New and Notable

Downhill All the Way: H^+ Gradients within Cardiac Myocytes

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It has been known since at least 1880 that acidosis decreases the strength of contraction of the heart (1). Since then it has become clear that acidosis alters almost all aspects of cardiac cell function, including electrical activity, ion handling, intracellular signaling, and the ability of the contractile proteins to generate force (2). These widespread effects are due largely to the effect of H^+ on the charge, and hence function, of proteins.

To maintain cell function during physiological and pathophysiological events that tend to alter intracellular pH from its normal value of ~7.1, the cell has a range of sarcolemmal ion transporters that either extrude or add acid equivalents to the cell cytoplasm, and are activated by an increase or decrease of cytoplasmic $[H^+]$ respectively (3). However, in order, for example, for acid to be extruded from the cell, it needs to move from where it is produced within the cell to the cell membrane, where the acid-extruding proteins are located. The article by Swietach, Spitzer, and Vaughan-Jones in this issue of *Biophysical Journal* describes a novel technique that allows H^+ movement within cells to be investigated, which they use to study the mechanisms that facilitate H^+ movement within cardiac myocytes.

The problem is that these cells, like other cell types, are rich in intracellular sites that buffer H^+, particularly on proteins, which have low mobility. This would result in very slow diffusion of H^+ to the membrane, and hence to the H^+ extrusion pathways. The authors have previously shown that this problem is overcome, in part, by intrinsic low molecular weight diffusible buffers that chaperone H^+ within the cell. Although this increases mobility of H^+ within the cell, it remains slower than in free solution and, perhaps surprisingly from a physiological point of view, becomes slower as $[H^+]$ increases.

In their study, Swietach et al. show that it is possible to use photolytic uncaging of H^+ from a membrane permeant donor, 2-nitrobenzaldehyde, in combination with confocal imaging of H^+ distribution within the same cell. This enables H^+ release to be localized within a small region of the cell, and the subsequent increase of $[H^+]$ in other regions of the cell to be monitored as H^+ move down their concentration gradient. Previous studies investigating H^+ gradients within cells have placed cells across streams of superfusate with different compositions, which is technically demanding and provides less precise control of the acid region, or have used a pipette sealed onto one end of the cell to provide an H^+ reservoir, which is also technically demanding and disturbs the intracellular milieu being investigated. In contrast, the technique of Swietach et al. is relatively simple and noninvasive, and allows precise control of where and how much H^+ is released. This method provides distinct advantages over those used previously, and has the promise of being widely applicable in other cell types.

Using this technique, the authors confirm the rate of diffusion of H^+ within and between cells determined using the techniques described previously. They also show slowing of the apparent rate of diffusion of H^+ within the cell as H^+ increased. In these experiments, the sarcolemmal flux pathways that normally regulate intracellular pH were inhibited; however, whole-cell sarcolemmal pH regulation plays little part in defining such gradients (4), although large acid fluxes across the cell membrane can create spatial nonuniformity of intracellular pH. The decrease in the apparent rate of diffusion is due in part to decreased mobility via the intrinsic buffer system described above. Importantly, however, the authors now also demonstrate H^+-induced slowing of H^+ movement due to the bicarbonate/CO_2 system they have described previously; this system utilizes carbonic anhydrase to catalyze the combination of H^+ and bicarbonate to form CO_2, which can diffuse within the cell and combine with water to reform H^+ and bicarbonate. Thus it now appears that both pathways identified for increasing H^+ mobility within cells are inhibited by acidosis.

This new technique has, therefore, allowed the mechanisms underlying H^+ movement within cells to be revealed more clearly. It will also facilitate further study of movement of H^+ within and between cardiac myocytes as well as other cell types, and the role of such movement in the regulation of H^+ and H^+ gradients within cells. Future challenges include determination of how and when intracellular pH gradients occur physiologically or pathophysiologically, whether intracellular organelles play a role in shaping short-term changes of intracellular pH, and further elucidation of the physiological role of intracellular H^+ movement in determining the response of cardiac myocytes to physiological and pathophysiological disturbances of intracellular pH: the decrease in the apparent diffusion coefficient of H^+ as $[H^+]$ increases is intriguing; simplistically, it might be expected that as $[H^+]$ within the cell increases, it would become more important for it to be transported rapidly to the membrane to be extruded. However, restricting H^+ movement may result in excessive loads being kept within the cell, thereby limiting exposure of adjacent cells to high, potentially detrimental, $[H^+]$. It will also be of interest to investigate whether this mechanism, and H^+ flux to adjacent cells via gap junctions, play different roles in the response to interventions that alter H^+ production within the cell and those that primarily decrease extracellular pH; the

Submitted September 19, 2006, and accepted for publication September 20, 2006.

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0006-3495/07/01/371/02 $2.00

doi: 10.1529/biophysj.106.097576

former will require H^+ transport to the cell membrane, whereas the latter will initially alter $[H^+]$ close to the cell membrane and hence the sarcolemmal proteins that regulate intracellular pH.

The study of Swietach, Spitzer, and Vaughan-Jones thus introduces an elegant new technique for the study of intracellular pH regulation, which has enabled the authors to reveal more clearly the intra- and intercellular movement of H^+. This technique will enable further elucidation of the regulation of intracellular pH, and the interaction of intracellular H^+ movement with the sarcolemmal proteins that regulate intracellular pH, and how this shapes the response to interventions that alter intracellular pH.

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