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THE INFLUENCE OF TEMPERATURE ON RYANODINE SENSITIVITY AND THE FORCE–FREQUENCY RELATIONSHIP IN THE MYOCARDIUM OF RAINBOW TROUT

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Summary

The relationship between stimulation frequency and contraction was established for ventricular strips from rainbow trout heart at 5, 15 and 25 °C. Compared to mammalian species, changes in temperature had little impact on force development in trout ventricle at physiologically relevant stimulation frequencies. However, the force–frequency relationship was changed from a biphasic response with a minimum around 0.2 Hz at 5 and 15 °C to a monophasic decline in force with increasing frequency at 25 °C. Ryanodine reversed the negative force–frequency relationship at 25 °C. Potentiation of twitch force after a 5 min rest period was increased from 121±4 % at 15 °C to 209±12 % at 25 °C. A similar augmentation was seen for the maximal rate of force development. Rest potentiation of both force and maximal rate of force development (dF/dT) was abolished by ryanodine at both 15 and 25 °C. The ryanodine concentration causing a half-maximal reduction in rest potentiation of force was 51 nmol l^{-1} at 25 °C and 483 nmol l^{-1} at 15 °C. Rest potentiation was maximally reduced by 10 μmol l^{-1} ryanodine to 50 and 79 % of the value in the absence of ryanodine at 25 and 15 °C, respectively. At 5 °C, rest potentiation was similar to that at 15 °C. At 5 °C, there was no rest potentiation of dF/dT and ryanodine did not reduce rest potentiation of force. Instead, rest potentiation was correlated with a potentiation of time to peak tension (TPT) at 5 °C. Thus, in trout ventricle, force correlates with TPT at 5 °C and seems to be regulated by a ryanodine-insensitive mechanism, while at 25 °C force is correlated with the maximal rate of force development and the sarcoplasmic reticulum appears to contribute significantly to excitation–contraction coupling.

Introduction

Although temperature is an important variable for cellular functions in ectothermic vertebrates, studies on the influence of temperature on the contrac-

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tility of the heart (Langer and Brady, 1968; Shattock and Bers, 1987; Bailey and Driedzic, 1990) have almost exclusively been concerned with endothermic species in which the heart muscle works at an essentially constant temperature *in vivo*. The relative contributions as well as the possibility of temperature-related effects of the Ca^{2+} flux across the sarcolemma and of Ca^{2+} release and uptake by the sarcoplasmic reticulum (SR) in the heart seem to differ in ectothermic and endothermic species (Fabiato and Fabiato, 1978; Chapman, 1983).

In mammalian species, it is generally believed that a calcium-induced release of Ca^{2+} from the SR plays a key role in excitation–contraction (E–C) coupling (Fabiato, 1983, 1985). However, the relative importance of the SR seems to vary among species (Fabiato and Fabiato, 1978; Bers, 1985). In contrast, contraction of the ectothermic heart is thought to depend more on Ca^{2+} fluxes across the sarcolemma while the SR seems to be of relatively little importance (Chapman, 1983; Morad and Cleemann, 1987) and is reported to be less well developed ultrastructurally (Page and Niedergerke, 1972; Santer, 1985). In teleost heart, the SR also seems to be unimportant in force development at physiologically relevant temperatures and stimulation frequencies (Driedzic and Gesser, 1988; Hove-Madsen and Gesser, 1989). Recent results (Tibbits *et al.* 1990) suggest that trans-sarcolemmal Ca^{2+} fluxes are a major mediator of the beat-to-beat regulation of cytosolic Ca^{2+} concentration in the salmonid myocardium. However, the rainbow trout myocardium exhibits rest potentiation of force after rest intervals of more than 5 s, a phenomenon that in the mammalian heart relies on Ca^{2+} released from the SR (Sutko and Willerson, 1980; Bers, 1985; Hryshko *et al.* 1990). Ryanodine, which inhibits closure of the SR Ca^{2+}-release channel (Meissner, 1986; Rousseau *et al.* 1987), inhibits this rest potentiation in trout ventricle at 15 °C, indicating that the SR may contribute to rest potentiation (Hove-Madsen and Gesser, 1989; El Sayed and Gesser, 1989). Furthermore, oxalate-dependent Ca^{2+} uptake in a crude homogenate of rainbow trout ventricle suggests that trout heart SR is well developed in comparison to that of other ectothermic species (Dybro and Gesser, 1986).

The aim of the present study was to evaluate the influence of temperature on contraction of the rainbow trout heart over a temperature range considered to be physiologically relevant, i.e. 5–25 °C (McCauley *et al.* 1977). Although being at the physiological limit, 25 °C was chosen as the high temperature for comparative purposes, as most mammalian data are at room temperature or above. Thus, it was of particular interest to compare the influence of temperature on SR function in the trout heart with that in the mammalian heart, where the Ca^{2+} load and the SR function increase with decreasing temperature, but a large hypothermic inotropy persists even when SR function is inhibited by ryanodine (Shattock and Bers, 1987).

Materials and methods

Rainbow trout *Oncorhyncus mykiss* (Walbaum) weighing 100–300 g were kept

in freshwater tanks at 10–15 °C. The fish were killed by decapitation and the heart was transferred to an oxygenated Ringer's solution. Ventricular strips with a diameter of less than 1 mm and a length of approximately 1 cm were prepared. The strips were mounted for measurements of the contraction variables: twitch force, maximal rate of contraction (dF/dT) and time to peak tension (TPT), as described previously (Gesser, 1977). Initially the strips were incubated at 15 °C and stimulated with two times the threshold voltage at 0.2 Hz in a standard Ringer containing (mmol l^{-1}): NaCl, 125; KCl, 5; $CaCl_2$, 1.25; $MgSO_4$, 0.94; Na_2HPO_4, 1; $NaHCO_3$, 15; glucose, 5; and gassed with 99 % O_2 and 1 % CO_2 by a gas-mixing pump (Wösthoff 1M 301/aF). The strips were stretched to 90 % of maximal contraction and stabilized for at least 45 min. After stabilization, temperature was thermostatically maintained at the desired temperature ±0.5 °C. The pH of the Ringer's solution was 7.70 at 5 °C, 7.78 at 15 °C and 7.82 at 25 °C. Free Ca^{2+} concentration was measured with with a Ca^{2+}-sensitive electrode (Radiometer, Copenhagen) and was found to be 1.1 mmol l^{-1}, slightly lower than values found in the blood of rainbow trout (Andreasen, 1985). However, rest potentiation and the force–frequency relationship are more pronounced at non-saturating Ca^{2+} concentrations (Driedzic and Gesser, 1985; Hove-Madsen and Gesser, 1989), and this reduced Ca^{2+} level was, therefore, chosen for the present experiments. Ryanodine, a generous gift from Merck Sharp and Dome, was stored at −20 °C in an aqueous stock solution at 1 mmol l^{-1}. In experiments with caffeine, 8 mmol l^{-1} caffeine was added directly to the bath.

Since there is a continuous decay in force development with time in the trout heart, two strips were run in parallel. One strip served as a control while the other was subjected to the experimental condition. This approach was applied since the variation between strips from the same ventricle is smaller than that between strips from different ventricles. Unless otherwise stated, twitch force was normalized to the steady-state twitch force at 0.2 Hz for the control strip to evaluate the relative effects of temperature or of pharmacological manipulations. Twitch force was also measured in mN mm^{-2}, by measuring the length and mass of the strips, which were assumed to be cylindrical rods with a specific mass of 1 g cm^{-3}. Thus, the twitch force (F) could be calculated in mN mm^{-2} as $F=C\times k\times$(length of strip/mass of strip), where C is the measured contraction (in mm) and k is a calibration factor obtained by hanging known weights on the force transducer (in mN mm^{-1}).

To evaluate the effects of temperature on the contractile variables, ventricular strips were stimulated at 0.2 Hz at 15 °C. Temperature was then increased to 25 °C or reduced to 5 °C, over a period of 30–45 min, until a new steady state was attained. In some strips, temperature was maintained at 15 °C to evaluate the decrease in twitch force with time. In 18 control strips, twitch force decreased to 89±2 % of its original value (mean±S.E.) over a 45 min period. The relative change in the contractile variables after a temperature change was obtained by normalizing the steady-state value at 5 or 25 °C to the steady-state value at 15 °C.

Dose–response curve for ryanodine

Since rest potentiation is the variable most sensitive to inhibition of SR function (Hove-Madsen and Gesser, 1989), the dose–response curve for ryanodine was obtained by measuring the effect of ryanodine on twitch force after a 5 min rest period. Two ventricular strips were stabilized at 0.2 Hz, 10 nmol l^{-1} ryanodine was then applied to one of the strips and equilibration at 0.2 Hz was allowed for 35 min, before a 5 min rest period was imposed. The strips were then stabilized at 0.2 Hz for 5 min, before ryanodine concentration was increased and the procedure repeated. To estimate the effect of ryanodine on twitch force after a 5 min rest period, the force of the first beat (FB) after the rest period was normalized to that of the last beat (LB) before the rest period (see Fig. 2A and B). If the ratio FB/LB is greater than 1, it is referred to as rest potentiation, while an FB/LB ratio of less than 1 is referred to as rest decay. When the dose–response curve for ryanodine had been generated, the effect of a given ryanodine concentration was normalized to the control value, i.e. $(FB/LB)_{ry}/(FB/LB)_c$ for the effect on rest potentiation and LB_{ry}/LB_c for the effect at 0.2 Hz (see Fig. 2A and B). This procedure allowed a comparison of the relative effects of ryanodine at 0.2 Hz and after a rest period, as well as at different temperatures. The maximal reduction of twitch force by ryanodine and the concentration needed to cause a half-maximal reduction of twitch force ($K_{1/2}$) were obtained by fitting the data with a sigmoid curve with a Hill coefficient of −1: $F=A+(B-A)/[1+(10^x/10^c)]$, where F is the twitch force, A is the maximal reduction of twitch force caused by ryanodine, B is the twitch force under control conditions, x is log[ryanodine] and c is $\log(K_{1/2})$, (Graph Pad 1985–89, by H. Motulsky, version 3.00).

When estimating the maximal effect of ryanodine on the contractile variables, 5 μmol l^{-1} ryanodine was applied to one of the strips after an initial stabilization at 0.2 Hz and 25 °C. After 40 min of incubation with ryanodine, a 5 min rest period was imposed and the FB/LB ratio was measured for force, dF/dT and TPT in the presence and absence of 5 μmol l^{-1} ryanodine.

Effect of ryanodine on the relationship between frequency and contraction

To evaluate the effect of ryanodine on the relationship between frequency and the contractile variables, two strips were run in parallel. The strips were stimulated at 0.2 Hz at the experimental temperature, 5 μmol l^{-1} ryanodine was then added to one of the strips, and equilibration was allowed for 40 min. The stimulation rate was set to a new value for 5 min, after which it was returned to 0.2 Hz and the twitch force was allowed to stabilize before the next stimulation rate was applied for 5 min. The relationship between frequency and force, dF/dT or TPT was established in the presence and absence of ryanodine, by normalizing the value at the end of the 5 min interval with altered stimulation frequency to the value at 0.2 Hz before addition of ryanodine. The order of stimulation frequencies applied was altered between experiments.

Results are given as means±S.E. and were evaluated using Student's t-test and linear regression analysis with the significance level set at 0.05.

Results

Effect of temperature on contraction

To evaluate the influence of temperature on the regulation of force of contraction, the variations of twitch force, dF/dT and TPT with temperature were examined. Fig. 1A shows the twitch force at 5, 15 and 25°C. Twitch force was normalized to the control value at 15°C. The twitch force showed no significant change when the temperature was raised from 15 to 25°C, but a lowering of temperature from 15 to 5°C increased twitch force significantly ($P<0.001$, $N=9$). dF/dT increased with increasing temperature (Fig. 1B) and TPT decreased with increasing temperature (Fig. 1C). Thus, twitch force seems to be a balance between dF/dT and the duration of the 'active state'. The effect of temperature on the rate of relaxation was similar to the effect on dF/dT whereas the time to half-maximal relaxation, like the TPT, was reduced with increasing temperature (data not shown).

Effect of ryanodine on contraction at different temperatures

The next question addressed was the influence of temperature on the importance of the SR to E–C coupling. In a series of experiments, the effect of increasing ryanodine concentration on force produced in the first contraction after a 5 min rest period was examined. Fig. 2A shows control traces of the force of the last beat (LB) before and the first beat (FB) after the 5 min rest period at 25°C. The average rest potentiation of the first beat was 209±12 % under control conditions ($N=7$). Fig. 2B shows that, in the presence of 10 μmol l^{-1} ryanodine at 25°C, the potentiation of twitch force after a 5 min rest period under control conditions is

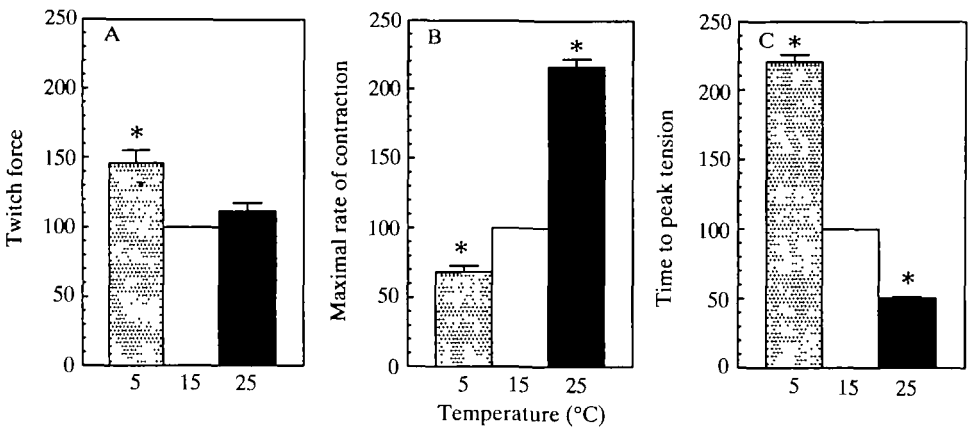

Fig. 1. Influence of temperature on (A) twitch force, (B) maximal rate of contraction (dF/dT) and (C) time to peak tension (TPT) in trout ventricular strips stimulated at 0.2 Hz. The variables were normalized to the steady-state control value at 15°C and 0.2 Hz. Each point represents the mean+S.E. for muscle strips from nine ventricles. A significant difference from the control value at 15°C is denoted by an asterisk ($P<0.001$). Stippled columns, 5°C; open columns, 15°C; filled columns, 25°C.

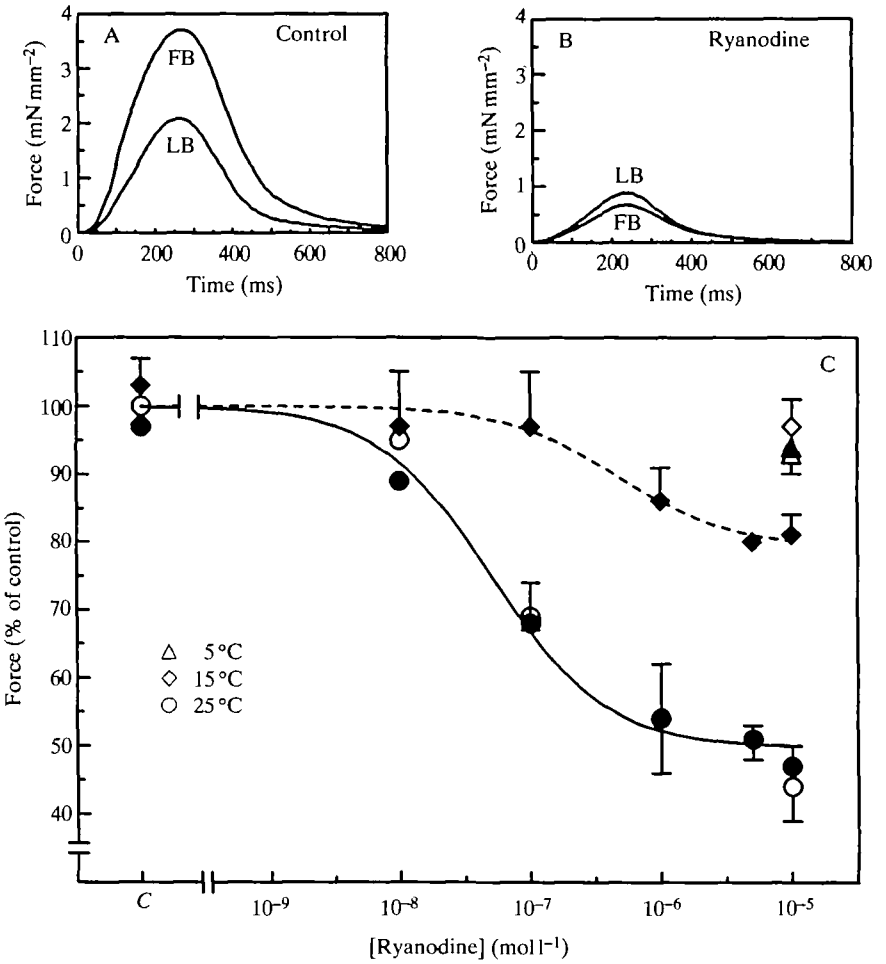

Fig. 2. (A, B) A comparison of the last beat at 0.2 Hz and 25 °C (LB) and the first beat after a 5 min rest period (FB) (A) in the absence and (B) in the presence of 10 μmol l^{-1} ryanodine. (C) Twitch force at 0.2 Hz (LB) and rest potentiation (FB/LB) in the presence of ryanodine normalized to the value in the control strip without ryanodine. The relative reduction of twitch force at 0.2 Hz (open symbols) and rest potentiation (filled symbols) are shown as a function of [ryanodine] at three temperatures. The data were fitted by a sigmoid curve with a Hill coefficient of −1. Dashed and solid lines are fitted to rest potentiation at 15 and 25 °C, respectively. Each point represents the mean±S.E. for 4–7 ventricles. *C*, control value.

reversed to a slight rest decay. In Fig. 2C, twitch force at 0.2 Hz and rest potentiation in the presence of ryanodine were normalized to their respective control values.

The maximal reduction of twitch force caused by ryanodine and the ryanodine concentration required to cause a half-maximal reduction of twitch force ($K_{1/2}$) were estimated by fitting the data with a sigmoid curve with a Hill coefficient of −1. The curve fitting resulted in values of ($K_{1/2}$) at 25 °C that were similar to those

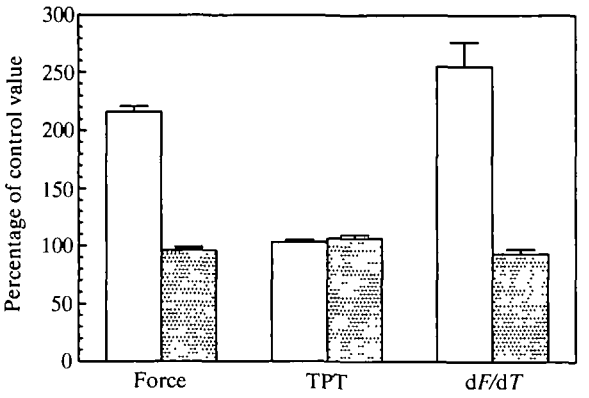

Fig. 3. The effect of 5 μmol l^{-1} ryanodine on the force of the first contraction after a 5 min rest period at 25 °C. The three contraction variables, force, dF/dT and TPT, were normalized to the value for the last beat at 0.2 Hz before the 5 min rest period. Each point represents the mean+S.E. for six ventricles. Open columns, control; stippled columns, with 5 μmol l^{-1} ryanodine.

obtained with continuous stimulation at 0.2 Hz and after a 5 min rest (75 and 51 nmol l^{-1}, respectively). Also, the estimated maximal reduction of twitch force by ryanodine at 0.2 Hz stimulation (48 % of control) was similar to the estimated maximal reduction of rest potentiation (50 % of control). At 15 °C, the maximal reduction of rest potentiation was estimated from the curve to be 79 % of the control value with a $K_{1/2}$ of 483 nmol l^{-1}. In contrast, no significant effect of the maximal [ryanodine] of 10 μmol l^{-1} could be demonstrated at 0.2 Hz and 15 °C. At 5 °C, no significant effect of ryanodine could be demonstrated on steady-state force either at 0.2 Hz or after a 5 min rest period, even with a preincubation at 25 °C with 10 μmol l^{-1} ryanodine.

Fig. 3 depicts the effects of 5 μmol l^{-1} ryanodine on twitch force, dF/dT and TPT after a 5 min rest period in tissues kept at 25 °C. In the absence of ryanodine, both force and dF/dT were potentiated by a rest period. These potentiations were abolished in the presence of ryanodine. In contrast, TPT was not altered by a 5 min rest nor was it significantly changed in the presence of ryanodine. Similar experiments were carried out at 25 °C with 8 mmol l^{-1} caffeine, which also reduced rest potentiation significantly ($P<0.001$) from a control value of 205±7 % to 111±9 % ($N=4$).

At a stimulation frequency of 0.2 Hz, a significant effect of ryanodine could only be demonstrated at 25 °C, where a maximal effective dose of 5 or 10 μmol l^{-1} decreased twitch force to 45±6 % of the control value ($N=15$). In contrast to ryanodine, 8 mmol l^{-1} caffeine increased the steady-state force at 0.2 Hz to 356±90 % in four strips. The steady-state force under control conditions at 25 °C was found to be 2.35±0.42 mN mm^{-2}, while dF/dT was 16.94±2.79 mN mm^{-2} s^{-1} and TPT was 187±5 ms ($N=9$).

Effect of ryanodine on the relationship between frequency and contraction at different temperatures

The relationships between frequency and force, dF/dT and TPT were established at 5, 15 and 25°C. In Fig. 4A, twitch force is shown as a function of stimulation frequency at 5, 15 and 25°C. There is a monophasic decline in twitch force with increasing stimulation frequency at 25°C, while twitch force at 5 and 15°C exhibit minima at 0.2 Hz. In the presence of ryanodine, twitch force is reduced significantly at stimulation frequencies below 0.4 Hz at 25°C ($P<0.0001$ at 0.2 Hz) and at stimulation frequencies below 0.2 Hz at 15°C ($P<0.035$ at 0.017 Hz). In contrast, ryanodine has no significant effect on twitch force at any stimulation frequency examined at 5°C (data not shown). It is also noticeable that at 25°C the negative force–frequency relationship is abolished in the presence of ryanodine. The relationship between frequency and dF/dT is illustrated in Fig. 4B. It shows that, both in the presence and in the absence of ryanodine, the relationship between dF/dT and frequency is strikingly similar to the force–

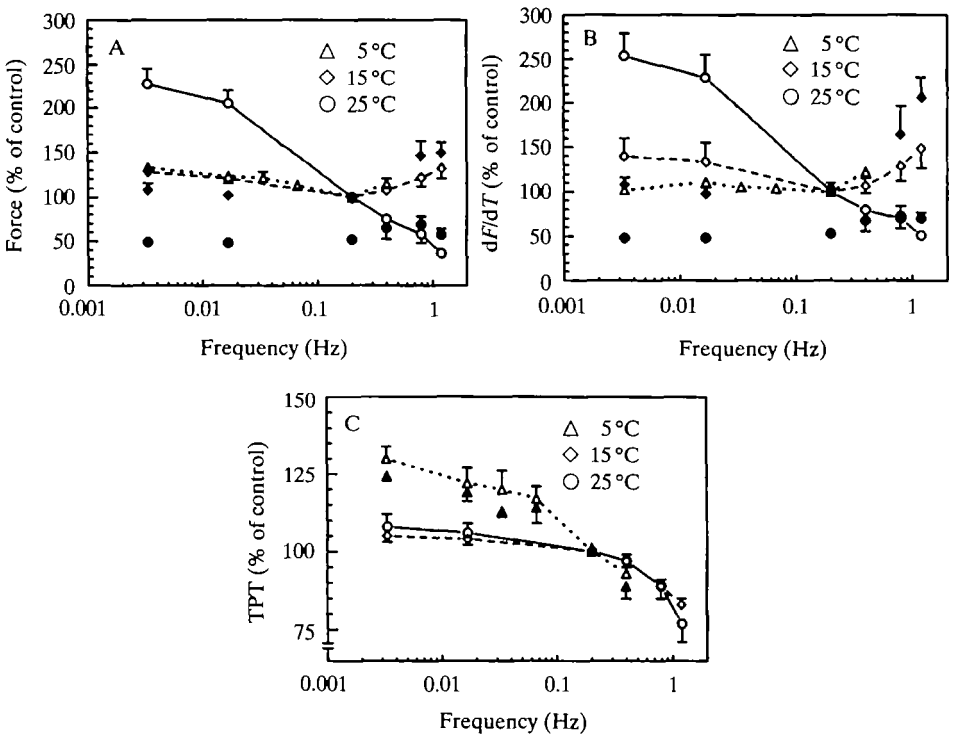

Fig. 4. The effect of temperature on the relationship between frequency and (A) twitch force, (B) dF/dT and (C) TPT in the absence (open symbols) or the presence (filled symbols) of 5 μmol l^{-1} ryanodine. Values were normalized to the control value at 0.2 Hz in the absence of ryanodine. All points represent the mean±S.E. for six ventricles.

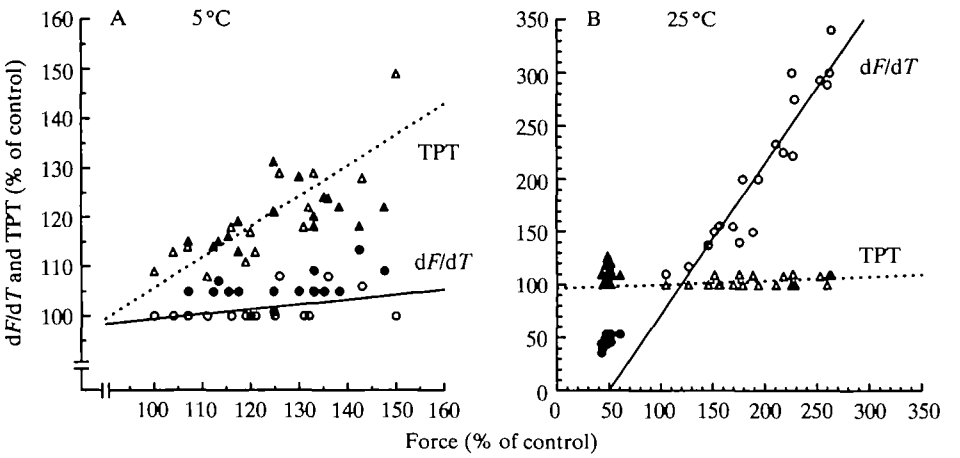

Fig. 5. The relationship between twitch force and dF/dT (circles) or TPT (triangles). Regression lines are drawn for control values of dF/dT *versus* force (solid line) and TPT *versus* force (dotted lines) at (A) 5°C and (B) 25°C. Values were obtained from six control ventricles (open symbols) and six ventricles treated with 5 μmol l^{-1} ryanodine (filled symbols) at stimulation frequencies below 0.2 Hz.

frequency relationship at 15 and 25°C (Fig. 4A). dF/dT was not changed significantly by ryanodine at any stimulation frequency at 5°C (only control data are shown for clarity). However, no increase is seen in dF/dT at stimulation frequencies below 0.2 Hz, while twitch force is increased significantly from the 0.2 Hz control value when frequency is lowered to 0.067 Hz or less ($P<0.008$ at 0.067 Hz). Fig. 4C shows the relationship between frequency and TPT. Ryanodine had no significant effect on TPT at any frequency or at any temperature (for clarity only control data are shown at 15 and 25°C). At 5°C, TPT showed a continuous decrease with increasing stimulation frequency, while frequency had little effect on TPT at 15 and 25°C. Finally, it should be noted that, at stimulation frequencies below 0.2 Hz at 5°C, there is a corresponding change in force and TPT with frequency, both in the presence and in the absence of ryanodine (Fig. 4A and C).

This point was further explored in Fig. 5A, which shows the relationship between force and TPT and dF/dT at 5°C. There is a significant correlation between force and TPT at 5°C both in control and in ryanodine-treated strips. When TPT was plotted against force, the slope increased with decreasing temperature from 0.02±0.02 at 25°C to 0.05±0.06 at 15°C (not shown) and 0.62±0.11 at 5°C, with the slope of the regression line being significantly different from 0 only at 5°C ($r=0.84$, $N=16$, $P<0.0001$).

As previously mentioned, ryanodine abolishes rest potentiation at 25°C. However, Fig. 5B shows that, although reduced, the values obtained in the presence of ryanodine lie close to the regression lines for control values of force *versus* dF/dT and TPT. It is also clear that at 25°C there is a strong correlation between force and dF/dT. The slope of the regression line for dF/dT *versus* force

decreased from 1.43±0.12 at 25°C to 1.20±0.18 at 15°C (not shown) and 0.10±0.05 at 5°C. Significant correlations between twitch force and dF/dT were found at 15 and 25°C only (r=0.87, N=20, P<0.0001 and r=0.94, N=20, P<0.0001, respectively).

Discussion

The present study presents three basic findings. First, the force–frequency relationship is changed from a response that shows a minimum at 0.2 Hz at 5 and 15°C to a monophasic negative response at 25°C (Fig. 4A). Second, ryanodine sensitivity increases with increasing temperature, and the monophasic decline in force with increasing frequency at 25°C is reversed to an increase in force in the presence of ryanodine. Furthermore, the changes in force brought about by ryanodine always correlate with changes in the rate of force development. Finally, the effect of temperature on force development in the trout myocardium is much smaller than that seen in the mammalian heart (Shattock and Bers, 1987).

Other ectothermic species such as frogs, as well as mammalian species such as rats and rabbits, show a monophasic decrease in myocardial force when temperature is increased (Shattock and Bers, 1987), whereas an increase in temperature from 15 to 25°C has no significant effect in the trout heart. It is possible that, when the temperature is raised from 15 to 25°C at a stimulation frequency of 0.2 Hz, the slight increase seen in trout ventricle twitch force, despite a significant decrease in the 'active state' (TPT), may be due to an increased contribution of the SR to force development. In agreement with this, rest potentiation, which is thought to be a sensitive indicator of the function of the SR (Bers, 1985), increased from 121 to 209 % when the temperature was increased from 15 to 25°C, and this increase could be abolished by ryanodine and caffeine.

The increase in the maximal reduction of twitch force by ryanodine at higher temperatures and the concurrent decrease in the ryanodine concentration required for a half-maximal effect ($K_{1/2}$) in the trout heart also agree with results for the mammalian heart (Shattock and Bers, 1987). The $K_{1/2}$ value of 51 nmol l^{-1} at 25°C also agrees with the dissociation constant (K_d) for ryanodine binding to the SR at this temperature both for mammalian species and for trout (Pessah *et al.* 1985; Meissner, 1986; G. F. Tibbits, personal communication).

The force–frequency relationship of the trout ventricle has previously been described at 15°C (Hove-Madsen and Gesser, 1989). The biphasic response seen at this temperature, exhibiting a minimum around 0.2 Hz, was found to depend on SR function at frequencies below 0.2 Hz. The rest potentiation of force is similar to that observed in species, such as mouse and rat, with a highly developed SR (Sutko and Willerson, 1980; Orchard and Lakatta, 1985). However, in the trout ventricle, the positive response of force to an elevation of frequency from 0.2 to 1.0 Hz was not affected by inhibition of the SR function with ryanodine. Thus, at physiologically relevant frequencies, i.e. above 0.2 Hz (Priede, 1974; Wood *et al.* 1979), the SR seems to be of less importance. In this respect, the trout heart resembles

mammalian species with a positive force–frequency relationship, such as rabbit and guinea pig, as well as ectothermic species such as turtle and frog (Hajdu, 1969; Lewartowski *et al.* 1984; Bers, 1985; Driedzic and Gesser, 1985).

When comparing the force–frequency relationships at different temperatures, the responses seen at 5 and 15 °C are similar. However, at 5 °C, rest potentiation of force cannot be inhibited by ryanodine even when strips are incubated with ryanodine at 25 °C for extended periods followed by a lowering of temperature to 5 °C. Thus, rest potentiation of force does not seem to depend on the SR. This is in agreement with the finding that, at 5 °C, force showed a correlation with TPT, which in turn may be due to a prolongation of the action potential duration (Rumberger and Reichel, 1972). Therefore, potentiation of twitch force after rest periods can apparently not be used as an indicator of SR function at 5 °C in the trout heart. The lack of effect of ryanodine at 5 °C agrees with results from other teleost and elasmobranch species (Driedzic and Gesser, 1988) as well as with data obtained from isolated sarcolemmal vesicles from the trout heart (Tibbits *et al.* 1990), which suggested that sarcolemmal Ca^{2+} flux plays a major role in E–C coupling at 21 °C.

In contrast to these data, at 25 °C the negative force–frequency relationship in the trout heart, seen over the entire range of frequencies examined, is similar to that seen in mammalian species with a highly developed SR (Stemmer and Akera, 1986; Orchard and Lakatta, 1985). In agreement with the results from mammalian cardiac muscle (Hajdu, 1969; Bers, 1985; Stemmer and Akera, 1986), ryanodine, although depressing twitch force, causes a reversal of the slope of the force–frequency relationship in the trout ventricle. That is, force increases with increasing frequency at 25 °C in the presence of ryanodine.

The rest potentiation of twitch force, which is generally thought to be a sensitive indicator of SR function (Bers, 1985; Hryshko *et al.* 1990), is increased strongly when temperature is increased from 15 to 25 °C in the trout heart. It is of interest that a corresponding temperature-dependent potentiation of dF/dT is seen, while the duration of the active state (TPT) after a 5 min rest period is not affected significantly by the increase in temperature. Thus, at 25 °C, rest potention of force is correlated to a potentiation of dF/dT, and both can be completely abolished by ryanodine.

In mammalian species, estimation of the SR Ca^{2+} content, by rapid cooling contractures, showed an increase in the SR Ca^{2+} content with a lowering of temperature (Shattock and Bers, 1987). This indicates that the SR is loaded with calcium at 25 °C in the mammalian heart. However, the reduction of twitch force by ryanodine declined with decreasing temperature (Shattock and Bers, 1987). In the ectothermic heart, the interpretation of rapid cooling contractures is complicated by the fact that contractions can be elicited at low temperatures. Thus, rapid cooling of trout ventricular strips to less than 5 °C increases the resting potential sufficiently to elicit an action potential and contraction (L. Hove-Madsen, unpublished observations). However, El Sayed and Gesser (1989) found that, in trout heart, ryanodine caused a reduction in the initial rate of ^{45}Ca uptake at all

stimulation frequencies examined, but twitch force was only reduced correspondingly by ryanodine with one stimulation at the end of the 5 min ^{45}Ca uptake. These results suggest that the SR accumulates Ca^{2+} at 15°C at various stimulation frequencies, but that this Ca^{2+} is not directly available for contraction at physiological stimulation frequencies.

One explanation of the above results for the trout myocardium is that the calcium-induced Ca^{2+} release from the SR depends on the rate of change of $[Ca^{2+}]$ around the SR, as suggested by Fabiato (1983), and that this release depends upon temperature. Thus, at 5°C the Ca^{2+} influx during the action potential may be too slow to induce a release of Ca^{2+} from the SR, whereas at 25°C the Ca^{2+} influx may be rapid enough to cause a calcium-induced release of Ca^{2+} from the SR. In agreement with this, rest potentiation of force can be correlated to rest potentiation of dF/dT at 15 and 25°C only and dF/dT is 4–5 times higher at 25°C than at 5°C. It should, however, be emphasized that dF/dT is a complex measure that also depends upon the Ca^{2+} sensitivity of the myofilaments, which is decreased by cooling in heart muscle (Harrison and Bers, 1990).

In parallel with a possible reduction of calcium-induced Ca^{2+} release from the SR, the Ca^{2+} content of the trout heart SR may be lowered as a result of a reduced accumulation of Ca^{2+} when frequency is increased (Orchard and Lakatta, 1985) or temperature falls. In favour of this, Ca^{2+} uptake in SR from mammalian skeletal muscle has been shown to increase with increasing temperature (Inesi and Watanabe, 1967). In trout ventricle, the maximal oxalate-dependent initial uptake rate of Ca^{2+} in crude homogenate increases when temperature is increased from 15 to 25°C, as does the oxalate-dependent filling capacity (H. Gesser personal communication). Furthermore, in trout heart, ryanodine is able to depress force development at stimulation frequencies up to 0.4 Hz at 25°C, while at 15°C it only causes a significant reduction of force when the rest period is longer than 15 s. It is striking that, at both temperatures, the reduction in twitch force caused by ryanodine occurs in the frequency interval where a negative force–frequency relationship is seen.

Thus, it is possible that at least two mechanisms regulate force development in the trout ventricle. First, an SR-dependent mechanism, the activity of which is reduced with increasing stimulation frequencies as a result of incomplete recycling of Ca^{2+} in the SR, as suggested by Orchard and Lakatta (1985). This component may become more active as the temperature rises, owing to an increased uptake and release of Ca^{2+} from the SR. Second, a ryanodine-insensitive mechanism, which may increase force with increasing stimulation frequency. This mechanism appears to depend on the Ca^{2+} flux across the sarcolemma and is affected by changes in the cellular sodium balance (Hove-Madsen and Gesser, 1989). Thus, at 25°C, it is possible that the SR dominates the regulation of force development at all frequencies, and twitch force decreases monophasically with increased frequency owing to incomplete recycling of Ca^{2+} in the SR. The frequency-dependent mechanism may be revealed at 25°C, when SR function is inhibited with ryanodine, and there is a positive force–frequency relationship. At 15 and

5°C, the SR-dependent mechanism becomes increasingly less important, leaving a frequency-stimulated increase in twitch force above 0.2 Hz at those temperatures.

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References

ANDREASEN, P. (1985). Free and total calcium concentrations in the blood of rainbow trout, *Salmo gairdneri*, during 'stress' conditions. *J. exp. Biol.* **118**, 161–168.

BAILEY, J. R. AND DRIEDZIC, W. R. (1990). Enhanced maximum frequency and force development of fish hearts following temperature acclimation. *J. exp. Biol.* **149**, 239–254.

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

CHAPMAN, R. A. (1983). Control of cardiac contractility at the cellular level. *Am. J. Physiol.* **245**, H535–H552.

DRIEDZIC, W. R. AND GESSER, H. (1985). Ca^{2+} protection from negative inotropic effect of contraction frequency on teleost heart. *J. comp. Physiol.* B **156**, 135–142.

DRIEDZIC, W. AND GESSER, H. (1988). Differences in force–frequency relationships and calcium dependency between elasmobranch and teleost hearts. *J. exp. Biol.* **140**, 227–241.

DYBRO, L. AND GESSER, H. (1986). Capacity of the sarcoplasmic reticulum (SR) and contractility in heart of 5 vertebrate species. *Acta physiol. scand.* **128**, p74.

EL SAYED, M. F. AND GESSER, H. (1989). Sarcoplasmic reticulum, potassium, and cardiac force in rainbow trout and plaice. *Am. J. Physiol.* **257**, R599–R604.

FABIATO, A. (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.* **245**, C1–C14.

FABIATO, A. (1985). Simulated calcium current can cause both calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J. gen. Physiol.* **85**, 291–320.

FABIATO, A. AND FABIATO, F. (1978). Calcium induced release of calcium from the sarcoplasmic reticulum and skinned cells from adult human, dog, cat, rabbit, rat and frog hearts and from fetal and newborn rat ventricles. *Ann. N.Y. Acad. Sci.* **307**, 491–522.

GESSER, H. (1977). The effect of hypoxia and reoxygenation of force development in myocardia of carp and rainbow trout: protective effects of CO_2/HCO_3. *J. exp. Biol.* **69**, 199–206.

HAJDU, S. (1969). Mechanism of the Woodworth staircase phenomenon in heart and skeletal muscle. *Am. J. Physiol.* **216**, 206–214.

HARRISON, S. AND BERS, D. M. (1990). Temperature dependence of myofilament Ca sensitivity of rat, guinea pig, and frog ventricular muscle. *Am. J. Physiol.* **258**, C274–C281.

HOVE-MADSEN, L. AND GESSER, H. (1989). Force frequency relation in the myocardium of rainbow trout. *J. comp. Physiol.* B **159**, 61–69.

HRYSHKO, L. W., STIFFEL, V. AND BERS, D. M. (1990). Rapid cooling contractures as an index of sarcoplasmic reticulum calcium content in rabbit ventricular myocytes. *Am. J. Physiol.* **257**, H1369–H1377.

INESI, G. AND WATANABE, S. (1967). Temperature dependence of ATP hydrolysis and calcium uptake by fragmented sarcoplasmic membranes. *Archs Biochem.* **121**, 665–671.

LANGER, G. A. AND BRADY, A. J. (1968). The effects of temperature upon contraction and ionic exchange in rabbit ventricular myocardium. *J. gen. Physiol.* **52**, 682–713.

LEWARTOWSKI, B., PYTKOWSKI, B. AND JANCZEWSKI, A. (1984). Calcium fraction correlating with contractile force of ventricular muscle of guinea-pig heart. *Pflügers Arch.* **401**, 198–203.

MCCAULEY, R. W., ELLIOT, J. R. AND READ, L. A. A. (1977). Influence of acclimation temperature on preferred temperature in the rainbow trout *Salmo gairdneri*. *Trans. Am. fish. Soc.* **106**, 362–367.

MEISSNER, G. (1986). Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. *J. biol. Chem.* **261**, 6300–6306.

MORAD, M. AND CLEEMANN, L. (1987). Role of Ca^{2+} channel in development of tension in heart muscle. *J. molec. cell. Cardiol.* **19**, 527–553.

ORCHARD, C. H. AND LAKATTA, E. G. (1985). Intracellular calcium transients and developed tension in rat heart muscle. *J. gen. Physiol.* **86**, 637–651.

PAGE, S. G. AND NIEDERGERKE, R. (1972). Structures of physiological interest in the frog heart ventricle. *J. Cell Sci.* **11**, 179–203.

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.

PRIEDE, I. G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. exp. Biol.* **60**, 305–319.

ROUSSEAU, E., SMITH, J. S. AND MEISSNER, G. (1987). Ryanodine modifies conductance and gating behaviour of single Ca^{2+} release channel. *Am. J. Physiol.* **253**, C364–C368.

RUMBERGER, E. AND REICHEL, H. (1972). The force–frequency relationship: A comparative study between warm- and cold-blooded animals. *Pflügers Arch.* **322**, 206–217.

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

SHATTOCK, M. AND BERS, D. M. (1987). Inotropic response to hypothermia and the temperature-dependence of ryanodine action in isolated rabbit and rat ventricular muscle: Implications for excitation–contraction coupling. *Circulation Res.* **61**, 761–771.

STEMMER, P. AND AKERA, T. (1986). Concealed positive force–frequency relationships in rat and mouse cardiac muscle revealed by ryanodine. *Am. J. Physiol.* **251**, H1106–H1110.

SUTKO, J. L. AND WILLERSON, J. T. (1980). Ryanodine alteration of the contractile state of rat ventricular tissues. *Circulation Res.* **46**, 332–343.

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.

WOOD, C. M., PIEPRZAC, P. AND TROTT, J. N. (1979). The influence of temperature and anemia on the adrenergic and cholinergic mechanisms controlling heart rate in the rainbow trout. *Can. J. Zool.* **57**, 2440–2462.