

**MITOCHONDRIAL ATP-SENSITIVE K⁺ CHANNELS (K_{ATP})
INFLUENCE FORCE DEVELOPMENT AND ANOXIC
CONTRACTILITY IN A FLATFISH, YELLOWTAIL FLOUNDER
(*Limanda ferruginea*), BUT NOT ATLANTIC COD (*Gadus morhua*)
HEART.**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Flatfishes often inhabit hypoxic waters and are known to exhibit substantial tolerance to cardiac acidosis and hypoxia. Studies on flounder suggest that flatfish have an atypical cardiovascular response to hypoxia. Winter flounder subjected to hypoxia exhibit increased cardiac output with no bradycardia (Cech et al., 1977). In isolated ventricular muscle from the European flounder, cardiac force development is initially potentiated following exposure to acidosis, before slowly declining over time (Gesser and Poupa, 1979). Gesser and Poupa have proposed that intracellular acidosis may trigger a release of stored mitochondrial Ca²⁺, subsequently enhancing force production.

Adenosine 5'-triphosphate sensitive potassium (K_{ATP}) channels have been identified in goldfish hearts (Ganim et al., 1998). K_{ATP} channels are activated by a decline in energy status and are most likely to affect cardiac function throughout periods of impaired ATP production, such as hypoxia. In mammalian heart K_{ATP} channels exist on the sarcolemmal membrane (sK_{ATP}) and inner

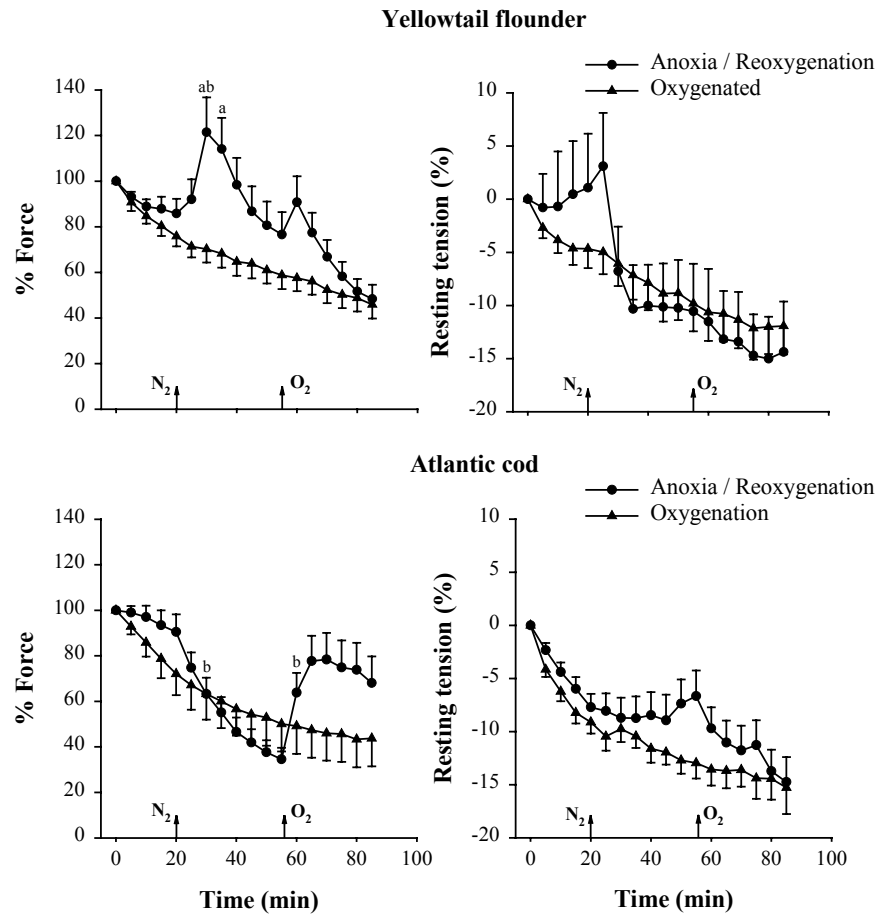
mitochondrial membrane (mK_{ATP}) and their activity has been linked with the cardioprotection afforded by preconditioning. sK_{ATP} channels facilitate cellular K^+ efflux while mK_{ATP} channels allow mitochondrial K^+ influx.

The objective of this study was to evaluate the possible involvement of K_{ATP} channels in hypoxic force potentiation in the yellowtail flounder heart, and to investigate their role in cardiac performance during anoxia and reoxygenation. Atlantic cod were chosen for comparisons as this species is considered to have poor cardiac anoxia tolerance. The contribution of K_{ATP} channels to heart performance during anoxia and reoxygenation was studied using isometrically contracting ventricular muscle preparations and pharmacological agents targeting sarcolemmal and mK_{ATP} channel activity.

Results

Force production in ventricular muscle from flounder is potentiated at the onset of anoxia, while force immediately declines in cod preparations (Fig. 1). Data on flounder preparations treated with agents to alter K_{ATP} activity are presented in Figure 2. Glibenclamide, a general K_{ATP} blocker, impaired oxygenated force development in flounder heart but was without effect on cod (data not shown). The mK_{ATP} specific blocker 5-hydroxydecanoic acid (5HD) improved oxygenated force production in yellowtail flounder heart without influencing contractility during anoxia or reoxygenation. The specific mK_{ATP} agonist diazoxide tended to preserve resting tension and significantly eliminated anoxic force potentiation in flounder preparations. Neither 5HD nor diazoxide affected contractility in cod preparations (data not shown).

Figure 1. Twitch force and resting tension for ventricular preparations from yellowtail flounder and Atlantic cod exposed to oxygenated conditions (flounder n = 6, cod n = 5) and to 35 min of anoxia followed by reoxygenation (n = 10, both species). a - significant difference between treatments. b - significant change from measurements within the treatment taken 5 min earlier.



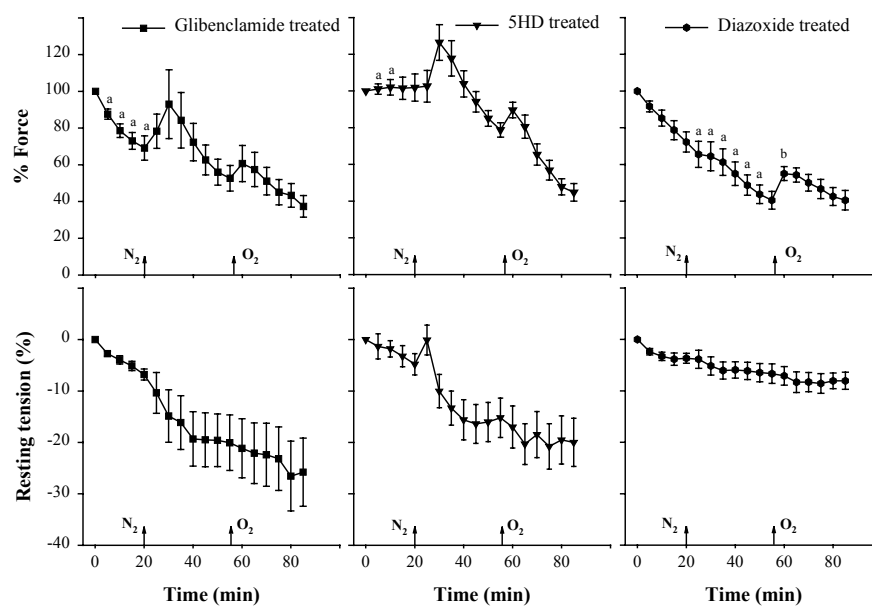
Discussion

Results suggest the presence of mK_{ATP} channels in ventricular muscle of yellowtail flounder. Under anoxic conditions, diazoxide eliminated the transient elevation in force development and stabilised resting tension. Acute activation of mK_{ATP} channels with diazoxide has been shown to depolarise the inner mitochondrial membrane in rat heart, leading to a rapid reduction in mitochondrial Ca^{2+} and inhibited Ca^{2+} uptake (Holmuhamedov et al., 1999). We suggest that in flounder heart, diazoxide releases mitochondrial Ca^{2+} more slowly than in rat heart due to the low temperature (6 °C) utilised in this experiment. Following a period of diazoxide treatment, mitochondrial Ca^{2+} content should already be reduced, so that when subjected to anoxia, force potentiation resulting from a bolus release of Ca^{2+} would be eliminated. If diazoxide does trigger a gradual release of mitochondrial Ca^{2+} to the cytoplasm, it may act to protect $[Ca^{2+}]_i$ during anoxia and overcome a net loss in activity, leading to the observed preservation of resting tension in diazoxide treated preparations.

Altering K_{ATP} channel activity in cod ventricle strips did not affect force development or resting tension under any of the conditions tested (data not shown). The data suggest that Atlantic cod do not have cardiac K_{ATP} channels sensitive to the pharmacological agents used, or that all of the factors needed to alter channel activity are not present in this tissue. Evolutionary differences within teleost fishes, and between fish and mammals may influence the sensitivity of K_{ATP} channels to pharmacological manipulation.

This study provides evidence for the presence of K_{ATP} channels in a fish heart and their potential importance in the control of cardiac function. The novel effects of mK_{ATP} channel modulators in yellowtail flounder heart imply differences exist in this channel's function over those known for mammalian systems. The influence of channel modulation on contractility revealed by this study also suggests a prospective role for these channels in excitation-contraction coupling in the fish heart.

Figure 2. Twitch force and resting tension for ventricular preparations from yellowtail flounder subjected to anoxia and reoxygenation and treated with agents to affect K_{ATP} channel activity. **A:** glibenclamide treated (n = 8). **B:** diazoxide treated (n = 5). **C:** 5HD treated (n = 6). *a*, significant difference between pharmacological treatment and appropriate control (untreated or vehicle DMSO treated). *b*, significant change from measurements within the treatment taken 5 min before hand.



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