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Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from bluefin tuna and Pacific mackerel

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Shiels, H. A., J. M. Blank, A. P. Farrell, and B. A. Block. Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from bluefin tuna and Pacific mackerel. *Am J Physiol Regul Integr Comp Physiol* 286: R659–R668, 2004. First published December 4, 2003; 10.1152/ajpregu.00521.2003.—Tunas are capable of exceptionally high maximum metabolic rates; such capability requires rapid delivery of oxygen and metabolic substrate to the tissues. This requirement is met, in part, by exceptionally high maximum cardiac outputs, opening the possibility that myocardial Ca^{2+} delivery is enhanced in myocytes from tuna compared with those from other fish. In this study, we investigated the electrophysiological properties of the cardiac L-type Ca^{2+} channel current (I_{Ca}) to test the hypothesis that Ca^{2+} influx would be larger and have faster kinetics in cardiomyocytes from Pacific bluefin tuna (*Thunnus orientalis*) than in those from its sister taxon, the Pacific mackerel (*Scomber japonicus*). In accordance with this hypothesis, I_{Ca} in atrial myocytes from bluefin tuna had significantly greater peak current amplitudes and faster fast inactivation kinetics (-4.4 ± 0.2 pA/pF and 25.9 ± 1.6 ms, respectively) than those from mackerel (-2.7 ± 0.5 pA/pF and 32.3 ± 3.8 ms, respectively). Steady-state activation, inactivation, and recovery from inactivation were also faster in atrial myocytes from tuna than from mackerel. In ventricular myocytes, current amplitude and activation and inactivation rates were similar in both species but elevated compared with those of other teleosts (Vornanen M. *Am J Physiol Regul Integr Comp Physiol* 272: R1432–R1440, 1997). These results indicate enhanced I_{Ca} in atrial myocytes from bluefin tuna compared with Pacific mackerel; this enhanced I_{Ca} may be associated with elevated cardiac performance, because I_{Ca} delivers the majority of Ca^{2+} involved in excitation-contraction coupling in most fish hearts. Similarly, I_{Ca} is enhanced in the ventricle of both species compared with other teleosts and may play a role in the robust cardiac performance of fishes of the family Scombridae.

Scombridae; ventricle; atrium; heart; calcium currents; excitation-contraction coupling; dihydropyridine receptor; fish; *Scomber japonicus*; *Thunnus orientalis*

CERTAIN SPECIES OF TUNA (family Scombridae) are apex predators, renowned for their unique thunniform swimming mode, high metabolic rates, and endothermic physiology (herein defined as the ability to elevate and retain metabolically produced heat in the swimming muscles and other organs, as well as the ability to physiologically control and reduce routes and rates of heat transfer with the environment) (6). For example, standard and active metabolic rates are at least twofold higher in yellowfin tuna (*Thunnus albacares*) than in other active teleosts (26). The high metabolic rates and endothermic physiology are supported by unique circulatory arrangements and cardiac characteristics that are exceptional even among athletic fishes. For example, the ventricles can be much larger relative to body size in tuna [0.3–0.4% body mass (8, 32)] than in rainbow trout [~0.1% body mass (9)], a modification required to produce ventricular pressures 50% higher than those in rainbow trout (2, 44). Furthermore, it has been estimated that maximum cardiac output in skipjack tuna (*Katsuwonus pelamis*) exceeds 200 $ml \cdot min^{-1} \cdot kg^{-1}$ (8), about twice the maximum cardiac output reported for salmonids at comparable temperatures. To achieve such high levels of maximum cardiac output, these fish have unusually high maximum heart rates (8). In view of these features, it is not surprising, therefore, that the aerobic capacity of the tuna heart exceeds that of other teleosts, as indicated by elevated biochemical indexes, e.g., high citrate synthase activity (12, 17), high mitochondrial oxygen consumption (31), and high myoglobin levels (18), as well as a highly developed coronary circulation (15).

The enhanced cardiac performance of tunas may also be related to specializations in excitation-contraction (E-C) coupling within the scombrid lineage. Because high heart rates in mammals are associated with increased expression of sarcoplasmic reticulum (SR) proteins (20), one hypothesis is that greater utilization of SR Ca^{2+} may enhance E-C coupling in tuna hearts and, thereby, play a role in the elevated cardiac performance of these fish (14). Evidence for elevated SR Ca^{2+} cycling in tuna hearts has emerged from ryanodine inhibition studies of isometric force development in isolated muscle preparations (25, 40) and measurements of enhanced SR Ca^{2+}-ATPase activity and Ca^{2+} uptake in ventricular vesicular preparations (28).

Despite the enhanced role for SR Ca^{2+} in E-C coupling, in tuna hearts, as in all vertebrates, the first step in E-C coupling in response to the change in membrane potential is the opening of L-type Ca^{2+} channels in the sarcolemma, resulting in an influx of Ca^{2+} from the extracellular space. Moreover, at physiologically relevant contraction frequencies and temperatures, the majority (>60%) of the Ca^{2+} necessary for contraction in skipjack and yellowfin tuna myocardium is extracellular in origin (25, 40). In cardiomyocytes from frog, rainbow trout (30, 45), and crucian carp (*Carassius carassius*) (47), most of the extracellular Ca^{2+} needed for contraction crosses the sarcolemma via L-type Ca^{2+} channels, although under certain conditions, the Na^+/Ca^{2+} exchanger can also transport significant quantities of Ca^{2+} across the sarcolemma (21, 46). In fact, force generated by isolated myocardial muscle from rainbow trout declines by >80% when L-type Ca^{2+} channels are inhibited with verapamil but barely 5% when the SR is

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inhibited with ryanodine (1). Furthermore, changes in action potential shape during rapid temperature changes have been shown to preserve transsarcolemmal Ca^{2+} flux in rainbow trout atrial myocytes (41). In sarcolemmal preparations from skipjack tuna ventricle, high L-type Ca^{2+} channel densities have been measured using dihydropyridine binding (42), and this leads to the hypothesis that increased L-type Ca^{2+} current (I_{Ca}) may play a role in the enhanced cardiac performance of tunas, especially because I_{Ca} is also important for triggering the release of SR Ca^{2+} stores (13). However, this hypothesis has not been tested, because I_{Ca} has not been recorded in myocytes from tunas or any other member of the Scombridae family.

In this study, we examine I_{Ca} in myocytes of the Pacific bluefin tuna. Despite the fact that little is known about their in vivo cardiac performance compared with other tunas, the bluefin group (including Atlantic, Pacific, and Southern bluefin) is of particular interest, because they display a wide thermal niche (2–31°C) (5, 19). This means that bluefin tuna may have to defend their endothermic skeletal muscle against large thermal gradients, and, at the same time, their cardiac tissue must operate at ambient water temperatures. The bluefin ventricle is proportionally larger and shows a higher SR Ca^{2+}-ATPase activity than ventricles of other teleosts, including other tuna species. In fact, recent biochemical evidence suggests that a gradient of expression of SR Ca^{2+}-ATPase activity exists among the *Thunnus* lineage, with bluefin tuna having higher levels than yellowfin tuna (28).

To place potential specializations in L-type Ca^{2+} current characteristics into the context of scombrid evolution, we compared I_{Ca} of atrium and ventricle of bluefin tuna with that of Pacific mackerel (*Scomber japonicus*), an ectothermic sister taxon to the *Thunnus* genus that shares several morphological and physiological features. In particular, ventricular aerobic capacity is elevated in Pacific mackerel and the tunas relative to other teleosts (17). However, mackerel have metabolic rates typical of other active fish (17). In addition, the hearts of mackerels are significantly smaller relative to body mass (0.1–0.2% body mass) than those of tunas, and the mackerel SR appears to play a lesser role in E-C coupling (28, 39). We hypothesized that I_{Ca} in bluefin tuna cardiomyocytes would be larger and have faster kinetics than those measured in mackerel to increase cytosolic Ca^{2+} directly and to trigger Ca^{2+} release from the SR, inasmuch as these would lead to the known enhancement of contractile strength. As this study constitutes the first cellular investigation into sarcolemmal Ca^{2+} flux in these species, it was deemed important to examine both atrial and ventricular myocytes. Significant differences in atrial and ventricular myosin isoforms (23), myosin ATPase activities (24), and SR Ca^{2+} uptake activities and isometric contraction parameters (1) are apparent in fish hearts. In addition, the spatial distribution of L-type Ca^{2+} channels differs in atrium and ventricle of mammals (10). Yet no studies have explicitly compared the atrium and ventricle of fish with regard to sarcolemmal Ca^{2+} transport.

The results of the present study clearly indicate that I_{Ca} is larger and faster in bluefin tuna atrium than in Pacific mackerel atrium, supporting the general idea that species-specific differences in I_{Ca} may contribute to the elevated cardiac performance of tunas among scombrid fishes. In ventricular myocytes, I_{Ca} is enhanced in both scombrid species compared with what is known for other teleost fishes (48). Thus the large density of I_{Ca}, coupled with the high surface area-to-volume ratio of the myocytes, may play key roles in the robust cardiac performance of the scombrid fishes.

METHODS AND MATERIALS

Fish origin and care. Bluefin tuna (fish mass = 14.3 ± 0.5 kg, heart mass = 52.1 ± 2.8 g, $n = 5$) were captured off San Diego, CA, and held aboard the F/V Shogun in large wells flooded with seawater before transport by truck to the Tuna Research and Conservation Center (Pacific Grove, CA). Bluefin tuna were held in a 109-m^3 tank at 20 ± 1°C and fed a diet of squid, sardines, and enriched gelatin, as previously described (16). Pacific mackerel (fish mass = 230.5 ± 10.9 g, heart mass = 0.57 ± 0.06 g, $n = 13$) were acquired from the Monterey Bay Aquarium (Monterey, CA), held in a 20-m^3 tank at 20 ± 1°C, and fed a diet of krill. Fish were held for ≥3 mo before experimentation. All procedures were in accordance with Stanford University Institutional Animal Care and Use Committee animal handling protocols.

Isolated myocyte preparation. Myocytes from mackerel and tuna were obtained by adaptation of the isolation protocol previously described for rainbow trout (45). Briefly, fish were euthanized by pithing, and the heart was excised. The heart was perfused with isolating solution from a height of 60 cm until it had stopped beating and was cleared of blood. This was accomplished by retrograde perfusion through the ventricular lumen for ~10 min in mackerel and ~20 min in tuna. In tuna, the coronary artery was additionally perfused to clear blood from the arteries and compact myocardial tissue. Proteolytic enzymes were then added to the isolating solution, and retrograde luminal perfusion was continued for ~20 min in mackerel and ~40 min in tuna. After enzymatic treatment, the atrium and ventricle were placed in separate dishes containing fresh isolating solution. Tissues were cut into small pieces with scissors and then triturated through the opening of a Pasteur pipette to free individual myocytes. Myocytes were stored in fresh isolating solution for up to 8 h at 20°C.

Solutions. The isolating solution contained (mM) 100 NaCl, 10 KCl, 1.2 KH_2PO_4, 4 $MgSO_4$, 50 taurine, 20 glucose, and 10 HEPES, with pH adjusted to 6.9 with KOH at 20°C. For enzymatic digestion, collagenase (type IA), trypsin (type IX), and fatty acid-free BSA were added to this solution. The extracellular solution for recording I_{Ca} contained (mM) 150 NaCl, 5.4 CsCl, 1.2 $MgSO_4$, 0.4 NaH_2PO_4, 3.2 $CaCl_2$, 10 glucose, and 10 HEPES, adjusted to pH 7.7 with CsOH. TTX (1 μM) was added to the perfusate to block fast Na^+ channels (see Fig. 2). Ryanodine (5 μM) was included to block SR Ca^{2+} release channels. The pipette solution contained (mM) 130 CsCl, 5 MgATP, 15 tetraethylammonium chloride, 1 $MgCl_2$, 5 oxaloacetate, 5 Na_2-phosphocreatine, 10 HEPES, and 0.03 Na_2GTP. The EGTA concentration was 0.025 mM unless stated otherwise. The pH was adjusted to 7.2 with CsOH. Inclusion of CsCl and tetraethylammonium chloride in our solutions inhibited K^+ currents. All drugs were purchased from Sigma.

Experimental procedures. A sample of myocytes was added to the recording chamber and allowed to settle on the bottom. Myocytes were superfused at a rate of ~2 ml/min with extracellular solution at 20°C. Whole cell voltage-clamp experiments were performed using a PC 505 amplifier (Warner Instruments, Hamden, CT) with a CV-4 1/100 head stage (Axon Instruments). Resistance of the pipette was 2.3 ± 0.1 MΩ when it was filled with pipette solution. Pipette capacitance (4.1 ± 0.1 pF) was compensated after formation of a gigaohm seal. Mean series resistance was 8.7 ± 0.4 MΩ. Membrane capacitance was measured using the membrane test function of the pClamp 8.0 software (Axon Instruments) and confirmed with the calibrated capacity compensation circuit of the amplifier (Table 1). Signals were leakage corrected using the P/N procedure of the software, low-pass filtered at a frequency of 2 kHz, and then analyzed offline using Clampfit 8.0 software (Axon Instruments).

Table 1. *Morphometric features of atrial and ventricular myocytes from bluefin tuna and Pacific mackerel*

	Atrial Myocytes					Ventricular Myocytes				
	Length, μm	Width, μm	Capacitance, pF	Vol, pl	Area/Vol, pF/pl	Length, μm	Width, μm	Capacitance, pF	Vol, pl	Area/Vol, pF/pl
Tuna	201.2±7.2 (32)	6.6±0.3 (32)	45.1±1.7 (53)	2.7±0.1 (53)	16.5 (53)	185.8±4.9 (31)	7.6±0.3† (31)	43.1±1.1 (36)	3.0±0.1 (36)	14.3 (36)
Mackerel	166.4±6.1* (21)	5.7±0.2 (21)	31.1±1.2* (28)	1.7±0.1* (28)	18.3 (20)	166.7±7.1* (20)	4.9±0.2* (20)	41.8±2.4*† (28)	2.0±0.1* (28)	21.9* (20)

Values are means ± SE of number of myocytes in parentheses; myocytes were pooled from 5 tuna (fish mass = 14.3 ± 5 kg, heart mass = 52.1 ± 2.8 g) and 13 mackerel (fish mass = 230.5 ± 10 g, heart mass = 0.57 ± 0.06 g). *Significant difference between species within each tissue; †significant difference between tissues within each species ($P < 0.05$, student's *t*-test).

Voltage-clamp waveforms and protocols used to study the characteristics of I_{Ca} are provided in Figs. 2, 5, and 6. The amplitude of I_{Ca} was calculated as the difference between the peak inward current and the current at the end of the depolarizing pulse. Kinetics describing the fast (τ_f) and slow (τ_s) components of inactivation of I_{Ca} were derived by fitting a second-order exponential function to the decaying portion of the current using the Chebyshev procedure (Clampfit 8.0 software, Axon Instruments). Steady-state kinetic parameters were obtained by fitting steady-state activation and inactivation data to Boltzmann equations to determine the half-activating or half-inactivating potential (V_h) and the slope of activation and inactivation (k), as previously described (41, 45). Recovery from inactivation of I_{Ca} was assessed by normalizing current amplitude at a constant test pulse (500 ms, −70 to 0 mV) to the constant prepulse value (500 ms, −70 to 0 mV) after variable interpulse durations (5–400 ms, −70 mV; see Fig. 6). Tissue- and species-specific differences were assessed with Student's *t*-tests, and significance was accepted at $P < 0.05$. When values are presented as means, the number of observations (n) is provided.

Myocyte morphometrics. The shape of the myocyte has important implications for E-C coupling (48). Detailed measurements of cell size were made using an Olympus BH2 microscope with an Olympus DP10 digital camera system. A sample of cells was placed on a glass slide and sealed with a coverslip. Cell length and width were measured with the calibrated optical graticule of the Olympus system. Width was determined at three levels, in the middle and halfway from the midpoint to the end, with the mean of the three values taken as cell width (43). Surface area and cell volume were calculated from cell capacitance, as well as cell length and width measurements, with the assumption that the cells were cylinders with elliptical cross sections (47).

RESULTS

Morphological characteristics of isolated myocytes. Isolated myocytes from the atrium and ventricle of the Pacific bluefin tuna and the Pacific mackerel are displayed in Fig. 1. A comparison of the morphometric characteristics of the isolated myocytes is provided in Table 1. Myocytes were long and thin, as described for amphibians and other fish species (48). A comparison of atrial cell size reveals significantly longer myocytes in the tuna, with a greater surface area and cell volume than in the mackerel (Table 1). The cell capacitance (pF)-to-volume (pl) ratio was 16.5 and 18.3 in tuna and mackerel atrial cells, respectively. A comparison of tuna and mackerel ventricular cells shows longer and wider myocytes in the tuna, with a significantly smaller cell capacitance-to-volume ratio than in the mackerel: 14.3 vs. 21.4 (Table 1). Sarcomeric striations are clearer in the tuna myocytes, probably reflecting a greater myofibril component (Fig. 1).

Characterization of I_{Ca}. Inward Ca^{2+} (I_{Ca}) and Na^+ current (I_{Na}) were present in atrial and ventricular myocytes from both species. I_{Na} was completely abolished by 1 μM TTX, as shown in Fig. 2*A* for a mackerel atrial myocyte. Similar results were obtained with mackerel ventricular myocytes and tuna atrial and ventricular myocytes (not shown). Because there is overlap between the active voltages for I_{Na} and I_{Ca}, 1 μM TTX was subsequently included in all extracellular solutions to isolate I_{Ca}. The specific L-type Ca^{2+} channel blocker nifedipine (2.5 μM) was used to confirm that I_{Ca} was the only remaining inward current in the presence of 1 μM TTX under our conditions in both tissues and both species (Fig. 2*B*).

Under whole cell patch-clamp conditions, the amplitude of I_{Ca} is sensitive to the amount of Ca^{2+} buffer included in the patch pipette [rat (36) and rainbow trout (22)]. We found that varying EGTA concentration from 0.025 to 5 mM had a large and significant effect on the amplitude of I_{Ca} over the range of activate voltages (Fig. 2*C*). At 0 mV, inclusion of 5 mM EGTA increased the amplitude of I_{Ca} nearly fourfold over the amplitude obtained with 0.025 mM EGTA. Although the intracellular buffering capacity of mackerel and tuna myoplasm is unknown, studies with rainbow trout cardiomyocytes suggest that inclusion of 0.025 mM EGTA in the pipette best mimics the in vivo buffering capacity (22). Furthermore, our experiments were short (<2 min for each myocyte), and I_{Ca} did not run down with 0.025 mM EGTA in the pipette over this time period. Therefore, in all subsequent experiments, we used 0.025 mM EGTA in the pipette solution.

Comparison of I_{Ca}-voltage relations. The current-voltage relation from both tissues and species showed activation of I_{Ca} beginning at −30 mV and peaking between 0 and +10 mV (Fig. 3). A comparison of atrial myocytes from both species reveals larger I_{Ca} in tuna than in mackerel (−4.4 ± 0.2 vs. −2.7 ± 0.4 pA/pF at 0 mV). The current-voltage relation showed that this difference persisted across the range of active voltages (Fig. 3*A*). In contrast to atrial myocytes, no significant difference in peak current amplitude was found between species in ventricular myocytes (−4.5 ± 0.3 and −5.6 ± 0.8 pA/pF in tuna and mackerel, respectively, at 0 mV). However, at more-negative voltages (−10 and −20 mV), current amplitude was significantly less in tuna than in mackerel ventricular myocytes (Fig. 3*B*).

Myocardial tissue comparisons revealed no significant differences in I_{Ca} in bluefin tuna. However, in mackerel, I_{Ca} was significantly larger and activated at more negative voltages in ventricle than in atrium (Fig. 3).

Comparison of I_{Ca} kinetics. In atrial myocytes, the kinetics of fast inactivation (τ_f) of I_{Ca} were significantly faster in tuna than in mackerel: 25.9 ± 1.6 vs. 32.3 ± 3.8 ms (Fig. 4, *A* and *B*). This may reflect greater Ca^{2+}-dependent inactivation from Ca^{2+} flowing through the channel, inasmuch as I_{Ca} density was greater in tuna atrial myocytes. In ventricular myocytes, there was a trend for faster τ_f of I_{Ca} in tuna than in mackerel (21.8 ±

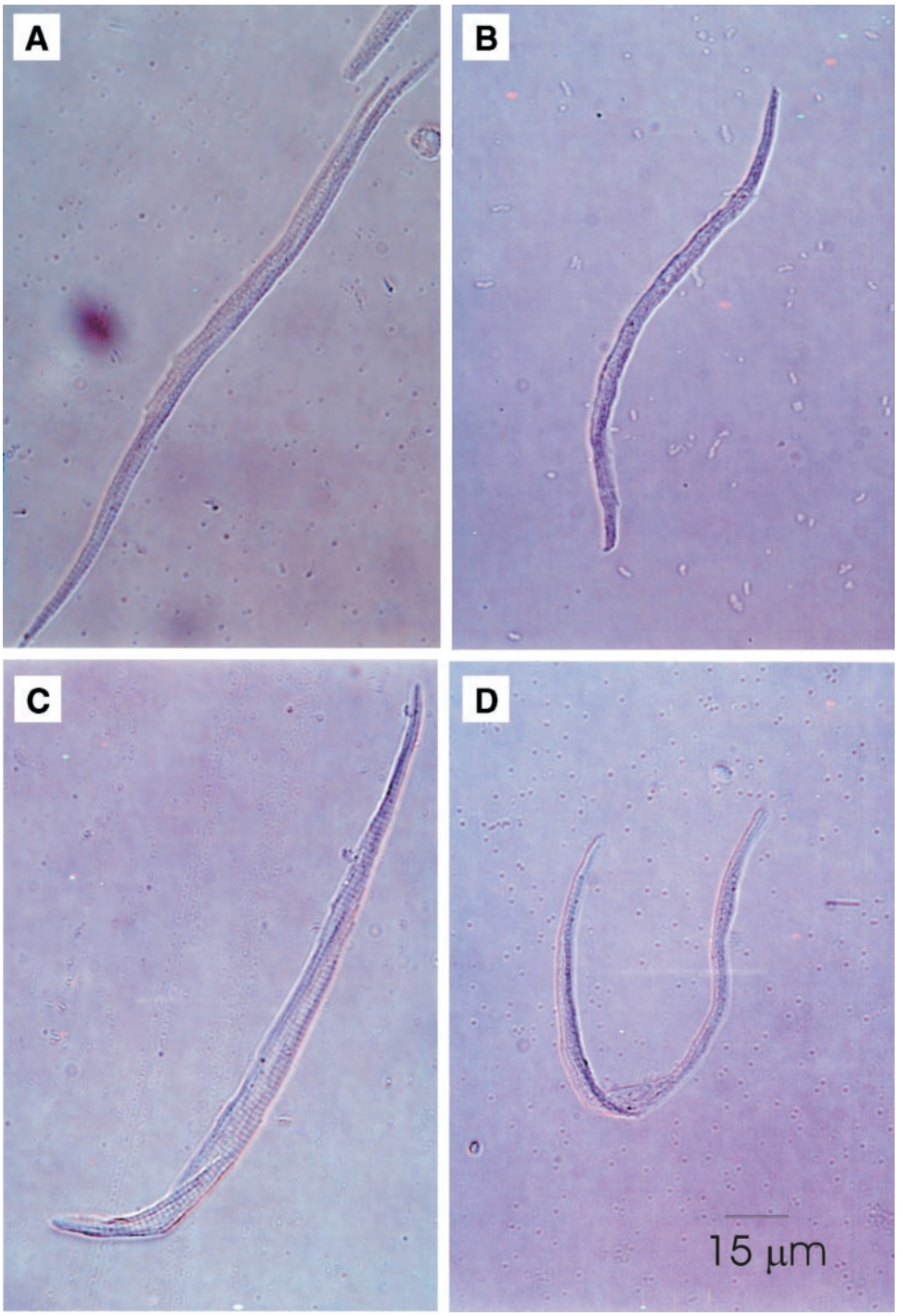

Fig. 1. Morphology of live cardiac myocytes from Pacific mackerel and bluefin tuna. *A* and *B*: atrial myocytes from tuna and mackerel, respectively. *C* and *D*: ventricular myocytes from tuna and mackerel, respectively. Note larger size and clearer sarcomeric striations in tuna than in mackerel myocytes. Mean morphometric data and statistical analysis are provided in Table 1. Scale bar applies to all images.

1.7 vs. 25.1 ± 0.8 ms), but this was not statistically resolvable (Fig. 4, *D* and *E*). No significant differences in τ_s were observed between species in atrial or ventricular myocytes (Fig. 4, *C*, *D*, and *F*). Myocardial tissue type did not significantly affect the kinetics of inactivation in either species.

Steady-state activation and inactivation kinetics. Steady-state activation and inactivation were faster in bluefin tuna than in Pacific mackerel atrial myocytes (Fig. 5*A*, Table 2). The slopes (*k*) of steady-state activation and inactivation were significantly steeper in tuna atrium (6.19 ± 0.16 and 5.29 ± 0.29 for activation and inactivation, respectively) than in mackerel atrium (7.69 ± 0.75 and 9.13 ± 0.57 for activation and inactivation, respectively). Activation of Ca^{2+} channels in tuna atria started positive to −40 mV and were half-maximal (V_h) at −8.13 mV. Similar values were observed in mackerel (Table 2). The inactivation variable (or channel availability) began decreasing positive to approximately −50 mV in tuna atria and was half complete at −26 mV. Mackerel atrial inactivation began decreasing earlier (positive to approximately −60 mV) but was half complete at a voltage similar to that in tuna (Table 2, Fig. 5). In contrast, the slopes of activation and inactivation were unchanged between species in ventricular myocytes (Fig.

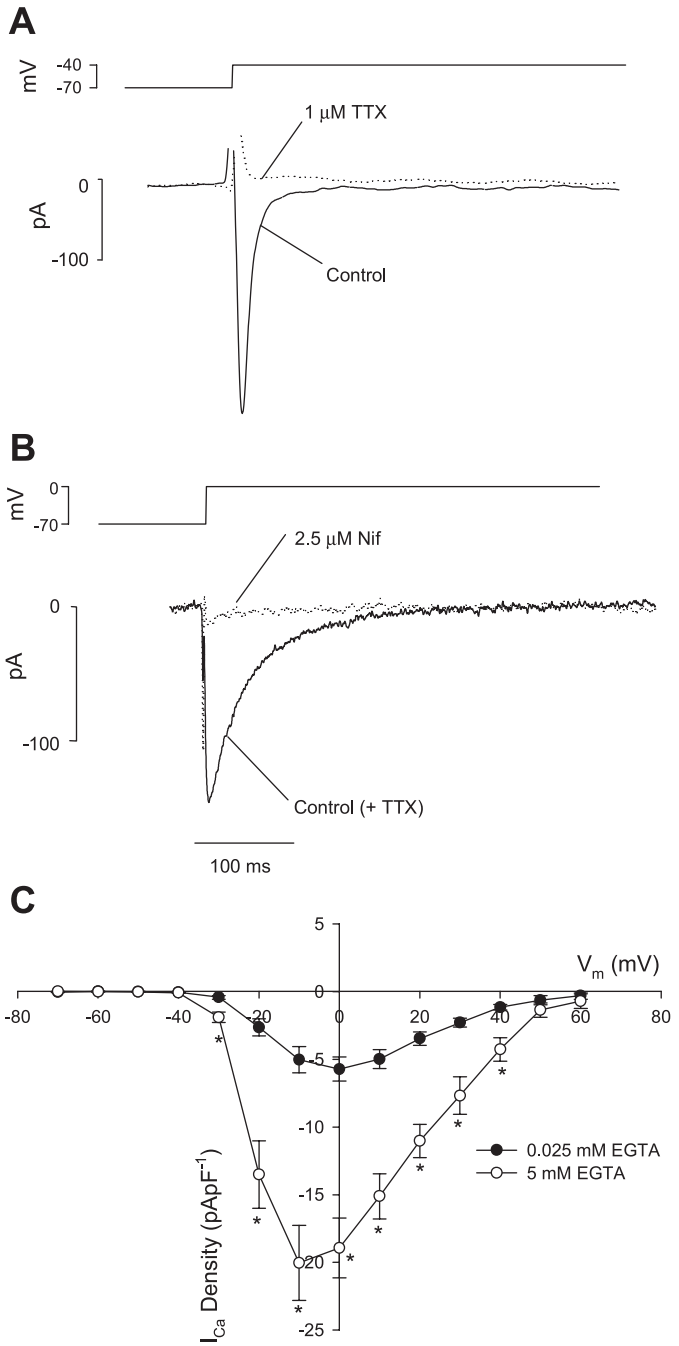

Fig. 2. Isolation of the L-type Ca^{2+} channel current (I_{Ca}) in scombrid cardiomyocytes. *A*: effect of 1 μM TTX on inward Na^+ current (I_{Na}) in an atrial myocyte (capacitance = 33 pF) from Pacific mackerel. A similar abolition of I_{Na} was seen in mackerel ventricular myocytes and in atrial and ventricular myocytes from bluefin tuna with 1 μM TTX (data not shown). *B*: effect of 2.5 μM nifedipine (Nif), a specific blocker of the L-type Ca^{2+} channel, on the remaining inward current in an atrial myocyte from bluefin tuna (capacitance = 56 pF) in the presence of 1 μM TTX. A similar inhibition of I_{Ca} was observed in tuna ventricular myocytes and myocytes from both tissues in the Pacific mackerel. Scale bar applies to *A* and *B*. Waveform protocol used to elicit I_{Na} and I_{Ca} is shown above each current recording. *C*: effect of increasing pipette EGTA concentration from 0.025 to 5 mM on Ca^{2+} current-voltage relation in mackerel ventricular myocytes. V_m, membrane potential. Values are means ± SE; n = 28 for 0.025 mM EGTA and n = 15 for 5 mM EGTA. *Significantly different at $P < 0.05$ (Student's *t*-test).

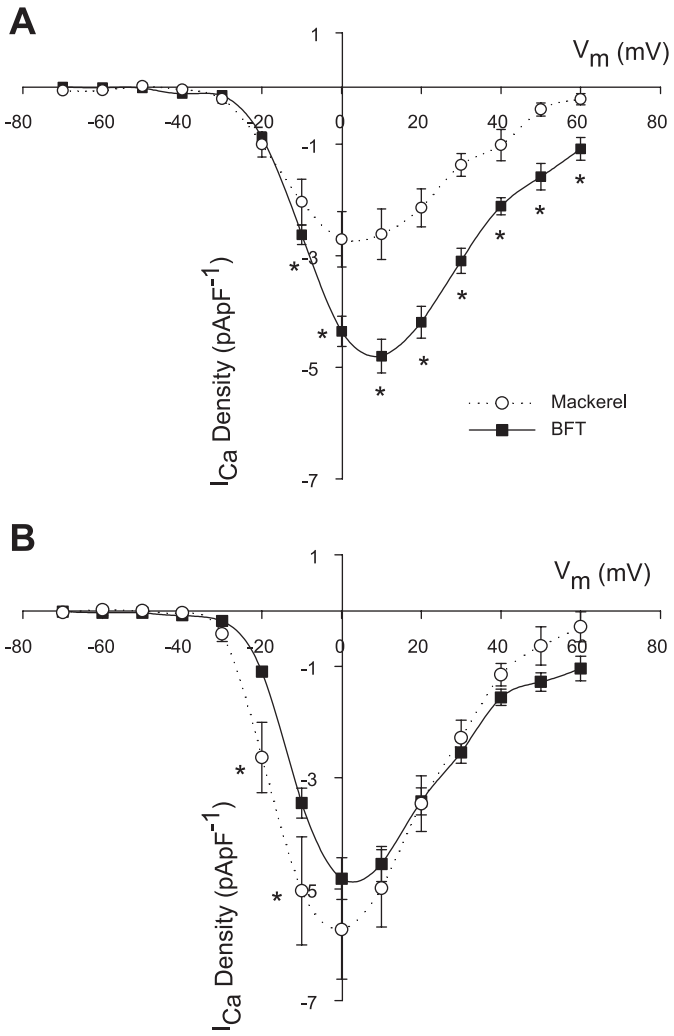

Fig. 3. Ca^{2+} current-voltage relations in cardiac myocytes from Pacific mackerel and bluefin tuna (BFT). Peak I_{Ca} (pA) was normalized to myocyte capacitance (pF) and, thus, expressed as peak current density (pA/pF). *A*: comparison of atrial myocytes from each species. *B*: comparison of ventricular myocytes from each species. Values are means ± SE; n = 21–53 myocytes from 5 tuna and 13 mackerel. *Significantly different at $P < 0.05$ (Student's *t*-test).

5*B*, Table 2). However, the voltage for half-maximal activation was significantly leftward shifted in mackerel ventricle to −14.3 ± 1.0 mV compared with −10.6 ± 0.6 mV in tuna. This is in accordance with the increased current amplitude at negative voltages observed in the current-voltage relation for mackerel ventricle (Fig. 3*B*).

Because of the overlap between activation and inactivation curves, a Ca^{2+} window current is present in both tissues and species (Fig. 5, *C* and *D*, *insets*). The Ca^{2+} window current was maximal at approximately −15 mV for both tissues in tuna, where it contributed 3–5% of the maximal conductance. In mackerel, the peak of the window current shifted from −20 mV in ventricle to −10 mV in atrium, where it contributed ~10% of maximal conductance.

Differences between atrial and ventricular cardiomyocytes are given in Table 2. I_{Ca} activated more quickly and was half-maximal at more negative voltages in the atrium of both

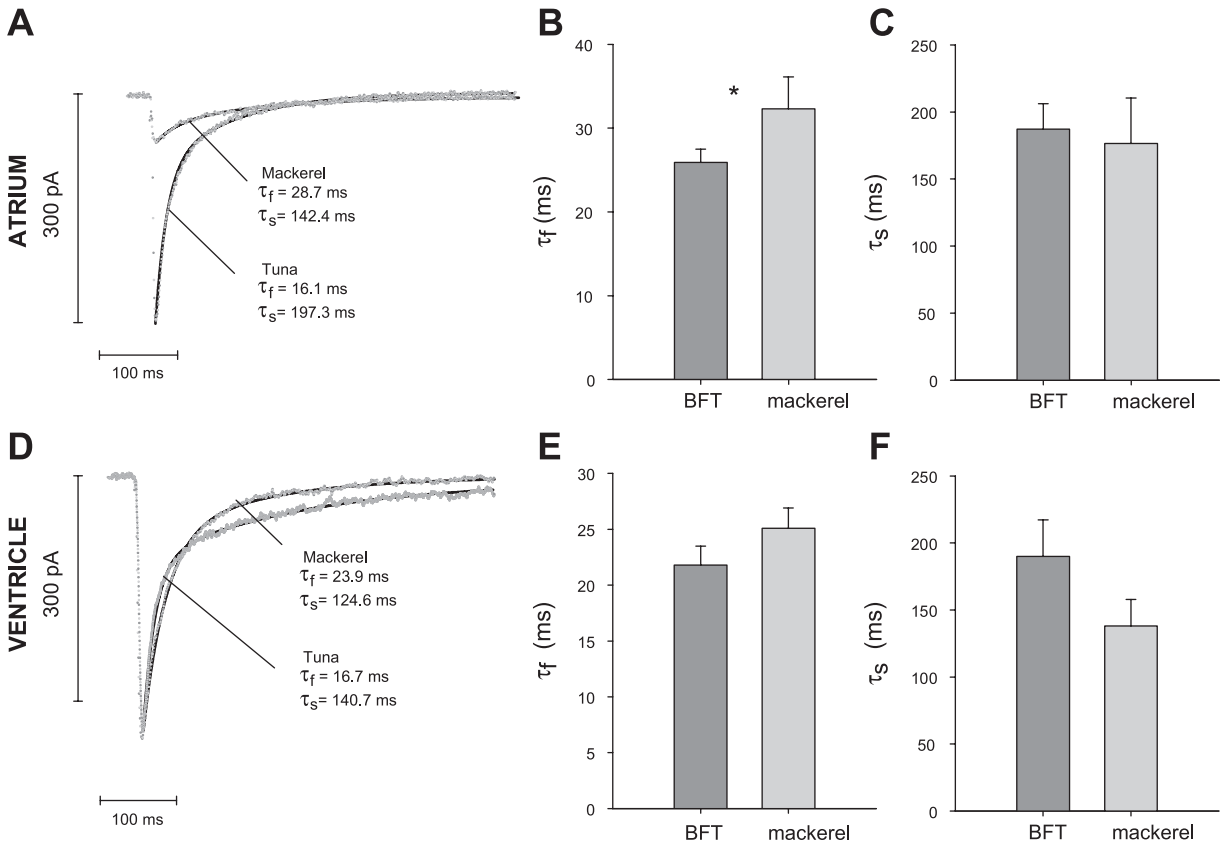

Fig. 4. Fast (f) and slow (s) inactivation kinetics (τ_f and τ_s) of I_{Ca} in cardiac myocytes from Pacific mackerel and bluefin tuna. *A–C*: inactivation kinetics of atrial myocytes from each species; *D–F*: inactivation kinetics of ventricular myocytes from each species. *A* and *D*: representative peak I_{Ca} (dotted gray lines) and τ_f and τ_s values calculated from double-exponential fit to decaying portion of the current (solid black lines). Values in *B*, *C*, *E*, and *F* are means ± SE; n = 21–53 myocytes from 5 tuna and 13 mackerel. *Significantly different at $P < 0.05$ (Student's *t*-test).

species. Steady-state inactivation was faster in mackerel ventricle but slower in tuna and was unchanged with respect to V_h.

Recovery from inactivation. The recovery of I_{Ca} from inactivation at −70 mV after 1-s prepulses to 0 mV is shown in Fig. 6. As the time between prepulse and test pulse was lengthened, the number of recovered channels increased in both species and both tissues. In atrial myocytes, the time constant of recovery from inactivation (τ) was significantly faster in tuna than in mackerel: 79.4 ± 5.7 vs. 134.4 ± 19.5 ms (Fig. 6*C*). Similarly, in ventricular myocytes, I_{Ca} recovered more quickly in tuna than in mackerel: 83.9 ± 5.8 vs. 116.3 ± 10.2 ms (Fig. 6*D*). In tuna ventricular myocytes, the amplitude of I_{Ca} did not fully recover after a 400-ms interpulse duration, as illustrated by the lower plateau of the normalized current amplitude in Fig. 6*D*.

There were no myocardial tissue-specific differences in recovery from inactivation within each species.

DISCUSSION

Practical challenges of maintaining research populations of tunas have limited rapid advances in tuna physiology. Tunas are challenging to acquire, difficult and expensive to maintain, and routinely kept at only a few laboratories worldwide (16). Establishment of a stable research population of Pacific bluefin tuna at the Tuna Research and Conservation Center enabled us to successfully isolate cardiomyocytes and investigate cellular aspects of E-C coupling.

Scombrid myocyte morphology. Cardiomyocytes of Pacific bluefin tuna and Pacific mackerel are long and thin (Fig. 1), similar to those of other teleosts and frogs (48). However, there are significant differences in morphology between species and tissues (Table 1). Atrial and ventricular myocytes from bluefin tuna were longer than those from mackerel. This difference may be partially due to cell growth, because the bluefin tuna was considerably larger than the mackerel (11). However, cardiomyocytes from a 14-kg bluefin tuna (Table 1) and ~100-g rainbow trout have similar dimensions, capacitances, and cell volumes: ~197 μm long, ~5.5 μm wide, ~36 pF, and 2.4 pl for trout atrium (22) and ~196 μm long, ~5.8 μm wide, ~46 pF, and 2.5 pl for trout ventricle (45). We suspect that such a small effect of mass on the size of cardiac cells reflects the possibility that hyperplasia, rather than hypertrophy, is the primary determinant of ventricular growth in fishes (11), including bluefin tuna. The surface area-to-volume ratio in mackerel and tuna cardiomyocytes is high (14–21 pF/pl; Table 1) compared with those in rabbit [4.5 pF/pl (35)] and rat [6.2 pF/pl (7)] but similar to those reported for rainbow trout [18.2 pF/pl (45)] and crucian carp [19.2 pF/pl (47)]. This high surface area-to-volume ratio emphasizes the efficacy of transsarcolemmal Ca^{2+} flux for modulating contractility in fish hearts.

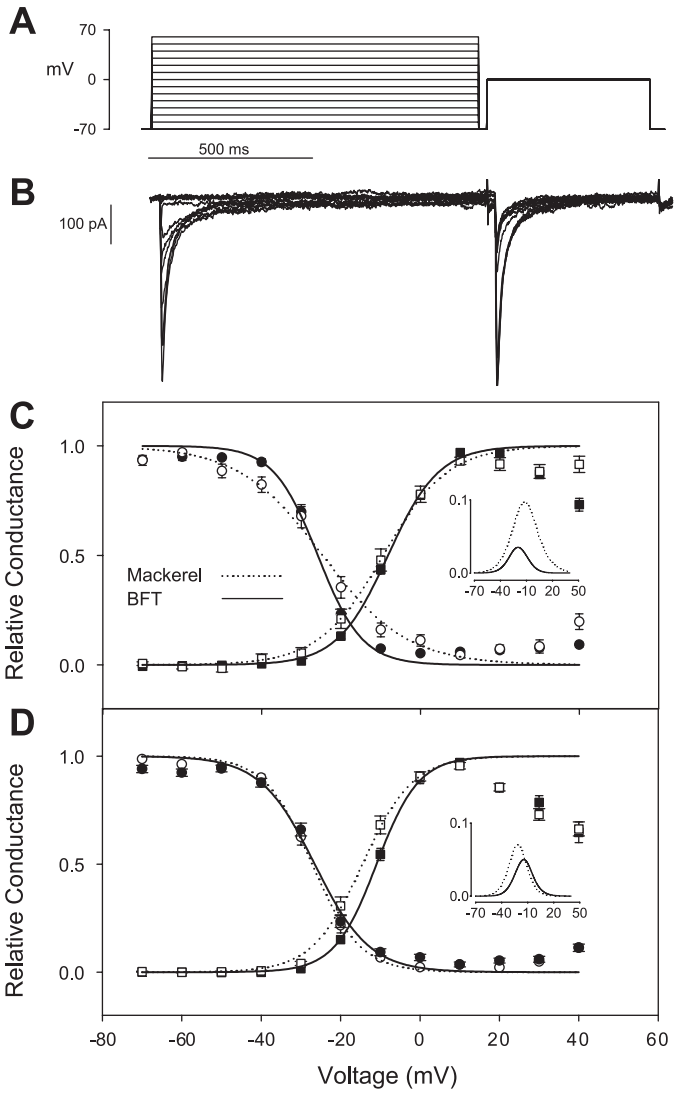

Fig. 5. Steady-state activation and inactivation of I_{Ca} in cardiac myocytes from Pacific mackerel and bluefin tuna. *A*: voltage protocol used to study steady-state activation and inactivation; *B*: corresponding representative recording from a bluefin tuna ventricular myocyte (capacitance = 50 pF). *C* and *D*: means ± SE for atria and ventricles, respectively, of both tissues and species. Inactivation [● (tuna) and ○ (mackerel)] is measured by depolarizing from −70 mV to a test potential for 1 s and then testing the remaining available I_{Ca} at 0 mV. Activation [■ (tuna) and □ (mackerel)] is measured by dividing peak current by apparent driving force [membrane potential (E_m) − reversal potential (E_{rev})] according to Ohm's law. Both curves are described by a Boltzmann relation (solid and dashed lines). *Insets*: L-type Ca^{2+} channel window current (product of activation and inactivation at each voltage). Statistical differences and *n* values are provided in Table 2.

Isolation of tuna myocytes is complicated by the highly aerobic nature of the myocardium (4, 31) and the occurrence of two myocardial layers (34). The Pacific bluefin tuna myocytes used in this study were thus obtained from the spongy myocardial layer only, which comprises the majority of the myocardium (34). Similarly, ventricular myocytes collected from Pacific mackerel heart were primarily from the spongy layer, and atrial muscle of both species is composed of spongy tissue, thus enabling comparisons between species and tissues.

Ca^{2+} flux through I_{Ca} is enhanced in tuna atrium. I_{Ca} plays many roles during E-C coupling; e.g., it is the primary pathway for transsarcolemmal Ca^{2+} influx in fish hearts (48) and for triggering SR Ca^{2+} release (see below). Our finding of larger and faster I_{Ca} in tuna myocytes than in mackerel (Figs. 3*A* and 4, *A* and *B*) and also than in nonscombrid fishes supports the idea that species-specific differences in I_{Ca} may be associated with species-specific differences in cardiac performance. I_{Ca} density in rainbow trout atrial myocytes was almost half [~2.6 pA/pF (22)] that observed in bluefin tuna atrial myocytes (~4.4 ± 0.2 pA/pF) under similar buffering conditions. The density of I_{Ca} is still lower in tuna than in mammals [~10 pA/pF in rabbit at 20°C (33)]. Nevertheless, the much higher surface area-to-volume ratio of the tuna myocytes means that a given sarcolemmal Ca^{2+} influx will produce a greater rise in total intracellular Ca^{2+}.

I_{Ca} activates and inactivates more rapidly in response to changes in membrane voltage in atrial myocytes of tuna than mackerel (Fig. 5*C*) and trout (41, 45). Because the rate of cycling of Ca^{2+} into and out of the cell is a key factor influencing the rate of cardiac contraction and relaxation, the faster transition between activation and inactivation in tuna may be an important factor in permitting their unusually high maximum heart rates. Additionally, I_{Ca} recovers more quickly from inactivation in tuna than in mackerel myocytes (Fig. 6*C*). This is the first measurement of recovery of I_{Ca} in cardiac myocytes from fish. Our values for the time constant of recovery (τ ~80 ms for tuna and ~125 ms for mackerel) compare favorably with what is known for other vertebrate species. The average time to 50% recovery from inactivation of I_{Ca} in myocytes from atrium and ventricle of frogs and a variety of mammals is ~100 ms at −80 and −100 mV at 22°C (29).

In mammals, the amount of Ca^{2+} released from the SR is graded with the amplitude of the I_{Ca} trigger (3). Although SR Ca^{2+} release has yet to be examined in Pacific bluefin tuna atrium, if it is similar to that of other members of the *Thunnus* genus, then the large density of I_{Ca} should trigger a larger SR Ca^{2+} release during atrial E-C coupling in tuna than in mack-

Table 2. *Steady-state activation and inactivation parameters for I_{Ca} in cardiac myocytes from bluefin tuna and Pacific mackerel*

	Atrial Myocytes				Ventricular Myocytes			
	Slope (*k*)		V_h, mV		Slope (*k*)		V_h, mV	
	Activation	Inactivation	Activation	Inactivation	Activation	Inactivation	Activation	Inactivation
Tuna	6.19±0.17	5.29±0.29	−8.13±0.5	−26.0±0.6	5.18±0.33†	6.55±0.54†	−10.6±0.6†	−26.5±0.7
Mackerel	7.69±0.75*	9.31±0.57*	−8.47±1.2	−24.3±1.8	5.19±0.30†	5.18±0.45†	−14.3±1.0*†	−27.1±0.8

Values are means ± SE of 16–39 myocytes from 5–13 fish. V_h, half-activating or half-inactivating potential; I_{Ca}, L-type Ca^{2+} channel current. *Significant difference between species within each tissue; †significant difference between tissues within each species ($P < 0.05$, student's *t*-test).

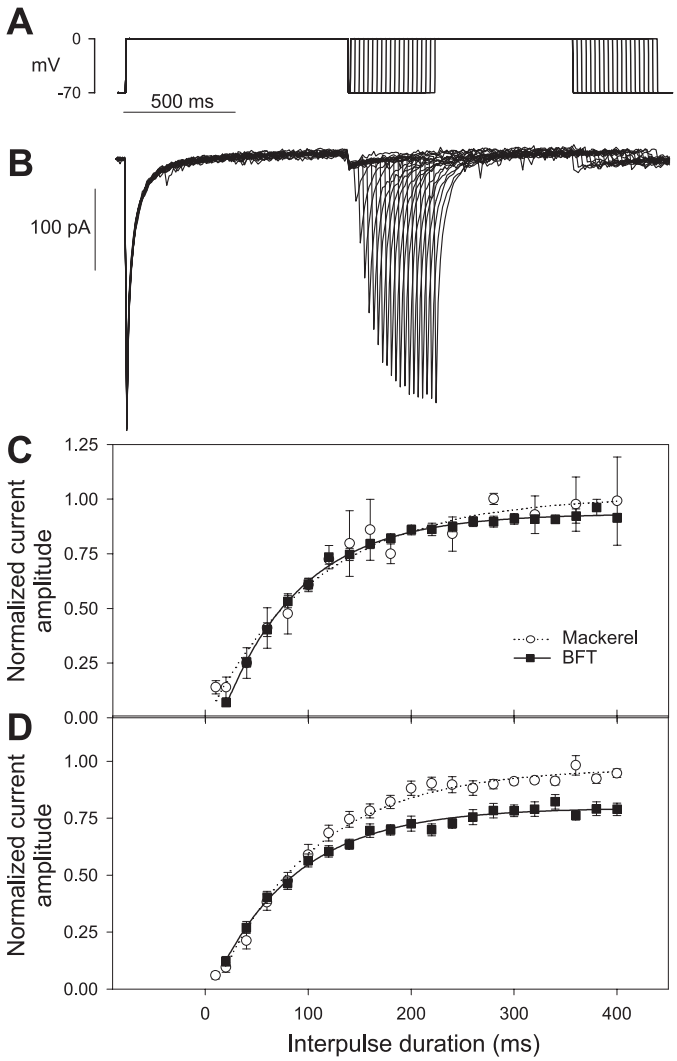

Fig. 6. Recovery of I_{Ca} from inactivation in cardiac myocytes from Pacific mackerel and bluefin tuna. *A*: voltage protocol used to study recovery from inactivation. *B*: representative recording from a bluefin tuna atrial myocyte (capacitance = 70 pF). *C* and *D*: relation between current recovery and interpulse duration for atrium and ventricle, respectively. Relation between amplitude of current recovery and interpulse duration was fit with a single exponential (dashed and solid lines, $r^2 > 0.975$ for each fit) to assess the time constant of recovery (τ). τ values were significantly faster for atrial and ventricular myocytes from tuna than from mackerel. Values are means ± SE, $n = 23$ for tuna atrium and ventricle and for mackerel ventricle; $n = 8$ for mackerel atrium.

erel. This suggestion conforms well with the known SR Ca^{2+} dependency of contraction in isolated atrial muscle from skipjack (25) and yellowfin tuna (40). Furthermore, the large peak I_{Ca} window current in mackerel atrium (~10% of the maximal Ca^{2+} conductance; Fig. 5*C*, *inset*) may indicate greater reliance on transsarcolemmal Ca^{2+} flux in this species.

I_{Ca} characteristics in ventricular myocytes from tuna and mackerel. Species-specific differences in the electrophysiological properties of I_{Ca} were less pronounced in ventricular myocytes. In both species, ventricular I_{Ca} had fast kinetics, and the density was larger than in other fish species examined under similar temperatures and buffering conditions (22, 45, 47). When coupled to the high surface area-to-volume ratio, the high density of I_{Ca} should enable large and rapid changes in total cellular Ca^{2+}, providing a mechanism for rapid myofibrillar activation. Rapid delivery of Ca^{2+} to the myofibrils may contribute to a faster excitation-contraction-relaxation cycle and, thus, may be an important factor in permitting the strong and rapid contractions of the tuna heart. Ventricular hyperplasia would also contribute by preserving a high cellular surface area-to-volume ratio while increasing ventricular mass. Indeed, Brill and Bushnell (8) noticed that although myocardial power output in yellowfin tuna heart is nearly seven times that of other teleosts per unit body mass, because of their enlarged ventricle, power output per unit ventricular mass is only twice that of other teleosts. It is also possible that the large I_{Ca} releases greater SR Ca^{2+}, which leads to greater contractile strength per myocyte. High oxidative enzyme activities and myoglobin levels relative to those of other active fish (2, 18) may also contribute to the high maximum power output of the tuna heart. Clearly, further studies are necessary to identify the pathways underlying the robust pumping ability of the tuna ventricle.

Perspectives

As a step toward understanding the evolution of cardiac E-C coupling in scombrid fishes, we compared I_{Ca} in two closely related members of the family Scombridae. We hypothesized that I_{Ca} in cardiac myocytes of Pacific bluefin tuna would be larger and have faster kinetics than those of Pacific mackerel. This was found to be the case. The basis for our hypothesis is that the evolutionary trend toward higher maximum heart rates and cardiac outputs in tuna is facilitated in part by adaptations related to E-C coupling in cardiac tissues. Our results, which show species-specific differences, support this hypothesis. For example, because I_{Ca} provides transsarcolemmal Ca^{2+} influx that directly activates the myofilaments, the elevated I_{Ca} we report here for bluefin tuna atria (compared with mackerel atria and the ventricles of both species compared with other fish) would result in an increase in the intracellular Ca^{2+} transient, leading to greater myofilament activation and stronger contraction. I_{Ca} also functions as the trigger for the release of Ca^{2+} from the SR and can modulate SR Ca^{2+} accumulation (38). Recent measurements show greater SR Ca^{2+} uptake rates in SR vesicles from bluefin ventricle than from Pacific mackerel ventricle (28). Likewise, atrial and ventricular muscle strips from yellowfin tuna show greater losses in isometric tension when SR function is inhibited than do preparations from Pacific mackerel (17, 40). Collectively, this evidence suggests that specializations in SR function, coupled with a strong I_{Ca} trigger, may play a key role in enhancing cardiac performance in the bluefin and yellowfin tuna and points toward a fruitful path for future investigations.

Similarly, I_{Ca} affects the rate of cardiac contraction, modulating the shape of the action potential, especially the voltage and duration of the plateau phase. The fast kinetics and recovery times of I_{Ca} indicate that this current does not contribute to the refractoriness of the heart and, thus, may be permissive in allowing tuna fast maximal hearts rates without overly compromising contractile strength. Interestingly, the inactivation kinetics were slowest for the mackerel atrium and, then, for a smaller I_{Ca}.

Bluefin tuna are endothermic predators that forage in the open sea on wide-ranging resources, often patchily distributed.

This metabolically demanding lifestyle places a premium on the ability of bluefin tuna to cope with the multiple energetic demands of locomotion, feeding, and digestion (27). Endothermy enhances these physiological processes and contributes to the ecological success and diverse thermal niches of the bluefin tuna group. However, endothermy also increases the metabolic rate of muscle, viscera, and cranial tissue, adding to the aerobic demand on the tuna heart. Thus physiological adaptations, such as those shown here, which augment contractile force and permit higher heart rates and cardiac outputs, can be seen as critical to the ecological success and niche expansion of the tunas. Increased understanding of the biophysics of the high-performance tuna heart and the link between cellular Ca^{2+} cycling and ecological success in the Scombridae lineage will benefit physiological ecology studies of this lineage.

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Some of the results have appeared previously in abstract form (37).

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