

**PRECONDITIONING AND ANOXIA TOLERANCE OF THE TROUT
HEART: NON-ADDITIVE EFFECTS AND THE ASSESSMENT OF
MYOCARDIAL DAMAGE.**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Although interspecific variation in myocardial hypoxia-tolerance has been extensively studied in fishes (Driedzic and Gesser, 1994), intraspecific differences have received little attention. In general, rainbow trout are considered to be a hypoxia-sensitive species. However, in recent years, we have identified groups of rainbow trout that vary greatly in their hypoxia-tolerance, and have used these fish to examine the relationship between inherent hypoxia-tolerance and preconditioning. Preconditioning is a phenomenon whereby a brief insult or stimulus to the heart greatly diminishes myocardial damage following a subsequent prolonged bout of hypoxia and/or ischemia, and the mammalian literature suggests that only hypoxia-sensitive hearts can be preconditioned; ie. these mechanisms of myocardial hypoxic protection are not additive.

This talk will focus on preconditioning experiments performed on hypoxia-sensitive trout hearts at Simon Fraser University (SFU) (Gamperl *et al.*, 2001) and on hypoxia-tolerant hearts at both Portland State University and SFU, and will incorporate recent data that questions whether both loss of function and cell death (irreversible damage) can be used as criteria to assess myocardial damage following severe hypoxic/anoxic exposure in fishes.

Methods

In situ heart preparations (Farrell *et al.*, 1986) at 10°C were used to determine myocardial hypoxia tolerance in three stocks of hatchery-reared rainbow trout, and to examine whether hearts from each stock could be preconditioned. In these experiments, the preconditioning stimulus was one or two brief (5 min) anoxic periods, and functional damage was assessed by comparing cardiac performance after and prior to experimental manipulations.

Irreversible myocardial damage (cell death) was evaluated by a number of techniques including: 1) measurement of creatine kinase (CK) activity, lactate dehydrogenase (LDH) activity and [myoglobin] in the perfusate being pumped by the *in situ* hearts at 5 min to 1h post-anoxia; 2) measurement of myocardial LDH activity in *in situ* hearts following reperfusion; and 3) incubation of small (4-6 mg) myocardial pieces with MTT (3-[4,5]-dimethylthiazol-2,5-diphenyltetrazolium bromide).

Results and Discussion

In the three groups of trout examined, there was a significant degree of variation in inherent-anoxia tolerance. Maximum cardiac function in the hearts used by Gamperl *et al.* (2001) was reduced by approx. 20-25% after just 15 minutes of anoxia (with only 5 min at physiological output pressure, 50 cm H₂O). In contrast, comparable reductions in myocardial function could only be achieved at PSU, and more recently at SFU, by significantly increasing both the duration of anoxia (to 20 or 30 min) and the amount of work performed by the heart during anoxia (by maintaining output pressure at 50 cm H₂O and/or attempting to maintain resting a cardiac output of 16 ml min⁻¹ kg⁻¹). Thus, a significant amount of intra-specific variation in myocardial hypoxia tolerance exists between populations (groups) of hatchery-reared rainbow trout. Although the reasons for this are unknown, water quality during rearing is likely to play a predominant role.

Of the three stocks of rainbow trout examined, it was clear that only the hypoxia-sensitive hearts used by Gamperl *et al.* (2001) could be preconditioned by anoxic pre-exposure. For example, although these authors report that preconditioning completely eliminated the myocardial dysfunction associated with 15 min of anoxia, the hypoxia-tolerant hearts tested at PSU were not protected, and appeared to be damaged further, by an identical preconditioning stimulus (Fig. 1). These results agree with studies on neonatal mammals (e.g.

Ostadal *et al.*, 1999; Baker *et al.*, 1999) which show that hearts with an innate level of myocardial hypoxia tolerance cannot be preconditioned (i.e. the protection afforded by inherent hypoxia-tolerance and preconditioning are not additive), and suggest that the cellular pathways and/or effectors that confer protection against ischemic/hypoxic damage are already maximally stimulated in hypoxia tolerant hearts.

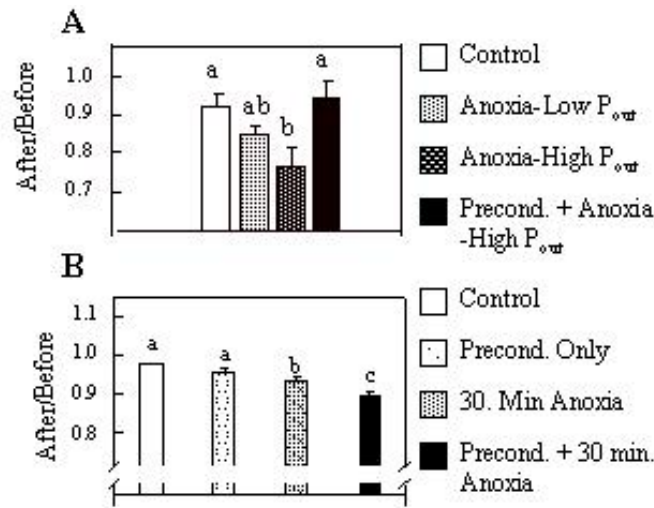
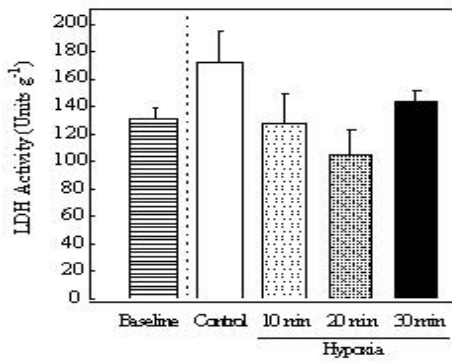


Figure 1. Effect of 5 min. of anoxic pre-exposure (preconditioning) on the recovery of maximum stroke volume in the hypoxia-sensitive hearts from Gamperl *et al.* (2001) (A) and the hypoxia-tolerant hearts used at PSU (B). For the hearts used in Gamperl *et al.* (2001), the main anoxic period was only 15 min. In both experiments, control hearts were only exposed to normoxic perfusate. Dissimilar letters indicate significant differences between groups ($P < 0.05$, one-way ANOVA). $N = 7 - 9$

To this point, none of the biochemical markers of myocardial damage that we have used has proven satisfactory for quantifying the degree of irreversible cell death in *in situ* hearts following anoxic exposure: LDH, CK and myoglobin were not detectable in the perfusate; no significant differences in myocardial LDH levels were found in trout hearts exposed to 0, 10, 20 or 30 min. of anoxia (maximum degree of functional damage approx. 20%) (Fig. 2A); and

preliminary experiments with MTT failed to reveal any differences in tissue damage between control and anoxia-exposed hearts. However, these experiments (Fig. 2B) with MTT staining of myocardial pieces incubated under various conditions suggest that the MTT assay works, and that trout myocardial cells may not die/become irreversibly damaged for some time even when exposed to prolonged (> 5 hrs) anoxia.

A



B

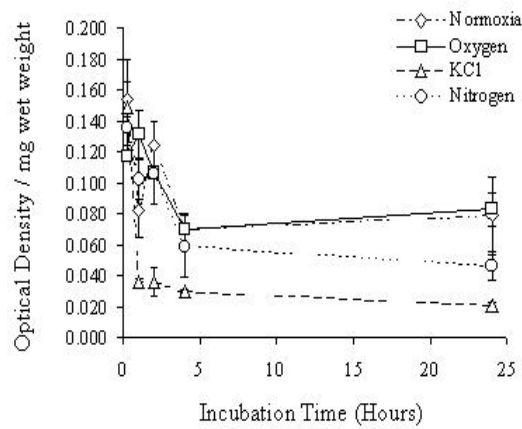


Figure 2. Biochemical assessment of myocardial cell death (irreversible damage) in the trout heart. (A) LDH activity in trout hearts exposed to

normoxia, or anoxia of increasing duration (N = 7 – 8). **(B)** MTT staining in myocardial pieces [4 – 6 mg; N = 1 (3 sub-samples per group)] exposed to saturated KCl for 10 min, or exposed to various levels of perfusate oxygenation. Note: Since the loss of MTT staining in the 3 non-KCl groups was identical during the first 4 h of incubation, we believe this represents mechanical damage related to cutting the myocardium into such small pieces.

When combined, our experiments suggest that: 1) the trout heart is merely “stunned” following brief periods of anoxic (severe hypoxic) exposure; and 2) that myocardial cell death only results following anoxic/severe hypoxic periods of several hours. These results are in contrast to those obtained with the mammalian heart, but not surprising given that trout generally live in cool water and that most of the trout heart is perfused with venous blood whose PO₂ can be reduced to < 10 mm Hg during intense exercise or aquatic hypoxia.

References

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