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Mitochondrial ATP-sensitive K^+ channels influence force development and anoxic contractility in a flatfish, yellowtail flounder *Limanda ferruginea*, but not Atlantic cod *Gadus morhua* heart

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Summary

The influence of ATP-sensitive K^+ channels (K_{ATP} channels) on cardiac performance during anoxia and reoxygenation was investigated in two species of fish showing different cardiac responses to anoxia. Force production in isometrically contracting ventricular muscle preparations from yellowtail flounder is potentiated at the onset of anoxia, while force immediately declines in Atlantic cod preparations. Glibenclamide, a general K_{ATP} blocker, impaired oxygenated force development in yellowtail flounder heart but was without effect on cod preparations. The mitochondrial K_{ATP} (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 preserved resting tension and eliminated anoxic force potentiation in yellowtail flounder heart preparations. Neither 5HD nor diazoxide affected contractility in cod ventricle preparations. Results indicate that K_{ATP} channels can modulate contractility in yellowtail flounder heart and are potentially important in cardiac hypoxia survival in this species.

Key words: adenosine 5′-triphosphate-sensitive potassium channel, fish heart, hypoxia, mitochondria, calcium, yellowtail flounder, *Limanda ferruginea*, Atlantic cod, *Gadus morhua*.

Introduction

Flatfishes often inhabit hypoxic waters and are known to exhibit substantial tolerance to cardiac acidosis and hypoxia (Gesser and Poupa, 1979, 1974). Flatfish (*Solea solea*) exposed to environmental hypoxia decrease spontaneous activity and stimulate glycolysis (Dalla Via et al., 1998). Escape behaviour seems to be reserved as a final option for survival, and is only employed if other compensatory reactions are insufficient to deal with hypoxia (Dalla Via et al., 1998). *In vivo* studies on the winter flounder (*Pseudopleuronectes americanus*) suggest that flatfish also have an atypical cardiovascular response to hypoxia. Winter flounder subjected to moderate hypoxia exhibit increased cardiac output with no bradycardia (Cech et al., 1977). In isolated ventricular muscle from the European flounder (*Platycthys flesus*), cardiac force development is initially potentiated following exposure to acidosis, before slowly declining over time (Gesser and Poupa, 1979). The observation of potentiated force in isolated cardiac muscle suggests the possibility that cellular mechanisms may contribute to increases in hypoxic cardiac output. Gesser and Poupa (1978, 1979) have proposed that intracellular acidosis may trigger a release of stored mitochondrial Ca^{2+}, subsequently enhancing force production, though this hypothesis remains untested.

Work by Ganim et al. (1998) on goldfish, as well as investigations on the Amazonian armoured catfish acari-bodo *Lipossarcus pardalis* (T. J. MacCormack, J. Treberg, V. M. F. Almeida-Val, A. L. Val and W. R. Driedzik, manuscript submitted for publication), have identified adenosine 5′-triphosphate-sensitive potassium (K_{ATP}) channels in fish hearts. K_{ATP} channels are activated by a decline in the ratio of ATP/ADP, and are therefore most likely to contribute to cardiac function throughout periods of impaired ATP production, such as hypoxia. The objective of this study was to evaluate whether K_{ATP} channels are involved in the phenomenon of hypoxic force potentiation in the yellowtail flounder *Limanda ferruginea* heart, and to investigate their role in cardiac performance during anoxia and reoxygenation in fish species with differing tolerances to cardiac anoxia. Atlantic cod *Gadus morhua* were chosen for comparisons, as this species is considered to have poor cardiac anoxia tolerance (Gesser and Poupa, 1974; Hartmund and Gesser, 1996).

In mammalian heart, K_{ATP} channels have been described on both the sarcolemmal membrane (sK_{ATP}) (Noma, 1983) and on the inner mitochondrial membrane (mK_{ATP}) (Inoue et al., 1991), and their activity has been linked with the cardioprotection afforded by various means of preconditioning. sK_{ATP} channels facilitate cellular K^+ efflux in mammalian cardiomyocytes, and can therefore alter membrane

electrical properties such as the action potential (Ganim et al., 1998) and extracellular K^+ concentrations (Kantor et al., 1990; Venkatesh et al., 1991; Wilde et al., 1990). mK_{ATP} channels allow mitochondrial K^+ influx, leading to decreasing inner mitochondrial membrane potential and swelling of the matrix in rat heart. Depolarisation also affects mitochondrial Ca^{2+} handling (Holmuhamedov et al., 1999), increases respiration and alters the rate of mitochondrial ATP synthesis (Holmuhamedov et al., 1998, Eells et al., 2000). Despite extensive study in mammals, it is still not clear whether the hypoxic cardioprotection associated with activated K_{ATP} channels is mediated by sarcolemmal or mitochondrial channels, or whether both play an important part (Sato et al., 2000; reviewed by Gross and Fryer, 1999).

Differences in excitation–contraction (E–C) coupling between fish and mammalian cardiac muscle may contribute to significant differences in the functional role of K_{ATP} channels in fish cardiomyocytes. Unlike mammalian cardiomyocytes, which derive the Ca^{2+} needed for contraction largely from intracellular stores such as the sarcoplasmic reticulum (SR), fish cardiomyocytes rely heavily on trans-sarcolemmal Ca^{2+} influx to achieve contraction (Vornanen, 1998, 1999). The dependence of fish cardiomyocytes on sarcolemmal Ca^{2+} flux enhances the importance of membrane-bound ion channels and transporters in controlling contractility. The role of K_{ATP} channels in fish cardiomyocytes may therefore, be quite different than that observed for mammals and could be important in beat-to-beat cardiac function in fish.

The contribution of K_{ATP} channels to heart performance during anoxia and reoxygenation was studied using isolated ventricular muscle strip preparations and pharmacological agents targeting sarcolemmal and mK_{ATP} channel activity. This study shows that agents altering K_{ATP} channel activity can impact on contractility in ventricular muscle from yellowtail flounder, but not Atlantic cod. Species-specific differences in fish cardiac K_{ATP} channels may have implications in anaerobic heart performance.

Materials and methods

Cultured yellowtail flounder *Limanda ferruginea* Storer (24 fish, body mass 108.5±5.1 g) and Atlantic cod *Gadus morhua* L. (22 fish, body mass 391.0±99.9 g) were maintained in aerated, flow-through seawater tanks at between 5 and 8 °C and natural photoperiod. Yellowtail flounder were held in either 215 or 1200 l tanks and fed commercial feed, while cod were held in 8100 l tanks and fed either commercial feed or frozen herring. All fish were acclimated for at least 1 month before use.

Tissue preparation

Animals were killed by a sharp blow to the head and doubly pithed. The heart was quickly excised and placed in cold, oxygenated bathing solution. The bathing medium was a standard solution for marine teleosts and included (in mmol l^{-1}): 150 NaCl, 5.0 KCl, 0.17 $MgSO_4$, 1.5 $CaCl_2$, 0.17 NaH_2PO_4, 2.33 Na_2HPO_4, 11.0 $NaHCO_3$, with pH set to 7.8 at 6 °C. Glucose (5.0 mmol l^{-1}) was added as a metabolic fuel (Driedzic and Bailey, 1994). The ventricle was dissected free of the atrium and bulbous arteriosus, bisected, and a strip approximately 1.5 mm wide and <10 mm in length was cut longitudinally from each section.

Preparations were mounted vertically in a tissue bath using a Plexiglas clamp and affixed to a Harvard Apparatus (South Natick, MA, USA) isometric force transducer (Model 60-2994) with 3-0 surgical silk. Each chamber contained 30 ml of bathing medium held at 6 °C and gassed with either 0.5 % CO_2, balance O_2 (oxygenated) or 0.5 % CO_2, balance N_2 (anoxia). Ganim et al. (1998) have shown that K_{ATP} channels are sensitive to acclimation temperature in fish, so in an attempt to reflect physiological conditions, experiments were run close to the acclimation temperature of the animals. Each preparation was subjected to only one treatment and run in parallel with appropriate control preparations in each instance.

Strips were positioned between platinum electrodes on the Plexiglas clamp and stimulated to contract by field stimulation using a Grass model S9 stimulator with voltage set at 150 % threshold and 5 ms duration. Strips were stretched to optimum length for maximum force production and allowed 30 min to stabilise at a pacing rate of 0.2 Hz. Pacing frequency was 0.2 Hz for all experiments and spontaneously contracting strips were eliminated from statistical analysis. Free-swimming Atlantic cod at a temperature similar to that used in the present study (6.4 °C) were found to have heart rates of approximately 0.33 Hz under normoxic conditions (Claireaux et al., 1995). Heart rate is not available for yellowtail flounder but in a similar species, *Pseudopleuronectes americanus*, heart rate was about 0.6 Hz under normoxia at 10 °C (Cech et al., 1977). Since ventricle strip preparations in the present study were made to contract at their maximum level of force development, a lower pacing frequency of 0.2 Hz was chosen to compensate for possible increases on energy demand in the tissue. This pacing frequency also facilitated comparisons with existing data on cardiac performance in other flatfish and Atlantic cod (Gesser and Poupa, 1974).

Anoxic conditions were induced rapidly and reversibly by replacing the oxygenated medium in the tissue bath with nitrogen-gassed medium. A reservoir of medium was maintained at 6 °C in a water-jacketed condenser and equilibrated with 0.5 % CO_2, balance N_2. During the switch to anoxia, the tissue bath was gassed with 0.5 % CO_2, balance N_2 and flushed with 150 ml of anoxic medium. Mechanical disturbance was minimal during the switch, and preliminary experiments using a reservoir of oxygenated medium found the process had no effect on force development or the contractile characteristics of the preparation. The switch from oxygenated to anoxic medium required <1 min and dissolved oxygen in the bath was routinely <0.1 mg l^{-1}. To achieve reoxygenation the bath was gassed with 0.5 % CO_2, balance O_2, resulting in saturation within approximately 1 min.

The response of ventricular muscle to anoxia and reoxygenation was first assessed in the absence of

pharmacological agents. Control strips were gassed with 0.5 % CO_2, balance O_2 for 85 min while treatment preparations were subjected to a 35 min period of anoxia followed by 30 min of reoxygenation.

The contribution of K_{ATP} channels to contractility in ventricular muscle from yellowtail flounder and cod during anoxia and reoxygenation was next assessed using glibenclamide, an inhibitor of both sarcolemmal and mK_{ATP} channels (Hu et al., 1999). Both control and treatment strips were subjected to a 35 min period of anoxia followed by 30 min of reoxygenation. Glibenclamide (5 μmol l^{-1}) was initially applied to the treatment bath during the first minute following stabilisation with the control bath receiving vehicle dimethylsulfoxide (DMSO). Chemicals were reapplied immediately following the switch to anoxia, to maintain a constant concentration in the bath.

The functional contribution of mK_{ATP} channels in the yellowtail flounder heart was next investigated using sodium 5-hydroxydecanoic acid (5HD), a highly specific inhibitor of mK_{ATP} channel function. Trials were as above with 100 μmol l^{-1} 5HD (Sato et al., 2000) added to the treatment bath in each instance and run parallel to untreated preparations.

Diazoxide (50 μmol l^{-1}; Hu et al., 1999), a specific mK_{ATP} channel opener, was also used to assess the effects of mK_{ATP} channels in anaerobic performance and recovery in ventricular strips from yellowtail flounder and Atlantic cod. Control preparations received vehicle DMSO. Doses for all agents were chosen from the lower end of the range of concentrations commonly used in mammalian studies, in order to minimise the risk of toxic side effects.

Drugs

All chemicals were purchased from Sigma (St Louis, MO, USA) with the exception of 5HD, which was purchased from ICN Biomedicals (Aurora, OH, USA). Stock solutions of glibenclamide (5 mmol l^{-1}) and diazoxide (18 mmol l^{-1}) were prepared in DMSO and stored at –20 °C in aliquots until just before use. A 100 mmol l^{-1} stock solution of 5HD was prepared in bathing medium and frozen in portions until just before use. All chemicals were pipetted directly into the tissue bath.

Data analysis and statistics

Force transducers were interfaced to a MacLab/2E computerised unit and data were collected online using the accompanying Chart software for Macintosh. Data were recorded for a duration of 30 s at 5 min intervals, and statistical analysis is based on the average of six contractions at each recording interval. Peak tension (% force) and resting tension were calculated using Microsoft Excel, and are expressed as a percent of initial tension development. Data from untreated, anoxia/reoxygenation trials were pooled for more accurate comparisons with untreated oxygenated preparations and 5HD-treated preparations. Anoxia/reoxygenation trials in which DMSO was applied to preparations were also pooled for comparisons against glibenclamide- and diazoxide-treated strips. Statistical analysis of data was performed using SPSS version 10.1 for Windows. The significance of changes in % force and resting tension between treatments was tested using a parametric repeated measures analysis. Within treatment differences were tested using a one-way analysis of variance (ANOVA). *P* values of less than 0.05 were considered to be statistically significant.

Results

Anoxia/reoxygenation

Yellowtail flounder

Fig. 1 shows peak tension and resting tension for untreated ventricular preparations from yellowtail flounder and Atlantic cod exposed to anoxia and reoxygenation. Ventricle strips from yellowtail flounder showed a consistent decay in force

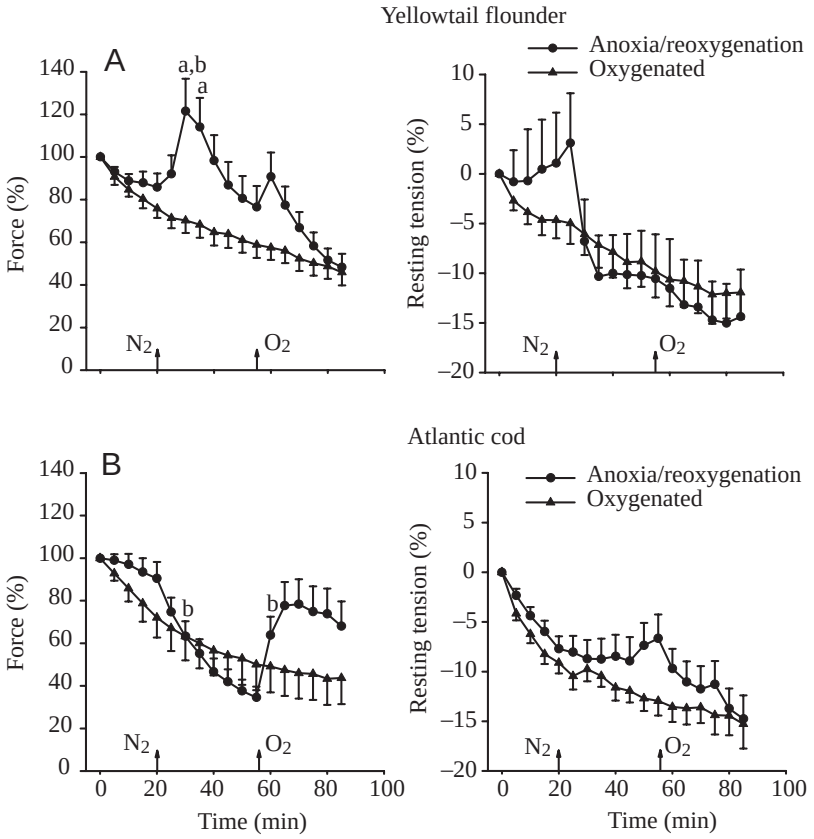

Fig. 1. Twitch force and resting tension for ventricular preparations from yellowtail flounder (A) and Atlantic cod (B) exposed to oxygenated conditions (▲) (flounder, *N*=6; cod, *N*=5) and to 35 min of anoxia followed by reoxygenation (●) (*N*=10, for both species). Arrows indicate times at which anoxia was induced (N_2) (20 min) and when preparations were reoxygenated (O_2) (55 min). 'a' indicates significant difference between treatments. 'b' indicates a significant decrease or increase from measurements within the treatment taken 5 min beforehand.

development under oxygenated conditions to approximately 50 % of initial after 85 min. Resting tension also fell by about 10 % during oxygenation. Resetting to the initial resting length of the ventricular strip after 85 min restored about 10 % of force, as the preparation moved back to the apex of the force–length curve (data not shown). In similar experiments on another species of flounder, *Platichthys flesus*, ventricle preparations under oxygenated conditions at 12 °C exhibited a decline in force development of approximately 16 % over 30 min (Gesser and Poupa, 1979; Gesser and Jørgensen, 1982).

Force production increased significantly above oxygenated levels in yellowtail flounder following exposure to anoxia, peaking at 122±13 % after 10 min. Force then declined over the balance of the anoxic period, but remained above levels observed for oxygenated preparations. Force recovered significantly above anoxic levels at reoxygenation before continuing to decline at a rate approximately equal to that observed before reoxygenation. Resting tension consistently fell rapidly by approximately 10 % during the initial 10 min of anoxia, before stabilising and diminishing at a rate similar to oxygenated controls. Reoxygenation did not affect resting tension.

Atlantic cod

Ventricle strips from cod exhibited a decay in force production and resting tension under oxygenated conditions similar to that observed for yellowtail flounder. In previous experiments on Atlantic cod, ventricle preparations under oxygenated conditions lost between 10 and 15 % of force development over 30 min (Gesser and Jørgensen, 1982; Hartmund and Gesser, 1996). Exposing preparations to anoxia resulted in a significant decline in force production relative to pre-anoxic levels, falling to 34.6±6.3 % of initial after 30 min. Anoxic force development was not significantly different from that observed for oxygenated controls. Following reoxygenation, force recovered to levels much higher than those observed for oxygenated controls (78.3±10.9 % compared with 46.0±15.4 %). Although preparations showed significant force recovery relative to anoxic levels, recovery was non-significant when compared with oxygenated preparations, owing to high variation in the recovering strips. Cod preparations did not exhibit the same rapid decay in resting tension at the onset of anoxia, as was observed for yellowtail flounder preparations, and were generally unaffected by anoxia or reoxygenation.

K_{ATP} contribution

Yellowtail flounder

Fig. 2 shows peak tension and resting tension data for yellowtail flounder ventricle preparations exposed to anoxia and reoxygenation and treated with agents to alter K_{ATP}-channel activity. Fig. 2A shows that the response to DMSO (vehicle for glibenclamide and diazoxide) alone is no different from untreated preparations, and confirms the biphasic pattern of force development following anoxia and reoxygenation. Blocking K_{ATP} channels with glibenclamide (Fig. 2B) decreased force production significantly under oxygenated conditions in preparations from yellowtail flounder. The inset graph (Fig. 2B) illustrates the specific effects of glibenclamide treatment on force development (% force from glibenclamide treated strips minus % force from DMSO treated strips over time). Despite an overall decrease in force development, preparations continued to respond similarly to anoxia and reoxygenation. Glibenclamide had no effect on resting tension in yellowtail flounder ventricle preparations.

The mK_{ATP} channel agonist diazoxide significantly eliminated the potentiation of force production observed in untreated preparations exposed to anoxia (Fig. 2C). The inset graph (Fig. 2C) shows the specific effects of diazoxide treatment on force development (% force from diazoxide-treated preparations – % force from DMSO-treated preparations over time). Diazoxide-treated preparations did show significant force recovery over anoxic levels when reoxygenated, but still tended to be weaker than untreated strips. Resting tension also tended to be more stable during anoxia in diazoxide-treated strips, with no rapid decline observed at the onset of anoxia. However, differences were not statistically significant. Inhibiting mK_{ATP} channels with 5HD (Fig. 2D) initially preserved force development under oxygenation in yellowtail flounder heart preparations. As 5HD was dissolved in bathing medium, the appropriate controls for this treatment are presented in Fig. 1A. 5HD did not significantly affect peak tension or resting tension during anoxia and reoxygenation. The inset graph (Fig. 2D) shows the specific effects of 5HD treatment on force development (% force from 5HD treated strips minus % force from untreated control strips over time).

Atlantic cod

Fig. 3 gives force and resting tension for Atlantic cod ventricular muscle preparations exposed to anoxia and reoxygenation and treated with agents to alter K_{ATP} channel activity. Glibenclamide, diazoxide and 5HD had no noticeable influence on force development or resting tension in cod ventricle preparations under the conditions tested. Force development in all preparations decreases under anoxia to approximately 33 % of the initial value. Upon reoxygenation, strips immediately recover to approximately 88 % of initial force development.

Discussion

Isolated ventricular preparations from yellowtail flounder and Atlantic cod were tracked for 85 min. Even under oxygenated conditions, preparations from both species showed a decrease in force production in association with decreased resting tension during this period. A component of force loss was recovered by resetting resting tension to initial levels, indicating that the preparation had lengthened and was no longer stretched to its optimum length for peak force production. Previous experiments on both flounder and Atlantic cod have shown similar declines in force development

under oxygenated conditions (Gesser and Poupa, 1979; Gesser and Jørgensen, 1982; Hartmund and Gesser, 1996). The mechanisms that underlie the observed force decay are unknown. Several possibilities that could contribute to force loss include osmotic pressure or the absence of something important from the perfusion medium. Overall, a decline in force should not detract from a qualitative assessment of the hypoxia/recovery response or the potential functionality of mK_{ATP} channels.

In Atlantic cod ventricular preparations, anoxia initially results in a loss in force development followed by a stabilisation. In previous investigations on Atlantic cod (Gesser and Poupa, 1974; Hartmund and Gesser, 1996) in which anoxia was simulated at 15 °C using sodium cyanide, a much more rapid decline in force development was observed, followed by a similar stabilisation. Given that anoxia should be induced more gradually using only N_2, and that test temperature (6 °C) was lower in the current study, the slower time course of force decay and stabilisation agrees well with existing data. Resting tension declined slightly during oxygenation in cod preparations, subsequently stabilising during anoxia. Force development under anoxia was not substantially different from control preparations. *In vivo*, Atlantic cod show a decrease in heart rate during environmental hypoxia but maintain cardiac output by increasing stroke volume (Fritsche and Nilsson, 1989). As such, power output both *in vivo* and with our ventricular strips is defended reasonably well, presumably through anaerobic metabolism under anoxia. Reoxygenating cod preparations led to restoration of force development under all treatment conditions.

The response of yellowtail flounder heart preparations to

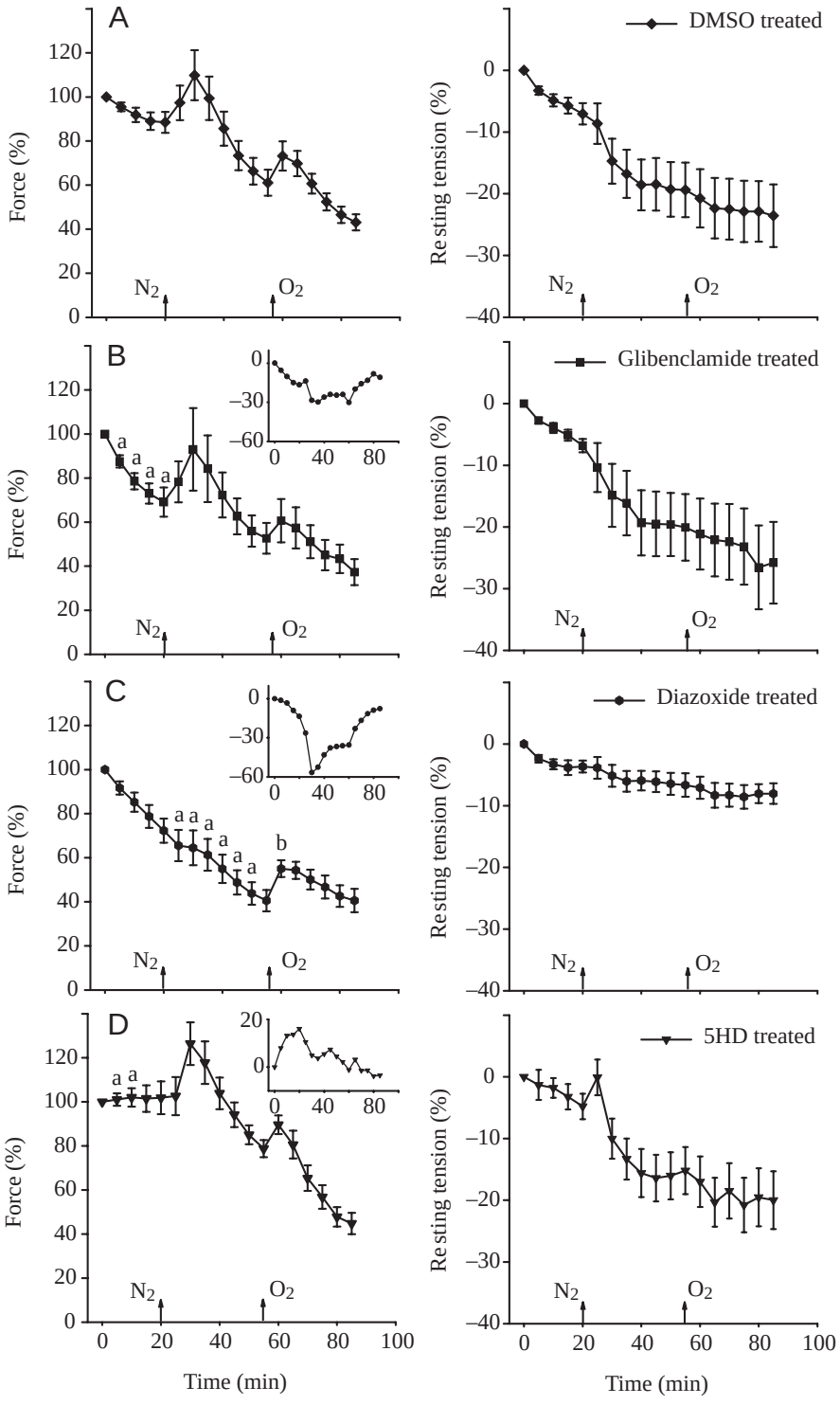

Fig. 2. Twitch force and resting tension for ventricular preparations from yellowtail flounder subjected to anoxia and reoxygenation and treated with agents to affect K_{ATP}-channel activity. Arrows indicate times at which anoxia was induced (20 min) and when preparations were reoxygenated (55 min). (A) DMSO treated (diamonds) (N=13). (B) Glibenclamide-treated (squares) (N=8). Inset graph shows change in twitch force associated with glibenclamide treatment against time (% force from glibenclamide-treated preparations – % force from DMSO-treated preparations). (C) Diazoxide treated (hexagons) (N=5). Inset graph shows alterations in twitch force associated with diazoxide treatment against time (% force from diazoxide-treated preparations – % force from DMSO-treated preparations). (D) 5HD-treated (triangles) (N=6). Inset graph shows alterations in twitch force associated with 5HD treatment against time (% force from 5HD treated preparations – % force from untreated preparations). ‘a’ indicates significant difference between pharmacological treatment and appropriate control. ‘b’ indicates a significant change from measurements within the treatment taken 5 min beforehand.

anoxia was quite different than that observed in Atlantic cod preparations. A common finding in almost all experiments with yellowtail flounder (with the exception of treatment with diazoxide) is a transient but substantial potentiation of force and a decline in resting tension at the onset of anoxia. Reoxygenation leads to a small and short-lived increase in force production followed by a decline in performance, similar to that seen in preparations maintained for an equivalent period under oxygenation. The potentiation of force shown by yellowtail flounder ventricle preparations exposed to nitrogen-induced anoxia is comparable with that shown by other species of flatfish subjected to acidosis (Gesser and Poupa, 1979; Hoglund and Gesser, 1987; Poupa and Johansen, 1975). These observations also agree well with *in vivo* studies on the winter flounder that do not display a bradycardic response and actually significantly increase cardiac output in response to hypoxia (Cech et al., 1977).

Increases in cardiac twitch force production in fish are generally agreed to result from increased intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) (Tibbits et al., 1991). Indirect evidence suggests that acidotic force potentiation in ectothermic vertebrates is due to a release of stored mitochondrial Ca^{2+} (Gesser and Poupa, 1978). We suggest below that the increase in force development of ventricular strips from yellowtail flounder under anoxia is also related to the release of Ca^{2+} from the mitochondria.

Our results show the presence of mK_{ATP} channels in ventricular muscle of yellowtail flounder. Under anoxic conditions, diazoxide completely 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 the rat heart at 30 °C, leading to a rapid reduction in mitochondrial Ca^{2+} content and inhibited mitochondrial Ca^{2+} uptake (Holmuhamedov et al., 1999). If the potentiation of force during anoxia in flounder is due to a bolus release of mitochondrial Ca^{2+}, then our results seem to contrast with those observed in mammals, in that the activation of mK_{ATP} channels in the flounder heart seems to stabilise $[Ca^{2+}]_i$ during anoxia. It is possible that in the flounder heart, diazoxide releases mitochondrial Ca^{2+} more slowly than in the rat heart, probably due to the relatively extreme low temperature (6 °C) used in this experiment. Following a period of diazoxide treatment, mitochondrial Ca^{2+} content should already be reduced, so that when subjected to anoxia, any large force potentiation resulting from a bolus release of mitochondrial Ca^{2+} will be eliminated. The observed preservation of resting tension in diazoxide-treated preparations supports this interpretation. Changes in resting tension are thought to reflect alterations in resting $[Ca^{2+}]_i$ (Driedzic and Gesser, 1994), therefore the observed decrease in resting tension at the onset of anoxia in untreated strips would presumably be due to a

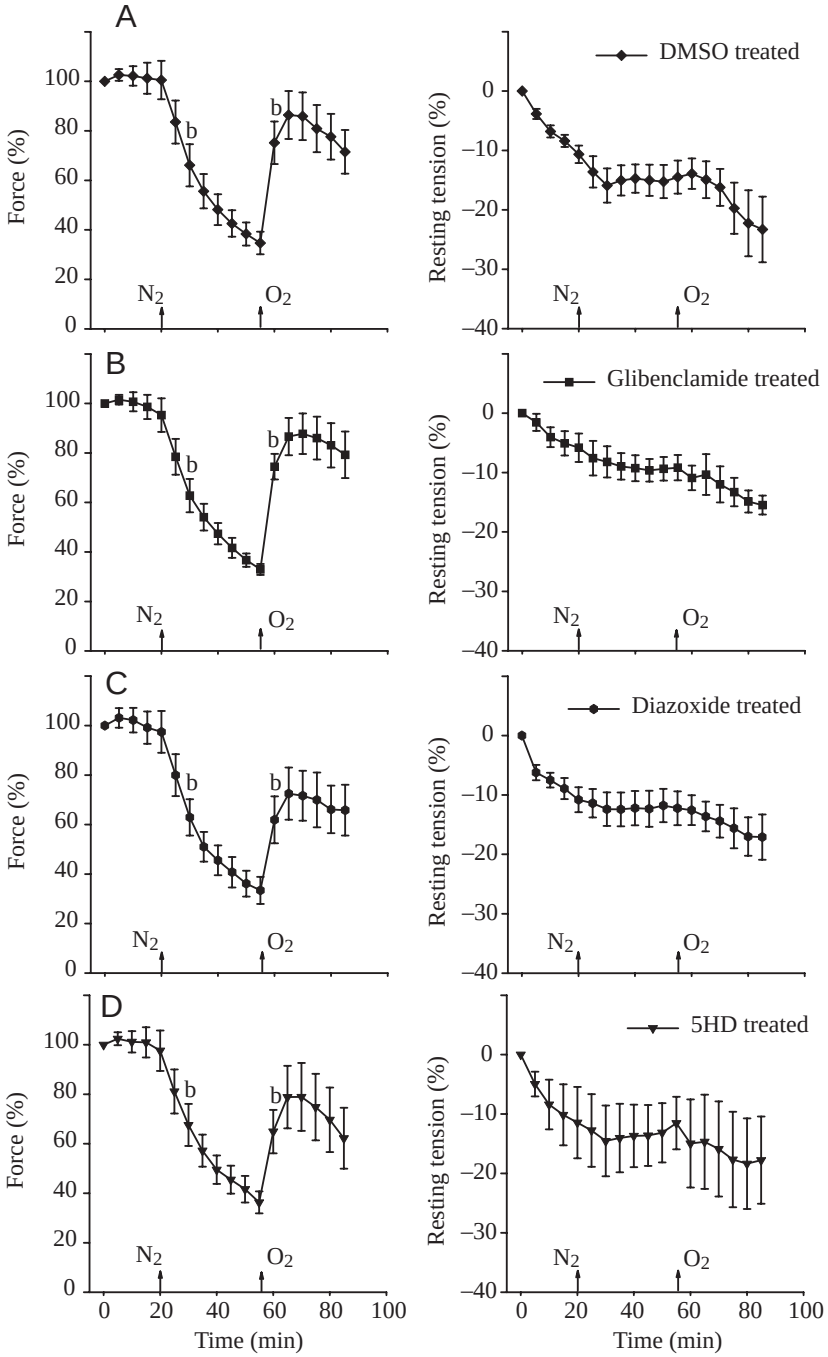

Fig. 3. Twitch force and resting tension for ventricular preparations from Atlantic cod subjected to anoxia and reoxygenation, and treated with agents to affect K_{ATP}-channel activity. Arrows indicate times at which anoxia was induced (20 min) and when preparations were reoxygenated (55 min). (A) DMSO treated (diamonds) (*N*=13). (B) Glibenclamide-treated (squares) (*N*=8). (C) Diazoxide treated (hexagons) (*N*=5). (D) 5HD-treated (triangles) (*N*=5). 'b' indicates a significant change from measurements within the treatment taken 5 min beforehand.

decrease in $[Ca^{2+}]_i$ activity. If diazoxide treatment triggers a more 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. This could lead to the observed preservation of resting tension.

5HD, a mK_{ATP} antagonist, protected against force loss under oxygenated conditions. On the basis of available information, we are unable to suggest the mechanism for this response. The important point though is that these observations provide evidence for mK_{ATP} channels in the yellowtail flounder heart. 5HD had no impact under anoxia suggesting that mK_{ATP} channels are already closed under these conditions. This implies that energy status is maintained under anoxia through a strong anaerobic metabolism

Glibenclamide, a general K_{ATP}-channel antagonist, significantly reduced force development in flounder ventricle preparations during oxygenation, but did not affect the characteristics of force development during anoxia or recovery. This, along with the observation that 5HD seemed to have the opposite effect on twitch force development in flounder ventricle strips, suggests that the force loss incurred with glibenclamide may be a result of a sarcolemmal rather than mK_{ATP} channel contribution. sK_{ATP} channel activity increases in isolated goldfish cardiomyocytes acclimated to low temperatures (7 °C) (Ganim et al., 1998). Glibenclamide had no effect on action potential duration in goldfish myocytes when tested at the acclimation temperature (Ganim et al., 1998), but we cannot rule out the possibility that it could affect the characteristics of the action potential in yellowtail flounder cardiomyocytes. Our results suggest that sK_{ATP} channels are normally active on a beat to beat basis in the yellowtail flounder heart at this temperature and may therefore be important in the regulation of contractility.

The observation of impaired force development in glibenclamide-treated preparations is unexpected and again difficult to explain using the available literature on either mammalian or fish heart. Theoretically, blocking sK_{ATP}-channel activity with glibenclamide should lengthen the duration of the action potential and enhance Ca^{2+} influx through L-type channels. Inhibiting sK_{ATP} activity should decrease net cellular K^+ efflux and cause the sarcolemmal membrane potential to become less polarised. A more positive membrane potential could, in turn, increase reverse Na^+/Ca^{2+} exchange, which has been shown to contribute a significant amount of activator Ca^{2+} at more depolarised membrane potentials in the fish heart (Vornanen, 1999). By all accounts, glibenclamide should facilitate increased twitch-force development through enhanced Ca^{2+} influx across the sarcolemmal membrane. Further investigations on the membrane events associated with sK_{ATP} channel opening are necessary to explain this observation.

Altering K_{ATP} channel activity in cod ventricle strips did not affect force development or resting tension under any of the conditions tested. The data suggest that Atlantic cod do not have cardiac K_{ATP} channels that are sensitive to the pharmacological agents used, or that all of the factors needed to alter channel activity are not present in this tissue. K_{ATP}-channel activity is sensitive to ATP concentration, Mg^{2+} and other nucleotide concentrations, as well as a host of other factors (Terzic et al., 1995). The characteristics of the intracellular environment in cod may lead to differences in the activation state of K_{ATP} channels, and hence the effectiveness of channel modulators in this animal. In addition, evolutionary differences within teleost fish, and between fish and mammals may influence the sensitivity of K_{ATP} channels to pharmacological manipulation.

K_{ATP} channels are known to exist in mammalian cardiac muscle; however, direct evidence of their presence in ectothermic myocardium has yet to be presented. Gamperl et al. (2001) have shown in the *in situ* rainbow trout *Oncorhynchus mykiss* heart that a 5 min period of anoxic preconditioning eliminates decreases in resting cardiac function, maximum cardiac output and maximum stroke volume associated with 15 min of anoxic exposure. Although not addressed in their study, K_{ATP} channels are generally agreed to play a key role in the cardioprotection afforded by hypoxic preconditioning in cardiac muscle (Gross and Fryer, 1999), suggesting that these channels are functional in the fish heart. Further evidence of the presence of K_{ATP} channels in ectothermic vertebrates has been provided by studies on isolated mitochondria from the frog *Rana temporaria* (St-Pierre et al., 2000). When isolated mitochondria were subjected to anoxia, membrane potential gradually declined to a new steady state. The activation of mK_{ATP} channels under hypoxia in isolated mammalian mitochondria results in a similar depolarisation of membrane potential (Holmuhamedov et al., 1998), again consistent with the contention that K_{ATP} channels are present in ectothermic vertebrates.

This study provides further evidence for the presence of K_{ATP} channels in a fish heart and the potential importance of these channels in the control of cardiac function. The novel effects of mK_{ATP}-channel modulators in yellowtail flounder heart imply differences exist in the function of this channel over those known for mammalian systems. Alterations in K_{ATP}-channel activity may be the cellular mechanism that underlies previous observations of increased hypoxic cardiac output in flatfish. For example, decreased resting tension may be associated with larger end diastolic volume. This, coupled with increased force, could lead to greater stroke volume and cardiac output. The influence of channel modulation on contractility revealed by this study also suggests a prospective role for these channels in E–C coupling in the fish heart. Future studies should address in more detail the exact means by which K_{ATP} channels influence contractility and their involvement in hypoxic cardioprotection.

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