H+ Ion Activation and Inactivation of the Ventricular Gap Junction

A Basis for Spatial Regulation of Intracellular pH

Pawel Swietach, Alessandra Rossini, Kenneth W. Spitzer, Richard D. Vaughan-Jones

Abstract—H+ ions are powerful modulators of cardiac function, liberated during metabolic activity. Among their physiological effects is a chemical gating of cell-to-cell communication, caused by H+-mediated closure of connexin (Cx) channels at gap junctions. This protects surrounding tissue from the damaging effects of local intracellular acidosis. Cx proteins (largely Cx-43 in ventricle) form multimeric pores between cells, permitting translocation of ions and other solutes up to ≈1 kDa. The channels are essential for electrical and metabolic coordination of a tissue. Here we demonstrate that, contrary to expectation, H+ ions can induce an increase of gap-junctional permeability. This occurs during modest intracellular acid loads in myocyte pairs isolated from mammalian ventricle. We show that the increase in permeability allows a local rise of [H+], to dissipate into neighboring myocytes, thereby providing a mechanism for spatially regulating intracellular pH (pHi). During larger acid loads, the increased permeability is overridden by a more familiar H+-dependent inhibition (H+ inactivation). This restricts cell-to-cell H+ movement, while allowing sarclemmal H+ transporters such as Na+/H+ exchange, to extrude the acid from the cell. The H+ sensitivity of Cx channels therefore defines whether junctional or sarcolemmal mechanisms are selected locally for the removal of an acid load. The bell-shaped pH dependence of permeability suggests that, in addition to H+ inactivation, an H+ activation process regulates the ensemble of Cx channels open at the junction. As well as promoting spatial pH regulation, H+ activation of junctional permeability may link increased metabolic activity to improved myocardial coupling, the better to meet mechanical demand. (Circ Res. 2007;100:1045-1054.)

Key Words: connexin 43 • gap junction channel • intracellular pH • ventricular myocytes

Protons generated within respiring tissue exert a profound influence on cellular function. In the heart, this includes effects on intracellular Ca2+ signaling,1 contraction,2 and electrical excitation.3 Indeed, much of the contractile failure and electrical arrhythmia associated with myocardial ischemia has been attributed to the ensuing decrease of pHi.4,5 In cardiac ventricular myocytes, an acute displacement of pHi causes junctional channels,7 suggesting an additional mechanism for regulating pHi. Gap junctions may dissipate localized acid/base disturbances, helping to maintain a more uniform pHi, within the myocardium.

Gap junctions consist of 2 hemichannels (connexons) coupled in series between adjacent myocytes. In ventricular tissue, the hemichannels are typically made of connexin 43 (Cx-43) subunits,8 arranged as a hexamer around a central pore,9 permitting passage of solutes <1 kDa.10,11 H+ permeation through ventricular gap junctions occurs readily but not via the movement of free H+ ions. Rather, it occurs via the permeation of mobile buffer molecules that carry the H+ ion through the channel.7 Typical mobile buffers are histidyl dipeptides, such as homocarnosine and acetylcarnosine (of molecular mass, 100 to 200 Da12). Because intracellular mobile buffer concentration is relatively high (~15 mmol/L12-14), significant quantities of H+ ions can be shuttled passively through the channel in response to a modest transjunctional pH gradient.7

One problem with the suggested role of myocardial gap junctions in mediating the spatial dissipation of H+ ions is that intracellular acidosis closes connexin channels.15-23 Such chemical gating is believed to be mediated directly by H+ titration of specific sites on the cytoplasmic C terminus of...
each Cx-43 subunit and indirectly by an H\(^+\)-induced rise of Ca\(^{2+}\), that also gates the channel.\(^{19,20}\) The extent to which this influences junctional H\(^+\) permeation is not known. By using techniques of cellular imaging, flash photolysis of an intracellular caged H\(^+\) compound, local cellular microperfusion, and cell-to-cell current injection, we have measured the ability of H\(^+\) ions and other solutes to flux through the gap junctions of isolated ventricular myocyte pairs, when pH\(_i\) was manipulated to various levels. Implications of the results for the spatial control of myocardial pH have then been explored using computational modeling. An intriguing finding is that an increase of [H\(^+\)], can enhance as well as inhibit gap-junctional permeability to various solutes, including the H\(^+\) ion itself. The results prompt a reevaluation of the chemical control of Cx channels by H\(^+\) ions and a reassessment of the functional effects of acidosis on metabolic and electrical coupling within the myocardium.

**Materials and Methods**

**Cell Preparation and pH\(_i\) Imaging**

Cell pairs were enzymically isolated from rat or guinea pig ventricles.\(^{6,7}\) Cells were loaded with 10 \(\mu\)mol/L acetoxymethyl (AM) carboxy-SNARF-1 or -4 (pK\(_{a}\) = 7.5 and pK\(_{a}\) = 6.5, respectively) for 10 minutes.\(^{24}\) In some experiments, cells were coloaded with 100 \(\mu\)mol/L BPAT-AAM to provide additional intracellular Ca\(^{2+}\) buffering. Myocytes were then transferred to a poly-L-lysine–coated glass, resistance 1 to 2 M\(\Omega\); filling solution: 113 mmol/L KCl, 113 mmol/L NaCl, 4.5 mmol/L KCl, 22 mmol/L NaHCO\(_3\), 11 mmol/L glucose, 2 mmol/L CaCl\(_2\), 1 mmol/L MgCl\(_2\). Solutions were bubbled with 5% CO\(_2\) at 37°C (pH 6.5, 7.5 and pK\(_{a}\) 6.5, respectively) for 10 minutes.\(^{24}\) In some experiments, cells were coloaded with 20 mmol/L Hepes (pH 7.4).

**Photolytic Uncaging of H\(^+\) Ions**

Rat ventricular myocytes were superfused with solutions containing 1 mmol/L membrane permeant, 2-nitrobenzaldehyde, a caged H\(^+\) compound, local cellular microperfusion, and cell-to-cell current injection rate (J\(_{inj}\)) was calculated from current (I) and the electrotonic changes in membrane potential (E\(_{m}\)).\(^{27}\) To elucidate the pH dependence of R\(_s\), the superfusion medium was changed, at constant pH\(_i\), to one containing 15 mmol/L NH\(_4\)Cl or 20 mmol/L trimethylamine to raise pH\(_i\) uniformly, or to one containing 80 mmol/L Na/acetate (plus 30 mmol/L cariporide) to reduce pH\(_i\) uniformly.

**Presetting pH\(_i\)**

It was often necessary to preset pH\(_i\) to different levels before an experiment. This was achieved by perfusing the cell pair with 10 to 30 mmol/L NH\(_4\)Cl-containing or 40 to 80 mmol/L Na/acetate-containing solutions for 3 to 8 minutes. On removal of these compounds, a uniform intracellular acidosis and alkalosis, respectively, was generated within 1 minute. The new pH\(_i\) level was then clamped by inhibiting membrane H\(^+\)–equivalent transport. In some experiments, C1\(^-\)/HCO\(_3^-\) exchange and Na\(^+\)/HCO\(_3^-\) cotransport (NBC) were blocked by using CO\(_2\)/HCO\(_3^-\)–free superfusates, and Na\(^+\)/H\(^+\) exchange inhibited by adding 30 mmol/L cariporide (as Cl\(^-\)/OH\(^-\) exchange activity is low, <2 mmol/L per minute, no inhibitory measures were taken). When using CO\(_2\)/HCO\(_3^-\)–buffered superfusates, NBC was inhibited pharmacologically by 10 mmol/L S0859\(^{28}\) and base transport at high pH, was blocked by replacing superfusate Cl\(^-\) with gluconate (and raising [Ca\(^{2+}\)]) to 8.5 mmol/L to compensate for Ca\(^{2+}\) binding to gluconate.

**Modeling Spatial pH\(_i\) Regulation**

Spatial pH\(_i\) regulation was simulated in a model myocyte expressing sarcosomal transporters and gap junctions. See Appendix for details.
Statistics and Curve Fitting

Quantitative data presented as mean±SEM; asterisks denote significance (* t test; 5% significance level). Data presented in Figures 1B, 2C, and 3B were fitted with biphasic curves that define junctional permeability ($P$) with $H^+$-binding constants $K$ and $Q$:

$$P = P_{\text{max}} \frac{[H^+]^n}{[H^+]^n + K^n} \cdot \frac{Q^n}{[H^+]^m + Q^m}$$

Results

Local Intracellular $H^+$ Uncaging to Measure Junctional $H^+$ Permeability

Repetitive UV flash photolysis of 2-nitrobenzaldehyde, a membrane-permeant caged $H^+$ compound, was used to introduce $H^+$ ions into one cell of an isolated pair of rat ventricular myocytes (Figure 1). Either side-by-side or end-to-end cell pairs were used. Progressive acidification of the distal cell of the pair indicated that cell-to-cell $H^+$ transmission was occurring (Figure 1A), the time course of which was fitted with a diffusion–permeation algorithm to estimate $P_{\text{app}}$, the apparent junctional $H^+$ permeability. By presetting the resting $pHi$ of cell pairs to different values (see Materials and Methods) and then locally uncaging $H^+$, the $pHi$ dependence of $P_{\text{app}}$ was revealed. In the presence of CO$_2$/HCO$_3^-$ buffer, a reduction of $pHi$ from 7.5 to 7.1 increased $P_{\text{app}}$ 5-fold (Figure 1B). A further reduction of $pHi$ from 7.1 to 6.3 reversed this effect. Note that, although the $pHi$ dependence of $P_{\text{app}}$ was revealed. In the presence of CO$_2$/HCO$_3^-$ buffer, a reduction of $pHi$ from 7.5 to 7.1 increased $P_{\text{app}}$ 5-fold (Figure 1B). A further reduction of $pHi$ from 7.1 to 6.3 reversed this effect. Note that, although

<table>
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<th>Data (Figure No.)</th>
<th>$P_{\text{max}}$ (cm/sec)</th>
<th>$K$ (mol/L)</th>
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<th>$Q$ (mol/L)</th>
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permeability was ~12-fold higher in end-to-end compared with side-by-side cell pairs, the fractional changes of permeability with pHi were the same. Comparable results were obtained when experiments were performed in the absence of CO2/HCO3 (Figure 1B), indicating that this buffer is not essential for the changes of PHapp. Reduced solute permeability during acidosis is well documented for gap junctions,15–23 but an increase has not commonly been reported.

As described in the introduction, almost all H+ ions that permeate the cardiac gap junction do so while bound reversibly to mobile buffers.7 Figure 1E illustrates this for H+ permeation on an intrinsic buffer such as acetylanserine, acetylcarnosine, or homocarnosine, whose collective intracellular concentration is ~15 mmol/L.12,13 The size of these buffers (100 to 200 Da) is well within the permeability limit of Cx-43 channels (~1 kDa).10,11 The slightly higher peak permeability seen with CO2/HCO3− (Figure 1B) indicates that carbonic buffer may also assist H+ permeation. Interpreting changes of H+ ion permeability can be problematic, as buffer capacity is pHi dependent,6 which will affect the pHi sensitivity of Pmob. Using the mean pK for mobile and fixed intrinsic buffers,12,13 and pH data gathered in the absence of CO2/HCO3−, we computed the average junctional permeability to the buffer molecules themselves (Pmob; see Materials and Methods). Any pHi sensitivity detected here is likely to be independent of the degree of buffer protonation. Results plotted in Figure 1C show that Pmob displays a biphasic dependence on pHi. Consistent with these permeability changes, the cell-to-cell H+ flux measured during the H+-uncaging procedure also varied biphasically with pHi (Figure 1D). Thus a modest fall of pHi from rest (gray arrow) enhanced H+ transmission, whereas a larger decrease impaired it.

**Steady-State pH Sensitivity of Junctional H+ Permeability**

H+-uncaging experiments provide an estimate of Pmob and Psub during dynamic changes of pHi. We also measured Pmob under steady-state pHi conditions, using a different technique. One end of a ventricular cell pair (this time, from a guinea pig rather than rat heart) was exposed to Hepes-buffered solution containing 30 mmol/L NH4Cl to elevate pHi locally25,26 (Figure 2Aii). Within ~30 seconds, this establishes an intracellular pH gradient along the cell pair, which drives tranjunctional H+ flux on the mobile buffer shuttle. The [H+], gradient is remarkably stable over time (many minutes) and displays a sharp discontinuity at the gap junction, where H+ flux is rate limiting (Figure 2Aii). In other experiments, NH4Cl was replaced by Na/acetate (Figure 2Bi) to generate local acidosis25,26 and drive H+ flux in the opposite direction.
This establishes a longitudinal pH gradient, distributed inversely to that seen with NH₄Cl (Figure 2Bii). In both cases, the amplitude of the H⁺ flux into one cell of a side-by-side pair (uniform pH) was measured from ratioed SNARF images (not shown). The permeability changes did not occur in the presence of 60 μmol/L α-glycyrrhetinic acid, a selective gap-junctional inhibitor (Figure 2C), confirming that permeation was via Cx channels. The changes were established within 1 to 3 minutes (the minimum time for presetting pH, and then applying a local [H⁺]i gradient). They were also reversible, as they were independent of the direction in which pH was preset in a cell pair (i.e., from 7.5 to 6.2 or 6.2 to 7.5; see Figure I in the online data supplement, available at http://circres.ahajournals.org). Furthermore, the changes persisted after Ca²⁺_i was clamped by intracellular BAPTA (loaded as the acetoxymethyl-ester; Figure 2C), although the decrease in P_mol in the pH range from 6.95 to 6.20 was attenuated at pH values <6.5. Thus, as reported previously, inactivation of junctional permeability at very low pH probably depends on a rise of [Ca²⁺], as well as a fall of pH. In contrast, however, the activation of P_mol as pH declines from 7.5 to 6.95 was unaffected by BAPTA, suggesting that activation is Ca²⁺ independent.

### pH-Sensitive Junctional Permeability Measured by Fluorescent-Dye Transfer

We investigated the pH sensitivity of junctional permeability applied to solutes other than H⁺ ions or intrinsic mobile buffer. In one set of experiments (Figure 3A), we monitored cell-to-cell permeation of a fluorescent marker dye by introducing SNARF-1 (453 Da) into one cell of a guinea pig ventricular pair from a cell-attached pipette. The rise of fluorescence in both cells was fitted with a diffusion–permeation algorithm to quantify junctional permeability to SNARF (P_SNARF). By presetting pH uniformly to different levels, P_SNARF was found to follow a biphasic relationship with pH (Figure 3B), similar in shape to the pH–P_mol relationship (Figure 2C).

### pH-Sensitive Junctional Permeability Measured by Current Injection

In other experiments (Figure 4Aii), we monitored electrical junctional resistance (R_j) by passing current (carried largely by intracellular K⁺ and Cl⁻ ions) between coupled cells while uniformly superfusing them with NH₄Cl (Figure 4Aii), trimethylamine-chloride, or Na/acetate to displace H⁺. Data pooled in Figure 4B show that R_j also varied biphasically with pH, rising at both high and low pH. By plotting R_j (inverted axis) and SNARF permeability on a common pH axis (Figure 4C), it is apparent that both parameters display a similar pH sensitivity. The biphasic pH sensitivity of permeability is thus likely to be a general feature of the ventricular gap junction.

### Computational Modeling of Cell-to-Cell H⁺ Movement

The H⁺-dependent activation and inactivation of junctional permeability has important implications for the spatial regulation of myocardial pH. Figure 5A superimposes the pH dependence of junctional permeability (P_mol) on the known pH dependence of sarcolemmal H⁺-equivalent transport that classically mediates pH regulation in cardiac cells. It is notable that the pH range for peak junctional permeability coincides with minimum transporter activity and vice versa. We have incorporated these parameters into a computational model of a myocyte surrounded by, and coupled to, other myocytes (see Appendix). Reducing pH of the central cell in the model from 7.25 (resting pH) to 6.0 induces an H⁺ efflux from that cell (Figure 5B). This is predicted to occur through gap junctions into surrounding cells (green trace; J_mol) and on sarcolemmal transporters into the extracellular space (red trace; J_mol). In the ventricular myocardium, gap junctions couple 1 myocyte to as many as 11 neighbors. In the model, junctional efflux (J_mol) predicted for maximal cell-to-cell coupling (continuous green trace) rises steeply to a peak at approximately pH=6.85, facilitated by activation of gap-junctional permeability. It declines at lower pH values because of inactivation of junctional permeability, whereas sarcolemmal H⁺ efflux (J_mol) activates steeply. At pH values 7.25 to 6.20, J_mol exceeds J_mol, indicating that most H⁺ efflux from the locally acidified zone is via junctional pathways. The dashed green line shows results predicted when only one end of the central cell is coupled in the model to the
surroundings (analogous to the cell pair experiments shown in Figure 1). Even under these restricted circumstances, \( J_{\text{junc}} \) is significantly larger than \( J_{\text{sarc}} \) for much of the pHi range (7.25 to 6.50). Junctional H\(^+/\)H\(_{11001}\) coupling in the intact myocardium is likely to lie between the two extremes illustrated in Figure 5B (continuous and dashed green lines). The model therefore predicts that, following a local fall of pHi, junctional H\(^+/\)H\(_{11001}\) permeability in the myocardium will provide a major mechanism for dispersing the acid load.

**Discussion**

**Spatial Regulation of pHi: Junctional Versus Sarcolemmal Control**

We have shown that H\(^+\) ions within ventricular tissue can activate as well as inactivate gap-junctional permeability. An important molecule that normally permeates the junction is the intracellular mobile H\(^+/\)H\(_{11001}\) buffer, which serves to carry H\(^+\) ions between adjacent myocytes.\(^7\) Thus intracellular H\(^+\) ions, by attaching to mobile buffers and also by modulating gap junctional permeability, can regulate their own cell-to-cell movement. The pHi sensitivity of junctional permeability provides a sophisticated mechanism for spatially regulating myocardial pHi. Our computational model indicates that a modest local H\(^+\) load (eg, pHi 6.90) in 1 or more cells (induced, for example, by local reduction of capillary perfusion) will enhance gap-junctional permeability, permitting the load to dissipate passively into neighboring cells: a “permissive” mode of local pHi regulation (Figure 5Ci). In contrast, a local H\(^+\) “overload” (eg, pHi 6.3; caused, for example, by extreme metabolic stress) will inhibit gap-junctional permeability, restricting the spatial spread of H\(^+\) ions: a “nonpermissive” mode of pHi regulation, as much junctional H\(^+\) transmission is barred (Figure 5Cii). Spatial dissipation of a modest H\(^+\) load will dilute it into an extended and highly buffered intracellular volume and will thus be an energy-efficient form of pHi regulation. The dispersed H\(^+\) ions will eventually be exported at a low rate across the sarcolemma. In contrast, following the isolation of a local H\(^+\) overload by gap-junctional inactivation, the H\(^+\) ions will be pumped rapidly into the extracellular space, as sarcolemmal extrusion is greatly stimulated at low pHi (Figure 5A). Given that H\(^+\) overload is potentially dangerous to the heart,\(^3,4\) restricting its spread while simultaneously removing it at source would seem a sensible strategy. But for more modest H\(^+\) loads, the pH sensitivity of gap junctions will enhance rather than inhibit the exchange of H\(^+\) ions between cells, tending to promote a myocardial pH syncytium.

**H\(^+\) Activation and Inactivation of the Ventricular Gap Junction**

The element orchestrating junctional versus sarcolemmal modes of pH regulation is the pH sensitivity of the ventric-
ular gap junction. We have yet to investigate if similar pH sensitivity is observed in other regions of the heart, such as the atria and conduction system. Although chemical H\(^+\)/H\(_{1000}\) inactivation of Cx-43 and Cx-45 channels, the dominant isoforms in ventricular myocardium,\(^8,29\) has long been known,\(^21–23,30\) the possibility of chemical H\(^+\)/H\(_{1000}\) activation of junctional permeability has received little attention. It is notable, however, that early work on amphibian blastomeres\(^31\) and on Cx-43 channels expressed in oocyte pairs\(^21,22\) documented a paradoxical and unexplained rise of junctional conductance during progressive acidosis that preceded the anticipated fall. H\(^+\) activation could account for such anomalous behavior.

How may H\(^+\) ions both activate and inactivate junctional permeability? The bell-shaped pH sensitivity of junctional permeability (Figures 2C and 3B) is consistent with distinct, overlapping H\(^+\) activation and H\(^+\) inactivation processes (Figure 6A) of similar pK (≈7.0), producing a steady-state window of ensemble Cx channel permeability (Figure 6B). In such a model, H\(^+\) titration of activation sites would increase junctional permeability, whereas simultaneous titration of inactivation sites would decrease it. Although acute H\(^+\) inactivation has been linked structurally to the cytoplasmic C terminus of Cx-43 proteins,\(^21–23\) the molecular correlates of H\(^+\) activation have yet to be identified. Preliminary work suggests that junctional permeability in HeLa cell pairs heterologously expressing Cx-43 channels shows a biphasic dependence on pH, qualitatively similar to that seen here in myocyte pairs.\(^32\) Furthermore, H\(^+\) activation and inactivation of junctional permeability appears to be greatly attenuated when mutant Cx-43 channels with truncated C termini are expressed.\(^32\) The H\(^+\)-induced increase of junctional permeability in myocytes, like the H\(^+\)-induced decrease, may therefore be related to a structural motif in the channel protein itself.

Acute H\(^+\) inactivation largely reflects the closure (ie, gating) of Cx channels.\(^21–23\) In contrast, there are at least three possible mechanisms for H\(^+\) activation that cannot yet be distinguished: increased Cx channel conductance, increased channel opening (or decreased closure), and insertion of additional Cx channel proteins from a mobilizable source,\(^33,34\) ie, a form of junctional remodeling. Indeed, the primary H\(^+\) activation site need not necessarily be located on the Cx channel itself. It could, for example, be located remotely on an accessory protein (as represented schematically in Figure 6A) or be part of a biochemical cascade, which targets the channel. H\(^+\) activation, however, must ultimately interact with the dynamic behavior of Cx channels to generate
increased junctional permeability. The relatively rapid kinetics and magnitude of H⁺ activation (up to 4-fold permeability increase within 1 to 3 minutes; Figure 2) suggest a regulation of channel gating and conductance, although a fast increase in channel density cannot be excluded. In contrast, H⁺-induced and ischemia-induced remodeling of glial34 and cardiac cell junctions33 is a slow process (>30 minutes) and has so far involved internalization rather than plasmalemmal insertion of Cx channels. The mechanism for H⁺ activation of Cx channels therefore remains unresolved.

Wider Implications for H⁺ Control of Junctional Coupling

A variety of connexin gap junctions, and functionally similar channels composed of innexin or pannexin proteins, is widely expressed in tissues throughout the animal kingdom.35 Whether H⁺ activation of junctional permeability is a common feature for these channels remains to be established. The possibility arises that, in addition to heart, H⁺ control of junctional coupling may direct the spatial distribution of pHᵢ in neural and glial networks, in smooth muscle, and in epithelial tissue. H⁺ activation may also help to explain recent reports that hypoxia and simulated ischemia can paradoxically open gap-junctional hemichannels in neurones36 and cardiac myocytes,19 as these conditions promote a fall of pHᵢ.

The acute increase of gap-junctional permeability with modest acidosis implies that, in ventricular myocardium, metabolic stimulation may commonly enhance electrical and biochemical communication between cells. This may be one means of increasing cardiac efficiency to match metabolic activity and hence mechanical demand. Only with more profound intracellular acidosis, such as occurs during severe ischemia, would this then be replaced by electrical and metabolic uncoupling.

Appendix

We used a mathematical model to assess the relative role of junctional and sarcolemmal H⁺-equivalent fluxes in controlling pHᵢ of a myocyte, coupled to neighboring cells in 3 dimensions, during a localized injection of acid (Figure 5). The simulations were performed assuming CO₂/HCO₃⁻ buffering. The model was based on

\[
\frac{\partial[H⁺]}{\partial t} = \frac{1}{r} \left( \frac{\partial}{\partial r} \left( r \cdot D_r[H⁺] \right) \right) + \frac{\partial}{\partial x} \left( D_x[H⁺] \right)
\]

where \(r\) and \(x\) are the radial and longitudinal axes, and \(D_r[H⁺]\) and \(D_x[H⁺]\) are the radial and longitudinal diffusion coefficients, respectively. The coefficient \(\rho\) accounts for permeation anisotropy (Figure 1B; \(\alpha = 12.1\)). The constant \(\rho\) is defined as the sum of intrinsic and carbonic buffering6,14 Junctional permeation H⁺ fluxes (\(J_{\text{junc}}\)) were defined as follows (note [mmol/L·sec⁻¹] for all equations hereafter):

\[
J_{\text{junc}}(\text{side-by-side}) = -\rho \cdot D_r[H⁺] \frac{\partial[H⁺]}{\partial r} = \rho \cdot P_{\text{r}}[H⁺] \Delta[H⁺]
\]

\[
J_{\text{junc}}(\text{end-to-end}) = -\rho \cdot D_x[H⁺] \frac{\partial[H⁺]}{\partial x} = \rho \cdot \alpha \cdot P_{\text{x}}[H⁺] \Delta[H⁺]
\]

In the simulation, H⁺ ions are injected into the whole volume of the central cell at a rate \(J_{\text{inj}}\) up to 125 mmol/L per minute. During injection, some H⁺ ions are buffered, some extruded by sarcolemmal carrier mechanisms \(J_{\text{bac}}\), and some transmitted to neighboring cells through gap junctions \(J_{\text{junc}}\). In the analysis shown in Figure 7A, the
central cell was H\(^+\) loaded at 100 mmol/L per minute. In the absence of sarcolemmal or junctional recovery mechanisms, this would predict a fall of central pH\(_i\) by \(>1.7\) U within 1 minute. The activation of \(J_{\text{junc}}\) and \(J_{\text{sarc}}\) reduces this acidosis to 0.8 U. The regulatory routes taken by the injected H\(^+\) ions were followed by plotting the cumulative rise in the amount of H\(^+\) ions in three domains (Figure 7B): (1) central-cell buffers, (2) neighboring cells (junctional transmission), and (3) the extracellular space (sarcolemmal extrusion). Over the first 60 seconds, \(J_{\text{sarc}}\) is far larger than \(J_{\text{junc}}\). By repeating the simulations for a range of \(J_{\text{junc}}\) values, it was possible to quantify, over a range of central pH\(_i\) values, the relative importance of \(J_{\text{junc}}\) and \(J_{\text{sarc}}\) for limiting the acid load (Figure 5B). These simulations reveal that \(J_{\text{sarc}}\) is the dominant regulatory mechanism during a physiological acid load (eg, pH\(_i\) 7.25 to 6.60), whereas \(J_{\text{junc}}\) becomes increasingly important at lower, more pathological pH\(_i\) values (eg, pH\(_i\) 6.60 to 6.00). Thus both \(J_{\text{sarc}}\) and \(J_{\text{junc}}\) will be key local controllers of pH\(_i\).

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None.

References
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**SUPPLEMENT**

**Figure S1: Reversibility of pH-dependent changes in $P_{mob}$**

$P_{mob}$ (junctional permeability-constant for mobile buffer) was measured using the dual microperfusion apparatus (i.e. by local microperfusion of 10-30mM ammonium or 40-120mM acetate at one pole of an end-to-end rat ventricular cell-pair), as described in the Methods, and illustrated in Figure 2 of the paper. The grey curve plotted in Figure S1 shows the best-fitting biphasic function for the pH-sensitivity of $P_{mob}$, reproduced from Figure 2C. In some experiments, $P_{mob}$ measurements at different pH values were made sequentially in the same cell-pair. Results for these individual experiments have been plotted in Figure S1. Arrows indicate the...
order in which $P_{mob}$ measurements were made. Measurements of $P_{mob}$ obtained by local microperfusion of acetate or ammonium were not statistically different, when compared at a common $pH_j$ (as also reported in the main text of the paper). In some experiments, local weak acid/base microperfusion was first performed at resting $pH_i$, to obtain $P_{mob}$ near 7.2. The local microperfusion was then removed, and $pH_i$ in the cell-pair was preset uniformly, by prepulsing it with 10-30mM ammonium or 40-80mM acetate. $P_{mob}$ was then sampled again (using local microperfusion of ammonium or acetate) at the new acidic or alkaline junctional $pH$ ($pH_j$), respectively. In other experiments, $pH_i$ in the cell-pair was first preset uniformly to an acidic or alkaline value (again by ammonium or acetate prepulsing), and $P_{mob}$ measured by local microperfusion. The microperfusion was then removed, and whole-cell $pH_i$ allowed to recover for a few minutes from its acidic or alkaline value. This recovery was mediated by sarcolemmal $H^+$-equivalent transport while superfusing the cell-pair with inhibitor-free, normal Tyrode. $P_{mob}$ was then sampled again when $pH_i$ (and hence $pH_j$) was closer to resting $pH_i$. Pooling results of the individual experiments in Figure S1 illustrates that the $pH_j$-versus-$P_{mob}$ relationship was essentially the same, irrespective of the direction in which $pH_i$ was manipulated. It can be inferred from this that, within the time-frame for $pH_i$ adjustment and subsequent $P_{mob}$ measurement (from one to several minutes), the $pH_i$-$P_{mob}$ relationship showed no obvious sign of hysteresis. It appears that acute $pH_i$-dependent changes in $P_{mob}$ are fully reversible.