Myocardial sarcolemma isolated from skipjack tuna, *Katsuwonus pelamis*

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The high cardiac outputs observed in tuna compared with other teleosts may imply differences in the regulation of myocardial contractility. Sarcolemma is a critical organelle in the teleost heart in the regulation of intracellular calcium concentration and hence contractility. A procedure is described for the isolation of large quantities of highly purified sarcolemma from the skipjack tuna heart by differential and sucrose-gradient centrifugation in the presence of multiple protease inhibitors. The preparation was characterized using the sarcolemma marker K^+-stimulated *p*-nitrophenylphosphatase (K^+*p*NPPase). From a starting ventricular wet weight of about 60 g, a yield of >20 mg sarcolemmal protein and a >43-fold purification over the crude homogenate were achieved. The percentage recovery of the total K^+*p*NPPase in the sarcolemma fraction was greater than 14%. Both the purification index and recovery were substantially greater than those achieved with a much smaller scale sarcolemma preparation recently described for the trout heart. The equilibrium binding of the dihydropyridine PN200-110 and ryanodine were determined in these preparations. In purified sarcolemma, the dihydropyridine receptor binding density (B_{max}) and dissociation constant (K_d) were found to be 0.48 ± 0.07 pmol · mg protein^{-1} and 0.09 nM respectively, similar to that found in mammals. In crude homogenates, the ryanodine receptor B_{max} was approximately 0.12 ± 0.04 pmol · mg protein^{-1} and thus was a little more than half the value observed for the dihydropyridine receptor. The large-scale sarcolemma isolation procedure described in this paper for tuna may be useful for developing a better understanding of the role of the sarcolemma in the regulation of ventricular function in teleosts.

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Les rendements cardiaques plus élevés chez les thons que chez d'autres téléostéens peuvent être attribuables à des différences dans le contrôle de la contractilité du myocarde. Le sarcolemme est un organite qui joue un rôle critique dans le coeur des téléostéens, puisqu'il contrôle la concentration de calcium intracellulaire et donc la contractilité. Nous décrivons ici une technique qui nous a permis d'extraire de grandes quantités de sarcolemme très purifié de coeurs de Thons à ventre rayé par centrifugation différentielle sur un gradient de sucrose en présence de multiples inhibiteurs de la protéase. La préparation a été caractérisée par l'utilisation d'un marqueur du sarcolemme la *p*-nitrophénylphosphatase stimulée par K^+ (la K^+*p*NPPase). A partir d'une masse ventriculaire fraîche d'environ 60 g, nous avons pu obtenir >20 mg de protéine de sarcolemme 43 fois plus pure que dans l'homogénat brut. Dans la fraction de sarcolemme obtenue, le pourcentage de marqueur récupéré était supérieur à 14%. L'indice de purification et la fraction récupérée étaient considérablement plus élevés que ceux qui peuvent être obtenus par une technique récemment décrite de préparation de sarcolemme de truite à échelle beaucoup plus petite. Les liaisons à l'équilibre de la dihydropyridine PN200-110 et de la ryanodine ont été déterminées pour ces préparations. Dans le sarcolemme purifié, le B_{max} du récepteur de la dihydropyridine et le K_d ont été évalués à 0,48 ± 0,07 pmol · mg protein^{-1} et 0,09 nM, valeurs semblables à celles qui prévalent chez les mammifères. Dans des homogénats bruts, le B_{max} du récepteur de la ryanodine était d'environ 0,12 ± 0,04 pmol · mg protein^{-1} et équivalait donc à un peu plus de la moitié de la valeur observée dans le cas du récepteur de la dihydropyridine. La technique d'extraction de sarcolemme sur une grande échelle, décrite ici dans le cas des thons, permettra sans doute d'obtenir une meilleure compréhension du rôle du sarcolemme dans le contrôle du fonctionnement ventriculaire chez les téléostéens.

[Traduit par la rédaction]

Introduction

Cardiac output, normalized per unit body mass, is substantially higher in the tuna relative to other teleosts and is comparable to that observed in mammals (Brill and Bushnell 1991). This is a consequence of both a higher operating heart rate (HR) and higher normalized stroke volume (SV) compared with other lower vertebrates. The observation that SV is substantially higher than that of other teleosts when expressed per unit body mass may be related to the following: (*i*) a ventricular mass that is several times higher than that of other teleosts when normalized per unit body mass, (*ii*) high venous return, and (*iii*) enhanced contractility. Clearly, the higher heart (or more importantly, left ventricle) to body weight ratio is well documented (Farrell 1991) and is an important factor in the determination of SV. However, the tuna heart may also exhibit important differences in myocyte contractile function that are consistent with both the higher HR and SV. These would include the rates of Ca^{2+} delivery to and removal from the contractile element that determine the strength of cardiac contraction and the minimum duration of the cardiac cycle. Virtually nothing is known about these functions in the tuna heart.

It is well documented that myocardial sarcolemma (SL) is an important regulator of the intracellular calcium concentration ($[Ca^{2+}]_i$), which is crucial in the regulation of contractility (Langer 1980). At least three SL proteins are involved in the

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regulation of $[Ca^{2+}]_i$ in the mammalian heart: Ca^{2+} ATPase, Na^+-Ca^{2+} exchange, and voltage-dependent Ca^{2+} channels (Carafoli 1987). Both Na^+-Ca^{2+} exchange and L-type voltage-dependent Ca^{2+} channels (LCC) were recently demonstrated in the trout heart (Tibbits *et al.* 1990). LCC are known to bind dihydropyridines (DHP) with high affinity (Mikami *et al.* 1989) and thus can be quantified by DHP ligand binding. Furthermore, in the mammalian heart, the DHP receptor of the SL comes in proximity to and is implicated in the opening of the Ca^{2+} release channel of the sarcoplasmic reticulum (SR). Thus, in the mammalian heart, the opening of the LCC and the subsequent Ca^{2+} influx are prerequisites for the release of SR Ca^{2+}, the major source of contractile Ca^{2+} (Fabiato 1983). In lower vertebrates, however, the importance of SR Ca^{2+} release in contractility is not well established (Klitzner and Morad 1983; Tibbits *et al.* 1991). Electron micrographs indicate that T-tubules are absent and that the SR is both sparse and poorly organized relative to that of higher vertebrates (Santer 1985). Furthermore, tension generation in cardiac tissue from lower vertebrates is insensitive to ryanodine in concentrations known to block the release of SR Ca^{2+} (Bers 1985; Driedzic and Gesser 1988).

The tuna heart, with a high HR and SV, may have a necessarily more complex system of Ca^{2+} regulation than other teleosts in order to meet these stringent demands. Thus this investigation was undertaken to obtain a better understanding of the molecular basis of Ca^{2+} regulation in the high-performance tuna heart. This article describes a suitable means of isolating large quantities of highly purified sarcolemma and some preliminary determinations of its properties.

Methods

Animal care and handling

Skipjack tuna, *Katsuwonus pelamis*, weighing 1–2 kg, were obtained from a local commercial fishing vessel and maintained in outdoor tanks at 24–26°C typically for 2–3 days at the Kewalo Research Facility in Honolulu.

Sarcolemmal isolation and characterization

Myocardial sarcolemma was isolated using modifications of techniques described for teleosts by Tibbits *et al.* (1990) and for mammals by Philipson and Ward (1987). The principal solution used in the isolation procedure was homogenizing medium (HM), which contained, in mM: sucrose, 350; *N*-tris(hydroxymethyl)-2,2-aminoethanesulfonic acid (TES), 20 (pH 7.6 at 25°C); and dithiothreitol (DDT), 1. A second solution (HMI) was produced by adding the following protease inhibitors (with final concentrations indicated in μM) to the HM: phenylmethylsulfonylfluoride (PMSF), 100; pepstatin A, 1; iodoacetamide, 1; benzamidine, 0.75; and the following (in mg/mL): trypsin soybean inhibitor, 0.1; leupeptin, 0.05; phenanthroline, 0.1; and aprotinin, 0.05.

After the tuna was killed by a sharp blow to the head and an incision made ventrally, the heart (approximately 5 g) was excised quickly and then rinsed in HMI maintained at 4°C. The heart was trimmed of connective tissue, atria, and fat and then weighed. Twelve tuna ventricles were pooled to give a mean starting wet weight of about 60 g for each of the three separate isolations. The pooled ventricles were minced in about three volumes of HMI with scissors to about 2 mm^3 and homogenized twice for 10 s each time in about four volumes of HMI in a chilled Waring blender set at high speed. The homogenate was brought to a volume of 290 mL with HMI, and 2 mL was removed for subsequent biochemical analyses of the crude homogenate (CH). The contractile proteins were solubilized by adding 32 mL of a KCl and $NaPPO_4$ solution to the 288 mL of CH, bringing the final concentrations of KCl and $NaPPO_4$ to 100 and 25 mM, respectively. The CH was spun at 20 000 × *g* for 35 min at 4°C. After the supernatants were discarded, the pellets were homogenized in HM with a Polytron homogenizer at setting 5–6 until the pellets were resuspended. This suspension was spun at 20 000 × *g* for 35 min, and once again the supernatant was discarded and the pellet resuspended in HM with a Polytron homogenizer at a setting of 5–6. The resultant suspension was spun at 38 000 × *g* for 45 min at 4°C.

These pellets were resuspended in six 5-mL aliquots of 45% sucrose. Six discontinuous sucrose gradients comprising 32, 30, 28, and 8% sucrose steps were layered on top. The gradients were spun in a swing bucket rotor (SW 28) at 122 000 × *g* for 16 h and each was fractionated into four tubes labeled F1 to F4 in order of increasing density. All fractions were diluted with roughly equal volumes of an ice-cold solution of 560 mM NaCl and 40 mM TES in order to avoid osmotic shock. The tubes were allowed to equilibrate for approximately 90 min on ice, after which they were slowly diluted with an ice-cold loading medium (LM) that contained 140 mM NaCl and 10 mM TES (pH 7.6 at 25°C). The suspensions were centrifuged at 180 000 × *g* for 1 h, and the pellets from F2 and F3 were resuspended in 3 mL LM (to give a final protein concentration of ~6 $mg \cdot mL^{-1}$), characterized as described below, and then frozen in liquid N_2. Protein determinations on all fractions were done by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. The SL marker K^+-stimulated *p*-nitrophenylphosphatase (K^+*p*NPPase) was assayed, as described by Tibbits *et al.* (1981), in the crude homogenate and all fractions derived from the sucrose gradient. Purification index (PI) was defined as the ratio of the specific activity of K^+*p*NPPase in the sarcolemmal fraction to that in the crude homogenate. Recovery was defined as the percentage of the total K^+*p*NPPase activity ($\mu mol \cdot h^{-1}$) in the SL fraction compared with the crude homogenate.

Dihydropyridine binding

The binding of the dihydropyridine [^{3}H](+)PN200-110 was measured in both the crude homogenate and the F2 (SL-enriched) fraction derived from the sucrose gradients. The tubes contained (in a final volume of 5 mL) 20 mM TES (pH 7.6 at 25°C), 0.05–0.8 nM [^{3}H](+)PN200-110, and 2.5 mM $CaCl_2$. The amount of protein in each tube was approximately 25 μg for F2 and 250 μg for CH, and the binding reaction was allowed to proceed for 90 min in the dark at room temperature. Assays were performed in both the presence and the absence of 1 μM unlabeled nifedipine to determine specific binding. All binding assays were conducted in dim (10 W) red light to prevent photoinactivation of the dihydropyridines. Binding was terminated by rapid vacuum filtration through GF/C (Whatman) filters. The filters were washed with three 4.5-mL aliquots of ice-cold buffer, placed in a vial, dried, and counted by standard liquid scintillation procedures.

Ryanodine binding

The binding of [^{3}H]ryanodine to crude homogenates was carried out with minor modifications of the procedure described by Chu *et al.* (1988). The binding reaction was carried out with 160 μg homogenate protein (at 28°C for 1 h) in a tube with a 1-mL final volume that contained the following: 11 nM [^{3}H]ryanodine, nonradioactive ryanodine (Calbiochem) in concentrations of 0–500 nM, 150 mM KCl, 20 mM TES (pH 7.6 at 25°C), and 25 μM $CaCl_2$. In pilot studies, we determined that these conditions resulted in equilibrium binding. No buffers were added in an attempt to regulate free Ca^{2+}. The ryanodine was dissolved in ETOH and the final [ETOH] was less than 0.5%, which we determined previously to have no effect on the binding. After 1 h, the binding reaction was stopped by filtration through 0.45-μm cellulose acetate filters. The filters were rinsed first with 2 mL of a solution containing 150 mM KCl and 10 mM TES (pH 7.6 at 25°C), and finally with 1 mL of a 10% ETOH solution. The dried filters were counted by conventional liquid scintillation procedures.

Materials

[^{3}H](+)PN200-110 and [^{3}H]ryanodine were purchased from New England Nuclear and were used without further purification. With the

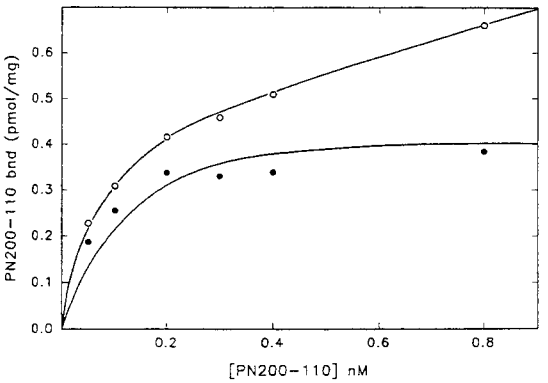

FIG. 1. Amount of [^{3}H]PN200-110 bound (bnd) to purified sarcolemma as a function of free [^{3}H]PN200-110 concentration. Both the total (○) and the specific (●) binding are indicated.

TABLE 1. Protein yield (normalized per unit of ventricular wet weight) and specific K$^+$*p*NPPase activity at 37°C (normalized per unit of protein) in the crude homogenate and various fractions from the sucrose gradient

	Protein yield	Specific K$^+$*p*NPPase activity
Homogenate	103.59±4.97	0.20±0.02
F2	0.31±0.03	8.66±0.96
F3	0.17±0.02	3.49±0.56
F4	10.45±2.56	0.21±0.06

NOTE: Values are given as means ± SE. Protein yield is measured as mg protein · g wet wt.$^{-1}$; specific K$^+$*p*NPPase activity is measured as μmol · mg protein^{-1} · h^{-1}.

TABLE 2. Recovery and purification index based on the activities of K$^+$*p*NPPase in the crude homogenate and various fractions from the sucrose gradient

	Recovery (%)	Purification index
F2	14.7	43.3
F3	3.3	17.5
F4	8.8	1.1

exception of cold ryanodine, all other biochemicals were of the highest purity available from the Sigma Chemical Company.

Results

The large-scale SL isolation procedure developed for the tuna heart resulted in the isolation of large quantities of highly purified SL with a yield of 0.31 mg SL protein · wet wt.$^{-1}$ (Table 1). The activities of the SL marker K$^+$*p*NPPase in the crude homogenate and SL fraction were 0.20 and 8.66 μmol · mg protein^{-1} · h^{-1} (Table 1). From these data, the calculated recovery of SL in F2 from the total SL pool was 14.7% and the purification index was 43.3-fold (Table 2).

Figure 1 illustrates the binding of the DHP [^{3}H](+)PN200-110 to highly purified SL as a function of the free concentration of [^{3}H](+)PN200-110 in a typical experiment. The specific binding between 0.05 and 0.20 nM [^{3}H](+)PN200-110 was greater than 81% of the total binding. Total [^{3}H](+)PN200-110 binding involved no more than 3% of the total ligand concentration. The specific binding data shown in Fig. 1 are presented as a Scatchard plot in Fig. 2. The correlation coefficient exceeded 0.95. Using three different SL preparations, the derived binding density (B_{max}) and dissociation constant (K_d) values for [^{3}H](+)PN200-110 equilibrium binding to SL were 0.48 ± 0.07 pmol · mg protein^{-1} and 0.09 ± 0.01 nM, respectively. The binding of [^{3}H](+)PN200-110 to crude homogenates from tuna ventricles was carried out in a identical manner, except more protein was included per tube. From a Scatchard plot (not shown) of these data, it was calculated that the B_{max} and K_d values in the crude homogenate were 0.24 ± 0.04 pmol · mg^{-1} and 0.15 ± 0.02 nM, respectively.

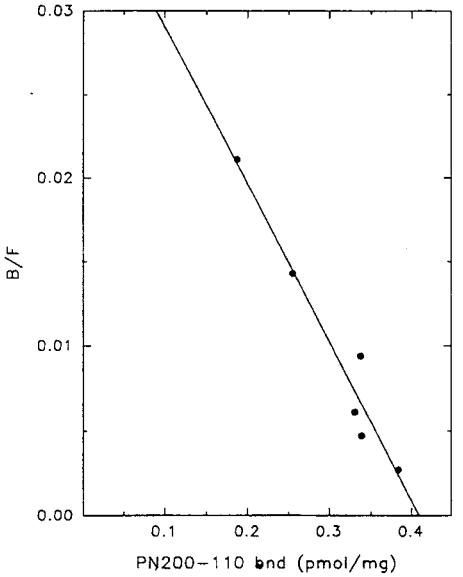

FIG. 2. Scatchard plot of the specific binding data shown in Fig. 1. A least-squares linear regression line is drawn through the data with a correlation coefficient of >0.95. B/F, bound/free; bnd, bound.

The [^{3}H]ryanodine binding was performed only with crude homogenate protein. The binding to crude homogenates proved to be difficult and not all experiments were successful, as judged by the consistency of the duplicates. The data presented represent those experiments in which the duplicates and goodness of fit were satisfactory. In Fig. 3, the specific binding of [^{3}H]ryanodine to crude homogenates is shown as a function of free ryanodine concentration from 22 to 511 nM. The specific binding at 22 nM represents more than 60% of the total binding of [^{3}H]ryanodine to this preparation. The Scatchard representation of these data is shown in Fig. 4, which has a correlation coefficient of >0.99. In three different preparations, the derived B_{max} of ryanodine binding to crude homogenates was 0.12 ± 0.04 pmol · mg protein^{-1}.

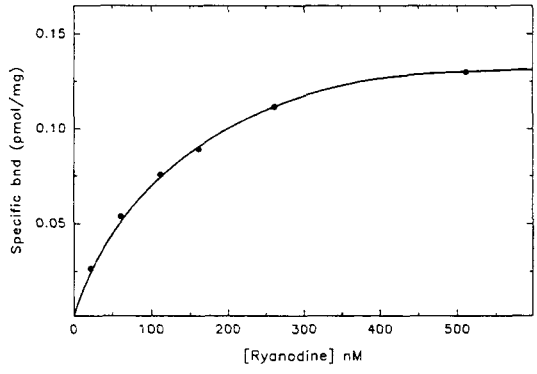

FIG. 3. Amount of ryanodine specifically bound (bnd) to crude homogenates as a function of the free ryanodine concentration.

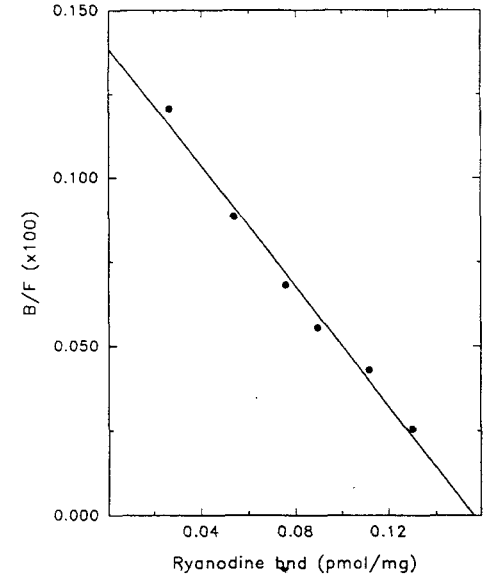

FIG. 4. Scatchard plot of the data shown in Fig. 3. A least-squares linear regression line is drawn through the data with a correlation coefficient of >0.99. B/F, bound/free; bnd, bound.

Discussion

The protein yield of the crude homogenate found in the tuna ventricle (103.6 mg · g wet wt.$^{-1}$) is similar to that found in trout (Tibbits *et al.* 1990) and mammalian (Tibbits *et al.* 1989) ventricles. The specific activity of K^+*p*NPPase in the crude homogenate, 0.20 μmol · mg protein^{-1} · h^{-1}, is only 31% of that observed in cold-adapted trout hearts (Tibbits *et al.* 1990). Both of these assays were conducted at 37°C for comparison with mammalian values; however, the temperature dependence of this enzyme was evaluated in both teleost species, to determine whether K^+*p*NPPase is denatured at 37°C and to determine the Q_{10} of the enzyme. There were no indications from the specific activities of K^+*p*NPPase from teleost species that the enzyme denatures at 37°C. The calculated Q_{10} values were 2.8 (21−37°C) and 2.4 (7−37°C) in tuna and trout ventricles, respectively, which are similar to that observed in mammals (G. F. Tibbits, unpublished observations). We have evidence that the high K^+*p*NPPase activity in the cold-adapted trout heart is a component of the adaptation to cold (McKnight *et al.* 1989). The specific activity of K^+*p*NPPase that we have observed in warm-adapted trout ventricles is about one-half that in cold-adapted heart (G. F. Tibbits and H. Kashihara, unpublished observations) and thus much closer to that observed in the tuna heart in the present study. These data probably reflect differences in densities of the enzyme in SL, but further experimentation is required to confirm this statement. Since the K^+*p*NPPase activity reflects a component of the Na^+-K^+ pump (Schwartz *et al.* 1975), this finding is consistent with the notion that the densities of ion pumps, which generally exhibit high Q_{10} values, must increase under colder conditions to maintain ionic gradients (Hochachka 1988). The tuna heart, functioning at ~25°C, therefore may not require the high pump densities that are apparent in the cold-adapted trout.

The SL isolation procedure described in this paper was successful in producing a reasonable yield of highly purified membranes from the tuna heart. The fraction enriched in SL (F2), had a yield of 0.31 mg protein · g wet wt.$^{-1}$, which is about two-thirds that achieved with a much smaller scale procedure using trout ventricles. However, the advantages of obtaining ~20 mg SL per isolation with this procedure versus ~3 mg with the small-scale approach are obvious. Furthermore, the recovery and purification of SL from the tuna ventricles are about 2 and 3 times greater, respectively, than those obtained with trout by means of the procedure described previously (Tibbits *et al.* 1990). The high specific activity of K^+*p*NPPase of the SL preparation indicates a high degree of purity. However, the extent of cross contamination from other organelles cannot be precisely stated without determinations of markers of SR and other organelles, which could not be performed because of prevailing conditions.

DHP binding was examined in both crude homogenates as well as in purified SL fractions from the tuna ventricles, using [^{3}H]PN200-110. The K_d values derived from Scatchard analyses of these binding curves were similar to those reported in trout (Tibbits *et al.* 1990) and mammalian hearts (Lee *et al.* 1984), suggesting a similarity in receptor structure across these phyla. The mean B_{max} values from homogenates and SL fractions were 0.24 and 0.48 pmol · mg protein^{-1}, respectively. The homogenate B_{max} is about 40% lower than that observed in the cold-adapted trout heart. Again, there is reason to believe that the very high DHP B_{max} observed in the cold-adapted trout heart homogenate is, at least in part, a consequence of the cold adaptation (G. F. Tibbits and H. Kashihara, unpublished observations). The B_{max} values in the warm-adapted (15°C) trout are similar to those observed in tuna in the present study. The DHP B_{max} observed in the SL-enriched fractions from tuna in the present study (0.48 pmol · mg protein^{-1}) is approximately 15% of that found in purified SL in the cold-adapted trout heart. There are probably two explanations for this disparity. The first is based on the differences in the homogenates. From these data, we would therefore expect the tuna SL DHP B_{max} to be about 40% that of trout. We propose that this is a species difference, an explanation of which is offered below. The second reason may be related to the

higher SL purification realized in the present study and the observation that the DHP receptor does not copurify strictly with conventional SL markers. This is supported by what we and others have observed in mammalian species. Thus, the high purification observed in this study may be at the expense of recovered DHP receptor and components of the junctional complex. However, despite the fact that the DHP B_{max} in SL in the tuna heart is lower than that of trout, it should be noted that it is comparable to that observed in mammals (Glossmann *et al.* 1984).

The SR Ca^{2+} release channel, or ryanodine receptor, controls the efflux of Ca^{2+} from the SR to the cytosol (Meissner 1986), which in the mammalian heart is the largest source of Ca^{2+} delivered to the myofilaments (Wier 1990). Ryanodine inhibits force production in the mammalian heart (Sutko and Kenyon 1983) by binding with very high affinity to the release channel and affecting the opening of the channel in a dose-dependent manner (Meissner 1986). The binding of [^{3}H]ryanodine to crude homogenates allowed us to determine the relative density of the ryanodine receptor in this tissue. The B_{max} observed in this study, 0.12 pmol · mg protein^{-1}, is similar to that observed by others (e.g., Pessah *et al.* 1985) in mammalian hearts.

Because of the difficulty with the ryanodine binding and a variety of other complications, we would prefer, at this stage, not to speculate about the stoichiometry of these receptors. We are not aware of ryanodine binding having been reported in cardiac tissue from other teleosts. These data suggest that there is a reasonable amount of SR Ca^{2+}-release channel protein in the tuna ventricle despite the apparent sparsity of SR observed in electron micrographs (Breisch *et al.* 1983). The function of the SR as a contributing source of Ca^{2+} is indicated further by the inhibition of atrial contractions by ryanodine in the study by Keen *et al.* (1992). As suggested previously (Tibbits *et al.* 1991), a reasonable hypothesis may be that the magnitude of SR releasable Ca^{2+} in the teleost heart is temperature dependent. It may be postulated that at lower (<20°C) temperatures the channel remains open and the SR is unable to sequester Ca^{2+}, thus eliminating the SR as a functional Ca^{2+}-release site. Preliminary evidence to support this hypothesis comes from studies (L. Hove-Madsen, unpublished observations) using ventricular strips from the trout heart studied at different temperatures.

We propose, therefore, that the tuna heart may have a higher reliance on SR Ca^{2+} for contraction than other lower vertebrates. This may be the consequence of a more fully developed SR or simply the fact that the tuna physiological temperature range (~25°C) allows the SR Ca^{2+}-release channel to function appropriately. This hypothesis offers an explanation for the lower DHP B_{max} observed in this study; namely, there is less dependence on SL Ca^{2+} influx for contraction in the tuna heart. However, considering the observation that the L-type Ca^{2+} channel has a high Q_{10} (~3) in mammals (Cavalie *et al.* 1985), it is also possible that elevated operational temperatures in tuna do not necessitate high channel densities for adequate Ca^{2+} current. The notion of greater reliance on SR Ca^{2+} release for contraction offers some advantages to the tuna heart, most notably, higher rates of delivery and removal of Ca^{2+} from the contractile element. The latter would be consistent with the shorter cardiac cycle required for the high heart rates observed in these species (Brill and Bushnell 1991). It should be stated that because of the limited data from this species, these inferences must be considered as no more than working hypotheses at this time. Much more extensive investigation (involving both biochemical and physiological approaches) is required before these hypotheses can gain acceptance.

In summary, we have been able to isolate highly purified SL in large quantities from the tuna ventricle. This procedure should permit experiments in the future that will offer a much greater understanding of the role of SL in myocardial function in teleosts than has heretofore been possible.

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Bers, D. M. 1985. Ca influx and sarcoplasmic reticulum Ca release in cardiac muscle activation during postrest recovery. Am. J. Physiol. **248**: H366–H381.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**: 48–254.

Breisch, R. E., White, F., Jones, H. M., and Laurs, R. M. 1983. Ultrastructural morphometry of the myocardium of *Thunnus alalunga*. Cell Tissue Res. **233**: 427–438.

Brill, R. W., and Bushnell, P. G. 1991. Metabolic scope of high energy demand teleosts, the tunas. Can. J. Zool. **70**: 2002–2009.

Carafoli, E. 1987. Intracellular calcium homeostasis. Annu. Rev. Biochem. **56**: 395–433.

Cavalie, A., McDonald, T. F., Pelzer, D., and Trautwein, W. 1985. Temperature-induced transitory and steady-state changes in the calcium current of guinea pig ventricular myocytes. Pfluegers Arch. **405**: 294–296.

Chu, A., Sumbilla, C., Scales, D., Piazza, A., and Inesi, G. 1988. Tryptic digestion of junctional sarcoplasmic reticulum vesicles. Biochemistry, **27**: 2827–2833.

Driedzic, W. R., and Gesser, H. 1988. Differences in force–frequency relationships and calcium dependency between elasmobranch and teleost hearts. J. Exp. Biol. **140**: 227–241.

Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac saracoplasmic reticulum. Am. J. Physiol. **245**: C1–C14.

Farrell, A. P. 1991. From hagfish to tuna: a perspective on cardiac function. Physiol. Zool. **64**: 1137–1164.

Glossmann, H., Ferry, D. R., Goll, A., and Rombusch, M. 1984. Molecular pharmacology of the calcium channel: evidence for subtypes, multiple drug-receptor sites, channel subunits, and the development of a radioiodinated 1,4-dihydropyridine calcium channel label, [^{125}I]iodipine. J. Cardiovasc. Pharmacol. **6**: S608–S612.

Hochachka, P. W. 1988. Metabolic-, channel-, and pump-coupled functions: constraints and compromises of coadaptation. Can. J. Zool. **66**: 1015–1027.

Keen, J. E., Farrell, A. P., Tibbits, G. F., and Brill, R. W. 1992. Cardiac physiology in tunas. II. Effect of ryanodine, calcium, and

adrenaline on force–frequency relationships in atrial strips from skipjack tuna, *Katsuwonus pelamis*. Can. J. Zool. **70**: 1211–1217.

Klitzner, T., and Morad, M. 1983. Excitation–contraction coupling in frog ventricle: possible Ca^{2+} transport mechanisms. Pfluegers Arch. **398**: 274–283.

Langer, G. A. 1980. The role of calcium in the control of myocardial contractility: an update. J. Mol. Cell. Cardiol. **12**: 231–239.

Lee, H. R., Roeske, W. R., and Yamamura, H. I. 1984. High affinity specific [^{3}H](+)PN200-110 binding to dihydropyridine receptors associated with calcium channels in rat cerebral cortex and heart. Life Sci. **35**: 721–732.

McKnight, K. J., Kashihara, H., and Tibbits, G. F. 1989. Na^+/Ca^{2+} exchange in sarcolemma from a salmonid heart: seasonal variations. Can. Fed. Biol. Soc. Proc. **32**: 150.

Meissner, G. 1986. Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. J. Biol. Chem. **261**: 6300–6306.

Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S., and Numa, S. 1989. Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. Nature (London), **340**: 230–233.

Pessah, I. N., Waterhouse, A. L., and Casida, J. E. 1985. The calcium–ryanodine receptor complex of skeletal and cardiac muscle. Biochem. Biophys. Res. Commun. **128**: 449–456.

Philipson, K. D., and Ward, R. 1987. Modulation of Na^+-Ca^{2+} exchange and Ca^{2+} permeability in cardiac sarcolemmal vesicles by doxylstearic acids. Biochim. Biophys. Acta, **897**: 152–158.

Santer, R. M. 1985. Morphology and innervation of the fish heart. Adv. Anat. Embryol. Cell Biol. **89**: 1–102.

Schwartz, A., Lindenmayer, G. E., and Allen, J. C. 1975. The sodium–potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. Pharmacol. Rev. **27**: 3.

Sutko, J. L., and Kenyon, J. L. 1983. Ryanodine modification of cardiac muscle responses to potassium-free solutions. J. Gen. Physiol. **82**: 385–404.

Tibbits, G. F., Sasaki, M., Ikeda, M., Shimada, K., Tsuruhara, T., and Nagatomo, T. 1981. Characterization of rat myocardial sarcolemma. J. Mol. Cell. Cardiol. **13**: 1051–1061.

Tibbits, G. F., Kashihara, H., and O'Reilly, K. 1989. Na/Ca exchange in cardiac sarcolemmal vesicles: modulation of Ca affinity by exercise. Am. J. Physiol. **256**: C638–C643.

Tibbits, G. F., Kashihara, H., Thomas, M. J., Keen, J. E., and Farrell, A. P. 1990. Ca^{2+} transport in myocardial sarcolemma from rainbow trout. Am. J. Physiol. **259**: R453–R460.

Tibbits, G. F, Hove-Madsen, L., and Bers, D. M. 1991. Calcium transport and the regulation of cardiac contractility in teleosts: a comparison with higher vertebrates. Can. J. Zool. **69**: 2014–2019.

Wier, W. G. 1990. Cytoplasmic [Ca^{2+}] in mammalian ventricle: dynamic control by cellular processes. Annu. Rev. Physiol. **52**: 467–485.