Cardiac responses to hypoxia and reoxygenation in Drosophila. New insights into evolutionarily conserved gene responses. Focus on “Cardiac responses to hypoxia and reoxygenation in Drosophila”

James T. Pearson

Monash Biomedical Imaging Facility, Melbourne, Australia, Department of Physiology, Monash University, Melbourne, Australia; and Australian Synchrotron, Melbourne, Australia

Submitted 29 September 2015; accepted in final form 29 September 2015

A SIMPLE HEART TUBE PROPELS hemolymph in the circulatory system of fruit fly Drosophila spp., and yet we find there is a great deal we can learn from these invertebrates regarding the regulation of cardiac contractility in the four-chambered hearts of mammals and how their myocardium responds to hypoxia. Indeed, in this issue of the American Journal of Physiology Regulatory, Integrated and Comparative Physiology, we learn that the cardiac responses of Drosophila to hypoxia depend greatly on time scale (10) and that Drosophila offer physiologists new insights into evolutionarily conserved gene responses.

The cardiovascular response to hypoxia in mammals has been shown to be complex and highly dependent on the severity of hypoxia and its duration. Importantly, the cardiovascular response is closely linked to changes in respiration (7). Acute exposure to low oxygen acts rapidly to directly modulate cardiac contractility and heart rate through protective oxygen-sensing mechanisms involving the peripheral nervous system. However, neurally mediated responses also interact with and frequently mask the local effects of continuous hypoxia on cellular function, integrity, and survival, making it difficult to dissociate extrinsic and local influences on the myocardium. Many vertebrates can routinely experience exposure to very low oxygen levels that vary from transient anoxia to sustained hypoxia associated with living in high-altitude environments. In humans, cardiopulmonary disease often evokes hypoxemia or ischemia resulting in tissue hypoxia, which can vary from being intermittent, sustained, or even chronic in duration. The changes in cardiac function that take place during continuous or sustained hypoxia have been shown to involve hypoxia-inducible factors (HIF), which are master transcription factors mediating a great many protective responses associated with oxygen homeostasis (8).

HIFs are transcription factors that are heterodimers comprising a constitutively expressed β-subunit and a hypoxia-inducible α-subunit. Three isoforms of the α-subunit are expressed (HIF-1α, HIF-2α, HIF-3α) in mammals, but only HIF-1α is ubiquitous. In insects, a single isoform encoded by sima is homologous with HIF-α-subunits, indicative of the highly conserved nature of oxygen sensing in metazoans (1). Under normoxic conditions, expressed HIF-1α protein is quickly degraded by ubiquitination and proteasome activity. However, negative regulation of HIF-1α expression is suppressed during hypoxia through stabilization of the oxygen-dependent degradation domain (ODD), leading to increased translocation to the nucleus and enhanced activation of HIF-1α target genes that are responsible for the response to hypoxia (3, 4). HIF-1α activates transcription of these genes by binding to a hypoxia response element (HRE) (8). Following sustained hypoxia, HIF-1α mediates changes such as promotion of glucose metabolism, erythropoiesis, angiogenesis, apoptosis, proliferation, and vascular remodeling. Many of these changes are generally believed to conserve ATP or act to promote vasculization and oxygen delivery to tissues. Zarndt et al. (10) now provide evidence to support a role for sima signaling in changes in heart rate and contractility in the core response to hypoxia.

Moreover, suppression of the contractions of the heart tube myofibrils during hypoxia by sima was demonstrated to be independent of neural and vascular changes in Drosophila by virtue of the characteristics of their model organism. That is, the authors utilized denervated preparations, and insects do not rely on their open circulatory system to satisfy the metabolic requirements of the heart tube.

Zarndt et al. (10) show with a video microscopy approach developed by the group that the cardiac response in wild-type, sima heterozygote and homozygote mutant Drosophila to moderate and severe hypoxia upon reoxygenation very much depends on the duration of hypoxia. Although the literature has predominantly shown that HIF-1α is important in the core hypoxia response when hypoxia is sustained for several hours, somewhat surprisingly, the authors reveal that the rapid reduction in fractional shortening during acute hypoxia (30 min at 1% O2) is partially dependent on sima, and, therefore, hypoxia-dependent HIF-α signaling (Fig. 2 in Ref. 10). In contrast, as hypoxia becomes sustained (18 h, 1% O2) or chronic in duration (3 wk, 4% O2) HIF-α signaling is responsible for suppressing heart rate and potentiating contractility in the flies. Measurements of the heart tube lumen dimensions reveal that hypoxia-induced sima effects are more pronounced in diastole, as relaxation time is increased, while contraction time is unaltered by the HIF-α signaling pathways (10). Reductions in fractional shortening were exaggerated by haploinsufficiency of sima (Figs. 3 and 4 in Ref. 10), while factors other than sima are responsible for constriction of the heart tube following sustained/chronic hypoxia and reoxygenation for hours to days in wild-type Drosophila. This suggests that when hypoxia becomes prolonged, HIF-α signaling acts to poten-
tiate contractility of the heart tube, in counter balance to the direct negative inotropic effect of hypoxia in *Drosophila*. Interestingly, when the authors examined the hypoxia responses in flies that were selected for survival at reduced oxygen levels over many generations, they found only a small but significant reduction in fractional shortening at 4% O₂ compared to control flies, three genetically distinct populations of outbred wild-type flies maintained at 21% O₂. However, the constriction of the heart tubes throughout the cardiac cycle and the increase in diastolic time interval were more striking in the hypoxia-selected flies (10). Myofibrillar organization was not affected by sustained or chronic hypoxia and reoxygenation, and yet hypoxia selection not only resulted in a smaller than expected heart tube (corrected for body size), but also more widespread abdominal fibrosis and myofibrillar disorganization. It is not known from this study whether *sima* induction target genes are more activated in hypoxia-selected flies. Zarndt et al. (10) in their article suggest that their findings, earlier studies involving HIF-1α and/or HIF-2α manipulation in mice, and investigations of HIF-1α in ischemia-reperfusion injury in humans, point toward a protective role for HIF signaling during acute and sustained hypoxia, but a maladaptive role when exposure to hypoxia becomes chronic. Thus, genetically amenable models such as *Drosophila* can aid in understanding fundamental mechanisms in signaling pathways, such as HIF, which are highly conserved across kingdoms.

Perhaps the most tangible advantages of the research approach of Zarndt et al (10) with *Drosophila* are that the combination of genetic recombination tools and high-speed optical imaging of beating hearts allows for introduction of this technique into laboratories at most institutes, and it will enable the study of other HRE and target genes in the HIF signaling pathways. The imaging approach developed by the authors (2) permits genetic dissection of heart function in semi-intact denervated preparations utilizing a microscope with an immersion lens and little other specialized equipment, in contrast to optical coherence tomography, which can also be used in *Drosophila* studies. Robust algorithms available in a freeware software package permit semi-automated tracking of heart rhythms and contraction-relaxation parameters. Although these authors (2, 10) advocate dissected preparations to remove the confounding influences of the insect nervous system, it can equally be argued that the study of intact flies might provide important information regarding the genetics of cardiac function. In the study of Tricoire et al. (9), cardiac imaging of *Drosophila* was achieved through the intact cuticle with the aid of RNA interference to introduce a inducible gene-switch driver to express green fluorescence protein specifically in the heart. The end result in this case was that it was possible to image cardiac function in anesthetized flies to study mitochondrial respiratory chain dysfunction in a *Drosophila* model of Freidreich’s ataxia. Both intact and semi-intact preparations are likely to provide new insights into the fundamental physiology of the heart.

It is noteworthy that HIF-1α transcription stimulates hundreds of genes that act to promote cell survival in response to hypoxia and during ischemia-reperfusion injury (8). Therefore, cardiac imaging of *Drosophila* might offer a time-efficient and fruitful means of examining the many genes that are activated by prolonged hypoxia or ischemia and their effects on cardiac function. For example, HIF-1α activation stimulates an increase in the protein levels of frataxin in the mitochondria of cardiomyocytes (6). The frataxin promoter has a HRE. Although the function of frataxin remains to be fully identified, it is clear that it is important for iron homeostasis in the mitochondrion, and it plays a role in regulating oxidative phosphorylation of enzymes and preventing reactive oxygen species (ROS) generation by acting as an iron storage protein. Frataxin might be an important antioxidant mechanism against ROS generation caused by excess iron accumulation in the mitochondria. Recently, Nanayakkara et al (6) showed that HIF-1α is responsible for this frataxin-mediated action, and HIF-1α activation prevents mitochondrial permeability transition pore opening and cardiomyocyte death in both hypoxia and ischemia-reperfusion injury of the mouse heart. Those authors were unable to establish the role of HIF-1α/frataxin signaling in the modulation of in vivo cardiac function. With the ability now to directly investigate changes in cardiac function and cardiac remodeling with a simple imaging approach, the *Drosophila* homologs of HIF-α and frataxin could prove important targets for future research. It is not inconceivable that a hypoxia fate-mapping approach (5) could be employed to irreversibly label hypoxic cells with a fluorescent protein marker to trace the lineage of cells exposed to prolonged hypoxia and, thereby, further characterize the mechanisms that determine HIF-dependent cardiac remodeling.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: J.T.P. drafted manuscript; J.T.P. edited and revised manuscript; J.T.P. approved final version of manuscript.

**REFERENCES**


