Cardiac physiology in tunas. II. Effect of ryanodine, calcium, and adrenaline on force–frequency relationships in atrial strips from skipjack tuna, *Katsuwonus pelamis*

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Force–frequency relationships and the dependence upon extracellular calcium as a source of activator calcium were investigated using atrial strips from the skipjack tuna (*Katsuwonus pelamis*). At 25°C, active force generation increased over a stimulation range of 0.2–1.6 Hz and declined at higher stimulation frequencies. Ryanodine, a blocker of calcium release from the sarcoplasmic reticulum, decreased active force by ~30% but did not alter the shape of the force–frequency curve. Uniform contractions were maintained up to stimulation rates of 3.0 Hz following ryanodine application, compared with 3.4 Hz under control conditions. Active force increased with increasing extracellular calcium concentration in a concentration-dependent manner with greatest sensitivity over the range of 1.0–5.0 mM calcium. Activation duration decreased with increasing extracellular calcium concentrations up to 5.0 mM and with increasing contraction frequency up to 1.0 Hz. Relaxation duration also was reduced with increasing extracellular calcium over a range of 0.5–4.0 mM, and over a stimulation range of 0.2–0.6 Hz. Adrenaline increased active force threefold over the range of 10^{-8} to 10^{-5} M, with an EC_{50} value of $4.90 \pm 0.57 \times 10^{-7}$ M. The EC_{50} value was not dependent upon stimulation frequency. These results indicate that contraction in the skipjack tuna atrium is partially dependent upon intracellular calcium release from the sarcoplasmic reticulum. In this manner, the tuna heart more closely resembles the mammalian model of excitation–contraction coupling than that suggested for other fishes in which activator calcium is almost exclusively derived from extracellular influx.

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Les relations force–fréquence et le rôle du calcium extracellulaire comme source de calcium d'activation ont été mesurés sur des bandes de tissu d'oreillette chez le Thon à ventre rayé, (*Katsuwonus pelamis*). À 25°C, la production d'une force active augmente entre les fréquences 0,2 et 1,6 Hz, mais diminue aux fréquences de stimulation plus élevées. La ryanodine, un inhibiteur de la libération du calcium dans le réticulum sarcoplasmique, diminue la force active d'environ 30%, mais ne modifie pas la forme de la courbe force–fréquence. Des contractions uniformes sont obtenues par l'application de taux de stimulation allant jusqu'à 3,0 Hz après l'application de ryanodine; cette valeur atteint 3,4 Hz dans des conditions témoins. La force active augmente en fonction de la concentration de calcium extracellulaire selon une relation de dépendance et la sensibilité est maximale entre 1,0–5,0 mM de calcium. La durée de l'activation est fonction inverse de la concentration de calcium extracellulaire jusqu'à concurrence de 5,0 mM et de la fréquence de la contraction jusqu'à 1,0 Hz. La durée de la relaxation diminue aussi lors d'une augmentation de calcium extracellulaire aux concentrations situées entre 0,5 et 4,0 mM et d'une augmentation de la stimulation dans l'étendue 0,2 à 0,6 Hz. L'adrénaline augmente la force active par un facteur de 3 dans l'étendue 10^{-8} à 10^{-5} M et la valeur du EC_{50} est de $4,90 \pm 0,57 \times 10^{-7}$ M. La valeur du EC_{50} est indépendante de la fréquence de stimulation. Ces résultats indiquent que la contraction de l'oreillette du Thon à ventre rayé est partiellement dépendante de la libération de calcium intracellulaire provenant du réticulum sarcoplasmique. Sous cet aspect, le coeur d'un thon ressemble donc plus au modèle de couplage excitation–contraction d'un mammifère qu'au modèle suggéré pour d'autres poissons dans lequel le calcium d'activation provient surtout d'un apport extracellulaire.

[Traduit par la rédaction]

Introduction

Tuna are the most athletic of any fish species and have cardiac features on a par with many mammals of equivalent size. Cardiac output (132 $mL \cdot min^{-1} \cdot kg^{-1}$) in spinally blocked skipjack tuna (*Katsuwonus pelamis*) (Bushnell 1988) compares more favorably with estimates in mammals (dog, 103 $mL \cdot min^{-1} \cdot kg^{-1}$, Liang and Huckabee 1973; rabbit, 193–300 $mL \cdot min^{-1} \cdot kg^{-1}$, White *et al.* 1974) than in other fishes (Atlantic cod (*Gadus morhua*), 17.3 $mL \cdot min^{-1} \cdot kg^{-1}$, Axelsson *et al.* 1986; rainbow trout (*Oncorhynchus mykiss*), 17.6 $mL \cdot min^{-1} \cdot kg^{-1}$, Kiceniuk and Jones 1977). Similarly, regulation of cardiac output has been suggested to be primarily mediated through control of heart rate (Farrell *et al.* 1992), as in mammals, rather than through major modifications in stroke volume, as in other fishes (Farrell 1991). In fact, the high heart rate is one of the more striking differences between the tuna and most other lower vertebrates. In most lower vertebrates examined so far (fishes, amphibians, and reptiles), heart rate does not exceed 120 beats per minute (bpm; Farrell 1991). Tuna, on the other hand, have heart rates more like

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those of small mammals than fish. In spinally blocked skipjack tuna, resting heart rates may average 120 bpm (117 ± 12 bpm; Bushnell *et al.* 1990), and maximal rates from swimming tuna have been measured to be greater than 200 bpm (Kanwisher *et al.* 1974; Brill 1987). The maintenance of a high heart rate is dependent upon appropriate activation and relaxation mechanisms. The former, at least, appears to be different in upper and lower vertebrates.

Mammalian cardiac excitation–contraction (E–C) coupling is considered to involve a small influx of calcium across the sarcolemma through voltage-dependent channels, which acts as a trigger for a greater release of stored calcium from the sarcoplasmic reticulum (SR). The intracellularly stored calcium is the primary activator of contractile processes (Fabiato 1983). Lower vertebrates, however, appear to have a greater reliance on extracellular calcium for direct activation of myofilaments. Although well-developed (Page and Niedergerke 1972; Santer 1974), the SR is comparatively sparse, by mammalian standards. Furthermore, the magnitude of extracellular calcium influx has been demonstrated in frog (Morad and Cleeman 1987) and suggested in fish (Vornanen 1989; Tibbits *et al.* 1991) to be sufficient to support myocyte contraction. Given the high heart rate found in tuna, it was of interest to determine the relative contribution of extra- and intra-cellular calcium to contractile function in tuna heart, i.e., whether E–C coupling in the tuna heart more closely resembles the pattern found in higher or lower vertebrates. This study examines the response of skipjack tuna atrial strips to agents affecting intracellular calcium concentration.

Materials and methods

Twelve skipjack tuna (1.39 ± 0.06 kg) were purchased from local fishermen and held in outdoor tanks (24–26°C) at the Kewalo Research Facility (Honolulu Laboratory, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Hawaii).

Tuna were stunned by a sharp blow to the head, and the heart was quickly excised and placed in an ice-cooled modified Cortland saline (pH 7.8 at 25°C) of the following composition (in mM): NaCl, 185.7; KCl, 7.0; $CaCl_2 \cdot 2H_2O$, 1.9; $MgCl_2 \cdot 6H_2O$, 1.1; *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid (TES, Na salt), TES (free acid), 2.8; dextrose, 5.5. This saline was used throughout all experiments except when calcium was changed as an experimental variable. All chemicals were reagent grade and were purchased from Sigma (St. Louis, Missouri) with the exception of ryanodine, which was purchased through Calbiochem (San Diego, California). Atrial strips were dissected using two parallel razor blades. Thin (5-0) silk threads were tied to both ends of the strip, which was mounted in a saline-filled, O_2-aerated and water-jacketed (25°C) organ bath (20 mL volume). One end was attached to a fixed post and the other to a Metrigram isometric force transducer (Gould, Cleveland, Ohio). Signals from the force transducer were suitably amplified and displayed on either a 220 or 8000S Analog chart recorder (Gould, Cleveland, Ohio). Strips were electrically paced by a Grass SD9 Student stimulator delivering current via two flattened platinum electrodes positioned on both sides of the strip. The voltage was 1.25 times the threshold required to produce a contraction (22.6 ± 1.4 V) and pulse duration was 10 ms. Muscle strips were stretched until active force (generated force) reached a peak and were allowed to equilibrate for 1 h at a basal stimulation rate of 1.0 Hz. Strips were discarded if passive force exceeded 30% of total generated force at this frequency. Bath saline was then replaced with fresh saline and, following an equilibration period, the strips were exposed to one of the three following protocols.

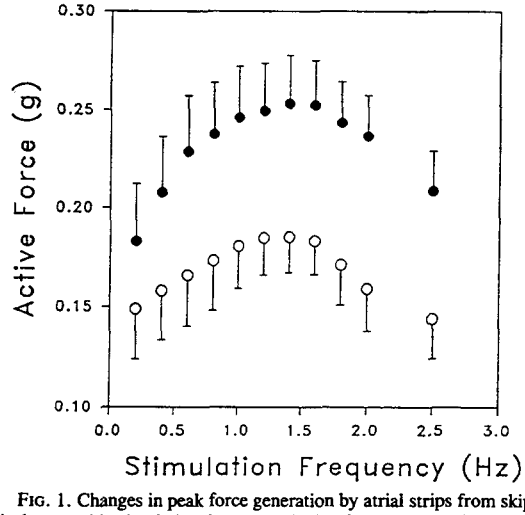

FIG. 1. Changes in peak force generation by atrial strips from skipjack tuna with stimulation frequency, in the absence (●) and presence (○) of 10 μM ryanodine. Pairwise comparisons of rate-dependent values were found to be significantly different in all cases ($p < 0.05$). Values are given as means + SE or − SE. $N = 5$.

Protocol 1

Stimulation frequency was changed to 0.2 Hz and increased stepwise (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 3.5, 4.0 Hz) until contractions displayed nonuniform twitches. At the conclusion of the trial, the control stimulation frequency (1.0 Hz) was reestablished. To examine the relative contribution of the SR to force development, ryanodine, a blocker of SR calcium release, was applied to a final concentration of 10 μM (Sutko and Kenyon 1983). The atrial strip was stimulated for 30 min after which the force–frequency trial was repeated.

Protocol 2

The dependency of force generation on extracellular calcium (Ca_o^{2+}) was examined over a calcium range of 0.5–10.0 mM Ca_o^{2+} and stimulation frequency range of 0.2–2.5 Hz. Bath saline was changed from control saline (1.9 mM Ca_o^{2+}) to one containing 0.5 mM Ca_o^{2+}. After equilibration at 1.0 Hz, stimulation frequency was changed to an initial test frequency of 0.2 Hz and the strip allowed to reequilibrate (1–2 min). Calcium concentration was then increased by sequential addition of a volume of saline-based stock calcium chloride solution. Following the test of the highest calcium concentration (10.0 mM), the bathing solution was replaced with the control saline (1.9 mM Ca_o^{2+}) and the muscle strip paced at the control stimulation frequency of 1.0 Hz. The bath solution was changed twice over a 10 min period to ensure complete return to control calcium levels; following equilibration, the atrial strip was reexposed to 0.5 mM Ca_o^{2+} saline and the protocol repeated at a new test frequency (0.4–2.5 Hz).

Protocol 3

The influence of adrenaline, an adrenergic agonist that operates, in part, through modifying the influx of Ca_o^{2+}, was examined through concentration–response curves generated at different test frequencies. Upon reaching steady-state force generation in control saline, stimulation frequency was changed from 1.0 Hz to a test rate (0.2–2.5 Hz). After equilibration, sequential addition of stock adrenaline produced a range of adrenaline concentrations from 10^{-10} to $\sim 10^{-4}$ M. Total added volume after final adrenaline application (at $\sim 10^{-4}$ M adrenaline) was 1.6% of the initial bath volume. After completion of the dose–response trial, the control stimulation frequency (1.0 Hz) was reimposed and the bath solution changed twice over a 10-min period.

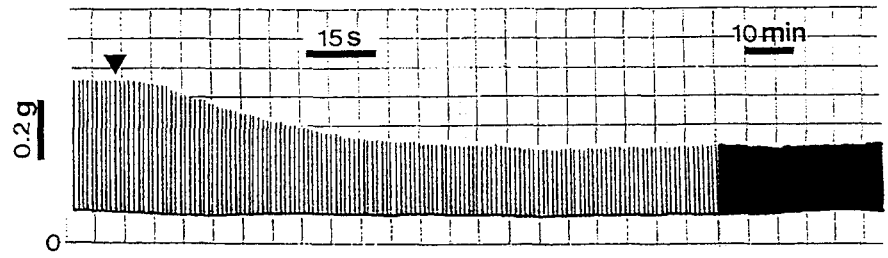

FIG. 2. Effect of ryanodine on force generation in atrial strips from skipjack tuna. The atrial strip was stimulated at 1.0 Hz in control saline. Ryanodine (10 μM) was added at the arrowhead. Time (in seconds and minutes) is indicated on the abscissa and force (in grams) on the ordinate.

At least 20 min (total time) was given between the return to control rate and the next test rate to ensure return of the muscle strip to a pre-adrenaline exposure condition and to minimize potential adrenoceptor desensitization phenomena.

After each manipulation the trace was run at a faster chart speed to estimate the time from 50% peak to peak force (activation duration) and the time from peak force to 50% of generated force (relaxation duration).

Muscle strips were weighed and the lengths measured using a micrometer and dissecting microscope. Widths were estimated assuming a specific gravity of 1.06 $g \cdot cm^{-3}$ and that the atrial strips approximated a cylinder. Mean length was 6.2 mm (range 3.9–7.8) and width estimates averaged 0.8 mm (range 0.6–0.9). As it is difficult to assess the number of dead or damaged fibers in muscle strip preparations, measurements are not presented per unit fiber weight or cross-sectional surface area.

Statistics were performed using paired and unpaired Student's *t*-tests where appropriate. A significance level of 0.05 was used in all instances.

Results

Force–frequency trials revealed active force generation to be biphasic (Fig. 1). Active force development increased with increasing stimulation frequency, with peak force (F_{max}) being developed at 1.4–1.6 Hz. Force generation declined at higher test frequencies. Following administration of ryanodine (10 μM), tension development quickly declined (Fig. 2) and a new steady state was essentially reached after 5 min of exposure. Rate trials were repeated after 30 min of exposure to ensure complete equilibration of ryanodine with the fibers. Ryanodine treatment (30 min incubation) significantly ($p < 0.05$) reduced active force production by ~30% at frequencies generating peak force (Fig. 1). Active force over this same time period in unexposed fibers was typically reduced by <5% of initial values. The decline in force in ryanodine-treated strips contrasts with previous studies (Driedzic and Gesser 1988) using ventricular strips from Atlantic cod and sea raven (*Hemitripterus americanus*), in which active force generation was not significantly modified by ryanodine.

The biphasic nature of the force–frequency curve was essentially unchanged by ryanodine application (Fig. 1). While not significantly affected ($p > 0.05$), the slope of the ascending arm of the curve was somewhat reduced, and this was reflected by a greater relative increase in F_{max} as a function of force generated at 0.2 Hz prior to ryanodine addition (48%, before; 37%, after). Passive force was unchanged following ryanodine application. Ryanodine also significantly ($p < 0.05$) depressed the maximal stimulation frequency generating uniform contractions (SF_{max}; not shown), from 3.4 ± 0.3 Hz (control) to 3.0 ± 0.2 Hz (ryanodine). Control SF_{max} values are in good agreement with *in vivo* estimates: swimming tuna have maximal heart rates of 180–210 bpm (3.0–3.5 Hz) (Brill and Bushnell 1991).

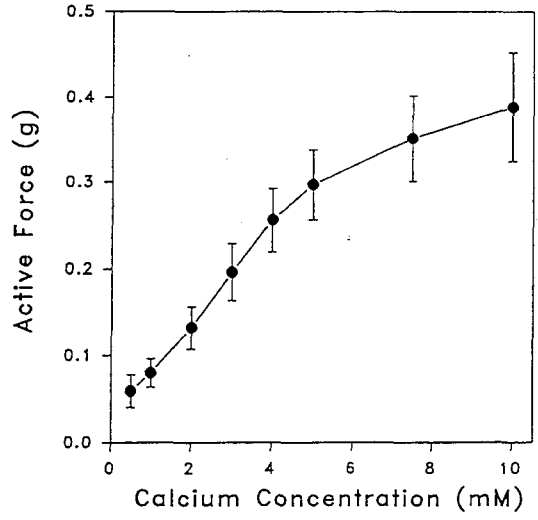

FIG. 3. Active force development as a function of external calcium concentration in atrial strips from skipjack tuna. Stimulation frequency did not have an effect on the slope (not shown). The depicted plot is from trials with a stimulation frequency of 0.2 Hz. Values are given as means ± SE. $N = 6$.

Active force generation increased with increased calcium concentration in the bathing solution (Fig. 3). Passive force was not significantly changed ($p > 0.05$). The "Ca_O^{2+}-response" curve was not altered by changes in stimulation frequency. The concentration producing half-maximal force development (EC_{50}) was similar at all stimulation rates and averaged 4.1 ± 0.2 mM Ca_O^{2+}. The slope of force development was greatest over the range of 1.0–5.0 mM Ca_O^{2+}. At the highest Ca_O^{2+} concentration (10.0 mM) force was, on average, 8.7 times greater than that at the lowest Ca_O^{2+} concentration (0.5 mM). At a stimulation rate of 0.2 Hz (Fig. 3), active force generation increased by an estimated 90% over the range of 3–9 mM. The sensitivity of skipjack tuna atrial strips to changes in Ca_O^{2+} is thus relatively greater than that found by Driedzic and Gesser (1988) for ventricular strips of spiny dogfish (*Squalus acanthias*,

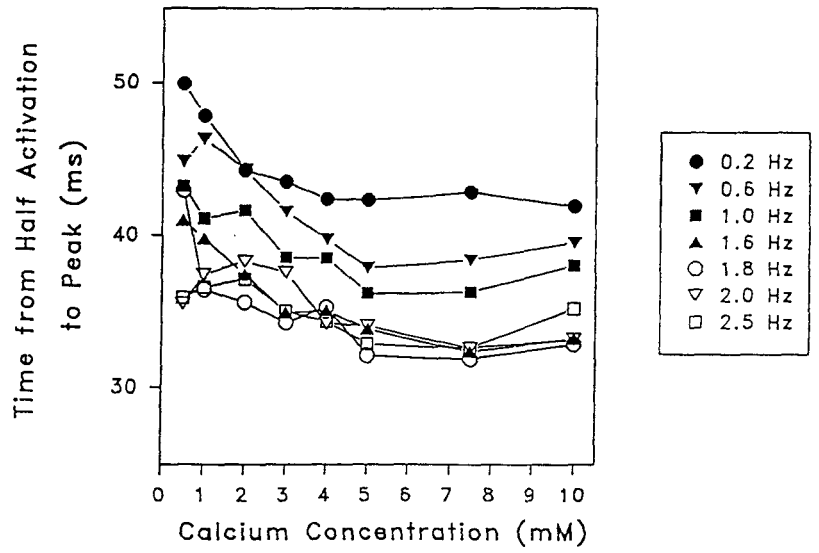

FIG. 4. Activation time (time from 50% of peak to peak force) as a function of extracellular calcium concentration and stimulation frequency (see legend) in atrial strips from skipjack tuna. Each point is the mean of six values. The SE (not shown for purposes of clarity) averaged less than 5% (range 1.6–9.6%) of the mean.

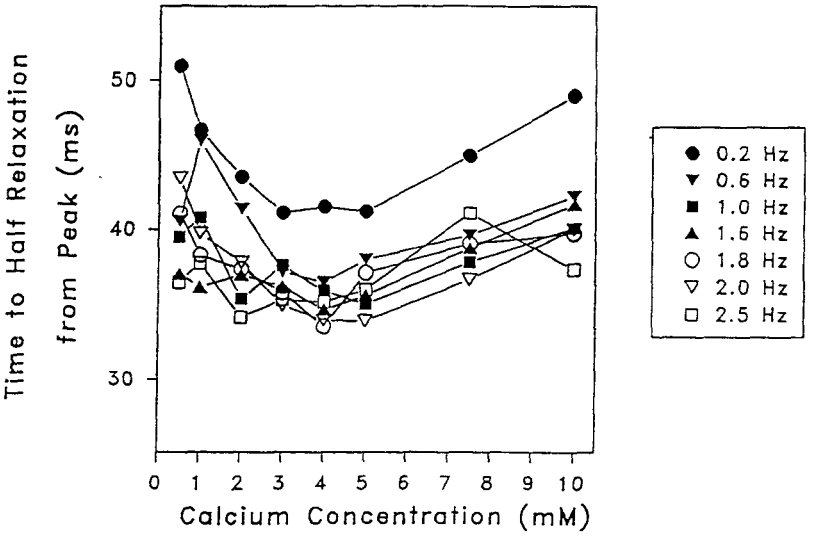

FIG. 5. Relaxation time (time from peak force to 50% peak force) as a function of extracellular calcium concentration and stimulation frequency (see legend) in atrial strips from skipjack tuna. Each point is the mean of six values, and the SE (not shown for purposes of clarity) averaged less than 6% (range 0.7–11.4%) of the mean.

20%) and hagfish (*Myxine glutinosa*, 40%), but less than that of little skate (*Raja erinacea*, 500%) and black dogfish (*Etmopterus spinax*, 580%).

The activation duration generally decreased with increasing calcium concentration over a range of 0.5–5.0 mM Ca_O^{2+} at all stimulation frequencies tested (Fig. 4). At concentrations greater than 5.0 mM Ca_O^{2+}, no further calcium-dependent reductions in activation duration were observed. Activation duration was also affected by stimulation frequency, being reduced with increasing stimulation rate over a range of 0.2–1.0 Hz (Fig. 4). Above 1.0 Hz, no further effects of frequency were found.

Relaxation times (Fig. 5) were generally reduced with increasing Ca_O^{2+} over the range of 0.5–4.0 mM. Increasing Ca_O^{2+} above 4.0 mM resulted in an increase in relaxation time. Like activation, increasing stimulation frequency decreased the duration of relaxation events, but only at the lowest frequencies tested (0.2–0.6 Hz).

Adrenaline concentration–response curves (Fig. 6) and EC_{50} values were not significantly affected by rate ($p > 0.05$). Force development was increased by adrenaline concentrations over a range of 10^{-8} to 10^{-5} M, with an EC_{50} value of $4.90 \pm 0.57 \times 10^{-7}$ M. In most instances, concentrations above 10^{-5} M decreased force somewhat, likely as a result of desensitization processes. In some trials, a slight decline in force production from control was observed at adrenaline concentrations below 10^{-8} M. This could reflect incomplete washout of adrenaline from the tissue after previous trials. Activation and relaxation durations were not found to be significantly ($p > 0.05$) altered by either adrenaline application or stimulation frequency (data not shown). No significant changes in passive force were observed with adrenaline application at any concentration.

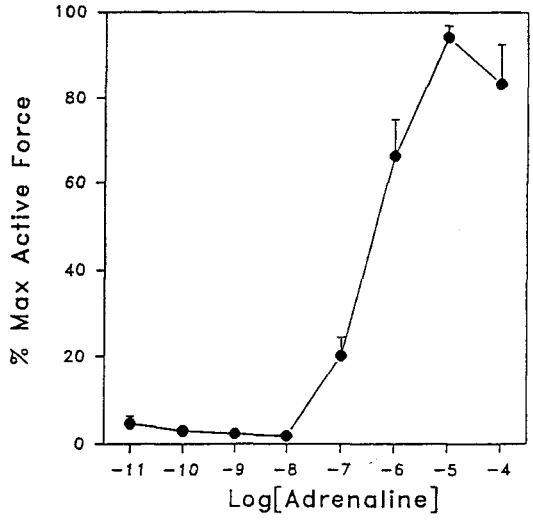

FIG. 6. Change in force development of skipjack tuna atrial strips as a function of adrenaline concentration. The concentration–response curve and estimated EC_{50} were not affected by stimulation frequency ($p < 0.05$). Adrenaline values are expressed as the log of the concentration. Force values are expressed as a percentage of maximal force and are given as means + SE unless error bars are smaller than symbol radius. Stimulation frequency in this instance was 1.0 Hz. Mean EC_{50} was estimated to be 5.13×10^{-7} M at this rate. $N = 6$.

Discussion

The force–frequency relationship for skipjack tuna atrial strips was unlike that of mammalian atrial preparations (Gibbons 1986). With increasing stimulation frequency, peak active force increased to a maximum and then declined at higher rates. The majority of mammalian atria demonstrate a three-component response to increasing frequency: force initially declines at low stimulation rates, then increases, and finally decreases at high frequencies. The tuna atrial curve was thus biphasic rather than triphasic and resembles, in this regard, results for ventricular preparations of mammals (Woodworth 1902; Blinks and Koch-Weser 1961), amphibians (Bowditch 1871; Niedergerke 1956), and elasmobranchs (Driedzic and Gesser 1988). It must be noted, however, that it is possible that the slowest stimulation rate used in this study was too high to detect force reductions associated with even lower frequencies.

The magnitude of the increase in force with increasing stimulation frequency was relatively modest, with peak force levels increasing by only about 50% over initial values (at 0.2 Hz). This is much less than the two- to three-fold increase in force that is typically found in atrial strip preparations from a variety of mammals (Gibbons 1986). In ventricular strips from Atlantic cod, sea raven (Driedzic and Gesser 1988), and carp (Vornanen 1989) only a decrease in force was found with increasing stimulation rate. The reasons underlying the differences in the force–frequency relationship are not readily apparent, but likely reflect species differences in processes involved in calcium storage and release (Blinks and Koch-Weser 1961). In this regard, we revealed, through the use of ryanodine, one important difference between skipjack tuna and all other fish species examined thus far. It appears contraction in the skipjack tuna is dependent to a significant degree on intracellular stores.

In mammals most of the activator calcium is thought to be derived from SR stores, with calcium influx across the sarcolemma serving primarily as the trigger for SR calcium release (Fabiato 1983). Ryanodine is a natural plant alkaloid that, at micromolar concentrations, blocks SR calcium release (Sutko and Kenyon 1983). Tissue sensitivity to ryanodine is considered to reflect the dependence of contractility on release from intracellular stores. Ventricular tissue from frog (Nayler 1963; Bers 1985) and rabbit (Sutko and Willerson 1980) is relatively insensitive to ryanodine treatment whereas contractility in rat (Bers 1985; Stemmer and Akera 1986) and mouse (Stemmer and Akera 1986) is greatly depressed. Cod and sea raven cardiac muscle strips are insensitive to ryanodine (Driedzic and Gesser 1988) whereas rainbow trout ventricular strips show limited sensitivity to ryanodine, depending upon the protocol (Gesser and Hoglund 1988; Hove-Madsen and Gesser 1989) and incubation conditions used (El-Sayed and Gesser 1989). Ryanodine application to tuna atrial stips was found to depress active force generation by approximately 20–30%, the magnitude being dependent upon the stimulation frequency. Assuming that, as in mammals, mitochondria are normally unimportant in supplementing contractile calcium and that the binding characteristics of ryanodine are similar in fishes and mammals, the calcium responsible for activation of force in the tuna heart must be derived from at least two sources. While the bulk of activator calcium is derived from extracellular sources, a significant fraction is supplied by internal stores (i.e., the SR).

Direct activation of myofilaments via calcium influx has been suggested in lower vertebrates (Morad *et al.* 1983; Vornanen 1989; Tibbits *et al.* 1990, 1991). It has been demonstrated in frog cardiac tissue by means of a variety of electrophysiological and pharmacological arguments that the magnitude of calcium influx across the sarcolemma is sufficient to directly activate the myofilaments (Morad *et al.* 1983; Morad and Cleeman 1987). Similar anatomical and biochemical features are apparent in fishes and frogs. In fishes (Santer 1985) and in frogs (Page and Niedergerke 1972), the SR is loosely organized and is sparse in comparison with that of mammals. Myocyte diameters in both frogs and fishes are typically much less than

10 μm (Page and Niedergerke 1972; Santer 1985). Lastly, under physiologically relevant conditions, ryanodine does not have a significant impact on force development in frog and all other fishes examined except the tuna. Thus, the tuna heart is like other fishes in that contraction is primarily dependent upon sarcolemmal influx rather than sarcoplasmic reticular release. However, unlike other fishes, a large contribution to the calcium pool responsible for contraction in the tuna heart is derived from the SR, as in many mammals.

Heart rates in most lower vertebrates do not exceed 120 bpm (Farrell 1991). While a number of components are likely involved in attainment and maintenance of high heart rates (nerve conduction velocities, densities and capacities of channels, exchangers and pumps, myosin isoforms, etc.), a contributing limitation may be the intracellular diffusion distance that calcium must cross to activate myofilaments. The diameter of cardiac myocytes of upper vertebrates generally exceeds 10 μm (Muir 1965; Sommer and Johnson 1979) and, given the heart rates found in most lower vertebrates, the implication is that it is too great to support high (>120 bpm) heart rates. This is circumvented in higher vertebrates by infoldings of the sarcolemma that invaginate the myocyte and form a junctional complex with the calcium-release sites of the terminal cisternae of the SR (McNutt 1975). Diffusional distances are secondly reduced by utilizing the SR, which is in close proximity to the myofilaments, as an intracellular source of activator calcium. The transverse tubule system found in mammals is not found in any fishes, including the tuna (Santer 1985); however, myocyte diameters in a related species of tuna, *Thunnus alalunga*, have been reported as 2.5–6.0 μm (Breisch *et al.* 1983) and the small intracellular diffusional distance, in conjunction with a functional store of SR calcium, may permit the high heart rates found.

The Ca_O^{2+}-response trials demonstrated changes in active force generation to be greatest over Ca_O^{2+} concentration range of 1.0–5.0 mM (Fig. 3). Plasma calcium concentration values for tuna are within this range (4.2–7.6 mM; Sather and Rogers 1967; Bourke *et al.* 1987) and, assuming free calcium to account for ~60% of the total plasma calcium pool (Driedzic and Gesser 1985), agents that alter calcium influx (such as adrenaline) represent an attractive method of increasing force production. This was also reflected by changes in activation and relaxation durations.

Increases in Ca_O^{2+} concentration were found to decrease the amount of time required to reach peak force generation (Fig. 4). Activation duration is a consequence of calcium influx and delivery to the myofibrils. With increasing Ca_O^{2+}, the time taken to fully activate contractile proteins should decline to a level at which force development is limited by diffusional distances (both to the SR and myofilaments) and not by any further increases in Ca_O^{2+}. This limiting concentration appears to approximate 5.0 mM calcium in tuna atrial strips.

Relaxation duration is related to the ability of efflux mechanisms to remove cytosolic calcium and its association with contractile proteins. Relaxation rates declined with increasing Ca_O^{2+} over a range of 0.5–4.0 mM (Fig. 5) and increased at higher concentrations. The apparent confounding relationship of a decrease in duration time despite an assumed greater cytosolic calcium load cannot be readily explained but may result from a balance between the affinity of efflux mechanisms for calcium and the capacity of the SR and mitochondria to store calcium. Indeed, were transport and storage mechanisms to approach saturation, the relaxation duration should increase as is found at Ca_O^{2+} levels in excess of 4.0 mM. This must be taken to be purely speculative at this juncture, as nothing is known about the action potential in tuna heart cells and the changes brought about by changes in Ca_O^{2+}.

Stimulation frequency also had an effect on activation and relaxation times, independent of Ca_O^{2+} levels. The mechanisms behind these events are beyond the scope of this paper but likely reflect alterations in both the quantity and rate of calcium flux to and from the myofilaments (Lewartowski and Pytkowski 1987). It is interesting to note, however, that the stimulation frequencies at which contractile cycle durations were minimized were ~1.0–1.6 Hz. This range of stimulation frequencies lies at or near the apex of the force–frequency relationship, and similar mechanisms may be responsible for both.

Lastly, the positive inotropism of cardiac muscle to adrenaline was examined through sequential addition to atrial strips. One of the actions of adrenaline is to increase the calcium influx through sarcolemma-bound L-type calcium channels (Reuter *et al.* 1986). Dose–response curves estimated an EC_{50} for adrenaline of 4.90×10^{-7} and demonstrated an operational range of 10^{-8} to 10^{-5} M. This is consistent with *in vivo* adrenaline levels in tuna (Watson 1990).

In summary, the tuna heart derives a large portion (~30% at resting stimulation frequencies) of activator calcium from intracellular stores, as shown in ryanodine trials, and resembles mammalian cardiac tissue in this regard more closely than other fishes. Perhaps not coincidentally, the heart rate of tuna is the highest of any lower vertebrate and approaches mammalian values. Unlike mammals, most of the contractile function appears to depend upon extracellular calcium supply rather than intracellular release, making modifications in calcium influx an attractive method of modifying force development in a tissue that is rather insensitive to rate changes in force production. This is supported by the high tissue sensitivity to changes in extracellular calcium at plasma calcium levels and to potential changes in intracellular calcium levels brought about by the action of adrenaline.

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