

# Oscillations at odds in the heart

James N. Weiss and Jun-Hai Yang

Division of Cardiology, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA 90095

Oscillations are everywhere in biology—the cell cycle, the heart beat, circadian rhythms, fertility cycles—life could not exist without them. They arise from time delays in the feedback circuits essential for regulating biological processes. As important as oscillations are to normal physiology, however, too many oscillators can spoil the broth of life. In the heart, the master oscillator is the sinus node, to which excitation–contraction coupling and the metabolic machinery supporting cardiac energetics are both entrained. In this issue, Ganitkevich et al. describe how a classical biological oscillator, the glycolytic oscillator, can come into play during ischemia, and in doing so, compound its pathophysiological consequences.

Previous studies have shown that glycolysis is capable of oscillating periodically, thought to be driven by the feedback loop in regulation of the key glycolytic enzyme phosphofructokinase by adenine nucleotides and other metabolites. Glycolytic oscillations have been most extensively characterized in yeast (Higgins, 1964; Hess and Boiteux, 1968), skeletal muscle extracts (Hess and Boiteux, 1971), and pancreatic  $\beta$  cells (Westermarck and Lansner, 2003; Silva and Yunes, 2006). Glycolytic oscillations in isolated cardiac myocytes were first described by O'Rourke et al. (1994), who showed that myocytes deprived of glucose developed oscillations in action potential duration (APD) mediated by activation of sarcolemmal ATP-sensitive K ( $K_{ATP}$ ) channels, metabolic sensors that shorten APD to reduce energy consumption when the ATP/ADP ratio falls. Recently, Yang et al. (2008) defined the conditions promoting glycolytic oscillations in cardiac myocytes more extensively. Using chemical metabolic inhibitors, they found that when the capacity of oxidative phosphorylation and the creatine kinase (CK) system to stabilize the cellular ATP/ADP ratio becomes compromised, glycolysis is enabled to oscillate due to the feedback of adenine nucleotides on phosphofructokinase when the ATP/ADP ratio is no longer clamped by normally dominant aerobic metabolism.

However, in these prior studies (O'Rourke et al., 1994; Ryu et al., 2005; Yang et al., 2008), metabolic

oscillations were induced under physiologically artificial conditions, involving complete glucose removal or chemical metabolic inhibition. Although some of the latter conditions are relevant to acute myocardial ischemia and anoxia, they are not identical to the real thing. The significance of the Ganitkevich et al. (2010) study is their demonstration that glycolytic oscillations occur under conditions directly relevant to clinical cardiac pathophysiology. Their elegant picrochamber technique allows a single isolated patch-clamped myocyte to be imaged with fluorescent dyes during severe anoxia. Because the myocyte is bathed in only a very small volume of extracellular fluid, anoxic metabolites and ions such as lactate, protons, and K accumulate progressively during anoxia. Thus, an isolated myocyte can be patch-clamped and imaged under conditions that recapitulate fairly accurately the features of genuine acute ischemia (anoxia with metabolite and ion accumulation) in intact cardiac muscle.

Under normoxic conditions, mitochondria produce >95% of ATP used by the beating heart, with glycolysis and glycogenolysis generating <5%. High levels of creatine phosphate in heart cells facilitate ATP delivery evenly throughout the cytoplasm by regenerating ADP locally via CK. Adenylate kinase, which converts two ADPs to AMP and ATP, serves a complementary role in preserving a high ATP/ADP ratio throughout the cytoplasm. However, during acute ischemia or anoxia, mitochondrial control of the ATP/ADP ratio is suppressed by lack of oxygen, and CK is progressively inactivated by reactive oxygen species (ROS) and other factors (Mekhfif et al., 1996; Dolder et al., 2001). Anaerobic glycolysis becomes the major source of energy production, despite an inherently limited capacity to meet the full energy needs of the beating heart. The onset of irreversible injury after 20–30 min of acute ischemia coincides with the progressive inhibition of glycolysis due to lactate accumulation and acidosis (Ichihara et al., 1984; Weiss et al., 1996; Geraldles et al., 1997; Schaefer and Ramasamy, 1997; Rehring et al., 1998). During anoxia with maintained coronary perfusion, removal of exogenous glucose as a substrate for glycolysis dramatically

Correspondence to James N. Weiss: jweiss@mednet.ucla.edu

Abbreviations used in this paper: APD, action potential duration; CK, creatine kinase;  $\Delta\Psi$ , mitochondrial membrane potential; ROS, reactive oxygen species.

© 2010 Weiss and Yang. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>).

accelerates cardiac injury (Runnman et al., 1990). These observations suggest that efficient coupling of glycolytic ATP production to cellular energy needs during acute ischemia and anoxia is crucial in protecting cells from irreversible injury. As long as glycolytic flux remains entrained to the heart beat, the myocyte has a chance to regulate energy balance appropriately. If glycolysis begins to oscillate independently, however, the myocyte loses its ability to match cellular energy demands to energy needs, further exacerbating energy supply–demand imbalance and potentially accelerating the onset of irreversible injury.

Ganitkevich et al. (2010) also show convincing evidence that when glycolysis oscillates under these conditions, the mitochondrial network secondarily oscillates in response. As glycolytic ATP production waxes, mitochondrial  $F_1F_0$  ATP synthase reverses and consumes ATP to support mitochondrial membrane potential ( $\Delta\Psi$ ). Conversely, as glycolytic ATP production wanes, mitochondria are less able to maintain  $\Delta\Psi$  and partially depolarize. The metabolic oscillations affect both cellular- and tissue-level functions. At the cellular level, consequences to excitation–contraction coupling are mediated through the activation of  $K_{ATP}$  channels, which shorten APD and depress the Ca transient when glycolytically derived ATP levels are low phase, but lengthen APD and increase Ca transient amplitude when glycolytic ATP production is in high phase (O’Rourke et al., 1994). The latter phase inappropriately accelerates energy requirements and may exacerbate intracellular Ca overload, a known factor promoting injury. At the tissue level, APD shortening during metabolic oscillations can lead to “metabolic sinks” that promote arrhythmias (Akar et al., 2005).

As multifunctional signaling proteins, glycolytic enzymes also influence cardioprotective signaling (Juhaszova et al., 2004). Evidence linking glycolysis to cardioprotection includes the finding that elevated extracellular glucose delays injury by preserving glycogen stores without improving overall high energy phosphate levels (Runnman et al., 1990), that the glycogenolytic enzyme GSK-3 $\beta$  is a key target protein mediating cardioprotective signaling (Juhaszova et al., 2004), and that enhanced glucose oxidation is cardioprotective (Lopaschuk and Stanley, 2006).

These observations raise the intriguing possibility that by inducing excitation–contraction–metabolism uncoupling, glycolytic oscillations may promote injury during ischemia and anoxia. In addition to glycolysis, the mitochondrial network can also escape its entrainment under conditions of increased oxidative stress (Romashko et al., 1998; Brady et al., 2004). In this setting, mitochondria become prone to periodic, cell-wide  $\Delta\Psi$  depolarization, which converts mitochondria into ATP consumers rather than producers, inducing  $K_{ATP}$  channel activation and APD shortening. The latter

oscillations are thought to be mediated by mitochondrial ROS–induced ROS release, involving either inner membrane anion channels (Romashko et al., 1998) or mitochondrial permeability transitions pores (Brady et al., 2004). Because of the requirement for ROS generation, mitochondrial  $\Delta\Psi$  oscillations are more likely to occur during reperfusion than ischemia, when reoxygenation stimulates a burst of ROS production. However, the evidence for mitochondrial  $\Delta\Psi$  oscillations during genuine ischemia is still indirect (Akar et al., 2005).

In summary, when excitation–contraction–metabolism uncoupling occurs, rebellious metabolic oscillators become potentially important factors compounding ischemia reperfusion injury in the heart. The study by Ganitkevich et al. (2010) documents that one form of these metabolic oscillations, arising from glycolysis, is directly relevant to acute anoxia and ischemia in isolated cardiac myocytes. The next frontier is to determine whether such metabolic oscillations can be detected during acute ischemia in the intact heart, and if so, whether they play a role in hastening cell death. Detection of metabolic oscillations in intact tissue is technically challenging. Due to electrotonic coupling, APD in tissue represents the average of thousands of cells, so that unless the phase of the underlying cellular metabolic oscillations is synchronized in a region of tissue, APD would not appear to oscillate. Attempts to directly visualize metabolic oscillations during acute ischemia or anoxia in intact cardiac muscle using confocal imaging have so far been unrevealing (Matsumoto-Ida et al., 2006). Moreover, changes in  $\Delta\Psi$  or NADH accompanying glycolytic oscillations are expected to be small. New genetically encoded fluorescent ATP/ADP sensors (Berg et al., 2009; Imamura et al., 2009) may be the ideal bioprobes to detect whether metabolic oscillations become at odds with the heart beat during acute ischemia/reperfusion in intact heart. If so, the relationship of such oscillations to cardioprotective signaling will become an interesting topic for study.

This work was supported by NIH grants 1P01 HL078931 and R01 HL095663.

Edward N. Pugh Jr. served as editor.

## REFERENCES

- Akar, F.G., M.A. Aon, G.F. Tomaselli, and B. O’Rourke. 2005. The mitochondrial origin of postischemic arrhythmias. *J. Clin. Invest.* 115:3527–3535. doi:10.1172/JCI25371
- Berg, J.Y., Y.P. Hung, and G. Yellen. 2009. A genetically encoded fluorescent reporter of ATP:ADP ratio. *Nat. Methods.* 6:161–166. doi:10.1038/nmeth.1288
- Brady, N.R., S.P. Elmore, J.J. van Beek, K. Krab, P.J. Courtoy, L. Hue, and H.V. Westerhoff. 2004. Coordinated behavior of mitochondria in both space and time: a reactive oxygen species-activated wave of mitochondrial depolarization. *Biophys. J.* 87:2022–2034. doi:10.1529/biophysj.103.035097

- Dolder, M., S. Wendt, and T. Wallimann. 2001. Mitochondrial creatine kinase in contact sites: interaction with porin and adenine nucleotide translocase, role in permeability transition and sensitivity to oxidative damage. *Biol. Signals Recept.* 10:93–111. doi:10.1159/000046878
- Ganitkevich, V., V. Mattea, and K. Benndorf. 2010. Glycolytic oscillations in single ischemic cardiomyocytes at near anoxia. *J. Gen. Physiol.* 135:307–319.
- Geraldes, C.F., M.M. Castro, A.D. Sherry, and R. Ramasamy. 1997. Influence of vanadate on glycolysis, intracellular sodium, and pH in perfused rat hearts. *Mol. Cell. Biochem.* 170:53–63. doi:10.1023/A:1006899932108
- Hess, B., and A. Boiteux. 1968. Mechanism of glycolytic oscillation in yeast. I. Aerobic and anaerobic growth conditions for obtaining glycolytic oscillation. *Hoppe Seylers Z. Physiol. Chem.* 349:1567–1574.
- Hess, B.A.B., and A. Boiteux. 1971. Oscillatory phenomena in biochemistry. *Annu. Rev. Biochem.* 40:237–258. doi:10.1146/annurev.bi.40.070171.001321
- Higgins, J. 1964. A chemical mechanism for oscillation of glycolytic intermediates in yeast cells. *Proc. Natl. Acad. Sci. USA.* 51:989–994. doi:10.1073/pnas.51.6.989
- Ichihara, K., N. Haga, and Y. Abiko. 1984. Is ischemia-induced pH decrease of dog myocardium respiratory or metabolic acidosis? *Am. J. Physiol.* 246:H652–H657.
- Imamura, H.K., K.P. Nhat, H. Togawa, K. Saito, R. Iino, Y. Kato-Yamada, T. Nagai, and H. Noji. 2009. Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. *Proc. Natl. Acad. Sci. USA.* 106:15651–15656. doi:10.1073/pnas.0904764106
- Juhaszova, M., D.B. Zorov, S.H. Kim, S. Pepe, Q. Fu, K.W. Fishbein, B.D. Ziman, S. Wang, K. Ytrehus, C.L. Antos, et al. 2004. Glycogen synthase kinase-3 $\beta$  mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J. Clin. Invest.* 113:1535–1549.
- Lopaschuk, G.D., and W.C. Stanley. 2006. Malonyl-CoA decarboxylase inhibition as a novel approach to treat ischemic heart disease. *Cardiovasc. Drugs Ther.* 20:433–439. doi:10.1007/s10557-006-0634-0
- Matsumoto-Ida, M., M. Akao, T. Takeda, M. Kato, and T. Kita. 2006. Real-time 2-photon imaging of mitochondrial function in perfused rat hearts subjected to ischemia/reperfusion. *Circulation.* 114:1497–1503. doi:10.1161/CIRCULATIONAHA.106.628834
- Mekhf, H., V. Veksler, P. Mateo, V. Maupoil, L. Rochette, and R. Ventura-Clapier. 1996. Creatine kinase is the main target of reactive oxygen species in cardiac myofibrils. *Circ. Res.* 78:1016–1027.
- O'Rourke, B., B.M. Ramza, and E. Marban. 1994. Oscillations of membrane current and excitability driven by metabolic oscillations in heart cells. *Science.* 265:962–966. doi:10.1126/science.8052856
- Rehring, T.F., J.I. Shapiro, B.S. Cain, D.R. Meldrum, J.C. Cleveland, A.H. Harken, and A. Banerjee. 1998. Mechanisms of pH preservation during global ischemia in preconditioned rat heart: roles for PKC and NHE. *Am. J. Physiol.* 275:H805–H813.
- Romashko, D.N., E. Marban, and B. O'Rourke. 1998. Subcellular metabolic transients and mitochondrial redox waves in heart cells. *Proc. Natl. Acad. Sci. USA.* 95:1618–1623. doi:10.1073/pnas.95.4.1618
- Runnman, E.M., S.T. Lamp, and J.N. Weiss. 1990. Enhanced utilization of exogenous glucose improves cardiac function in hypoxic rabbit ventricle without increasing total glycolytic flux. *J. Clin. Invest.* 86:1222–1233. doi:10.1172/JCI114828
- Ryu, S.-Y., S.-H. Lee, and W.-K. Ho. 2005. Generation of metabolic oscillations by mitoKATP and ATP synthase during simulated ischemia in ventricular myocytes. *J. Mol. Cell. Cardiol.* 39:874–881. doi:10.1016/j.yjmcc.2005.08.011
- Schaefer, S., and R. Ramasamy. 1997. Glycogen utilization and ischemic injury in the isolated rat heart. *Cardiovasc. Res.* 35:90–98. doi:10.1016/S0008-6363(97)00087-4
- Silva, A.S., and J.A. Yunes. 2006. Conservation of glycolytic oscillations in *Saccharomyces cerevisiae* and human pancreatic beta-cells: a study of metabolic robustness. *Genet. Mol. Res.* 5:525–535.
- Weiss, R.G., C.P. de Albuquerque, K. Vandegaer, V.P. Chacko, and G. Gerstenblith. 1996. Attenuated glycogenolysis reduces glycolytic catabolite accumulation during ischemia in preconditioned rat hearts. *Circ. Res.* 79:435–446.
- Westermarck, P.O., and A. Lansner. 2003. A model of phosphofructokinase and glycolytic oscillations in the pancreatic beta-cell. *Biophys. J.* 85:126–139. doi:10.1016/S0006-3495(03)74460-9
- Yang, J.H., L. Yang, Z. Qu, and J.N. Weiss. 2008. Glycolytic oscillations in isolated rabbit ventricular myocytes. *J. Biol. Chem.* 283:36321–36327. doi:10.1074/jbc.M804794200