Bradycardia in zebrafish heart failure: a true physiological response or anesthetic-induced red herring?

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Bradycardia in zebrafish heart failure: a true physiological response or anesthetic-induced red herring?

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Dear Editor,

In the human heart, chronic anemia induces a hyperdynamic state due to volume overload, which, over time, predisposes to cardiac chamber enlargement and contractile dysfunction.\(^1\)

In their recent article, Ernens \textit{et al.} reported that similar responses to anemia occur in the zebrafish heart.\(^2\) We note with interest that Ernens \textit{et al.} found that heart rate was depressed, in contrast to the relative tachycardia that is usually present in anemia-associated human heart failure.\(^1\) These findings raise a thought-provoking conundrum regarding the cause of bradycardia and its impact on cardiac function in this zebrafish model.

One possibility is that bradycardia represents a primary response to heart failure. Since cardiac output = heart rate × stroke volume, an adaptive increase in heart rate serves as an important physiological response to maintain cardiac output in settings of contractile dysfunction. A true bradycardia would therefore accelerate a decline in overall cardiac performance in low cardiac output states where myocardial contractile function is already compromised. The possibility that zebrafish are incapable of developing a compensatory increase in heart rate seems unlikely, however, given that tachycardia has been documented in a heart failure model associated with a sarcomere protein mutation.\(^3\) Moreover, in our hands, we have noted a dose-dependent tachycardia during epinephrine administration in wild-type \textit{TE} fish aged 6 months (Fig. 1A, 1B), demonstrating that zebrafish are able to mount an appropriate chronotropic response to increased sympathetic stimulation.

Another possibility is that bradycardia might be indicative of advanced disease and a decompensated state. Phenylhydrazine (2.5 µg/mL) results in profound hemolytic anemia (relative hemoglobin <10% that of untreated animals).\(^2,4\) It is notable that Sun \textit{et al.}, who first reported the phenylhydrazine protocol used by Ernens \textit{et al.}, observed an initial tachycardia in unanesthetized \textit{tr265/tr265} transgenic fish with severe anemia at 5 days and 10 days of..
age, but bradycardia from 3 weeks.\textsuperscript{4} Interestingly, this time-point also coincided with the need for anesthesia to facilitate measurements.

Anesthetic agents and temperature are known to influence heart rate during echocardiography,\textsuperscript{5,6} and Ernens \textit{et al}. suggested that the dose of anesthetic used (tricaine methane-sulfonate, 160 mg/L), and/or the performance of their studies at room temperature, may have contributed to their heart rate results.\textsuperscript{2} These factors would apply equally to wildtype and anemic fish, however, and thus do not explain the observed differences between groups.\textsuperscript{2} A parsimonious explanation is that anemic zebrafish have a substantially greater susceptibility than healthy zebrafish to cardiodepressant effects of anesthesia. We also found that a similar dose of tricaine caused bradycardia in anemic zebrafish, with progressive reductions of heart rate occurring with increasing duration of anesthetic exposure (Fig. 1C, 1D).

Optimizing anesthetic regimens to gain adequate sedation and image acquisition at the same time as minimizing side effects is challenging, and the use of anesthesia during echocardiography may confound interpretation of the underlying disease phenotype. It should be noted that in addition to negative chronotropic effects, anesthetic drugs can also directly depress myocardial contraction. Collectively, these considerations argue for a critical need to use minimum doses of anesthetic drugs when performing \textit{in vivo} cardiac phenotype analysis, particularly in zebrafish heart failure models.

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Disclosure Statement

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References


Figure Legend

**FIG. 1.** Heart rate responses to epinephrine and anesthesia. Heart rate was measured by observing cardiac contraction over a 15 s period on B-mode echocardiography which was performed at room temperature (25°C) in male wild-type TE fish anesthetized using tricaine methane-sulfonate 200 mg/L (Sigma-Aldrich, St. Louis, MO, USA).  (A) Scatter plots showing heart rate at baseline and following sequential exposure (15 min each step) to epinephrine (Sigma-Aldrich), 100 µM then 500 µM, in fish aged 6 months (n=10). (B) Bar graphs showing mean increment in heart rate compared with baseline following increasing doses of epinephrine. (C) Scatter plots showing changes in heart rate with increasing time after induction of anesthesia in fish aged 3 months (n=8) that were studied at the end of an 18-day hemolytic anemia protocol. (D) Bar graphs showing mean reduction in heart rate compared with baseline following increasing anesthetic time. Experiments were approved by the local institutional animal ethics committee. Data shown are mean ± s.e.m. ** P<0.01; *** P<0.001, paired t test, two-tailed.
Figure 1

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