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Review article

H^+-activated Na^+ influx in the ventricular myocyte couples Ca^{2+}-signalling to intracellular pH

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ABSTRACT

Acid extrusion on Na^+-coupled pH-regulatory proteins (pH-transporters), Na^+/H^+ exchange (NHE1) and Na^+-HCO_3^- co-transport (NBC), drives Na^+ influx into the ventricular myocyte. This H^+-activated Na^+-influx is acutely up-regulated at $pH_i < 7.2$, greatly exceeding Na^+-efflux on the Na^+/K^+ ATPase. It is spatially heterogeneous, due to the co-localisation of NHE1 protein (the dominant pH-transporter) with gap-junctions at intercalated discs. Overall Na^+-influx via NBC is considerably lower, but much is co-localised with L-type Ca^{2+}-channels in transverse-tubules. Through a functional coupling with Na^+/Ca^{2+} exchange (NCX), H^+-activated Na^+-influx increases sarcoplasmic-reticular Ca^{2+}-loading and release during intracellular acidosis. This raises Ca^{2+}-transient amplitude, rescuing it from direct H^+-inhibition. Functional coupling is biochemically regulated and linked to membrane receptors, through effects on NHE1 and NBC. It requires adequate cytoplasmic Na^+-mobility, as NHE1 and NCX are spatially separated (up to 60 μm). The relevant functional NCX activity must be close to dyads, as it exerts no effect on bulk diastolic Ca^{2+}. H^+-activated Na^+-influx is up-regulated during ischaemia–reperfusion and some forms of maladaptive hypertrophy and heart failure. It is thus an attractive system for therapeutic manipulation.
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Contents

Abbreviations: pH_i, intracellular pH; pH transporters, pH regulatory proteins; NHE, Na^+/H^+ exchange; NBC, Na^+-HCO_3^- co-transport; NCX, Na^+/Ca^{2+} exchange; SR, sarcoplasmic reticulum; SERCA, sarcoplasmic reticular Ca^{2+} ATPase; PMCA, plasmalemmal Ca^{2+} ATPase; LTCC, sarcolemmal L-type Ca^{2+} channel; RyR, ryanodine receptor; MCT, monocarboxylic acid transporter; CBE, Cl^-/HCO_3^- exchange; CHE, Cl^-/OH^- exchange; t-tubules, transverse tubules; CaT, Ca^{2+} transient; DAD, delayed after-depolarisation; CA, carbonic anhydrase; MAPK, mitogen activated protein kinase; PKC, protein kinase C.

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1. Introduction

Intracellular Na^+ and H^+ ions are important signalling messengers in cardiac cells. H^+ ions exist in cytoplasm at a concentration of ~60 nM (equivalent to a pH_i of 7.2). They are highly reactive end-products of metabolism, arising from multiple sources, such as lactic acid, ketone bodies, CO_2-hydration (generating H^+ and HCO_3^- ions), and net ATP hydrolysis. H^+_i levels can fluctuate on a seconds-to-minutes time-scale during physiological manoeuvres (e.g. changes of heart-rate and cardiac work-load), and under pathophysiological conditions like myocardial ischaemia. H^+ ions interact with, and strongly modulate, the function of many intracellular proteins. In cardiac myocytes these include the myofilaments and their regulatory protein, troponin [1], resulting in a decrease in myocyte contraction [2]. They also include numerous proteins associated with intracellular Ca^{2+} ion signalling, such as the sarcolemmal Na^+/Ca^{2+} exchanger (NCX) [3,4], the sarcoplasmic reticular (SR) Ca^{2+} ATPase (SERCA) (plus its regulatory protein, phospholamban) [5–7], plasmalemmal Ca^{2+} ATPase (PMCA) [8], sarcolemmal L-type Ca^{2+} channels (LTCCs) [9] and ryanodine receptors (RyRs, the SR Ca^{2+}-release channels) [10–12]. Not surprisingly, therefore, H^+ ions induce complex changes in Ca^{2+} signalling. In contrast, intracellular Na^+ ions reside in ventricular myocytes at a concentration of 7–10 mM. Their levels can also fluctuate on a seconds-to-minutes timescale, although they exert little or no direct physiological effect on the myofilaments. But they exert a powerful indirect effect on intracellular Ca^{2+} signalling. This is because they bind to, and are transported by NCX, at the sarcolemmal membrane. As a result, $[Na^+]_i$ secondarily modulates $[Ca^{2+}]_i$, and hence Ca^{2+}-signalling and the cardiac myocyte's inotropic state [13,14].

It is notable that Na^+ ions are key counter and co-ions on the principal intracellular pH (pH_i) regulatory membrane transporters (pH transporters), Na^+/H^+ exchange (NHE) and Na^+-HCO_3^- co-transport (NBC) [15]. During acidosis, this creates an intimate link between $[H^+]_i$, $[Na^+]_i$, intracellular Ca^{2+} signalling and contraction, mediated through a functional coupling among the activities of NCX, NHE, and NBC proteins [2,14] (for review see [16]). Furthermore the physiological range of H^+ and Ca^{2+} ions, at either end of this ionic sequence, is of a comparable order of magnitude, operating in the tens to hundreds of nanomolar units. H^+, Na^+ and Ca^{2+} form a triumvirate of intracellular ions with intricate cross-talk. This can be regarded as a supra-signalling system that dictates cardiac function. When considering the role of cardiac pH transporters in the regulation of $[Na^+]_i$, it is important to assess not only their expression and associated Na^+ flux, but also their role in coupling Ca^{2+} with H^+ ion signals in the heart.

2. NHE, NBC and the regulation of pH_i

2.1. General model of pH_i regulation

The schematic diagram shown in Fig. 1A has been derived from experiments on mammalian ventricular tissue or enzymically isolated myocytes, for a variety of species, including human [17,18]. Two of the generic pH transporters, NHE and NBC, are Na^+-coupled. These proteins operate by directly exporting H^+ ions (NHE), or by importing HCO_3^- anions (NBC) that neutralise cytoplasmic H^+ ions (generating CO_2 that fluxes from the cell). NHE and NBC are secondary active transport proteins, coded for by different gene families. NHE1 (SLC9A1) [19], the only NHE isoform expressed at the sarcolemma, electroneutrally counter-transports $1Na^+$ for $1H^+$ (but cf [20]). Generic NBC co-transports Na^+ with HCO_3^-. At least two isoforms are expressed at the sarcolemma, NBCn1 (SLC4A7) [21], which exhibits an electroneutral $1Na^+$:$1HCO_3^-$ stoichiometry, and NBCe1 (SLC4A4) which is electrogenic, with a $1Na^+$:$2HCO_3^-$ stoichiometry [22–24]. There is some dispute that the transported anion may be CO_3^{2-} rather than HCO_3^- [25], but pH_i data are interpreted in terms of membrane HCO_3^- flux. NBCn1 expression has only recently been confirmed in ventricular myocytes [26], although its atrial expression has long been known [27]. Protein expression of isoform NBCe2 in mammalian cardiac myocytes has yet to be conclusively demonstrated.

The other three generic pH transporters shown in Fig. 1A, Cl^-/HCO_3^- exchange (CBE), Cl^-/OH^- exchange (CHE), and a monocarboxylic acid transporter (MCT), also contribute to pH_i regulation (see legend to Fig. 1), but these transporters are not Na^+-coupled, and so are not discussed further. For more details, see [16].

2.2. NHE1 and NBC activity is controlled by pH_i

Acutely acid-loading an isolated ventricular myocyte (i.e. raising $[H^+]_i$, thus reducing pH_i) promotes global acid extrusion from the cell. Cytoplasmic pH then recovers to control levels within a few minutes, as shown in Fig. 1B. This recovery is due to the activity of NHE1 and NBC. For example, it is inhibited by the removal of Na^+ from the extracellular medium (not shown). To gain insight into Na^+ influx during pH_i homeostasis, one can quantify acid efflux through each transporter-type in the native cell, and then translate this into Na^+ influx, knowing the transport stoichiometry.

Because the intracellular compartment is so highly buffered [28], the nM changes of $[H^+]_i$ indicated in Fig. 1B are actually achieved through the export of mM quantities of acid, and thus the import of comparable quantities of Na^+. Acid efflux has been quantified in panel C, which plots H^+-equivalent efflux versus pH_i. Dissection of flux components due to NHE1 and NBC has been achieved by selectively inhibiting either NHE1 or NBC, using extracellular ion-substitution, or inhibitor drugs like amiloride and the highly selective analogue, cariporide (for NHE1) [29], and the N-cyanosulphonamide drug, S0859 (for generic NBC) [30], or selective inhibitory antibodies (for NBCe1 [31]). Note that global acid efflux is greatly enhanced at low pH_i, where the flux is dominated by NHE1 activity. In contrast, both NHE1 and NBC transporters operate at comparable but low acid efflux rates (about 0.5 mM/min) when pH_i is at its normal steady-state value of ~7.2 [15]. Because of their coupling to extracellular Na^+, NHE1 and NBC will therefore mediate an intracellular H^+-activated Na^+-influx across the sarcolemma. Given that H^+ ions are universal metabolic end-products, this Na^+ influx can become linked to biochemical H^+ ion production, and hence metabolic stress. The subsequent Na^+ load induced by the influx must then be extruded by the sarcolemmal Na^+/K^+ ATPase.

2.3. Spatial sarcolemmal distribution of NHE1 and NBC

Immunofluorescent antibody staining (Fig. 1D) indicates that NBCe1 and NBCn1 proteins are expressed in all sarcolemmal membrane zones of the ventricular myocyte, most notably in the transverse tubules. In contrast, NHE1 is largely excluded from transverse tubules but is evident in lateral sarcolemma, and particularly prominent at the ends of the cell (intercalated disc regions). This transporter distribution has been confirmed in functional experiments where isolated, ventricular cells were detubulated (by transient osmotic shock using 1.5 M formamide; Fig. 2A). Detubulated cells displayed no change in the magnitude of acid extrusion through NHE1, but a 40% reduction in generic NBC activity, consistent with NBC mediating acid extrusion from transverse tubular (t-tubular) regions [26]. T-tubules are also prominent sites of NCX expression, as also shown in Fig. 1D, a feature that is relevant to functional NHE1–NBC/NCX coupling, as discussed later (Section 4).

Further immunofluorescent studies have established that NBCs co-localise with LTCCs in the t-tubules, while NHE1 proteins co-localise with Cx43 protein, the subunit of the main gap-junctional channel in ventricular myocardium [26], expressed prominently at intercalated discs. NBC will thus have privileged access to pH_i-control in the vicinity of the couplons. These are t-tubular sites of excitation–contraction coupling in the ventricular myocyte, where surface membrane LTCCs are

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