Online Supplementary text for:

Yagi et al. Sustained currents through ASIC3 ion channels at the modest pH changes that occur during myocardial ischemia

Mechanism of Lactate Action

Lactate has been shown to shift ASIC activation curves because it binds extracellular divalent ions. This allows ASICs to open at higher pH. To ask whether a similar mechanism explains the effect of lactate on the bell-shaped activation curve of the sustained current, we made solutions that reproduce the free divalent ion concentration expected in lactate-containing solutions. From lactate binding constants, 15 mM lactate is expected to decrease free extracellular Ca\(^{2+}\) from 2 mM to 1.71 mM, and free extracellular Mg\(^{2+}\) from 1 mM to 0.88 mM. In Supplementary Figure 1A, the solutions with these lower divalent ion concentrations are called “mimic”. The sustained current evoked by pH 7.2 is enhanced identically by 15 mM lactate and by the mimic solution (Supplementary Figure, Aa), whereas neither affects sustained current at pH 7.0. The mimic solution perfectly reproduces the lactate at each pH tested (Supplementary Figure, Ab). Evidently, the change in divalent ion concentration explains lactate modulation of the sustained ASIC current.

Supplementary Figure 1B asks why lactate and divalent ions broaden the bell shape rather than simply shift it. The action of a large (ten-fold) decrease in Ca\(^{2+}\) on activation and inactivation curves is shown. If the activation and inactivation curves shifted identically, their overlap would simply shift. However, decreased Ca\(^{2+}\) both shifts the activation curve and relieves a channel block, resulting in greater maximal ASIC current. This increases the area of overlap of activation and inactivation (Supplementary Figure 1Ba), thereby broadening the predicted window current (Supplementary Figure 1Bb). 15 mM lactate is more subtle than this, presumably because it causes a smaller
change in divalent concentration.

The variety of acid-evoked currents in cardiac afferents

We previously showed that most acid-evoked current in cardiac afferents from dorsal root ganglia passes through channels containing ASIC3, with the remainder consistent with ASIC1-containing channels. The variety of responses among different cardiac afferents is illustrated in Supplementary Figure 2, which shows a current evoked by pH 5.0 from each of the 30 cardiac sensory neurons that we recorded in this study. This particular stimulus emphasizes the presence of ASIC1, which manifests as a slowly decaying current. Substantial currents were seen at pH 7 in all but the two cells that were acid-insensitive (not shown). Because rat ASIC1 fails to evoke current at pH 7, we take the current at pH 7 to be ASIC3-like. In contrast to the large fraction of cardiac afferents that have currents at pH 7.0, less than 50% of randomly selected sensory neurons exhibit current at pH 7. This emphasizes the unique phenotype of cardiac afferents.

Acid stimulation of action potentials in cardiac sensory neurons

We tested whether small, sustained acid stimuli evoke action potentials in cardiac sensory neurons. Unsurprisingly, results varied widely among the 15 neurons tested. The reason this is unsurprising is that whole cell recording modifies the rest potential in unpredictable ways, and because the seal leak differs among different neurons and this greatly affects threshold measurements. Moreover, action potential threshold is exceedingly sensitive to cell geometry and the soma is a grossly different structure from the nerve ending. Thus, measurements of action potential firing using patch clamp at the soma have questionable relevance to sensory endings.

Nine of 15 cardiac sensory neurons exhibited action potentials in response to 20
second applications of modest pH ranging from 7.2 to 6.8. Four of these exhibited sustained firing, whereas the others fired just at the onset of the increases in acidity. The four are shown in Supplementary Figure 3A. The average number of action potentials increased with decreasing pH (Supplementary Figure 3B).
References


4. Immke DC, McCleskey EW. Protons open acid-sensing ion channels by catalyzing relief of Ca2+ blockade. *Neuron.* 2003;37:75-84.


Legend Supplementary Figure 1. Lactate increases pH sensitivity of sustained ASIC3 current by decreasing divalent ion concentration. A, (a) Sustained ASIC3 currents evoked by either pH 7.2 (upper) or pH 7.0 (lower). 15 mM lactate and a lactate “mimic” solution have identical effects. Control and lactate solutions had 2 mM total Ca\(^{2+}\) and 1 mM Mg\(^{2+}\); the mimic solution had 1.71 mM Ca\(^{2+}\) and 0.88 mM Mg\(^{2+}\), which are the free divalent concentrations expected in a 15 mM lactate solution that has 2 and 1 mM total Ca\(^{2+}\) and Mg\(^{2+}\). (b) Percent change in current evoked by the indicated pH with 15 mM lactate or the mimic solution. B, (a) Dropping extracellular Ca\(^{2+}\) ten-fold has 3 effects: 1) it shifts both the inactivation and activation curves to more basic pH; 2) it increases the maximum current in the activation curve; 3) and it increases the area of overlap (inset). Stars are the values used for normalization. (b) The calculated window currents show a broader, greater window current at the low Ca\(^{2+}\) concentration.

Legend Supplementary Figure 2. Current evoked by stepping pH from 7.4 to 5.0 in the 30 cardiac afferents recorded for this study. A, The 15 cells from which we also obtained voltage and current clamp data using prolonged pH changes (as in Supplementary Figure 3). B, The 15 other cells.

Legend Supplementary Figure 3. A, Four cardiac sensory neurons that exhibited persistent action potentials in response to sustained acid stimuli. Currents evoked by a brief step to pH 5 are shown at left; all are above 10 nA. Currents and action potentials evoked by prolonged stimuli to modest pH are shown at right. B, The average number of action potentials evoked by the indicated pH (firing frequency for the final 15 seconds of a 20 second stimulus).
Supplementary Figure 1

A

\[ \text{activation (2 mM Ca}^{2+}) \]
\[ \text{inactivation (2 mM Ca}^{2+}) \]
\[ \text{activation (0.2 mM Ca}^{2+}) \]
\[ \text{inactivation (0.2 mM Ca}^{2+}) \]

B

\[ \text{15 mM lactate} \]
\[ \text{mimic} \]

\[ \text{pH} \]

\[ \text{% change} \]

\[ \text{pH} \]

\[ \text{0.2 mM Ca}^{2+} \]
\[ \text{2 mM Ca}^{2+} \]
Supplementary Figure 3

A

B

Number of action potentials

7.4  7.2  7.0  6.8  7.4

mV

| 50 pA

0  30  60  90 sec

7.4  7.2  7.0  6.8  7.4

mV

| 50 pA

0  30  60  90 sec

7.4  7.2  7.0  6.8  7.4

mV

| 50 pA

0  30  60  90 sec

7.4  7.2  7.0  6.8  7.4

mV

| 50 pA

0  30  60  90 sec

Number of action potentials

7.2  7.0  6.8

0  5  10

pH

5 nA

1 sec