COMPARATIVE ASPECTS OF CARDIAC CONTRACTILITY IN TELEOST FISH: CLUES ABOUT E-C COUPLING FROM ISOMETRIC STUDIES.

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Isometric force measurements have had a long-standing application in the study of cardiac contractility and more recent studies of this ilk have been directed at elucidating excitation-contraction (E-C) coupling in fish hearts. This paper brings together recent discoveries, emphasizing differences among species and the effects of temperature. The importance of such an overview is two-fold. Foremost, the isometric studies have resulted in models of E-C coupling that are currently being tested using other techniques, some of which form important contributions to this symposium. Second, emphasis on exceptional species and experimental conditions has increased and this may blur the broader picture. Therefore, we hope to clearly establish what is known, set the stage for some of the other talks, and act as a pointer for future research.

Isometric force has been measured a number of species, including: hagfish (*Myxine glutinosa*), little skate (*Raja erinacea*), spiny dogfish (*Squalus acanthias*), black dogfish (*Etmopterus spinax*), white sturgeon (*Acipenser transmontanus*), Atlantic cod (*Gadus morhua*), ocean pout (*Macrozoarces americanus*), goosefish (*Lophius americanus*), sea raven (*Hemitripterus americanus*), lumpfish (*Cyclopterus lumpus*), longhorn sculpin (*Myoxcephalus octodecimspinosus*), smallmouth bass (*Micropterus dolomieui*), yellow perch

(*Perca flavescens*), crucian carp (*Carassius carassius*), alewife (*Alosa pseudoharengus*), rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), Atlantic salmon (*Salmo salar*), Atlantic mackerel (*Scomber scombrus*), Pacific mackerel (*Scomber japonicus*), skipjack tuna (*Katsuwonus pelamis*), and yellowfin tuna (*Thunnus albacares*). Rainbow trout account for by far the largest number and greatest breadth of studies. The focus here will be on teleost species, but this does not imply that other fish species are uninteresting and undeserving of further attention.

Isometric force is dependent on pacing frequency. For most of the teleost species listed above, isometric force decreases with increased pacing frequency (a negative force-frequency relationship) (Driedzic and Gesser, 1988). Just before contractions become arrhythmic at high pacing frequencies, isometric force is typically reduced by 50% to 90%. Thus, calcium delivery to the myofilaments is reduced by 2- to 10-fold, if we assume that active force is a reasonable estimate of calcium delivery.

Isometric force is also modulated by temperature. Maximum pacing frequency decreases with temperature, as does the rate of contraction and relaxation. However, if isometric force also decreases with temperature, as it does in some but not all species, the rate of calcium delivery may not change appreciably. Also, an asymptotic relationship exists between temperature and contraction time and 50% relaxation time, with the asymptotes for ventricular tissue (0.2 s and 0.1 s, respectively) being about twice those for atrial tissue. These differences in isometric force and kinetics likely relate to the shape of the action potential and to SR-ATPase activity, both of which vary with temperature and between atrial and ventricular tissue (Møller-Nielsen and Gesser, 1992; Aho and Vornanen, 1999; Shiels et al., 2000).

Increasing temperature can alter the shape of the force-frequency relationship. For rainbow trout, a plateau can develop at low pacing frequencies with increasing temperature (Shiels and Farrell, 1997). For skipjack tuna (Keen et al., 1992), yellowfin tuna (Shiels et al., 1999) and Pacific mackerel (Shiels and Farrell, 2000), which have a positive and then negative (biphasic) force-frequency relationship, the plateau can shift to the right with increasing temperature. The finding that peak isometric force occurs at a higher frequency with increasing temperature represents a useful compensatory mechanism given that *in vivo* heart rate also increases with temperature. Caution must be used, however, when comparing force-frequency relationships in the absence of absolute force data. Technical difference such as damaged muscle fibres, variable orientation of muscle fibres and a hypoxic core in the muscle strip could confound comparisons. Isolated trabecular muscle preparations minimize these problems.

Extracellular calcium, rather than intracellular calcium from sarcoplasmic reticulum (SR) stores, is the major source of activator calcium for cardiac contraction for most fish species studied to date. Three lines of experimental evidence support this notion. Foremost, isometric force increases anywhere from 50% to 500%, depending on the species, when extracellular calcium is increased (Driedzic and Gesser, 1985). However, extracellular calcium is more likely modulated by the open state probability of the L-type Ca-channel than calcium concentration because extracellular calcium concentration rarely varies by >1mM. This highlights the second line of evidence; isometric force increases up to two-fold with adrenaline, a known agonist of L-type Ca-channel open probability and decreases by 4- to 6-fold with the antagonist verapamil (Aho and Vornanen, 1999). The inotropic response of the teleost myocardium to adrenergic stimulation is also temperature-dependent, with cold-acclimation increasing the number of - adrenoreceptors on the sarcolemma of rainbow trout (Keen et al., 1993). The third line of evidence comes from using ryanodine on muscle strips to inactivate SR-calcium release channels.

Ryanodine has been tested on six fish species to date. Atlantic cod and sea raven were ryanodine-insensitive (Driedzic and Gesser, 1988) while ryanodine sensitivity in rainbow trout depended on temperature, pacing frequency, and the tissue type. With rainbow trout held at 10-15^{o}C, ryanodine sensitivity of ventricular strips occurred at sub-physiological pacing frequencies (up to 0.2 Hz) when tested at 15^{o}C and 25^{o}C but not at all at 5^{o}C (Hove-Madsen, 1992). Similarly, with 8^{o}C- and 18^{o}C-acclimated rainbow trout, ryanodine sensitivity of ventricular strips occurred only at low pacing frequencies at 18^{o}C and not at all at 8^{o}C (Keen et al., 1994); acute temperature changes exacerbated this difference. Ventricular trabeculae from rainbow trout held at 12^{o}C or 22^{o}C and again tested at both temperatures but under tonic adrenergic stimulation yielded somewhat different results (Shiels and Farrell, 1997). Ryanodine sensitivity occurred at both test temperatures for 12^{o}C-acclimated fish, but again only at low pacing frequencies. With 22^{o}C-acclimated fish tested at 22^{o}C, ryanodine sensitivity was present at all pacing frequencies, but when tested at 12^{o}C, ryanodine sensitivity was present only at low pacing frequencies. Three important points emerge from these rainbow trout studies, despite underlying concerns about methodological differences and preparation quality. Foremost, at routine temperatures for rainbow trout, ryanodine sensitivity is present only at sub-physiological pacing frequencies and so extracellular calcium normally predominates in ventricular E-C coupling. Second, ryanodine sensitivity changes with acute temperature changes. Third, the ryanodine-sensitive component of calcium delivery usually accounts for 5-30% of the isometric force. An apparent exception is when cold-acclimated hearts are tested at warm temperatures and this percentage increases.

Atrial tissue from rainbow trout has a greater, broader and more physiologically relevant ryanodine-sensitivity compared with ventricular tissue (Gesser, 1996, Aho and Vornanen, 1999; Shiels et al., 1999). Atrial sensitivity to ryanodine is still augmented by cold temperature and low frequency, but to a lesser extent than the ventricle. Further, there appears to be a slightly different relationship between adrenergic stimulation and ryanodine sensitivity in rainbow trout atrium than in ventricle (Gesser, 1996; Shiels et al., 1999).

Atrial tissue from the sub-tropical skipjack tuna (Keen et al., 1992) and yellowfin tuna (Shiels et al., 1999) demonstrates the greatest ryanodine sensitivity studied to date and may reflect interspecific differences, in addition to tissue specific differences. This is because there are marked differences in the contractile properties of scombrid myocardium compared with salmonid myocardium. All scombrid species tested so far have exhibited biphasic force-frequency relationships (Keen et al. 1992; Shiels et al. 1999; Shiels and Farrell, 2000). Second, significant ryanodine sensitivity occurs at all pacing frequencies. In ventricular trabeculae from 15^{o}C Pacific mackerel, ryanodine reduced isometric force by 20-30% when tested at 20^{o}C but not at all at 15^{o}C. In atrial strips from skipjack tuna held and tested at 25^{o}C, ryanodine reduced isometric force by 20-40%. In atrial trabeculae from yellowfin tuna held at 20^{o}C and tested at 15, 18 and 25^{o}C under tonic adrenergic stimulation, isometric force was reduced by 20-70%.

For rainbow trout and scombrids, the percentage reduction in isometric force as a result of ryanodine application suggests that the quantitative contribution of SR-calcium to E-C coupling is usually minor compared with that of extracellular calcium. Yet for certain physiologically relevant situations with atrial tissue and some unusual situations with ventricular tissue, the contribution of SR-calcium to E-C coupling can equal (and perhaps slightly exceed) the contribution of extracellular calcium. However, a danger exists when comparing percentages between species and experimental conditions because total calcium requirement is not considered. To remedy this, existing data for absolute force development were reanalysed to indicate total calcium requirement and compared to examine the effects of ryanodine and experimental conditions. Among the new insights is the finding that the estimated SR-calcium contribution in yellowfin tuna atrium exceeded that of the rainbow trout ventricle by as much as 10-fold, while the extracellular contribution was only 2-fold higher. In addition, SR-calcium contribution was largely independent of acute temperature changes in rainbow trout, Pacific mackerel and yellowfin tuna.

In summary, limited data suggest that a number of factors diminish the role of extracellular calcium and increase the role of SR-calcium for isometric force development in fish hearts. Foremost, there are species-specific factors. Fish that tend to lead more active life styles (salmonids and tunas) apparently have evolved mechanisms, notably SR-calcium stores, to supplement or replace extracellularly derived calcium for contraction. Second, there are tissue-specific factors, with the atrium apparently relying on SR-calcium to a greater degree than the ventricle. Third, there are frequency dependent factors because the rainbow trout ventricle is sensitive to ryanodine at low, but not high pacing frequencies. Fourth, there are temperature-specific factors. Clearly, as we move to a more integrated understanding of E-C coupling in teleost fish, consideration needs to be given to spatial and temporal constraints, as well as the overall calcium demand of the heart during contraction. This paradigm will mean that the usefulness of isometric force studies alone will decrease and will be replaced by cellular level studies of calcium movements, some of which we will hear about in this symposium.

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