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DIFFERENCES IN FORCE–FREQUENCY RELATIONSHIPS AND CALCIUM DEPENDENCY BETWEEN ELASMOBRANCH AND TELEOST HEARTS

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Summary

Ventricle strips from little skate (*Raja erinacea*), spiny dogfish (*Squalus acanthias*), black dogfish (*Etmopterus spinax*), sea raven (*Hemitripterus americanus*), cod (*Gadus morhua*), hagfish (*Myxine glutinosa*) and white sturgeon (*Acipenser transmontanus*) were mounted for isometric force recording. Force development was assessed as a function of external calcium concentration and frequency of contraction. Post-rest potentiation was determined in skate and the teleost species to assess indirectly calcium storage capacities. Sea raven and cod preparations were also treated with ryanodine to assess the importance of calcium release from the sarcoplasmic reticulum.

Ventricle strips from skate and black dogfish showed a five-fold increase in force development when external calcium was raised from a physiological to a saturating level. Force development by ventricle strips from other species tested increased by only about 50 % over the same range of calcium concentration.

For all elasmobranchs tested, an elevation in frequency of contraction of ventricle strips resulted first in an increase and subsequently in a decrease in force development. The apices of the curves were well within the physiological range of heart rates exhibited by these species. Preparations from teleosts showed only a decrease in force development when contraction frequency was elevated.

Skate ventricle strips exhibited a very marked post-rest potentiation at $3\,mmol\,l^{-1}$ external calcium. This protocol is considered to reflect the importance of intracellular calcium stores in the beat-to-beat maintenance of contractility. Sea raven and cod ventricle strips did not show any major post-rest potentiation, suggesting that calcium storage in hearts of these species is minimal. Ryanodine treatment had no effect upon sea raven and cod heart preparations. This approach further implies that calcium release from sarcoplasmic reticulum is not critical in these species.

Key words: calcium sensitivity, elasmobranch hearts, force–frequency, post-rest potentiation, ryanodine, teleost hearts.

Introduction

In isolated cardiac muscle from many mammals, turtles and frogs, stepwise transitions from low (e.g. 20 contractions min^{-1}) to high frequencies result in first an increase and subsequently a decrease in twitch tension development (Johnson, 1979). The prime determinants of force development by heart muscle are intracellular Ca^{2+} activity and the time available for Ca^{2+} to interact with the contractile system (Yue, 1987). On a beat-to-beat basis, Ca^{2+} may arise from the extracellular space or from internal stores, especially the sarcoplasmic reticulum. The apex of the force–frequency curve is modified by $[Ca^{2+}]_o$ (external calcium concentration) and occurs at rates which are within the physiological range experienced by ectothermic animals. As such, the force–frequency relationship and response to changes in $[Ca^{2+}]_o$ may have special significance in the operation of fish myocardia.

Ventricle strip preparations from numerous teleost fishes show only a negative change in response to small stepwise increases in frequency. However, elevation of $[Ca^{2+}]_o$ can compensate for any decrease in force development imposed by frequency alone (Driedzic & Gesser, 1985). According to Ruben & Bennett (1981), plasma Ca^{2+} level increases in response to strenuous activity in some teleosts but not in elasmobranchs. However, this finding was not confirmed in a later study on rainbow trout, *Salmo gairdneri* (Andreasen, 1985). In a preliminary report Maylie *et al.* (1979) indicated that trabeculae from an elasmobranch, *Squalus acanthias*, exhibit a positive force–frequency relationship over the range from 10 to 60 contractions min^{-1}. These potential class-associated differences motivated us to examine the influence of $[Ca^{2+}]_o$ and frequency on force development by isometrically contracting ventricle strips from selected elasmobranchs, hagfish and sturgeon. In addition, cellular Ca^{2+} handling was indirectly assessed by determining post-rest potentiation and by treatment with the natural plant alkaloid ryanodine. The actions of ryanodine on cardiac muscle are complex; it may impede Ca^{2+} efflux from sarcoplasmic reticulum or, under some conditions, may cause the sarcoplasmic reticulum to discharge its Ca^{2+} stores (Rousseau *et al.* 1987; MacLeod & Bers, 1987). Ryanodine allows expression of a positive force–frequency relationship in rat heart under conditions which otherwise result in a negative response (Stemmer & Akera, 1986).

The present studies show that elasmobranch hearts exhibit the typical mammalian positive, followed by negative, force–frequency relationship, whereas teleost hearts show only the negative component. Moreover, calcium storage capabilities appear to be much better developed in skate hearts than in hearts from two species of teleosts.

Materials and methods

Animals

Sea raven (*Hemitripterus americanus*) Gmelin (280–600 g), cod (*Gadus morhua*) L. (50–500 g), spiny dogfish (*Squalus acanthias*) L. (753–1650 g), little skate (*Raja*

erinacea) Mitchell (36–850 g) and hagfish (*Myxine glutinosa*) L. (36–66 g) were captured in waters off St Andrews, NB, Canada. Black dogfish (*Etmopterus spinax*) L. (7–288 g) were caught in waters off Bergen, Norway. White sturgeon (*Acipenser transmontanus*) Richardson (19–23 g) were provided by the Bergen Aquarium.

Experimental preparations

Animals were killed by a blow to the head, hearts were excised and placed in cold bathing medium containing 1 mmol l^{-1} $[Ca^{2+}]_o$ or 1·25 mmol l^{-1} $[Ca^{2+}]_o$ (for teleosts). Ventricle strips of approximately 1 mm in diameter were mounted for isometric recording of force as previously described (Gesser, 1977). Preparations were stimulated to contract *via* two platinum electrodes, one on each side of the strip. Strips were stretched until peak force did not increase any further and allowed to stabilize at 12 contractions min^{-1}.

Protocols

1. *Force–frequency relationships in elasmobranchs, hagfish and sturgeon*

Paired strips from spiny dogfish, skate and hagfish were allowed to stabilize in media containing 1 mmol l^{-1} $[Ca^{2+}]_o$; thereafter $[Ca^{2+}]_o$ was increased to 3 mmol l^{-1} for both preparations. $[Ca^{2+}]_o$ was further increased stepwise for one preparation while the other remained in 3 mmol l^{-1} $[Ca^{2+}]_o$. After stabilization of the preparation at 7 or 9 mmol l^{-1} $[Ca^{2+}]_o$, the stimulation frequency for both preparations was decreased to 6 contractions min^{-1}. Thereafter, frequency was increased stepwise until the preparation ceased to respond in a regular fashion. In experiments with black dogfish and sturgeon, the same strip was utilized in a sequential fashion for a frequency challenge at low $[Ca^{2+}]_o$, $[Ca^{2+}]_o$ increases, and finally a frequency challenge at high $[Ca^{2+}]_o$. Skate and hagfish preparations took many minutes to reach new steady-state conditions after any perturbation. With other species in this series, frequency shifts resulted in a new steady-state level after a few contractions. Only data obtained under steady-state conditions are presented and they are expressed as a percentage of the maximum force development attained.

2. *Post-rest potentiation in skate*

Paired strips from skate were allowed to stabilize at 18 contractions min^{-1} in either 3 or 9 mmol l^{-1} $[Ca^{2+}]_o$. Thereafter, frequency was increased to 36 contractions min^{-1} for 10 min. Preparations were allowed to rest for 3 s and a 'test contraction' was assessed. Frequency was subsequently lowered in decrements of 6 contractions min^{-1} to a minimum of 6 contractions min^{-1}, and then elevated in increments, with determination of a test contraction after each 10-min step. The rationale of the experiment was to assess whether there was storage of Ca^{2+} during the 10 min conditioning period which could be released subsequently in response to a single stimulation (see Rumberger & Reichel, 1972). The optimal rest period which resulted in maximal test contraction force development was determined in

preliminary studies. The patterns of change in force development were similar under conditions of decreasing or increasing frequency transitions. Average values were calculated and expressed as a percentage of force development at the frequency which resulted in maximum steady-state performance. This frequency was preparation-dependent and varied from 18 to 30 contractions min^{-1}.

3. *Force–frequency and post-rest potentiation in teleosts*

Paired strips from sea raven and cod were mounted in medium containing either 1·25 mmol l^{-1} or 5·0 mmol l^{-1} $[Ca^{2+}]_o$. Following a stabilization period, strips were paced at 12 contractions min^{-1} for 10 min, allowed to rest for 20 s and a test contraction was assessed. Strips were then paced for 1 min at 12 contractions min^{-1} and subsequently elevated to the next rate for 10 min and the procedure repeated. Preliminary studies determined the optimal rest period which resulted in maximal test contraction force development.

4. *Ryanodine treatment of teleost hearts*

Force–frequency experiments described above were repeated with paired strips from sea raven and cod in medium containing 1·25 mmol l^{-1} $[Ca^{2+}]_o$ with or without ryanodine (10 μmol l^{-1}). Strips were allowed to equilibrate with pacing at 12 contractions min^{-1} for 40 min in the presence of ryanodine prior to the frequency challenge.

Bathing media

Protocol 1

The initial media contained 1 mmol l^{-1} $[Ca^{2+}]_o$. In studies with spiny dogfish and skate the medium contained (in mmol l^{-1}): NaCl, 280; KCl, 5; $MgCl_2$, 2·5; NaH_2PO_4, 0·034; Na_2HPO_4, 0·466; $MgSO_4$, 0·5; urea, 350; glucose, 5; $NaHCO_3$, 11; $CaCl_2$, variable. The medium was equilibrated with 0·5 % CO_2 in O_2 and had a final pH of 7·7 at 10 °C. Preliminary studies with a medium containing 80 mmol l^{-1} trimethylamine showed that it did not influence the parameters under investigation and it was not included in further work. In experiments with black dogfish the medium was aerated and contained 2·5 mmol l^{-1} $NaHCO_3$. The pH was 7·6 at 15 °C. The medium for hagfish hearts contained (in mmol l^{-1}): NaCl, 550; KCl, 10; $MgCl_2$, 14; $MgSO_4$, 1; NaH_2PO_4, 0·034; Na_2HPO_4, 0·466; glucose, 5; $NaHCO_3$, 11; $CaCl_2$, variable. Gassing, pH and temperature were the same as those for spiny dogfish and skate studies. The medium for sturgeon hearts contained (in mmol l^{-1}): NaCl, 150; KCl, 5; $MgCl_2$, 5; $MgSO_4$, 0·5; NaH_2PO_4, 0·034; Na_2HPO_4, 0·466; glucose, 5; $NaHCO_3$, 2·5; $CaCl_2$, variable. Gassing, pH and temperature were the same as those for black dogfish studies.

Protocol 2

Media were gassed with 1 % CO_2: 99 % O_2 and $[NaHCO_3]$ was increased to 15 mmol l^{-1} to provide a pH of 7·8 at 10 °C.

Protocols 3 and 4

The initial medium contained (in mmol l^{-1}): NaCl, 150; KCl, 5; $MgSO_4$, 2; NaH_2PO_4, 1; $CaCl_2$, 1·25; glucose, 5; $NaHCO_3$, 15; and was equilibrated with 1 % CO_2: 99 % O_2 to provide a final pH of 7·8 at 10°C. In some experiments ryanodine (Progressive Agri-Systems, Wind Gap, PA, USA) was added to a final concentration of 10 μmol l^{-1}. The efficacy of the ryanodine was checked against rat atrial strips and papillary muscle. Treatment with 1 μmol l^{-1} ryanodine resulted in rapid failure of these preparations.

Data analysis

Force development between peak and resting level was expressed as a percentage of indicated conditions. In Ca^{2+} sensitivity experiments (protocol 1) all values are expressed as a function of force development in medium containing 3 mmol l^{-1} $[Ca^{2+}]_o$. Force–frequency experiments with skate, dogfish and sturgeon yielded positive followed by negative responses. Data are expressed as a percentage of the condition under which maximal force generation was observed. Due to variability in the apex of the curves, data for individuals are presented. Force–frequency data for sea raven and cod are expressed as a percentage of the steady-state force developed at 12 contractions min^{-1}. Wherever possible data are expressed as mean ± S.E.M.

Results

Ca^{2+} sensitivity

An increase in $[Ca^{2+}]_o$ resulted in an increase in force development for ventricle strips from all species tested (Fig. 1). There was extreme species variability in

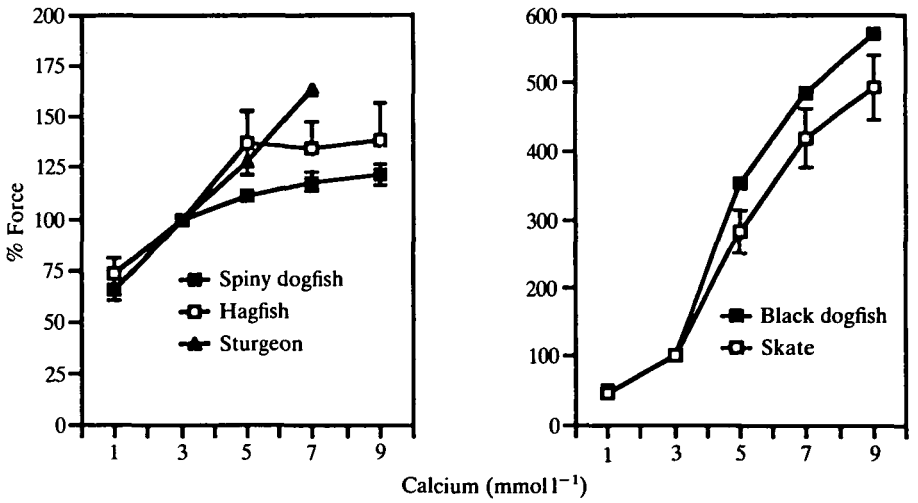

Fig. 1. Relative force development of isometrically contracting ventricle strips as a function of $[Ca^{2+}]_o$. All preparations were paced at 12 contractions min^{-1}. Sample sizes: spiny dogfish (5), hagfish (3), sturgeon (2), black dogfish (2), skate (3).

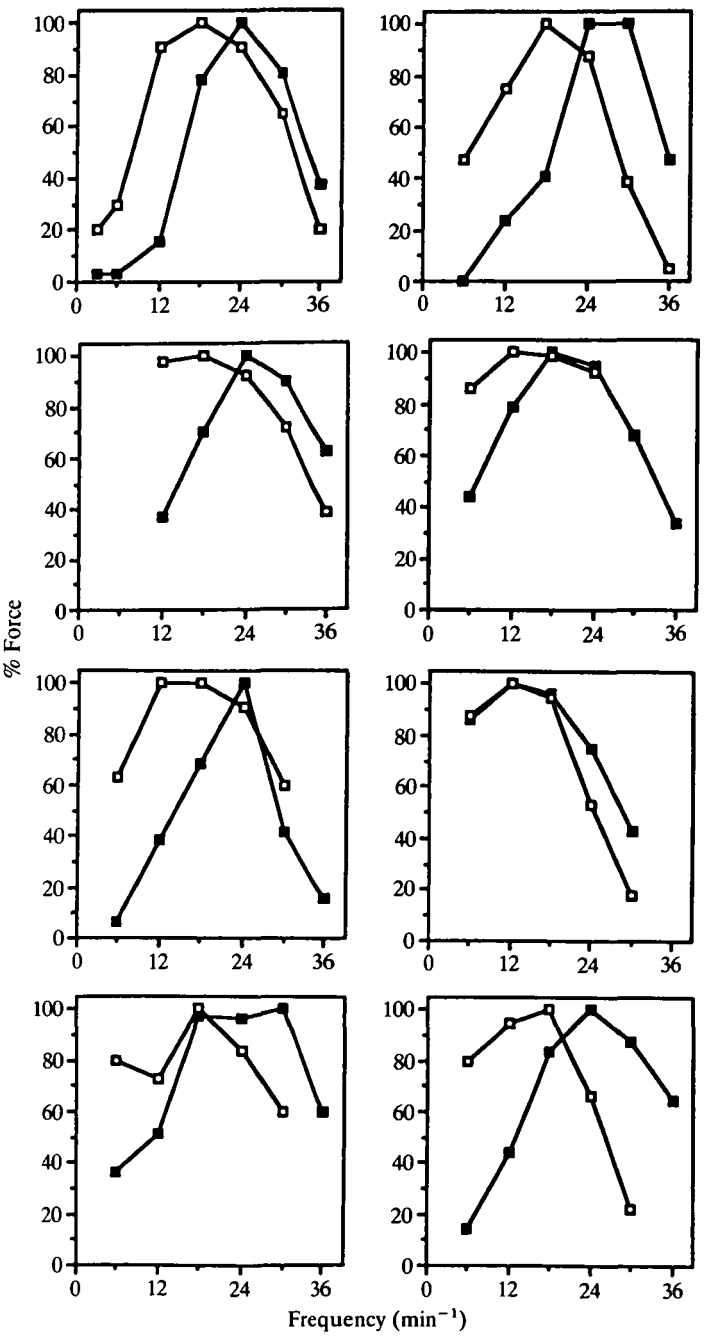

Fig. 2. Force–frequency relationships for ventricle strips from little skate at 3 (■) and 9 mmol l^{-1} (□) $[Ca^{2+}]_o$. Data are presented for paired strips from individuals.

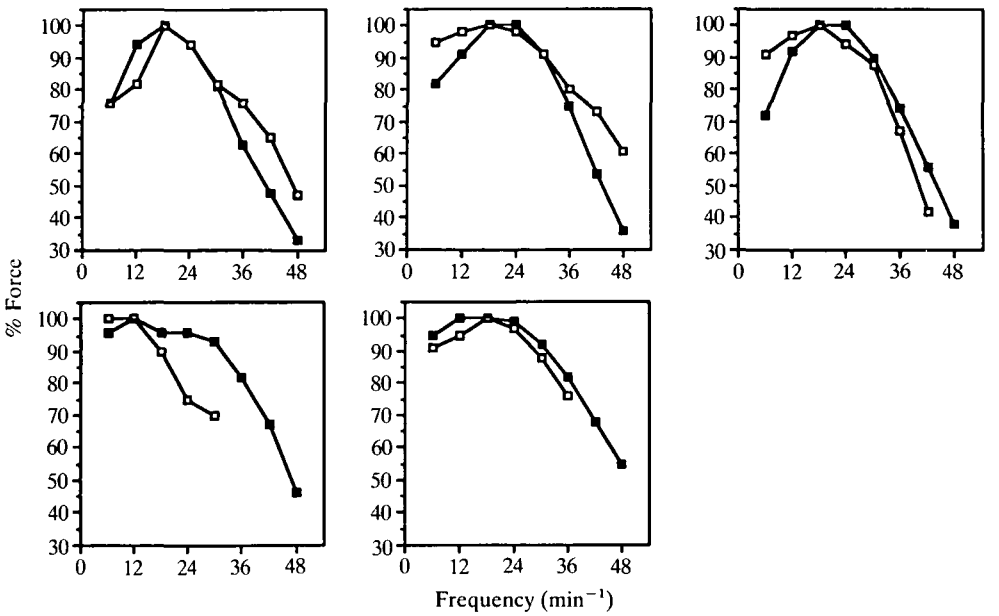

Fig. 3. Force–frequency relationships for ventricle strips from spiny dogfish at 3 (■) and 9 mmol l^{-1} (□) $[Ca^{2+}]_o$. Data are presented for paired strips from individuals.

sensitivity to $[Ca^{2+}]_o$. Black dogfish and skate showed a five-fold increase in force development at 9 mmol l^{-1} compared with that at 3 mmol l^{-1} $[Ca^{2+}]_o$. For the other species tested the increase over the same range was about 25 %.

Force–frequency relationships in elasmobranchs

In all cases with skate (Fig. 2), an increase in frequency initially resulted in an increase in tension development. After an apex had been reached, force development declined severely as frequency was increased. The same general relationship was observed at both 3 and 9 mmol l^{-1} $[Ca^{2+}]_o$. In seven out of eight experiments the curve was shifted to the left at the elevated $[Ca^{2+}]_o$ level. Most preparations failed to respond in a consistent fashion to imposed frequencies above 36 contractions min^{-1}. One experiment was conducted with thorny skate (*Raja radiata*) at 3 mmol l^{-1} $[Ca^{2+}]_o$ (data not shown). The pattern of response was similar to that of little skate. In experiments with spiny dogfish (Fig. 3), nine out of 10 preparations showed increases in force frequency when contraction frequency was increased from 6 to 18 contractions min^{-1}. The increase in tension development was small but consistent. Further increases in frequency resulted in a decline in force development. At 48 contractions min^{-1} only about 50 % of the maximum force was developed. Most preparations failed to respond in a consistent fashion at higher rates. The data for black dogfish are less extensive (Fig. 4) but the same general pattern occurred: an increase in force development was followed by a decline as contraction frequency was increased.

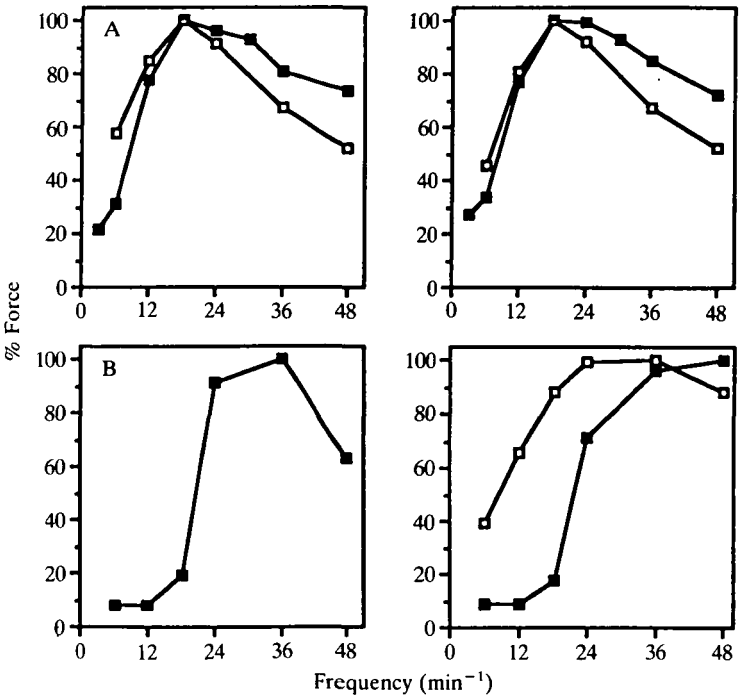

Fig. 4. Force–frequency relationships for ventricle strips from black dogfish at 3 (■) and 9 mmol l^{-1} (□) $[Ca^{2+}]_o$. (A) Two preparations from the same animal; (B) second and third individuals.

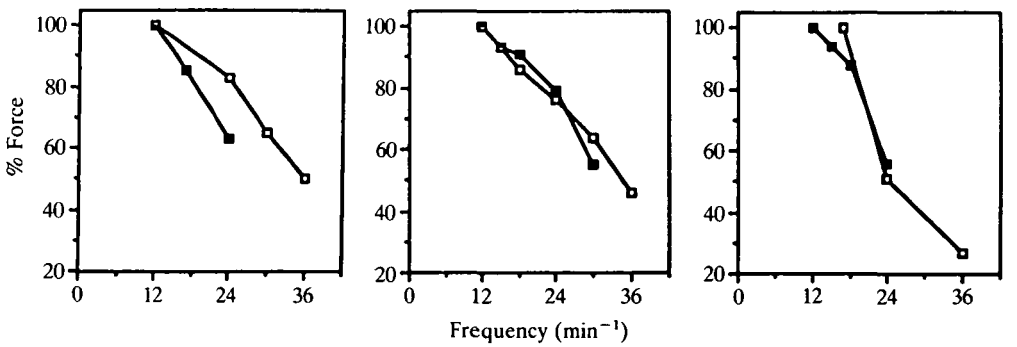

Fig. 5. Force–frequency relationships for ventricle strips from hagfish at 3 (■) and 9 mmol l^{-1} (□) $[Ca^{2+}]_o$. Data are presented for paired strips from individuals.

Force–frequency relationships in hagfish and sturgeon

Hagfish (class Agnatha) showed only negative responses under the conditions tested (Fig. 5). A similar negative response was observed in one preparation which was bathed overnight and subsequently tested in medium containing 0·1 mmol l^{-1} $[Ca^{2+}]_o$ (data not shown). The preparations were spontaneously active at rates of

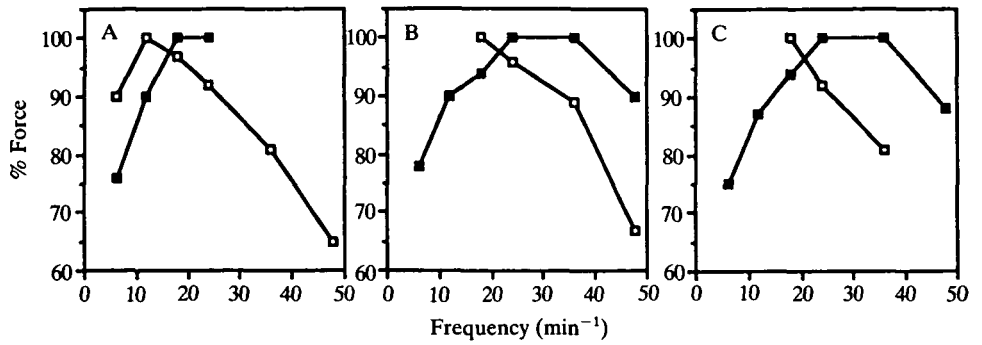

Fig. 6. Force–frequency relationships for ventricle strips from white sturgeon at 1 (■) and 7 mmol l^{-1} (□) $[Ca^{2+}]_o$. (A) Data from one animal; (B,C) data from a second animal.

about 8 contractions min^{-1}. Therefore, it was not possible to assess the force–frequency relationship at extremely low rates. White sturgeon (order Acipenseriform) showed a positive response in each of three preparations assessed in medium containing 1 mmol l^{-1} $[Ca^{2+}]_o$. Two preparations became spontaneously active in high $[Ca^{2+}]_o$ and showed only a negative response, the third preparation exhibited a small positive response (Fig. 6).

Force–frequency relationships in teleosts

Sea raven ventricle strips exhibited only negative responses at both low and high $[Ca^{2+}]_o$ (Fig. 7A). The situation with cod was more complex as some preparations in low $[Ca^{2+}]_o$ did exhibit a small increase in force development upon the transition from 12 to 24 and 36 contractions min^{-1} (Fig. 7B). Mean percentage force, however, did not differ significantly from 100 % under these conditions. The inclusion of ryanodine in the bathing media (Fig. 7C,D) did not alter the general response for either sea raven or cod preparations.

Post-rest potentiation

Following 10 min at each of the imposed frequencies, strips were allowed to rest for either 3 s (skate) or 20 s (sea raven and cod). Preparations were then induced to contract. For skate ventricle strips (Fig. 8) at a low $[Ca^{2+}]_o$, the pre-test stimulation frequency influenced the strength of the 'test contraction' so that force development increased as the conditioning frequency was increased from 6 to 24 contractions min^{-1}. The experimental protocol was applied to sea raven and cod hearts at both 1·25 and 5·0 mmol l^{-1} $[Ca^{2+}]_o$ (Fig. 9A,B) and with or without ryanodine in the bathing media at 1·25 mmol l^{-1} $[Ca^{2+}]_o$ (Fig. 9C,D). No major post-rest potentiation could be detected.

Discussion

The sensitivity of ventricle strips from elasmobranchs, sturgeon and hagfish to

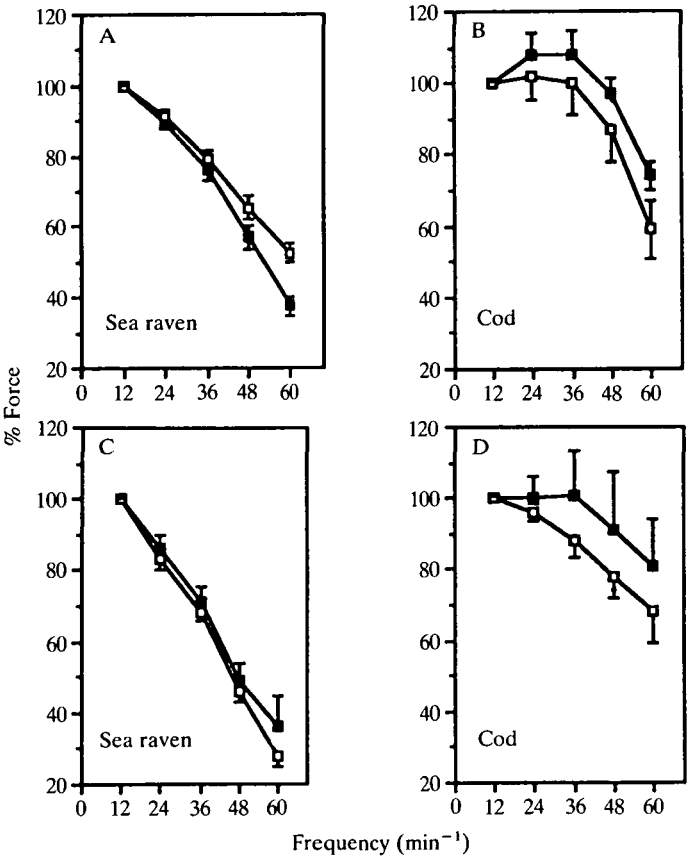

Fig. 7. (A,B) Force–frequency relationships for ventricle strips from sea raven ($N = 8$) and cod ($N = 5$) at 1·25 (■) and 5 mmol l^{-1} (□) $[Ca^{2+}]_o$. Data are presented for paired strips. (C,D) Force–frequency relationships for ventricle strips from sea raven ($N = 7$) and cod ($N = 7$) without (■) or with (□) ryanodine (10 μmol l^{-1}) in bathing media. Data are presented for paired strips.

$[Ca^{2+}]_o$ over the 1–3 mmol l^{-1} range is similar to that previously described for seven teleost species performing at the same rate of contraction (Driedzic & Gesser, 1985). Two species of elasmobranchs, little skate and black dogfish, showed extreme sensitivity to further elevations in $[Ca^{2+}]_o$. Total plasma calcium concentration in elasmobranchs is generally in the range 3–7 mmol l^{-1} (Holmes & Donaldson, 1969), so the steep part of the calcium sensitivity curve may be in the physiological range for some species.

Each of the three elasmobranchs tested showed a positive response as contraction frequency was increased. The apex of the curve was in the range 18–24 contractions min^{-1}. The general nature of this response is similar to that occurring in most mammalian species (Koch-Weser & Blinks, 1963). The two species, skate and black dogfish, which showed the highest sensitivity to $[Ca^{2+}]_o$ also exhibited the steepest positive response. The positive response in little skate

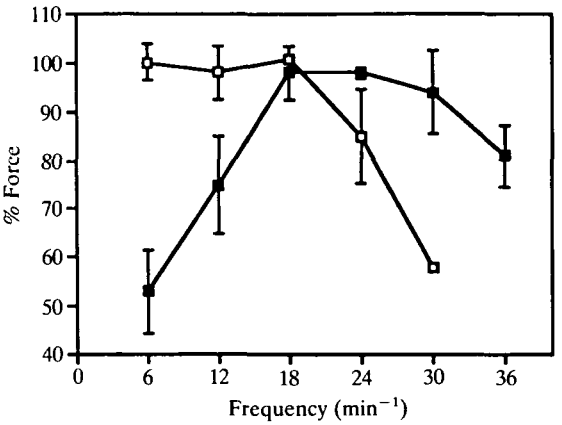

Fig. 8. Relative force of the first contraction of skate ventricle strips after 10 min at the imposed frequencies followed by a 3 s rest; 3 mmol l^{-1} $[Ca^{2+}]_o$ (■), 9 mmol l^{-1} $[Ca^{2+}]_o$ (□). Force is expressed as a percentage of the maximal steady-state force developed during the frequency challenge. Data are presented for paired strips from three individuals.

and spiny dogfish was followed by a very marked decline in force development to about 50% of the maximum at 36–48 contractions min^{-1}. The steep decline in these species may be particularly relevant to control of heart rate *in situ*. In the dogfish *Scyliorhinus* the resting heart rate is about 40 beats min^{-1} in normoxic water but declines to about 20 beats min^{-1} in hypoxic water (Piper *et al.* 1970; Butler *et al.* 1978). *In situ*, there is an increase in stroke volume associated with the bradycardia. The inherent capacity of the cardiac muscle to contract with greater vigour at the moderately reduced rate may, in part, be responsible for the increase in stroke volume. The extent of the bradycardia *in situ* may be limited by the apex of the force–frequency curve so as not to compromise contractility. During exercise, a number of elasmobranchs have been shown to hold heart rate fairly constant (Johansen *et al.* 1966). Again, this may be associated with the steep decline in force development, as any increase in heart rate could impair cardiac performance.

Sea raven and cod heart preparations did not exhibit an increase in force development in response to increases in frequency. This is consistent with previous findings (Driedzic & Gesser, 1985). Neither incubation in 0·1 mmol l^{-1} $[Ca^{2+}]_o$ (sea raven and alewife) nor initiation of the frequency challenge at 6 contractions min^{-1} (trout) resulted in a positive response (Driedzic & Gesser, 1985). Moreover, flounder ventricle strips tested in $[Ca^{2+}]_o$ from 0·1 to 9 mmol l^{-1} and paced at frequencies starting as low as 1 contraction min^{-1} showed only a negative response (H. Gesser, unpublished data). Thus it has not been possible to show positive force–frequency responses in a wide range of teleost hearts, in marked contrast to the situation with the elasmobranchs. Sturgeon hearts appear more similar to elasmobranch than teleost hearts in that a positive response may be

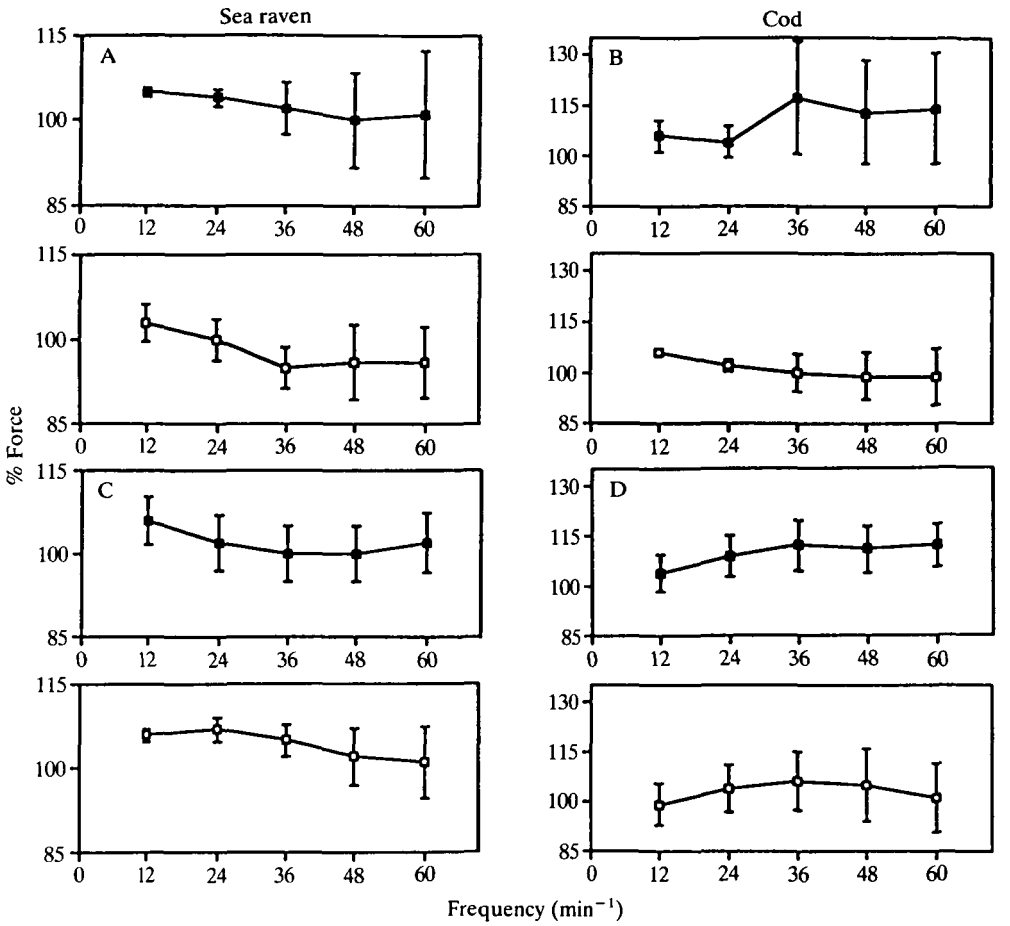

Fig. 9. Relative force of the first contraction of sea raven and cod ventricle strips after 10 min at imposed frequencies followed by a 20 s rest. Data are expressed as a percentage of force developed under steady-state conditions at 12 contractions min^{-1}. Experiments are for paired strips from individuals. (A,B) Tests at different $[Ca^{2+}]_o$ levels: 1·25 mmol l^{-1} $[Ca^{2+}]_o$ (■), 5 mmol l^{-1} $[Ca^{2+}]_o$ (□); sea raven, $N = 8$; cod, $N = 5$. (C,D) Tests without (■) or with (□) ryanodine (10 μmol l^{-1}) in bathing media; sea raven, $N = 7$; cod, $N = 7$.

induced. The question cannot be fully resolved with hagfish since preparations could not be paced at frequencies below 12 contractions min^{-1} owing to spontaneous contractility. The differences in the cardiac force–frequency relationships amongst species cannot be explained at present. The force–frequency relationship probably involves several mechanisms associated with Ca^{2+} uptake and storage (Wohlfort & Noble, 1982). The relative importance of individual components of the process may be species-variable.

The frequently used drug ryanodine is considered to block calcium release from sarcoplasmic reticulum or to cause the sarcoplasmic reticulum to discharge its Ca^{2+} stores (Sutko *et al.* 1986; Rousseau *et al.* 1987; MacLeod & Bers, 1987).

Treatment of mammalian hearts with nanomolar levels of ryanodine results in a decrease in twitch tension (Stemmer & Akera, 1986). Most mammalian species exhibit a positive followed by a negative response when subjected to protocols similar to the type utilized in these studies. Exceptions to this are rat and mouse hearts which exhibit only a negative response. Treatment of these hearts with $2\,\text{nmol}\,l^{-1}$ ryanodine induces the normal mammalian pattern during frequency transitions (Stemmer & Akera, 1986). Ryanodine apparently blocks a major component of the negative response which, in turn, allows expression of the positive response at low frequencies. These views are consistent with the hypothesis that the negative response is in part due to the calcium recycling capabilities of sarcoplasmic reticulum (Orchard & Lakatta, 1985). Ryanodine at a concentration of $10\,\mu\text{mol}\,l^{-1}$ had no effect upon either sea raven or cod hearts. It neither decreased force development under the initial conditions (data not shown) nor altered the negative force–frequency relationship. The findings suggest that beat-to-beat force development in sea raven and cod heart is not dependent upon calcium release from the sarcoplasmic reticulum.

Calcium storage capabilities in skate, sea raven and cod heart were indirectly assessed by determining post-rest potentiation. According to this protocol, strips were primed at a steady rate for 10 min, allowed to rest for a fixed period and then a test contraction was assessed. In mammalian heart, the higher the priming frequency, the greater the strength of the test beat. This has been related to a greater accumulation of calcium at the higher priming frequencies which, in turn, is released during the test beat (Edman & Johannsson, 1976; Rumberger & Reichel, 1972). Skate ventricle strips showed a very marked post-rest potentiation at $3\,\text{mmol}\,l^{-1}$ $[Ca^{2+}]_o$, suggesting an enhanced storage of calcium. At $9\,\text{mmol}\,l^{-1}$ $[Ca^{2+}]_o$, the calcium sites were probably close to saturation even at low contraction frequencies. We have no explanation for the possible decline in post-rest potentiation at $9\,\text{mmol}\,l^{-1}$ $[Ca^{2+}]_o$ and elevated priming frequencies. However, such an occurrence has been reported for turtle hearts (Rumberger & Reichel, 1972). Sea raven and cod hearts exhibited only minimal post-rest potentiation, and only at low contraction frequencies. Ryanodine had no effect on the development of post-rest potentiation. This experimental approach also implies that the calcium storage capabilities of sea raven and cod hearts are not well developed.

In conclusion, elasmobranch hearts exhibit the classical positive force–frequency response seen in most mammalian, turtle and frog hearts. In contrast, teleost hearts exhibit only a negative response and, at least in the case of sea raven and cod, this cannot be converted into a positive response by treatment with ryanodine. Post-rest potentiation studies suggest calcium storage capabilities in skate hearts but not in sea raven or cod hearts. Calcium requirements for beat-to-beat regulation in the teleost hearts tested here appear to be derived primarily from the extracellular space. The findings of this study are not immediately consistent with comparative microstructure, as the sarcoplasmic reticulum is generally considered to be much better developed in teleost than in elasmobranch

hearts (Santer, 1985). The discrepancy between the observed richness of sarcoplasmic reticulum in teleost hearts, and the lack of a functional importance in handling calcium is yet to be resolved.

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