced for the APD$_{50}$ than for the APD$_{90}$, possibly reflecting the higher driving force for HCO$_3^-$ influx through the NBC at plateau potentials than at more repolarized potentials.

The change of a superfusate from a Hepes-buffered to a CO$_2$/HCO$_3^-$-buffered solution induces a transient intracellular acidification due to the rapid CO$_2$ permeation into the cell, followed by a recovery to control values within 5–10 min after changing from Hepes to CO$_2$/HCO$_3^-$ solutions (Dart & Vaughan-Jones, 1992, Camilón de Hurtado et al. 1995). Since membrane currents are sensitive to pH$_i$ changes, these effects may partially explain the APD alterations observed in CO$_2$/HCO$_3^-$-buffered

![Figure 1](image1.png)  
**Figure 1.** APD shortening after switching the extracellular solution from Hepes to HCO$_3^-$-buffered solution  
A. AP recordings under current-clamp mode before (Hepes) and after 1, 2, 5 and 10 min of superfusion of a cat ventricular myocyte with external HCO$_3^-$$. The presence of HCO$_3^-$ in the external solution produced a gradual APD shortening. B, typical time course of beat-to-beat APD$_{50}$ measured before and after switching the extracellular solution from Hepes to HCO$_3^-$.
solution. In order to evaluate the time course of pHj changes induced by the switch from Hepes to CO₂/HCO₃⁻ solution, we performed experiments in isolated cat myocytes in which pHj and cell length shortening were measured simultaneously. Figure 3A shows the average changes in pHj after the acid load induced by replacement of the Hepes-buffered superfusate with the HCO₃⁻-buffered solution. An early decrease in pHj, followed by a recovery, can be seen. Initially, CO₂ entry causes intracellular acidification but, within 2–4 min, pHj started to recover towards control values. After 10 min, pHj was completely recovered to values not different from those obtained before the replacement of the extracellular solution. The time course of the cell-length-shortening changes induced by the replacement of Hepes-buffered with CO₂/HCO₃⁻-buffered solution was associated to that of pHj (Fig. 3B). As previously observed in cat papillary muscles (Camilioni de Hurtado et al. 1995), the changes in contractility were slightly delayed with respect to the changes in pHj, suggesting that the former are secondary to the latter, probably due to alterations in myofibrillar Ca²⁺ sensitivity and/or changes in sarcoplasmic reticulum Ca²⁺ content or fractional release (Bers, 2001).

We next recorded cell length shortening and APs simultaneously as an indirect method to evaluate whether the time course of pHj changes observed in intact cat cardiomyocytes is similar to that of the perforated patch-clamped cat cardiomyocytes. As observed in the intact myocytes, when the superfusate was switched from Hepes to HCO₃⁻-buffered solution, the perforated patch-clamped myocyte contractility transiently decreased, but within 6–8 min recovered towards control values, reaching steady-state after 8 min (Fig. 4A). These changes in contractility were faster than those observed in the epifluorescence setup, possibly due to slight differences in superfusion of the cells (i.e. different chamber sizes). However, if we consider that the changes in contractility are secondary to those of pHj (see above), we can speculate that pHj would be totally recovered after 6–8 min of switching the superfusate of the clamped myocytes from Hepes to CO₂/HCO₃⁻. Thus, after 6–8 min of switching to CO₂/HCO₃⁻, despite full recovery of pHj, APD remained shortened (Fig. 4B), indicating that

![Figure 2](image1.png)

**Figure 2.** Hyperpolarization of RMP and shortening of the APD induced by external HCO₃⁻.

A, time course of the average changes in RMP after switching the superfusate from Hepes- to HCO₃⁻-buffered solution (n = 9). B, time course of the percentage average changes (with respect to the value in Hepes measured immediately before the switch to HCO₃⁻) in APD₅₀ and APD₉₀ induced by external HCO₃⁻ (n = 9). External HCO₃⁻ induced a slight RMP hyperpolarization and a significant and relevant shortening of the APD.

![Figure 3](image2.png)

**Figure 3.** Simultaneous measurements of pHj and cell shortening in the absence and presence of external HCO₃⁻.

A, average pHj values obtained in five myocytes during the switch of the extracellular solution from Hepes to HCO₃⁻. B, average cell shortening (n = 5, expressed as percentage change with respect to the value in Hepes measured immediately before the switch to HCO₃⁻) recorded simultaneously with pHj.
both phenomena are not related. However, taking into account that it was previously reported that acidosis causes APD lengthening (Coraboeuf et al. 1976; Poole-Wilson & Langer, 1975; Spitzer & Hogan, 1979), we cannot rule out the possibility that the APD shortening would have been delayed during the first minute in $\text{CO}_2/\text{HCO}_3^-$ due to the transient intracellular acidification.

We next evaluated the effect on APD of a smaller concentration of $\text{HCO}_3^-$ (5 mM) in the absence of $\text{CO}_2$ bubbling ($\text{HCO}_3^-$ added to the Hepes-buffered solution), which should minimize the $\text{CO}_2$-induced transient intracellular acidification. Under these conditions RMP hyperpolarized from $-73.8 \pm 1.2$ mV to $-76 \pm 1.2$ mV ($n = 9$, $P < 0.05$) after 8 min of exposure of the myocytes to $\text{HCO}_3^-$ (Fig. 5). Figure 5 shows the average values of APD$_{50}$ and APD$_{90}$ in the absence of $\text{HCO}_3^-$ and after 8 min in the presence of 5 mM $\text{HCO}_3^-$ in the extracellular solution. This concentration of $\text{HCO}_3^-$ induced an APD$_{50}$ and APD$_{90}$ shortening of 19.3 ± 4.6% and 11.9 ± 3.8% ($n = 9$) of the values in the absence of $\text{HCO}_3^-$, respectively, values that are lower than those observed with 20 mM $\text{HCO}_3^-$ (Fig. 2B). These results demonstrate that the magnitude of the APD shortening observed in $\text{HCO}_3^-$ is dependent on the extracellular concentration of $\text{HCO}_3^-$.

The APD shortening induced by exposure of the myocytes to 20 mM $\text{HCO}_3^-$ was reversed (Fig. 6A and B) and prevented (Fig. 6C and D) by the non-specific blocker (general anionic blocker) of NBC, SITS (0.1 mM). As observed in Fig. 6, there is a tendency of APD to be slightly prolonged with SITS, but this effect did not attain statistical significance. The RMP hyperpolarization was also reversed (Hepes: $-73.3 \pm 2.2$ mV; $\text{HCO}_3^-$: $-76.4 \pm 2.4$ mV, $P < 0.05$; $\text{HCO}_3^-$ + SITS: $-73.4 \pm 2.3$ mV; $n = 6$) and prevented by SITS (Hepes + SITS: $-75.6 \pm 0.5$ mV; $\text{HCO}_3^-$ + SITS: $-76.5 \pm 0.5$ mV; $n = 6$).

Figure 7A shows representative traces of APs recorded in Hepes zero $\text{Na}^+$ and $\text{HCO}_3^-$ zero $\text{Na}^+$. $\text{Na}^+$ was entirely replaced with $\text{Li}^+$, a cation that can be carried by the $\text{Na}^+$ channels (Le Guennec & Noble, 1994) and by the $\text{Na}^+$/H$^+$ exchanger (NHE) (Paris & Pouyssegur, 1983; Aronson, 1985), but can only minimally substitute for extracellular $\text{Na}^+$ on NBC transport (Jentsch et al. 1985; Dart & Vaughan-Jones, 1992; Amlal et al. 1998; Sciortino & Romero, 1999). Changing the superfusate from Hepes to $\text{HCO}_3^-$-buffered solution in the absence of extracellular $\text{Na}^+$ did not produce significant changes on the AP (Fig. 7A and B). As shown on Fig. 7B, after 10 min in the presence of $\text{HCO}_3^-$ the APD$_{50}$ and APD$_{90}$ were 109.6 ± 3.9% and 107.5 ± 3.1% of the value in Hepes ($n = 6$), respectively. These results indicate that the $\text{HCO}_3^-$-induced APD shortening is dependent on the presence of extracellular $\text{Na}^+$, as expected for the involvement of the electroneutral NBC in this effect.

Figure 8A shows the effects of changing the extracellular superfusate from Hepes to $\text{HCO}_3^-$ on steady-state

---

**Figure 4.** Simultaneous measurements of cell shortening and APD in the absence and presence of external $\text{HCO}_3^-$

A, average cell shortening of seven myocytes during the switch of the extracellular solution from Hepes to $\text{HCO}_3^-$; B, average APD$_{50}$ ($n = 7$) recorded simultaneously with cell shortening. The values were expressed as percentage change with respect to the value in Hepes measured immediately before the switch to $\text{HCO}_3^-$ (Fig. 4A and B). The $\text{HCO}_3^-$-induced APD shortening remained present despite the transient decrease and postponed recovery of cell contractility.

**Figure 5.** APD shortening induced by 5 mM $\text{HCO}_3^-$

Average values of APD$_{50}$ and APD$_{90}$ in Hepes and after 8-10 min with 5 mM $\text{HCO}_3^-$ in the extracellular solution ($n = 9$). This concentration of $\text{HCO}_3^-$ induced an APD$_{50}$ and APD$_{90}$ shortening of 19.3 ± 4.6% and 11.9 ± 3.8% ($n = 9$) of the values in the absence of $\text{HCO}_3^-$, respectively. *Significantly different from Hepes.
currents evoked by 8 s duration voltage-clamped ramps from −130 to +30 mV, from a holding potential of −75 mV. A novel component of an outward current was detected in the presence of HCO₃⁻, consistent with an electrogenic influx of this anion. This HCO₃⁻-induced outward current was cancelled by addition of SITS to the extracellular medium (Fig. 8A). Figure 8B shows the difference currents, obtained by subtraction of the current in Hepes or the current in HCO₃⁻ + SITS to the current in HCO₃⁻. The average current–voltage relationship for these two difference currents is shown in Fig. 8C. Although there was a tendency for the SITS-sensitive current to be greater than the HCO₃⁻-sensitive current, the difference did not attain statistical significance. The HCO₃⁻-sensitive current and the SITS-sensitive current reversed at around −85 mV (HCO₃⁻-sensitive current: −87 ± 4.8 mV, n = 7; SITS-sensitive current: 88 ± 6 mV, n = 7). The reversal potential ($E_{\text{IBC}}$) of the NBC current ($I_{\text{IBC}}$) with a HCO₃⁻/Na⁺ stoichiometry of 2:1 was calculated with the following equation:

$$E_{\text{IBC}} = \frac{RT}{F(n-1)} \ln \left[ \frac{[Na^+]_i}{[Na^+]_o} \right] \frac{[HCO_3^-]_i}{[HCO_3^-]_o}$$

(see Newman, 1991)

Considering that $[Na^+]_i = 8 \text{ mM}$, $[Na^+]_o = 138 \text{ mM}$, $[HCO_3^-]_i = 14.5 \text{ mM} \ (pH_i \sim 7.2)$, $[HCO_3^-]_o = 20 \text{ mM}$ ($pH_o = 7.35$) and that the experiments were performed at 20–22°C, $E_{\text{IBC}}$ was calculated to be −89.4 mV, a similar value to the one measured in our experiments. Thus, these results suggest that the HCO₃⁻-sensitive current and the SITS-sensitive current detected herein represents $I_{\text{IBC}}$.

Finally, in order to investigate the physiological implication of the electrogenic NBC in the electrical properties of cat cardiomyocytes, the impact of HCO₃⁻ on APD was evaluated at 30°C and 37°C. Figure 9 shows representative traces of AP before and after 10 min of switching the extracellular solution from Hepes to HCO₃⁻ at 30°C (Fig. 9A) or 37°C (Fig. 9C). In both cases, RMP hyperpolarization and APD shortening were observed in the presence of the physiological buffer. Average values of APD₅₀, before and after HCO₃⁻, are shown in Fig. 9B (30°C) and D (37°C). On average, we detected a HCO₃⁻-induced APD₅₀ shortening of 14.2 ± 1.6% ($n = 8$) at 30°C and of 16.1 ± 2.3% at 37°C (n = 4). In Hepes, the average values of RMP were −77.5 ± 2.3 mV at 30°C and −80.7 ± 2.5 mV at 37°C (n = 4). After exposure of the myocytes to 10 min of HCO₃⁻, RMP hyperpolarized to −81.1 ± 2.2 mV ($n = 8$, $P < 0.05$) and −84.1 ± 2.2 mV ($n = 4$, $P < 0.05$) at 30°C and 37°C, respectively. These data support the notion that the electrogenic NBC plays a

**Figure 6. Effects of the anionic blocker SITS on the HCO₃⁻-induced changes in APD**

A, representative traces of APs recorded from a myocyte exposed successively to Hepes, HCO₃⁻ (10 min) and HCO₃⁻ + SITS (0.1 mM) (10 min). B, average values of APD₅₀ obtained from six myocytes subjected to the conditions of A. C, representative traces of APs recorded from a myocyte exposed successively to Hepes, Hepes + SITS (0.1 mM) (10 min) and HCO₃⁻ + SITS (0.1 mM) (10 min). D, average values of APD₅₀ obtained from six myocytes subjected to the conditions of C. The APD shortening induced by 20 mM HCO₃⁻ was reversed and prevented by 0.1 mM SITS. *Significantly different from Hepes.
relevant role in the electrical modelling of the ventricular AP at physiological temperatures.

**Discussion**

The experiments reported herein demonstrate that cat cardiac ventricular cells possess an electrogenic NBC that has a relevant impact on APD. Changing the myocytes' bathing superfusate from a HCO$_3^-$-free (Hepes-buffered) to a HCO$_3^-$-containing solution at constant pH$_{2}$, induces RMP hyperpolarization, APD shortening, and development of an outward current, consistent with the influx of HCO$_3^-$ into the cell. These changes are sensitive to the extracellular concentration of HCO$_3^-$, and are blunted by anionic blockade or by sodium deprivation. Accordingly, the RMP hyperpolarization and APD shortening induced by HCO$_3^-$ seem to have an underlying anionic current which is dependent on the presence of extracellular Na$^+$, sensitive to the extracellular

Figure 7. Effects of Na$^+$ deprivation on the HCO$_3^-$-induced changes in APD

A, representative traces of AP recordings before and after 10 min of exposure of a cat myocyte to HCO$_3^-$ in the absence of extracellular Na$^+$. No HCO$_3^-$-induced effects on RMP and APD were observed under these conditions. B, average change in APD$_{50}$ and APD$_{90}$ after 10 min in HCO$_3^-$ free Na$^+$ (n = 4), expressed as percentage change with respect to the value in Hepes (100%) measured immediately before the switch to HCO$_3^-$.

Figure 8. Effects of external HCO$_3^-$ on steady-state currents, perforated whole-cell configuration

A, steady-state currents evoked by 8 s-duration voltage-clamp ramps ranging from $-130$ to $+30$ mV, from a holding potential of $-75$ mV, recorded from a cardiomyocyte exposed successively to external Heps, HCO$_3^-$ (10 min), and HCO$_3^-$ in the presence of SITS (10 min). B, HCO$_3^-$-sensitive difference current (HCO$_3^-$ — Heps) and SITS-sensitive difference current (HCO$_3^-$ — (HCO$_3^-$ + SITS)). C, average current–voltage relationship for the HCO$_3^-$-sensitive difference current (HCO$_3^-$ — Heps) (n = 7) and SITS-sensitive difference current (HCO$_3^-$ — (HCO$_3^-$ + SITS)) (n = 7). These two difference currents reversed at $-85$ mV, a value close to the expected $E_{NBC}$ of an electrogenic NBC with a stoichiometry ratio of 2HCO$_3^-$ : 1Na$^+$. © 2007 The Authors; Journal compilation © 2007 The Physiological Society
concentration of HCO$_3^-$ and reverses at a potential close to the estimated $E_{\text{NBC}}$ with a HCO$_3^-$:Na$^+$ ratio of 2:1. Thus, these results strongly suggest that the relevant HCO$_3^-$-induced changes in RMP and APD observed in the present study are due to the activation of the electrogenic NBC present in the cardiomyocyte.

The results presented here are in agreement with early experiments performed in canine Purkinje fibres by Spitzer & Hogan (1979). The authors reported that lowering extracellular HCO$_3^-$ at constant pH$_j$ produced depolarization of RMP and APD shortening. These authors suggested that these effects were due to changes in a background HCO$_3^-$ current. In the light of the present results, it seems likely that the previously reported background HCO$_3^-$ current of canine Purkinje fibres could have been carried by the electrogenic NBC.

Although the effects of acidosis on the cardiac action potential are relatively modest, they are manifested as a depolarization of RMP and prolongation of APD (Coraboeuf et al. 1976; Poole-Wilson & Langer, 1975; Spitzer & Hogan, 1979). In our experiments, the transient acidosis caused after changing the superfusate from Hepes- to HCO$_3^-$-buffered solution did not seem to have significant effects on the membrane currents that control RMP or AP configuration, since in the presence of SITS or in the absence of extracellular Na$^+$, exposure of the myocytes to CO$_2$/HCO$_3^-$ did not significantly alter RMP or APD. It is important to note that in the SITS and the zero Na$^+$ (replaced with Li$^+$) experiments, the recovery of pH$_j$ upon changing from Hepes to CO$_2$/HCO$_3^-$ solution, would be partially impaired due to block of NBC, depending exclusively on the activity of the NHE. Thus, a persisting acidosis might be present at the time chosen to measure APD and RMP (10 min after changing the solutions). In order to evaluate the time course of pH$_j$ changes in the presence of SITS or in the absence of extracellular Na$^+$, we measured pH$_j$ under these conditions. In the presence of SITS, the average

![Figure 9. APD shortening induced by external HCO$_3^-$ at 30°C and 37°C](image)

A, AP recordings before (Hepes) and after 10 min of superfusion of a cat ventricular myocyte with external HCO$_3^-$ at 30°C. B, average APD$_{50}$ (n = 8) measured before and after 10 min of HCO$_3^-$-buffered solution at 30°C. At 30°C, HCO$_3^-$-induced an APD shortening of 14.2 ± 1.6% (n = 8). C, AP recordings before (Hepes) and after 10 min of superfusion of a cat ventricular myocyte with external HCO$_3^-$ at 37°C. D, average APD$_{50}$ (n = 4) measured before and after 10 min of HCO$_3^-$-buffered solution at 37°C. At 37°C, HCO$_3^-$-induced an APD shortening of 16.1 ± 2.3% (n = 4).
The HCO$_3^-$-induced hyperpolarization was small but consistent and, with the obvious cell-to-cell variability, it was present in most of the experiments performed. Moreover, just a small hyperpolarization is expected because at RMP values the driving force for HCO$_3^-$ influx through the NBC with a stoichiometry ratio of 2 HCO$_3^-$ : 1 Na$^+$ is not large.

In the presence of external HCO$_3^-$, we were able to record in isolated cat myocytes a SITS-sensitive current which reverses at approximately −85 mV. This value is very close to the estimated $I_{\text{NBC}}$ with a HCO$_3^-$/Na$^+$ stoichiometry ratio of 2:1. The same stoichiometry was reported for native $I_{\text{NBC}}$ recorded in rat (Aiello et al. 1998; Yamamoto et al. 2005), rabbit and guinea pig (Yamamoto et al. 2005) ventricular myocytes, amphibian optic nerve and mouse cerebral astrocytes (Aston & Orkand, 1988; Brookes & Turner, 1994), leech glial cells (Deitmer & Schlue, 1989) and frog retinal epithelium (Hughes et al. 1989). In contrast, a stoichiometry of 3:1 has been estimated for mammalian proximal tubule (Boron & Boulpaep, 1983) and salamander retinal glia (Newman, 1991). This variation in stoichiometry may reflect differences in cotransporter function in different systems. A cotransporter stoichiometry of 2:1 in ventricular myocytes suggests that HCO$_3^-$ is normally transported in an inward direction in these cells, generating an outward current that contributes to hyperpolarizing RMP and shortening APD.

An electrically silent NBC was reported to be present in sheep Purkinje fibres and isolated guinea pig myocytes, by Dart & Vaughan-Jones (1992) and Lagadic-Gossman et al. (1992), respectively. The authors failed to record $I_{\text{NBC}}$ using a standard whole-cell configuration of patch-clamp technique in guinea pig ventricular myocytes (Lagadic-Gossman et al. 1992). However, a recent work did report the presence of $I_{\text{NBC}}$ in guinea pig, rat and rabbit myocytes, using perforated-patch instead of standard whole-cell techniques (Yamamoto et al. 2005). Since our previous (Aiello et al. 1998) and present experiments were also performed with perforated patch, it seems mandatory that minimal altered intracellular milieu conditions (perforated-patch configuration) are necessary to detect this novel anionic current.

Yamamoto et al. (2005) reported that rat $I_{\text{NBC}}$ recorded at 0 mV and 36°C was two-fold and five-fold greater than $I_{\text{NBC}}$ recorded in guinea pig and rabbit cardiomyocytes, respectively. Thus, it seems likely that $I_{\text{NBC}}$ is much smaller in larger animals, compared to the rat. However, in disagreement with this hypothesis, the magnitude of $I_{\text{NBC}}$ of cat cardiomyocytes recorded herein at 0 mV and room temperature was almost two-fold higher than the one reported for rat cardiomyocytes by Yamamoto et al. (2005). Interestingly, if we consider the density of $I_{\text{NBC}}$ recorded at +30 mV, cat $I_{\text{NBC}}$ (Fig. 8) was similar to that of rat $I_{\text{NBC}}$ (Yamamoto et al. 2005), due to the strong rectification of cat $I_{\text{NBC}}$ at potentials positive to 0 mV, in comparison to rat $I_{\text{NBC}}$, which seems to rectify at more positive potentials (Yamamoto et al. 2005). Although these modest variations in the biophysical properties of $I_{\text{NBC}}$ among these two species could be explained by the disparity in the recording temperature, it is also possible that the rectification at less positive voltages could be typical of larger mammals, since a similar phenomenon was observed in guinea pig and rabbit $I_{\text{NBC}}$ (Yamamoto et al. 2005). Although both electrogenic isoforms of NBC, NBC1 and NBC4, were reported to be present in normal human heart (Ch’en et al. 1999; Pushkin et al. 2000), no studies have yet shown the presence of native $I_{\text{NBC}}$ in human cardiomyocytes. Thus, the evaluation of human cardiac $I_{\text{NBC}}$ and its influence on APD and RMP represents a relevant matter to be addressed in the near future.

We presented herein that the HCO$_3^-$-induced APD shortening was also present at physiological temperatures. However, although the NBC activity was reported to be slowed by reduced temperature (Ch’en et al. 2003), the magnitude of the HCO$_3^-$-induced APD shortening at physiological temperatures was not greater, but actually smaller, than at room temperature. Although we do not have data that can certainly explain the reason for this difference, we can speculate that the expected decrease in membrane resistance and increase in other ionic currents at higher temperatures could account for the smaller effect of $I_{\text{NBC}}$ on APD.

Yamamoto et al. (2005) have shown that the cardiac NBC activity is associated with a net Na$^+$ influx to the cell. After the acidification induced by ischaemia, Na$^+$-coupled acid extrusion during ischaemia and/or reperfusion contributes to net H$^+$ efflux. The associated Na$^+$ entry may contribute to a rise in [Na$^+$]i and therefore Ca$^{2+}$ overload via Na$^+$/Ca$^{2+}$ exchange. Vandenberg et al. (1993) showed that the NBC contributes to about 20% to the total pH$_i$ recovery during reperfusion. Schafer et al. (2000) demonstrated that during reoxygenation of rat myocytes exposed to 70 min of anoxia, the NBC also plays an important role in pH$_i$ recovery (approximately 50% of the total pH$_i$ recovery). Furthermore, these authors showed that the calcium oscillations that cause hypercontracture of the cells were diminished by DIDS, a
blocker of the NBC (Schafer et al. 2000). More recently, Khandoudi et al. (2001) have shown in perfused rat hearts that the presence of a selective antibody against cardiac NBC1b significantly improved the post-ischaemic functional recovery. These authors also showed that NBC1 but not NBC3 protein expression in human myocardium from patients with heart failure was markedly increased in comparison to control hearts (Khandoudi et al. 2001). In addition, an upregulation of NBC1 mRNA and protein was reported to be present after myocardial infarction in the rat heart (Sandmann et al. 2001). Finally, since the NBC1b is a Na\(^{+}\)-loading mechanism, the overexpression of this transporter might contribute to increase the incidence of arrhythmias in the failing heart (Verdonck et al. 2003). Accordingly, the contribution of \(I_{\text{NBC}}\) to the shape of the AP in pathophysiological states represents an interesting forthcoming issue to be resolved.

In summary, the present study demonstrates the presence of an electrogenic NBC in isolated cat ventricular myocytes that participates in the modulation of RMP and APD. These effects originate from the presence of a SITS-sensitive outward current that can be seen only when HCO\(_3\)- and Na\(^{+}\) are present in the media. Although HCO\(_3\) is the physiological buffer, HCO\(_3\)-buffered solutions are not usually employed in patch-clamp experiments, masking the observation of these effects. From these results it is clear that \(I_{\text{NBC}}\) should be considered as part of the currents contributing to the configuration of the cardiac AP.

References


Acknowledgements

This work was partly supported by a grant from the Agencia Nacional de Promoción Científica y Tecnológica (PICT 25495 to E.A.A.). The authors wish to thank Mónica Rando for excellent technical assistance.