Hypercapnic Acidosis Reduces Contractile Function in the Ventricle of the Armored Catfish, *Pterygoplichthys pardalis*

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ABSTRACT

The armored catfish, *Pterygoplichthys pardalis* (formerly *Liposarcus pardalis*), is a freshwater, facultative air-breathing teleost that experiences seasonal hypercapnia in the water systems of South America. We studied the tolerance of the *P. pardalis* heart to hypercapnic acidosis using an isolated ventricular muscle strip preparation. Force generation and kinetic variables were examined across a range of contraction frequencies under normocapnic and hypercapnic conditions in the absence and presence of sarcoplasmic reticulum (SR) inhibitors. *Pterygoplichthys pardalis* ventricle exhibited robust contractile force, on par with athletic fish species such as trout and tuna and a relatively flat force-frequency relationship between 0.2 and 1.5 Hz under normocapnic conditions (1% CO_2, pH 7.78 ± 0.02). Hypercapnic acidosis (7.5% CO_2, pH 7.1 ± 0.03) did not alter the shape of the force-frequency response but reduced force by ~50% across all frequencies tested, with only partial recovery upon return to normocapnic conditions. A subsequent and more severe acidotic challenge (15% CO_2, pH 6.77 ± 0.05) caused an additional 20% decrease in force. Force recovered to the level at which it had stablized after the first hypercapnic insult. SR inhibition had no steady state effect on force production at 0.2 Hz but resulted in a negative force-frequency relationship, suggesting that SR Ca^{2+} is recruited to a greater extent at high contraction frequencies. Surprisingly, SR-inhibited muscle was more resistant to hypercapnic acidosis (force decreased by ~40% across all frequencies) and displayed improved recovery upon return to normocapnic conditions. The significance of this latter finding is not clear. In aggregate, our results demonstrate robust contractile force, which extends across a range of frequencies and appears to be supported by SR Ca^{2+} cycling. Hypercapnic acidosis reduced contractile force but may provide preconditioning-like protection from subsequent insults.

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Introduction

Most fish do not routinely experience hypercapnic acidosis of body tissues because CO_2 diffuses readily across the gills and into the surrounding water. However, environmental hypercapnia (high P_{CO_2}) is a common feature of Neotropical aquatic environments such as the Amazon and Pantanal river systems of South America. Environmental hypercapnia is a product of the extensive vegetation that covers the stagnant ponds created by receding water during the dry season. Fish living in these stagnant waters exhibit a host of responses to cope with the physiological stress of hypercapnia including air breathing (Brauner et al. 1995), increased anaerobic metabolism (MacCormack and Driedzic 2007), and behavioral responses (MacCormack et al. 2003*a*). The armored catfish, *Pterygoplichthys pardalis* (Castelnau) (formerly *Liposarcus pardalis*), is indigenous to such waters and is tolerant of environmental hypercapnia (Brauner et al. 1995, 2004).

The whole-animal tolerance of aquatic hypercapnia in *P. pardalis* is not related to preservation of extracellular pH. Brauner et al. (2004) show acidemia (low blood pH; from ~7.9 to ~6.9) within 2 h of environmental hypercapnic exposure, and blood pH remained depressed for 4 d. This whole-animal tolerance extends to the organ level, where maximal in situ cardiac performance of *P. pardalis* is not compromised by hypercapnia until extracellular (perfusate) pH is decreased to 7.1 (from a normocapnic pH of 7.8; Hanson et al. 2009). Furthermore, this study also found that cardiac performance fully recovered upon restoration of P_{CO_2} (and thus pH) to normocapnic values (Hanson et al. 2009).

The ability of *P. pardalis* to maintain cardiac performance during hypercapnic acidosis may be related to tight regulation of intracellular pH (pH_i) in the face of extracellular acidosis (Brauner et al. 2004). In mammals, extracellular acidosis results in intracellular acidosis, which has numerous deleterious consequences including reduced myocardial contractility and the development of fatal arrhythmias. Contractility falls primarily because low pH decreases the Ca^{2+} sensitivity of the myofilaments (Fabiato and Fabiato 1978; Orchard and Kentish 1990) such that less force is generated for a given intracellular Ca^{2+} transient. The cellular ion flux pathways associated with hypercapnia and acidosis have not been investigated in fish hearts. However, studies with rainbow trout (*Oncorhynchus mykiss*)

troponin C (Gillis et al. 2000) and chemically skinned catfish (*Pterygoplichthys*) ventricular muscle preparations (Meadows et al. 1998) clearly show that intracellular acidosis decreases the responsiveness of the contractile element to Ca^{2+}.

In most air-breathing vertebrates, the initial loss of contractile force is recovered during persistent acidosis through the following cascade: intracellular protons are extruded by the Na^+-H^+ exchanger, resulting in an increase in intracellular Na^+ ($[Na^+]_i$). Increased $[Na^+]_i$ limits Ca^{2+} extrusion and even promotes Ca^{2+} influx through the Na^+-Ca^{2+} exchanger. The increased Ca^{2+} can directly interact with myofilaments, offsetting the reduced Ca^{2+} sensitivity and recovering contractile force. In animals with an active sarcoplasmic reticulum (SR), this increased Ca^{2+} can also be pumped into the SR and, when released, can further increase the amplitude of the systolic Ca^{2+} transient (Orchard 1987). However, these mechanisms that permit recovery of force and protect contractility during acidosis can become toxic during return to physiological pH, inducing Ca^{2+} overload and reperfusion injury (Maxwell and Lip 1997). Whether similar processes underlie the hypercapnic tolerance and recovery of cardiac performance in fish in general (and in *P. pardalis* in particular) has not been investigated. However, previous work using an isolated ventricular muscle preparation revealed a role for SR Ca^{2+} cycling in the maintenance and recovery of myocardial contractility during anoxia in *P. pardalis* (MacCormack et al. 2003*b*). Furthermore, a mammalian-like recovery of force during persistent acidosis has been observed in some fish (Gesser and Poupa 1983). Alternatively, if the myocardium of *P. pardalis* is able to regulate pH_i, it could avoid the deleterious effects of intracellular acidosis on cardiac contractility and the subsequent injury associated with reperfusion altogether.

The aim of our study was to gain mechanistic insight into the hypercapnic tolerance of *P. pardalis* by investigating myocardial function at the tissue level. Further, by examining force generation in the presence and absence of SR inhibition, we aimed to assess the role of SR Ca^{2+} cycling during repeated and severe hypercapnic acidosis and following return to normocapnia.

Methods

Experimental Animals

Adult armored catfish (*Pterygoplichthys pardalis* (Castelnau); $n = 7$, mean mass = 584.3 ± 107.6 g) were caught in the Pantanal, Mato Grosso do Sul, Brazil, and transported to the Jacarezário animal facility at the Rio Claro campus of the São Paulo State University (UNESP), where they were housed in large outdoor ponds supplied with continuous fresh water. Water temperature was ~25°C, and the animals were fed ad lib. All protocols adhered to local guidelines.

Preparation of Ventricular Strips

Pterygoplichthys pardalis was killed by pithing, and the heart was removed ventrally and placed in cooled physiological saline. The ventricle was cut away from the rest of the heart and weighed (0.283 ± 0.059 g, $n = 7$). Four muscle strips, roughly cylindrical in shape and no more than 1.5 mm in width, were cut from the ventricle of a single animal. Where possible, strips were dissected out as trabecular bundles to promote similar fiber orientation. Muscles were hung from 25-g force transducers in a Myobath II Multi-Channel Tissue Bath System (World Precision Instruments, Sarasota, FL), lowered into four separate saline baths, gassed with 1% CO_2 (normocapnic conditions), and left 10 min before stimulation commenced (at 0.2 Hz, 10 ms, ~55 V; Grass SD9B Stimulator, Grass Medical Instruments, Quincy, MA). Analog signals were amplified (Transbridge 4M; World Precision Instruments), A/D converted (LT4/16-S; World Precision Instruments), and then stored on a computer, using the DataTrax data acquisition/analysis program (World Precision Instruments).

The four strips were randomly designated strip 1, 2, 3, or 4. All four muscle preparations were then stretched to L_{max} (the length which produced optimal isometric tension). Once L_{max} was reached, SR inhibitors (ryanodine [10 μM] and thapsigargin [2 μM]) were administered to strips 2 and 4, and all four preparations were left for 40 min before commencement of the force-frequency trials.

All muscle strips were subjected to five sequential force-frequency trials under the following conditions: (1) normocapnia: all strips, 1% CO_2, pH 7.78 ± 0.02; (2) hypercapnia; strips 3 and 4, 7.5% CO_2, P_{CO_2} = 57 mmHg, pH 7.14 ± 0.03 (strips 1 and 2 remained normocapnic); (3) recovery 1: all strips, 1% CO_2, pH 7.77 ± 0.02; (4) severe hypercapnia: strips 3 and 4, 15% CO_2, P_{CO_2} = 114 mmHg, pH 6.77 ± 0.05 (strips 1 and 2 remained normocapnic); (5) recovery 2; all strips, 1% CO_2, pH 7.77 ± 0.02. Thus, strip 1 was maintained normocapnic; strip 2 was normocapnic and also exposed to SR inhibitors; strip 3 was made hypercapnic; and strip 4 was hypercapnic and exposed to SR inhibitors.

In each force-frequency trial, stimulation frequency was increased from 0.2 to 0.5 to 1.0 to 1.5 and then decreased to 0.8 and 0.2 Hz. The muscle was held at each new frequency until force (F_{max}) stabilized, except at 1.5 Hz, where frequency was changed to 0.8 Hz after 10 contractions. On average it took 10 min for the pH to stabilize after switching the gas mixtures and 10 min to perform each force-frequency trial. At the end of the experiment, the length of the muscle strip between the clips was measured and then cut down from the apparatus and blotted dry, and wet weight was measured to the nearest milligram.

Drugs and Solutions

All drugs were purchased from Sigma (St. Louis, MO). Adrenaline, ryanodine, and thapsigargin were prepared daily from stock solutions. The combination of 2 μM thapsigargin and 10 μM ryanodine should inhibit SR Ca^{2+} uptake and release in isolated muscle, respectively (Maier et al. 2000; Rousseau et al. 1987; Sagara and Inesi 1991). A tonic level of adrenaline (3 nM) was maintained in the physiological saline of all prepa-

rations throughout the experiments to preserve cardiac tonus and minimize muscle fatigue (Farrell et al. 1986; Milligan et al. 1989). Lights were dimmed throughout the experiment to minimize degradation of adrenaline. The composition of the physiological saline was (in mM): NaCl 125, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2.5, glucose 5.6, $NaHCO_3$ 11.9, pH 7.78 ± 0.02 when gassed with 1% CO_2, balance O_2. Hypercapnia was induced via mixing O_2 and CO_2 with a gas-mixing flowmeter (GF-3MP; Cameron Instrument, Port Aransas, TX). Throughout the experiment, pH was monitored using a digital TEC-2MP pH meter (Tecnal, São Paulo, Brazil).

Calculations and Statistical Analysis

F_{max} is expressed as mN mm^{-2}. Mean cross-sectional area was calculated using wet muscle mass, trabecular length, and an assumed muscle density of 1.06 g cm^{-3} (Layland and Altringham 1995). Measurements of force (F_{max}), time to peak tension, time to 50% relaxation, and rates of contraction and relaxation were achieved using DataTrax software. Rates were calculated by dividing F_{max} by rise or decay times and were thus average rates of rise and fall. Because there is fatigue of the preparation over the course of the experiment, loss in force of the normocapnic strip (a maximal loss of 20% of original force) was compensated for in the experimental strips by adding on the mean percentage lost (see Shiels and Farrell 2000). One-way repeated-measures ANOVA or repeated-measures ANOVA on ranks followed by Student-Newman-Keuls post hoc tests were used to assess the effect of frequency and drug treatments as indicated in the figure legends. Significance was accepted at $P < 0.05$. All statistical analyses were performed using SigmaStat 3.5 (Systat Software, San Jose, CA).

Results

Effect of Hypercapnia and Recovery on F_{max} *at 0.2 Hz*

Raw data traces showing the effect of hypercapnia and recovery on F_{max} are given in Figure 1*A*. Isolated ventricular muscle from *Pterygoplichthys pardalis* generated strong isometric twitch force (F_{max}) under normocapnic conditions (1% CO_2, pH 7.78 ± 0.02; Fig. 1*A*, 1*B*; Table 1). Hypercapnia at 7.5% CO_2 (pH 7.1 ± 0.03) significantly decreased F_{max} (by ~50%) and remained stable during persistent hypercapnic exposure (Fig. 1*A*). Restoration of normocapnic pH (1% CO_2, pH 7.77 ± 0.02) caused F_{max} to increase and then decrease again before stabilizing at a new steady state. This transient "hump" in F_{max} during recovery (see brackets in Fig. 1*A*) was observed in five out of six preparations. The subsequent and more severe hypercapnic insult (15% CO_2, pH 6.77 ± 0.05) produced a further significant drop in F_{max} of approximately 20% (~70% below normocapnic F_{max}), which recovered to the level achieved after the first hypercapnic insult upon return to normocapnic conditions (1% CO_2, pH 7.76 ± 0.02). A similar but more pronounced hump in F_{max} was observed during recovery from the second hypercapnic insult (second bracket in Fig. 1*A*).

Effect of Hypercapnia and Recovery on the Force-Frequency Relationship

An increase in stimulation frequency from 0.2 to 1.5 Hz had little effect on F_{max} under normocapnic conditions (1% CO_2, pH 7.78 ± 0.02), resulting in a flat force-frequency relationship (Fig. 1*B*, 1*C*; Fig. 3*B*). This response to frequency was not altered by hypercapnia, however the amplitude of the twitch was significantly decreased (by ~50%) across all frequencies and only partially recovered upon return to normocapnic conditions (1% CO_2, pH 7.77 ± 0.02; Fig. 1*B*, 1*C*, *right panel*). Severe hypercapnia (15% CO_2, pH 6.77 ± 0.05) produced a further drop in F_{max} of approximately 20% (~70% below normocapnic F_{max}) across all frequencies, which recovered to the level achieved after the first hypercapnic insult upon return to normocapnic conditions (1% CO_2, pH 7.76 ± 0.02; Fig. 1*B*, 1*C*, *left panel*). Resting tension was not affected by hypercapnia (Fig. 1*A*) or frequency (not shown).

Under normocapnic conditions, the time required to reach peak F_{max} decreased as stimulation frequency was increased (Fig. 2*A*). Hypercapnia, either at 7.5% (Fig. 2*A*) or 15% (data not shown) had no effect on the time required to reach F_{max}, except at 0.2 Hz, where it was prolonged. Neither frequency nor hypercapnia had a significant effect on the time for relaxation (Fig. 2*B*). Thus, differences in the rates of rise and fall of contractions during hypercapnia and recovery (Table 1) were largely a result of changes in F_{max}.

Effects of SR Inhibition on the Force-Frequency Relationship

Because the SR has been implicated in contributing Ca^{2+} to force generation in the heart of *P. pardalis* in anoxia (MacCormack et al. 2003*b*) and has been implicated in the recovery of force during persistent acidosis in mammals (Orchard 1987; Orchard et al. 1987), the next series of experiments examined how combined inhibition of SR Ca^{2+} release (10 μM ryanodine) and SR Ca^{2+} uptake (2 μM thapsigargin) affected the force-frequency relationship under normocapnic conditions. Figure 3*A* shows that inhibiting the SR had no effect on F_{max} at 0.2 Hz, suggesting a limited role for the SR in contributing Ca^{2+} to force development. This result is supported by the lack of either postrest potentiation or rest decay of force after a 3-min pause in stimulation (not shown). However, it should be noted that in four out of 12 muscle preparations, addition of SR inhibitors caused an initial but transient decrease in F_{max} that recovered to predrug levels by the end of the 40-min incubation period. This suggests that at 0.2 Hz, loss of SR function may have been compensated for by other Ca^{2+} cycling mechanisms.

Despite the lack of a steady state effect of SR inhibition on F_{max} at 0.2 Hz, SR inhibition had a significant effect on the shape of the force-frequency relationship (Fig. 3*B*; Fig. 4*B*, 4*C*). The force-frequency relationship was negative under SR inhibition over the entire frequency range tested, suggesting that the SR may function to maintain force production at higher heart rates. This was associated with a slowing of relaxation

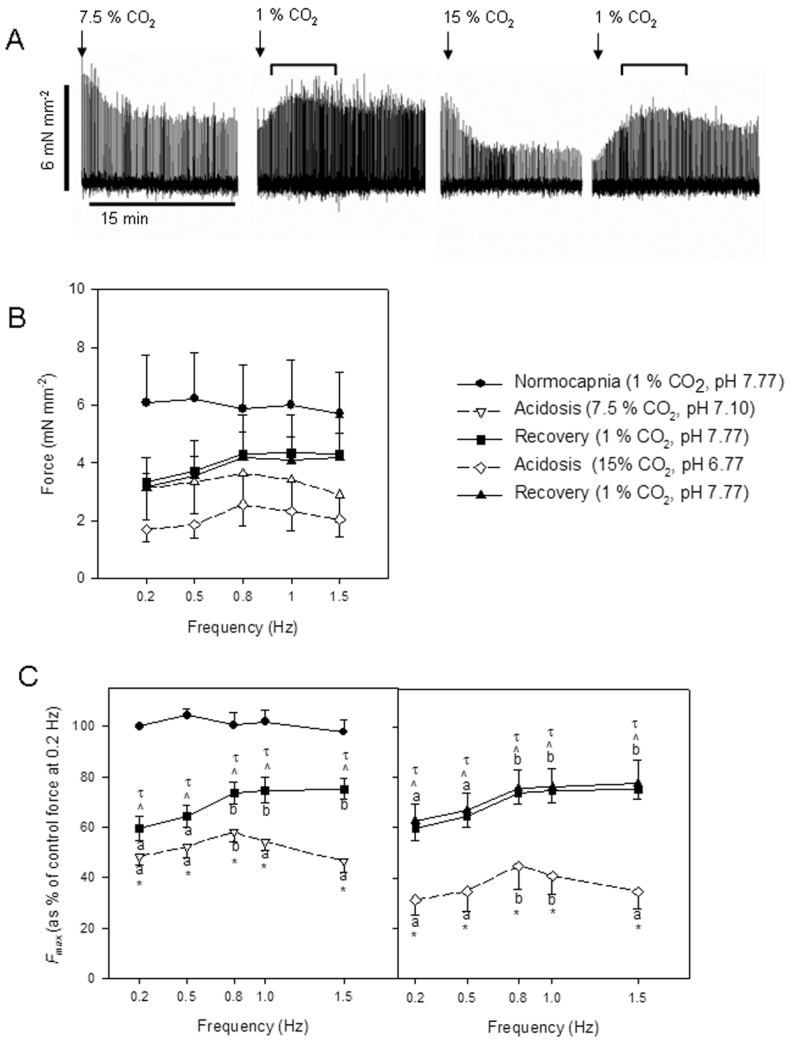

Figure 1. Effect of hypercapnic acidosis and recovery on isometric force production (F_{max}) in ventricular muscle from *Pterygoplichthys pardalis*. *A*, Representative raw data trace showing isometric contraction on a slow time base at 0.2 Hz. Arrows indicate when gas mixture was changed. Bracket indicates "hump" in F_{max} after change in pH before settling to a new steady state level. Breaks in traces are where the force-frequency trials (see below) have been removed (see text for details). Resting tension was not affected by hypercapnia. *B*, Mean data showing the progressive effect of acidosis and recovery on absolute F_{max} across the range of test frequencies. *C*, Mean data normalized to 0.2 Hz normocapnic F_{max}. The effect of the first acidotic challenge (drop in pH from 7.78 to 7.1; *open triangles*) and recovery (to pH 7.77; *squares*) on F_{max} is shown in the left panel. The right panel shows the effects of the second acidotic challenge (from recovered pH of 7.77 [*squares*] to 6.77 [*diamonds*]) and the second recovery period (to pH 7.76; *filled triangles*) on F_{max}. For clarity, statistics are shown for normalized data only. Dissimilar letters indicate significant effect of frequency within a given CO_2 treatment ($P < 0.05$; repeated-measures ANOVA on ranks). An asterisk indicates significant effect of acidosis from normocapnic or recovered conditions at a given stimulation frequency. A circumflex indicates that the recovered F_{max} is significantly different from hypercapnic, and τ indicates that recovered F_{max} is significantly different from normocapnic at a given stimulation frequency ($P < 0.05$, repeated-measures ANOVA on ranks or Mann-Whitney test). All data are means ± SEM; $n = 6$ preparations from six animals.

kinetics at 0.2 Hz (Fig. 3*D*) but no change in the time to the peak of contraction (Fig. 3*C*) or in resting tension (not shown).

The Role of the SR during Hypercapnia and Recovery

The purpose of this series of experiments was to assess whether the SR played a role in the response of the myocardium to severe and repeated hypercapnia and recovery. In SR-inhibited strips, hypercapnia (7.5% CO_2, pH 7.1 ± 0.03) significantly decreased F_{max} by ~40%, which remained stable and depressed throughout hypercapnic exposure at 0.2 Hz (Fig. 4*A*). Recovery of F_{max} was improved in SR-inhibited muscle compared with normocapnic muscle strips, and there was little evidence of a hump in F_{max} (only observed in one out of six preparations). The second and more severe hypercapnic insult (15% CO_2, pH 6.77 ± 0.05) resulted in a similar reduction in F_{max} (~40% across all frequencies) which also recovered to the level achieved after the first hypercapnic insult with return to normocapnia.

Table 1: Contractile force and kinetic parameters for isolated ventricular muscle from *Pterygoplichthys paradalis* during hypercapnic acidosis and recovery

	F_{max} (mN mm^{-2})	TPF (s)	Average Rate of Rise (mN mm^{-2} s^{-1})	THR (s)	Average Rate of Fall (mN mm^{-2} s^{-1})
Normocapnia (1% CO_2, pH 7.78)	6.07 ± 1.66^{A}	.38 ± .02	16.2 ± 3.5^{A}	.18 ± .01	−17.1 ± 3.7^{A}
Hypercapnia (7.5% CO_2, pH 7.10)	3.12 ± 1.08^{B}	.43 ± .02*	7.8 ± 2.0^{B}	.19 ± .01	−8.7 ± 2.0^{B}
Recovery 1 (1% CO_2, pH 7.77)	3.02 ± .58^{B}	.36 ± .03	8.2 ± 1.3^{B}	.17 ± .01	−9.3 ± 1.5^{B}
Severe hypercapnia (15% CO_2, pH 6.77)	1.69 ± .40^{C}	.37 ± .02	4.1 ± .8^{C}	.21 ± .02	−4.3 ± 1.1^{C}
Recovery 2 (1% CO_2, pH 7.77)	3.16 ± .38^{B}	.32 ± .02	8.1 ± 1.3^{B}	.19 ± .02	−6.7 ± 1.3^{B,C}

Note. TPF = time to peak force; THR = the time required to relax by 50%. The rates of rise and fall were calculated by dividing F_{max} by TPF or THR. All parameters were measured at 0.2 Hz. Data are means ± SEM ($n = 6$). All data are paired. Different letters indicate statistically significant differences ($P < 0.05$, one-way repeated-measures ANOVA).

* Statistically significant differences $P < 0.05$, one-way repeated-measures ANOVA.

Inhibiting the SR during hypercapnia had no effect on resting tension (not shown) or on contractile kinetics (Fig. 2*C*, 2*D*). Further, hypercapnia did not alter the negative shape of the force-frequency relationship (Fig. 4*B*, 4*C*).

Discussion

The Force-Frequency Relationship

Most teleost species demonstrate a negative force-frequency relationship across their in vivo range of heart rates (see review by Shiels et al. 2002). The in vivo range of heart rates for *Pterygoplichthys pardalis* is not known; however, previous work has reported an in vivo heart rate of 1.0 Hz at 28°C (MacCormack et al. 2003*a*). We report robust isometric force production in *P. pardalis* ventricle at 1.0 Hz and show that it is relatively unaffected by changes in stimulation frequency (between 0.2 and 1.5 Hz). Thus, unlike most teleosts, *P. pardalis* demonstrates a flat force-frequency relationship. Highly active pelagic fishes of the family Scombridae, including mackerels and tunas, also demonstrate a flat (Pacific mackerel, *Scomber*

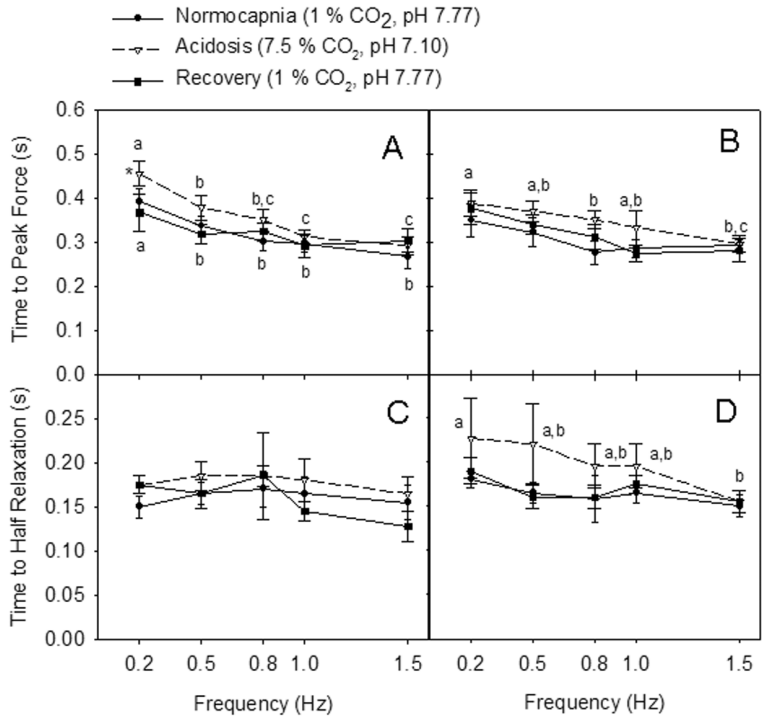

Figure 2. Effect of hypercapnic acidosis on the kinetics of F_{max} in ventricular muscle from *Pterygoplichthys pardalis*. *A* and *C* show the response to hypercapnic acidosis (pH 7.1) and the return to normocapnic conditions (recovery pH 7.77). *B* and *D* show the same responses but in ventricular muscle pretreated with SR inhibitors. Dissimilar letters indicate significant effect of frequency within a given CO_2 treatment ($P < 0.05$, repeated-measures ANOVA) under normocapnic conditions and during acidosis. There was no effect of frequency during recovery. An asterisk indicates significant effect of acidosis at any given frequency ($P < 0.05$, repeated-measures ANOVA). All data are means ± SEM; $n = 6$ preparations from six animals.

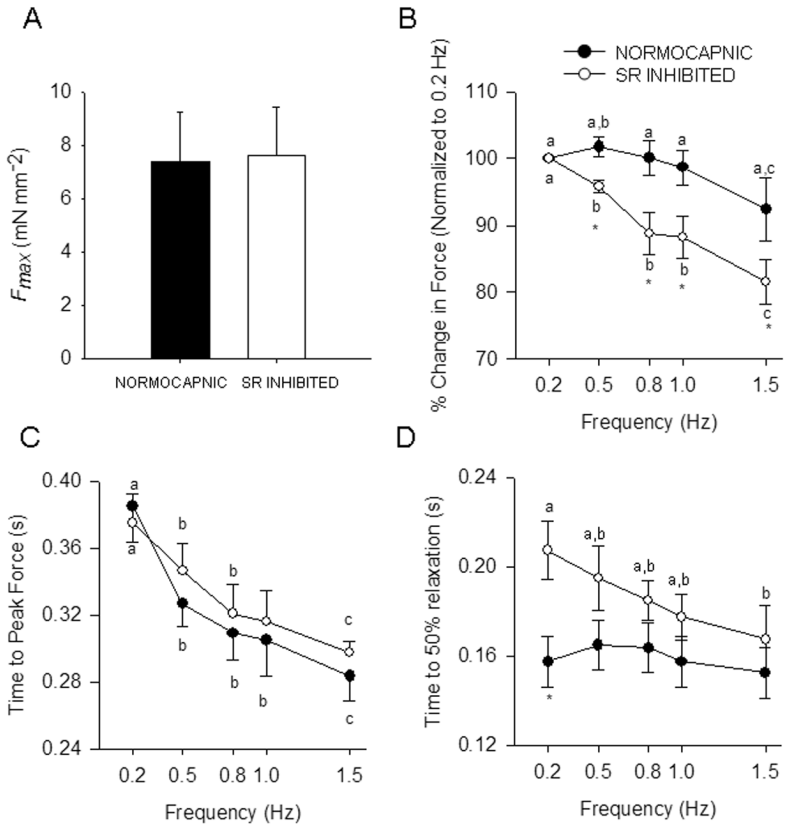

Figure 3. Effect of SR inhibition on F_{max} and contractile kinetics in ventricular muscle from *Pterygoplichthys pardalis*. *A*, SR inhibition had no effect on steady state F_{max} at 0.2 Hz. *B*, SR-inhibited muscle displayed a pronounced negative force-frequency relationship. *C*, Time to peak tension was unchanged by SR inhibition. *D*, Time to reach 50% relaxation was slowed only at 0.2 Hz by SR inhibition. Dissimilar letters indicate significant effect of frequency within a given CO_2 treatment ($P < 0.05$, repeated-measures ANOVA or repeated-measures ANOVA on ranks). An asterisk indicates significant difference between normocapnic and SR-inhibited muscle at any given frequency ($P < 0.05$, Student's *t*-test or Mann-Whitney test). All data are means ± SEM; $n = 12$ preparations from six animals.

japonicus [Shiels and Farrell 2000]) or slightly positively biphasic (yellowfin tuna, *Thunnus albacares* [Shiels et al. 1999]) force-frequency relationship with exact shape determined by the frequency range, temperature, and cardiac tissue type (see Shiels et al. 2002). Similar to the active scombrids, many (but not all; see Anelli et al. 2004) Neotropical fishes show a relatively flat force-frequency relationship across physiological heart rates and temperatures (*Oreochromis niloticus* [Costa et al. 2000]; *Lepidosiren paradoxa* (Dipnoi) [Costa et al. 2005]; *Synbranchus marmoratus* [Rocha et al. 2007]; *Bathygobius soporator* (Gobiidae) [Rantin et al. 1998]). The reason for the strong and maintained isometric force production across a range of frequencies in these diverse groups of fishes may relate to cardiac adaptations that allow them to maintain contractility in a harsh and unstable environment (Shiels and Farrell 2000; Galli et al. 2009).

Effects of Hypercapnic Acidosis

Isometric force was depressed by hypercapnia in *P. pardalis* myocardium. A similar (~50%) reduction in whole-heart performance has been recently reported for the in situ *P. pardalis* heart in response to hypercapnia (7.5% CO_2, pH 7.1; Hanson et al. 2009). Depressed contractility is a common feature of vertebrate muscle subjected to hypercapnic acidosis. However, many air-breathing vertebrates (amphibians, reptiles, mammals) show a rebound in contractile force during persistent acidosis (Gesser and Jorgensen 1982). In contrast, water-breathing vertebrates show a greater loss of force in response to acidosis and only partially recover force upon return to normocapnia. Thus, our data suggest that the facultative air-breathing *P. pardalis* heart is more similar to other fish species than air breathers in the response to hypercapnic acidosis (Gesser and Poupa 1983).

The mechanism underlying depressed myocardial contractility during hypercapnia has not been directly investigated in fish. The most probable mechanism is that an intracellular acidosis follows the extracellular acidosis that reduces myofilament Ca^{2+} sensitivity (Orchard and Kentish 1990). This interpretation is supported in the current study by the lack of an effect of hypercapnia on the kinetics of contraction or on the shape of the force-frequency relationship, which would occur with a Ca^{2+}-cycling-dependent change in force. Furthermore, earlier work has shown that acidosis reduces the Ca^{2+} sensitivity of the teleost contractile element (Meadows et al.

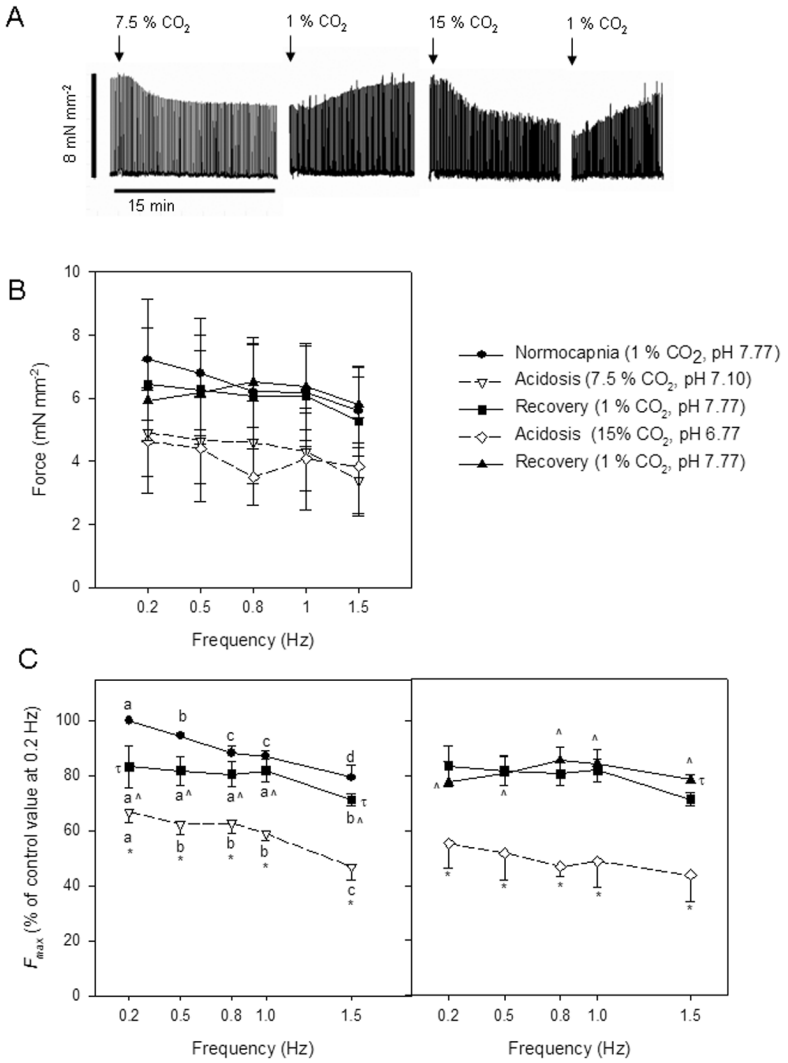

Figure 4. Effect of hypercapnic acidosis on isometric force production (F_{max}) in ventricular muscle from *Pterygoplichthys pardalis* pretreated with SR inhibitors. *A*, Representative raw data trace showing isometric contraction on a slow time base at 0.2 Hz. Arrows indicate where gas mixture was changed. Breaks in the traces are where the force-frequency trials (see below) have been removed. Resting tension was not affected by hypercapnia. *B*, Mean data showing the progressive effect of acidosis and recovery on absolute F_{max} across the range of test frequencies. *C*, Mean data normalized to 0.2 Hz normocapnic F_{max}. The effect of the first acidotic challenge (drop in pH from 7.78 to 7.1; *open triangles*) and recovery (to pH 7.77; *squares*) on F_{max} is shown in the left panel. The right panel shows the effects of the second acidotic challenge (from recovered pH of 7.77 [*squares*] to 6.77 [*diamonds*]) and the second recovery period (to pH 7.77; *filled triangles*) on F_{max}. For clarity, statistics are shown for normalized data only. Dissimilar letters indicate significant effect of frequency within a given CO_2 treatment ($P < 0.05$; repeated-measures ANOVA on ranks). An asterisk indicates significant effect of acidosis from normocapnic or recovered conditions at a given stimulation frequency. A circumflex indicates that the recovered F_{max} is significantly different from hypercapnic, and τ indicates that recovered F_{max} is significantly different from normocapnic at a given stimulation frequency ($P < 0.05$, repeated-measures ANOVA on ranks or Mann-Whitney test). All data are means ± SEM; $n = 6$ preparations from six animals.

1998; Gillis et al. 2000). What is not clear is (1) the mechanism underlying the lack of force recovery during persistent acidosis in *P. pardalis* (and most other fish species) and (2) why force does not recover fully upon return to normocapnic conditions in this study. Each of these questions will be considered briefly below.

The lack of force recovery during persistent acidosis may merely reflect the fact that ~15 min is insufficient for recovery. However, this seems unlikely because myocardial force is known to recover during persistent acidosis with a fairly rapid time course (~5 min) in both mammals (Orchard 1987) and amphibians (Salas et al. 2006). Alternatively, the lack of force recovery during persistent acidosis may be because acid extrusion mechanisms were not activated (or sufficiently activated). These extrusion mechanisms are necessary to restore pH_i or initiate the ion flux cascade that results in an increased Ca^{2+} influx, which offsets the negative effect of acidosis on myofilament Ca^{2+} sensitivity. These possibilities cannot be directly

resolved from the current study; however, our results allow for speculation. The hump in F_{max} that often occurred during restoration of normocapnic pH suggests a transitory increase in the amplitude of the systolic Ca^{2+} transient in *P. pardalis* during recovery. The fact that this hump was absent when the SR was inhibited suggests the SR could be loading (DeSantiago et al. 2004) but not releasing (Rousseau and Pinkos 1990) Ca^{2+} throughout the acidotic bout. Upon resumption of normocapnic conditions, the SR may release its stored Ca^{2+}, thereby causing the hump in recovery. This would support the idea that acid extrusion mechanisms are activated during acidosis but are not adequate to restore myocardial force.

Isometric force does not fully recover upon return to normocapnic pH in the *P. pardalis* heart. This observation is in line with earlier teleost studies that show that regardless of whole-animal acidotic tolerance, isometric force of isolated cardiac tissue does not fully recover from a hypercapnic insult (Gesser and Jorgensen 1982). In contrast, most mammalian hearts subjected to hypercapnia recover isometric force following return to normocapnic conditions (Orchard and Kentish 1990), as do turtles (Yee and Jackson 1984). A number of explanations may account for the limited recovery in *P. pardalis* heart in the current study. The simplest is that pH_i does not recover and cellular Ca^{2+} cycling is not sufficiently enhanced to make up for the loss of myofilament Ca^{2+} sensitivity. In disagreement with this idea, Hanson et al. (2009) showed that whole-heart performance completely recovers after a reduction of ~50% in response to hypercapnia (7.5% CO_2, pH 7.1) upon return to normocapnic pH (1% CO_2, pH 7.76). Furthermore, they found that during recovery, pH_i was unchanged from that measured before the acidotic insult (measured as off-line endpoint mean tissue pH_i; Pörtner et al. 1990). Interestingly, reductions in pH_i may have had a relatively greater effect on isometric force than on whole-heart function, which may help to explain the discrepancy between the two studies. Support for this contention comes from mammalian experiments that show acidosis has less of an effect on shortening velocity (i.e., ejection) than isometric force development (Orchard and Kentish 1990).

Alternatively, hypercapnia may have caused permanent damage to the contractile apparatus or induced a state of poststress contractile dysfunction akin to myocardial stunning (Bers 2001). This could have occurred through the production of reactive oxygen species or from Ca^{2+} overload during return to normocapnic pH. However, this seems unlikely because recovery was significantly improved in the muscle treated with SR inhibitors (see below), and these muscles did not demonstrate a hump in force during recovery.

Interestingly, recovery of contractile force following hypercapnia was improved after the second, more severe, hypercapnic bout (Fig. 1*B*). The explanation for this phenomenon is not clear. It is possible that mechanisms that protect the heart against hypercapnia in the catfish heart are activated only during severe, rather than a modest, hypercapnia. Alternatively, our results could point toward a preconditioning-like response in the catfish heart. The nature of our experimental protocol does not allow us to test this idea directly but points toward an interesting avenue for future work.

The Role of the SR

This is the first study to directly investigate the effect of SR inhibition on force production in *P. pardalis* ventricle. When the SR was inhibited, the ventricle was not able to maintain force as frequency was increased, but no steady state effect of SR inhibition was observed at low pacing frequencies. This suggests that other Ca^{2+} flux pathways are able to compensate for the loss of SR Ca^{2+} cycling at 0.2 Hz but that these pathways are insufficient when Ca^{2+} cycling demands are increased by higher contraction rates. This finding is in contrast to the depressive effects of SR inhibition at low frequencies in two other Neotropical air-breathing fish (*S. marmoratus*, Rocha et al. 2007; *L. paradoxa*, Costa et al. 2005) but similar to that observed in the yellowfin tuna (Shiels et al. 1999).

Recruitment of SR Ca^{2+} stores during stressful or adverse conditions has been hypothesized for fishes (e.g., Shiels et al. 2002). Indeed, the relative importance of the SR increases in our study with increased contraction frequency, and previous work with *P. pardalis* revealed a role for the SR during anoxia (MacCormack et al. 2003*b*). It was therefore surprising to observe SR-inhibited muscle maintained and recovered force to a larger extent than control muscle during hypercapnic acidosis. Mechanisms underlying the improved recovery of *P. pardalis* ventricle when the SR was inhibited are not clear. Cellular studies are required to interpret these findings and to adequately reveal the underlying mechanisms.

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