Molecular pH Probes
Mediators of Angina and Ischemic Preconditioning?

James K. Bubien, Dale J. Benos

Physiology is an old (some today may consider it an ancient) discipline. However, physiology is, at its core, the discipline that merges structure and function in biological organisms. Over the past 30 years, as technology has advanced, the discipline has gravitated to smaller and smaller systems and structures, going from the whole body to the cell, to subcellular structures, and eventually ending up at the genes. The time has come for a reverse in direction so that the knowledge gained from this progression can now be applied to the multitude of medical conditions that exist at a very macroscopic level.

We now have the means to investigate disease mechanisms with technologies that were not available 30 years ago. Physiology has made tremendous progress in advancing this technology. The Nobel Prize for the invention of the patch clamp is one shining example. One of the articles that this commentary reports on uses this technology at its most effective level, and actually resolves the cellular mechanism that underlies the pain associated with ischemic angina.

In contrast to physiology, molecular biology can still be considered to be in its infancy. While the tools of molecular biology are formidable, the understanding of the outcomes is also formidable. Molecular tools can be used to identify gene products, localize these products to specific tissues, and make an attempt to verify that these gene products perform a physiological function. However, the last point has been problematic. Also, because gene products have been observed in multiple tissue types, assigning specific functions to the myriad of proteins being investigated has lacked specificity, leading to a fair amount of ambiguity in the function of any given protein.

Two articles in this issue of Circulation Research reflect these differences, and point out the need to have a greater convergence between these two disciplines. The electrophysiological studies by Yagi et al reach a definitive conclusion and prove a point with scientific rigor. This is physiology at its best. The studies of Barnes et al suggest possibilities, but do not prove any of the hypotheses. Thus these two studies reflect the difference in maturity of the disciplines. It is our hope that by pointing out these differences, a more potent collaboration between physiology and molecular biology can be forged, and that through this collaboration the shortcomings of each discipline can be minimized for the benefit of everyone.

Lactic acid has long been postulated to be a critical mediator of the pH reduction associated with cardiac ischemia. It is also known that sensory neurons that innervate the heart express ion channels that are activated by pH reductions. However, the physiological role of these acid sensing ion channels (ASIC) in neurons had not been proven. The article by Yagi et al describes a systematic electrophysiological study that provides convincing evidence showing that ASIC3 expressed by cardiac sensory neurons plays a major transducing role in the sensation of angina. The main reason that these studies are compelling is that the stimuli for activation of a sustained current through these channels are physiologically appropriate and directly associated with metabolic changes that are known to occur during ischemia. Of particular interest is the high sensitivity of ASIC to lactate-induced acidosis.

Another important facet of the work is the clear demonstration of graded effects with modest decreases in pH. Thus, while pH shifts from 7.4 to 5.0 produce large transient currents, the shift may be too large to be physiologically relevant. However, a pH shift from 7.4 to 6.7 is entirely plausible, and well within the physiological range that occurs during cardiac ischemia. Moreover, because ischemic heart pain is persistent, and individual ASIC currents inactivate, these large transient currents are unlikely to mediate pain sensation. The authors show quite nicely that a modest pH shift produces a significant activation of a sustained ASIC-mediated current.

The sensation of pain is mediated via nerve transmission mainly through modulation of the frequency of action potentials. Although currents activated under voltage clamp conditions can give an idea of what is happening to the cell, the technique still is a voltage clamp and therefore not physiological. In the case of a sustained activation of an ASIC-mediated sodium permeability, one would predict a depolarization of the plasma membrane potential. In a neuron, it is possible that such a depolarization could alter the intrinsic firing rate of the neuron, and therefore modulate the transmission of information. In the case of cardiac sensory neurons, this could certainly result in the sensation of angina pain. However the proof is in the action potential, not the current. The authors (in Figure 6Bc) demonstrate an increase in action potential frequency with changes in pH. This more macroscopic measure is the piece of information that links ion channel function to the physiological outcome, and the
hypothesized role for the channels in angina pain. Few studies of non–voltage-gated ion channels have been able to make such a direct connection between channel function and physiological function. In this article, Yagi et al.\(^1\) provide a clear link between the channel function and the physiological consequence, and for making that connection in a convincing way, the authors have made significant contributions to our understanding of both ASIC function and the cellular mechanisms responsible for angina.

Barnes et al.\(^2\) describe a “novel cardiac adenosine transporter activated by acidic pH.” It is possible that this transporter has some physiological function and could play a role in postinfarct reperfusion injury. However, in contrast to the definitive findings that were integrated from pH change to the generation of angina pain demonstrated by Yagi et al.\(^1\) the Barnes et al.\(^2\) article is only suggestive. Some of the conclusions are based on deductions, and even the specificity of the transporter is unclear in that the protein appears to be able to transport organic cations in general,\(^6,7\) as well as serotonin and adenosine more specifically. Based on these observations, the authors hypothesize that hENT4 is “a dual-function” transporter. But because of this lack of substrate specificity, it is difficult to assign any specific, physiological function to the transporter. A further complication is that the transporter is highly expressed in tissues other than heart, such as brain, intestine, pancreas, kidney, liver, bone marrow, and lymph nodes. It is hard to conclude that a protein that is so widely expressed can play a significant role specifically and exclusively in cardiac reperfusion injury, based only on the observation that at extremely low pH the protein appears to have a reasonable affinity for the transport of adenosine. If this were correct, it begs the question of what the function of the protein is in intestine, kidney, and in lymph nodes. Measuring the relative expression of gene products is a routine part of many studies. The implication is that the more a protein is expressed the more important it must be. Without specific knowledge of a function known to occur in vivo, and proved to be mediated by the gene product being investigated, this assumption is not warranted.

Barnes et al.\(^2\) speculate that ENT4 plays “an important role in ischemic conditions,” that role being to regulate adenosine levels during times of “ischemic preconditioning.” However, there is no direct evidence (ie, no experiments akin to the action potential frequency changes shown by Yagi et al.) to support the authors’ speculation. Barnes et al.\(^2\) state that maximum activity of hENT4 is achieved at a pH of 6.0. However, they also state that the lowest pH observed in ischemic tissue is \(\approx\) 6.6. In contrast to the Yagi et al.\(^1\) data showing the pH induced current changes within the appropriate pH range, and that the ensuing depolarization caused by the increased sodium permeability also occurs in the same range, the observations on ENT4 do not fit precisely. Thus, it remains unclear how much of a role this transporter actually plays in ischemic preconditioning. The authors finish their article by stating “ENT4 represents a possible future therapeutic target for cardiac disease.” One has to ask the questions: What is it a target for? What particular facet of cardiac disease will be targeted? Will ENT4 be inhibited? Will its activity need to be enhanced to be therapeutically relevant? Thus although the observations presented by Barnes et al.\(^2\) are intriguing, they are by no means definitive and proffer no specific answers to normal physiological or pathophysiological processes that can be specifically attributed to ENT4.

The differences between the work of Yagi et al.\(^1\) and Barnes et al.\(^2\) do not arise from differences in scientific rigor of the investigations, but rather they arise from differences in the maturity and breadth associated with the main disciplines used to investigate the two problems. The Barnes et al.\(^2\) study is the first report of its kind. The study of Yagi et al.\(^1\) is the culmination of over 7 years of work investigating the role of ASIC in cardiac ischemia. It is reasonable to expect that it will take a similar amount of time and a similar number of incremental studies on ENT4 before Barnes et al.\(^2\) are able to provide definitive conclusions relating ENT4 to ischemic preconditioning.

**Sources of Funding**

This work was supported by NIH grants DK037206, CA101952, and P50 CA97247.

**Disclosures**

None.

**References**


**Key Words:** ASIC3 • ENT4 • ischemia • angina • ion channels • transporters
Molecular pH Probes: Mediators of Angina and Ischemic Preconditioning?
James K. Bubien and Dale J. Benos

Circ Res. 2006;99:453-454
doi: 10.1161/01.RES.0000241052.33145.54
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/99/5/453

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/