Oscillations at odds in the heart

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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 β cells (Westermark 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 picochamber 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. Adenlyate 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 (Mekhfi 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; Geraldes 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

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

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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_1-F_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-3β 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.

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