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THE ENERGETIC COST OF ACTIVATION OF WHITE MUSCLE FIBRES FROM THE DOGFISH *SCYLIORHINUS CANICULA*

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Summary

The energetic cost of activation was measured during an isometric tetanus of white muscle fibres from the dogfish *Scyliorhinus canicula*. The total heat production by the fibres was taken as a measure of the total energetic cost. This energy consists of two parts. One is due to crossbridge interaction which produces isometric force, and this part varies linearly with the degree of filament overlap in the fibres. The other part of the energy is that associated with activation of the crossbridges by Ca^{2+}, mainly with uptake of Ca^{2+} into the sarcoplasmic reticulum by the ATP-driven Ca^{2+} pump. Total heat production was measured at various degrees of filament overlap beyond the optimum for force development. Extrapolation of heat *versus* force production data to evaluate the heat remaining at zero force gave a value of 34±5 % (mean ± S.E.M., *N*=24) for activation heat as a percentage of total heat production in a 2.0 s isometric tetanus. Values for 0.4 and 1.0 s of stimulation were similar. Comparison with values in the literature shows that the energetic cost of activation in dogfish muscle is very similar to that of frog skeletal muscle and it cannot explain the lower maximum efficiency of dogfish muscle compared with frog muscle. The proportion of energy for activation (Ca^{2+} turnover) is similar to that expected from a simple model in which Ca^{2+} turnover was varied to minimize the total energy cost for a contraction plus relaxation cycle.

Key words: activation, activation heat, calcium, Ca^{2+}, muscle contraction, energetics, dogfish, *Scyliorhinus canicula*, heat production, efficiency.

Introduction

An important feature of muscle energetics is how efficiently the muscle can perform work. The efficiency of muscular contraction can be defined as the ratio of the mechanical power to the rate of total energy output (mechanical power plus heat rate). The efficiency of muscular contraction varies among different species of animals. For example, the maximum efficiency of dogfish white muscle is lower than that of frog skeletal muscle, 0.33 (Curtin and Woledge, 1991) and 0.45 (Hill, 1964*b*), respectively. Here we examine whether energy expenditure on activation processes is responsible for this difference in efficiency.

The heat production during contraction consists of two parts. One is due to the interaction between myosin and actin filaments, the process that produces work and force-dependent or crossbridge heat. The remainder is activation heat, which is force-independent or non-crossbridge heat. The activation heat is associated with the cycling of Ca^{2+} during contraction, which includes the liberation of Ca^{2+} into the sarcoplasm, its binding and release from various proteins, and the ATP-driven reuptake of Ca^{2+} by the sarcoplasmic reticulum, which makes the largest contribution to activation heat.

Dogfish muscle might be less efficient than frog muscle because of a larger expenditure of energy by activation processes or a less efficient conversion of chemical energy into mechanical power by the crossbridges themselves. To distinguish between these two possibilities, one needs to know the energy cost of the activation process in these two species of animals. Activation heat has been measured in frog skeletal muscles (Smith, 1972; Homsher *et al.* 1972; Rall, 1979; Curtin and Woledge, 1981; Burchfield and Rall, 1985, 1986; Barclay *et al.* 1993). The present experiments measure the activation heat in white myotomal muscle fibres from dogfish.

Materials and methods

Dogfish, *Scyliorhinus canicula* (L.), from the Plymouth Marine Laboratory, were kept in circulating artificial sea water (density 1.26 g ml^{-1}) at approximately 12 °C for at least several days before use. The length of the fish, measured from the pectoral fin to the tail tip, ranged from 53 to 56 cm. They were killed by decapitation followed by destruction of the brain and spinal cord. Thin slices of white myotomal muscle were taken

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from the immediate post-anal region. Bundles of 11–14 fibres were dissected under saline, which contained (in mmol l^{-1}): NaCl, 292; KCl, 3.2; $CaCl_2$, 5.0; $MgSO_4$, 1.0; Na_2SO_4, 1.6; $NaHCO_3$, 5.9; urea, 483; and tubocurarine, 1.5 mg l^{-1}.

The experiments were performed at approximately 11 °C. A piece of myoseptum at each end of the muscle bundle was held in a platinum foil T-clip. The muscle bundle was mounted horizontally between a force transducer (Cambridge Technology, Inc., model 400A) and a motor (Cambridge Technology, Inc., model 300B). The preparation was in contact with a thermopile that measured changes in fibre temperature from which heat output was determined. The thermopile was made by deposition of constantan and chromel to form a series of thermocouples on a Kapton substratum. Each thermocouple produced 37.8 μV $°C^{-1}$. The thermopile was 12 mm long and there were four thermocouples per millimetre along the length of the thermopile. Either a 3 or a 4 mm length of thermopile was used to record the output; the range of L_0 values (fibre length optimal for force production) for the fibre preparations we used was 4.4–6.0 mm (N=7).

The fibre bundle was stimulated end-to-end with 0.2 ms pulses. At the beginning of each experiment, the relationship between stimulus voltage and twitch tension was investigated to establish the number of live fibres and the supramaximal stimulus strength (voltage range 3–6 V). The diameters of dogfish white fibres are relatively large and unusually uniform. Thus, the steps in the stimulus strength–response relationship are of quite constant size and can easily be counted (4–12 live fibres in this study) in preparations containing a modest number of fibres. Typically two-thirds of the fibres in the preparation were alive. The length–tension relationship was also investigated, and L_0, the length at which tetanic force was maximum, was identified. The maximum force produced at L_0 is referred to as P_0.

The muscle fibres were stimulated every 10 min to produce a 2.0 s isometric tetanus at the fusion frequency, approximately 30 Hz. We report values based on the force and heat produced during 0.4, 1.0 and 2.0 s of stimulation during a 2.0 s tetanus. The experiment was not designed to include the heat produced during relaxation because the aim was to compare the activation heat during stimulation with the energy produced during stimulation in efficiency experiments (Curtin and Woledge, 1991). The length of the fibres was set to values between 92 % and 140 % of L_0. Alternate tetani were at L_0. Force and thermopile output were recorded simultaneously during each tetanus.

At each of the fibre lengths, a Peltier calibration record was obtained by passing a known current through the thermopile (Kretzschmar and Wilkie, 1972, 1975; Woledge *et al.* 1985) from which the time constant for heat loss and the heat capacity of the material on the recording part of the thermopile were found. The records of thermopile output were corrected for stimulation heat, which amounted to $0.03735V^2t$ per stimulus (μJ), where V is the stimulus strength (in volts) and t is the pulse duration (in milliseconds) (Curtin and Woledge, 1996).

At the end of each experiment, the muscle preparation was removed from the thermopile and fixed in ethanol at L_0. The length of the muscle fibres was measured under a dissecting microscope. The fixed fibres were carefully separated from the myosepta and any other non-fibre material, dried at room temperature, and weighed on a Cahn microbalance. The fibres were then soaked in glycerol. The fibre bundle was separated into individual fibres. The sarcomere lengths of the fibres were measured using diffraction of laser light. A mean value of the sarcomere lengths of the fibres at L_0 (SL_0) was obtained for each preparation. The dry mass of live fibres was calculated from the number of live fibres (determined from the strength–response curve), the total number of fibres weighed and the total dry mass. The wet to dry mass ratio is 4.90 in dogfish white muscle fibres (Curtin and Woledge, 1993). To take account of differences due to variations in muscle size, force is expressed as force production × L_0/mass of live fibres, and heat as heat production/mass of live fibres. See Table 1 for mean values.

Over the period of an experiment (usually longer than 2 h) there was some decline in both maximum force and heat output, 27.5±6.4 % (N=7) and 21.4±4.6 % (N=7), respectively. In order to minimize the possible error due to this decline, alternate tetani were used as controls. All the data points shown were normalized to the mean values of the adjacent controls.

Where stated, errors are expressed as standard errors of the mean (S.E.M.). Student's t-test was used for statistical comparisons.

Results

Force and heat production at different fibre lengths

Fig. 1 shows sample recordings of force, stimulation and heat production from a bundle of muscle fibres during a 2 s isometric tetanus at three different fibre lengths. The maximum tetanic force and heat production were reduced at lengths beyond L_0.

The force rose rapidly at the start of stimulation at all fibre lengths. In two of the seven fibre preparations, the force at long

Table 1. *Mean values for fibre bundles*

L_0 (mm)	5.26±0.24
SL_0 (μm)	2.21±0.04
Dry mass of live fibres (μg)	236.3±26.1
Dry mass per fibre (μg)	30.0±2.6
P_0 (N m g^{-1} dry mass)	1.444±0.067
P_0 (N m g^{-1} wet mass)	0.295±0.014
Heat (J g^{-1} dry mass)	0.454±0.018

Values are means ± S.E.M.; the sample size in each case is seven muscle fibre preparations.

L_0 and SL_0 are the fibre length and sarcomere length giving maximum isometric force.

P_0 is the peak isometric force during a 2 s tetanus at L_0, multiplied by L_0 and divided by the mass of live fibres.

Heat is that produced during 2 s of stimulation.

The ratio of wet mass to dry mass is 4.90 (see text).

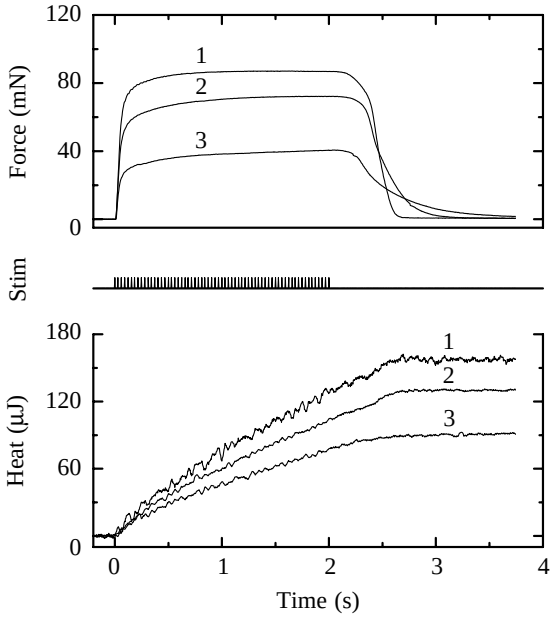

Fig. 1. Sample recordings of force and heat output during isometric tetani. Superimposed records of tetani at (1) the length for maximal force (for this preparation SL_0=2.39 μm), (2) 1.16SL_0 and (3) 1.40SL_0. Stim shows tetanic stimulation for 2 s at 30 Hz. Temperature was 12 °C (P_0=1.322 N m g^{-1}, heat produced during 2 s of stimulation at SL_0=0.465 J g^{-1}).

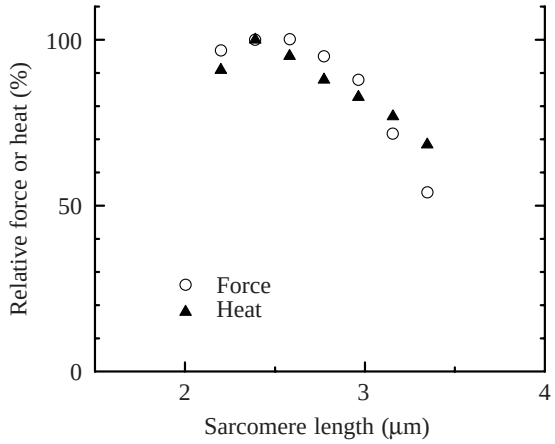

Fig. 2. The length–tension (○) and length–heat output (▲) relationships for the same fibre bundle as shown in Fig. 1. Data were normalized to the force and heat output values at the length for maximum force (SL_0, 2.39 μm).

lengths continued to increase gradually throughout stimulation; this later phase of increasing force has been called 'creep' (Gordon *et al.* 1966). It is a consequence of different degrees of filament overlap along the length of the muscle fibres; such non-uniformity occurs particularly at lengths beyond L_0 (for references, see Edman and Reggianni, 1984). Force creeps up during stimulation because sarcomeres in one part of a fibre are gradually elongated by shortening of sarcomeres in other parts of the fibre where there is more filament overlap. The values of force we report here do not include a contribution from creep; in the two cases where creep occurred, the force records were extrapolated to remove its contribution before measuring force (see Fig. 3 in Gordon *et al.* 1966). The mean correction was 10±2.0 % (N=10, force expressed as a percentage of that at SL_0). The force records in Fig. 1 also show that the relaxation was markedly slower at long fibre lengths.

The heat records in Fig. 1 show that at all fibre lengths the rate of heat output was high at the start of stimulation and gradually declined during the first second of stimulation to a lower, steady value. The muscle continued to produce heat for a brief period after the end of stimulation.

The dependence of force and heat production on sarcomere length for the same preparation (2.0 s stimulation) are summarized in Fig. 2. The experiments were carried out at various sarcomere lengths from 92 to 140 % SL_0. Both force and heat output are shown expressed relative to the values at SL_0. Fig. 2 shows that the length at which heat output was greatest was the same as the length at which force production was greatest (SL_0). This was the case in six out of the seven muscle fibre preparations. The amount of heat produced was reduced at sarcomere lengths greater than SL_0, but the dependence of heat on length was different from that of force. This pattern was typical, as will be shown by the comparisons of heat with force production discussed below.

Relationship between heat and force production

Results for seven different muscle fibre preparations are summarized in Fig. 3. Fig. 3A shows the relationship between force and heat production for isometric tetani at sarcomere lengths between 100 and 140 % SL_0. Both force and heat output are shown expressed relative to the values at SL_0; see Table 1 for absolute values at SL_0.

Over most of the range of forces, there is a constant linear relationship between heat production and force, but at high force output the relationship becomes steeper. The data above 70 % P_0 are plotted on a larger scale in Fig. 3B. The change in the heat:force relationship can also be seen in Fig. 2 where, as sarcomere length increased beyond SL_0, the heat output first declined faster than did force, and then slower than did force. This phenomenon is similar to that found by Aubert and Gilbert (1980) in frog muscle.

We have tested the possibility that the change in slope in Fig. 3 is due to 'creep' in force at long sarcomere length, since creep or the correction for it might give an incorrect force value. However, this change in slope of the relationship between heat and force is apparent whether or not force is corrected for creep, so it seems unlikely that the change in slope is due to creep.

Activation heat

Our determination of activation heat is based on the following reasoning. As sarcomere length increases, filament overlap decreases; consequently, force and force-related crossbridge heat are reduced. When sarcomere length is long enough for there to be no filament overlap, the force would be

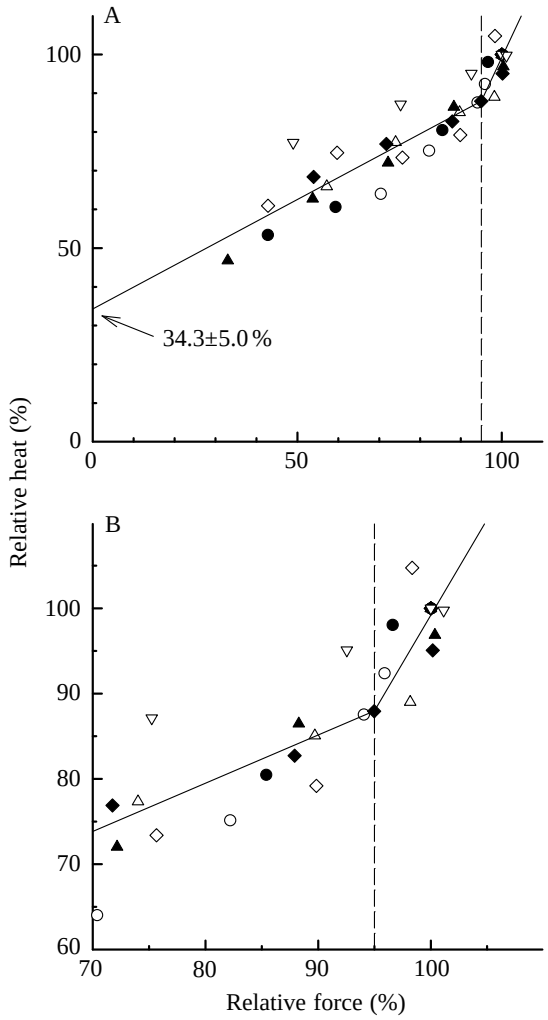

Fig. 3. (A) Relationship between heat output and force production for 2 s stimulation under isometric conditions from seven muscle fibre bundles. The dashed vertical lines are drawn at 95 % P_0, where P_0 is the maximum force produced; the two regression lines shown are: y=0.565x+34.3, for relative force less than 95 % P_0, and y=2.246x −12540, for relative force greater than 95 % P_0. (B) The values near P_0 plotted on a larger scale.

zero and there would be no crossbridge heat. Any remaining heat production would be purely activation heat. Thus, the amount of activation heat can be found by extrapolating the relationship between heat and force to zero force.

Fig. 3A shows lines fitted to the data points for forces below and above 95 % P_0. The value of 95 % P_0 was chosen as the 'break point' because it gave the minimum total error of the intercept and 'root-mean-square' scatter of the points around the lines. Activation heat was found by extrapolating the relationship for forces below 95 % P_0 to zero force, where the intercept on the heat axis is the activation heat. Its value is 34.3±5.0 % (mean ± S.E.M., N=24) of the heat output during 2.0 s of stimulation under isometric conditions.

Since heat is not produced at a constant rate during the first second of stimulation (Fig. 1), the question arises whether activation heat, expressed as a percentage of total heat output, is constant during a tetanus. To examine this, the same analysis was carried out using values from the same records, but measured at 0.4 and 1.0 s of stimulation. The regression lines for relative force less than 95 % P_0 and the corresponding activation heats were: y=0.5x+0.403, activation heat=40.3±8.4 % (mean ± S.E.M., N=20) for 0.4 s and y=0.55.0x+0.356, activation heat=35.6±5.5 % (mean ± S.E.M., N=24) for 1.0 s. Thus, we have no evidence that activation heat expressed as a proportion of the total isometric heat varies with tetanus duration.

Discussion

Activation heat as a fraction of isometric heat

The present experiments investigated the relationship between force and heat output, and quantified the force-independent activation heat in dogfish white muscle. Our results show that activation heat, based on extrapolation of the force–heat relationship, is approximately 35 % of the heat produced during an isometric tetanus at the optimal length. This value is not unusual compared with values reported for muscles from other species under a variety of conditions. These values range from 18 to 41 % (amphibians, *Rana temporaria* and *Rana pipiens*, close to 0 °C, Homsher *et al.* 1972; Smith, 1972; Rall, 1979; Burchfield and Rall, 1985, 1986; Barclay *et al.* 1993; chicken muscle at 21 °C, Rall and Schottelius, 1973; and mammalian muscle at 27 °C, Gibbs and Gibson, 1972; Wendt and Gibbs, 1973; Wendt and Barclay, 1980).

Activation heat and crossbridge efficiency

The maximum value of efficiency (power/rate of total energy output) during steady-velocity shortening is 0.33 in dogfish white muscle (Curtin and Woledge, 1991) and 0.45 in frog muscle (Hill, 1964*b*). Is dogfish muscle less efficient than frog muscle because dogfish muscle uses more energy for activation processes or because its crossbridge efficiency is lower? This question can be answered on the basis of our measurement of activation heat reported here and other information from the literature. We measured activation heat in an *isometric* tetanus of dogfish muscle to be 34.3 % of the energy output during stimulation; the corresponding value for frog muscle is 27 % (Homsher *et al.* 1972; Smith, 1972; Burchfield and Rall, 1986; Barclay *et al.* 1993). However, our aim here is to compare activation heat during shortening under maximum efficiency conditions. Activation heat will constitute a considerably smaller proportion of the energy output during shortening, because the rate of energy output is larger during shortening, mainly due to high mechanical power output, than during isometric contraction (the Fenn effect; Fenn, 1923). In dogfish muscle, the rate of energy output is 3.25 times higher during shortening (maximum efficiency conditions) than during isometric contraction (Curtin and Woledge, 1991) and in frog muscle it is 3.00 times higher (Hill, 1964*a*,*b*). Thus, activation heat is 11 % of the energy output during shortening at maximum efficiency in dogfish muscle and 9 % in frog muscle.

Work, activation heat and crossbridge heat, which are the

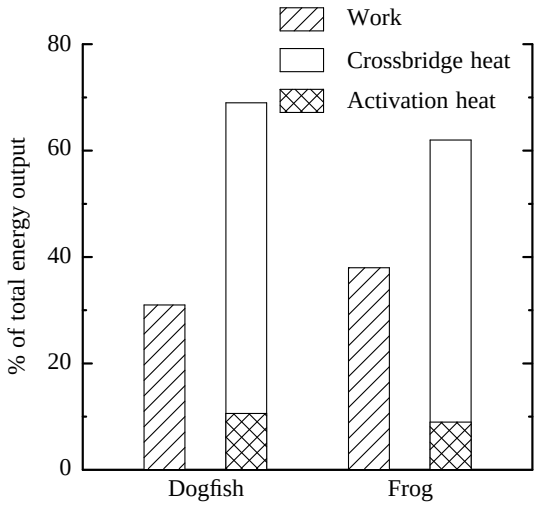

Fig. 4. Comparison of the three components of total energy output during shortening at maximum mechanical power in dogfish white muscle and frog skeletal muscle. Efficiency is expressed as the percentage of work in the total energy output; activation heat has been calculated from the percentage of isometric heat as described in the text; the rest of heat production is crossbridge heat.

three parts of the total energy production at maximum mechanical power, are shown in Fig. 4 for dogfish and frog muscle. The values are expressed as a percentage of the total energy output by the muscle. Work expressed in this way is equivalent to efficiency. The rest of the energy output is the heat production, which includes the crossbridge heat and the activation heat. As can be seen, the activation heat in these two species of animals is very similar. The lower efficiency of dogfish white muscle is largely due to a lower crossbridge efficiency. That is, more crossbridge heat is produced during work performance in dogfish white muscle than in frog muscle. At the crossbridge level, dogfish white muscle is less efficient at converting chemical energy into mechanical work.

Minimizing total cost: how much for activation?

Can we understand what factors determine the energetic cost of the process of Ca^{2+} uptake (activation heat)? During a contraction (stimulation plus relaxation), most of the energy turnover is due to ATP use by the interaction of myosin crossbridges with actin; this occurs during stimulation and continues at a diminishing rate after the end of stimulation. In addition, Ca^{2+} is being both released into and removed from the sarcoplasm. The amount of energy used for Ca^{2+} uptake by the sarcoplasmic reticulum (SR) depends on the amount of this Ca^{2+} turnover which can, in principle, be varied. If Ca^{2+} turnover were made slower, then the turnover of Ca^{2+} during any given period of stimulation would be less and would need less energy. However, at the end of the period of stimulation, the removal of Ca^{2+} from the sarcoplasm would then be slower because the slower turnover means reduced rates of both Ca^{2+} release and uptake; during this slower relaxation, more energy would be used by the crossbridges.

For each duration of stimulation, there will be a certain rate of Ca^{2+} turnover that requires the minimum energy usage for the cycle of stimulation and relaxation. When Ca^{2+} turnover is slower, the energy saved through having less Ca^{2+} to remove from the sarcoplasm is more than compensated by the extra energy used by the crossbridges during the prolonged relaxation.

We have modelled these processes for different durations of isometric tetani and have varied the Ca^{2+} turnover rate to find the rate that gives the minimum total energy usage.

As in many animals, the white muscle fibres of dogfish contain high concentrations of the Ca^{2+}-binding protein parvalbumin ($0.61\pm0.05\,mmol\,l^{-1}$, mean ± S.E.M., N=28; J. A. Rall, T. T. Hou and L. D'Anniballe, personal communication). In such muscle, there are two ways that Ca^{2+} is removed from the sarcoplasm: by binding to parvalbumin (PA) and by uptake into the SR by the Ca^{2+} pump. The eventual energetic cost of Ca^{2+} removal by either route is the same, however, since all the Ca^{2+} bound to PA must be returned to the SR by the pump (although this does not occur immediately). It is also the case that the amounts of heat produced by Ca^{2+} uptake by PA and that produced by the Ca^{2+} pump are approximately the same per mole of Ca^{2+} removed from the sarcoplasm (Tanokura, 1990, and references therein). Thus, the process of transferring Ca^{2+} from PA to the SR is approximately thermally neutral.

For brief tetani, most of the Ca^{2+} removal from the sarcoplasm is due to binding to PA, whereas during long tetani, e.g. more than a few seconds at low temperature, the parvalbumin becomes saturated with Ca^{2+} and, after this, relaxation is wholly dependent on the uptake by the SR Ca^{2+} pump (Jiang *et al.* 1996, and references therein). Since dogfish white myotomal muscle is mostly used for brief tetani, only Ca^{2+} uptake by PA is included in our model. The rate of Ca^{2+} removal from the sarcoplasm was varied by changing the amount of PA present at the start of stimulation. Each parvalbumin molecule contains two binding sites for Ca^{2+} (the Ca^{2+}–Mg^{2+} sites) which are relevant here. Under resting conditions approximately half of the Ca^{2+}–Mg^{2+} sites have Mg^{2+} bound to them, and the concentration of these sites is approximately the same as the PA concentration (Hou *et al.* 1991). Here we use PA–$Mg^{2+}{}_o$ to mean the concentration of Ca^{2+}–Mg^{2+} sites with Mg^{2+} bound to them before stimulation. We make the following simplifying assumptions. (1) The Ca^{2+}-specific troponin (TN) sites ($0.18\,mmol\,l^{-1}$; Yates and Greaser, 1983) are immediately saturated with Ca^{2+} when stimulation starts and they remain saturated during stimulation. This seems justified since the rate constant for Ca^{2+} binding to TN is $30\,s^{-1}$ (Johnson *et al.* 1981) which is fast on the scale being considered here. (2) The amount of free Ca^{2+} in the sarcoplasm is negligible compared with the amounts bound to PA and to TN. This assumption is reasonable for periods of stimulation too brief to saturate all of the PA sites with Ca^{2+}. Fig. 5 shows the fluxes of Ca^{2+} during stimulation and relaxation that are included in the model. The rate of Ca^{2+} uptake by PA is known to be limited by the rate at which Mg^{2+} dissociates from the Ca^{2+}–Mg^{2+} binding sites on PA (Hou *et al.* 1991, 1992; Jiang *et al.* 1996). Using a rate constant of $1.76\,s^{-1}$ for this process

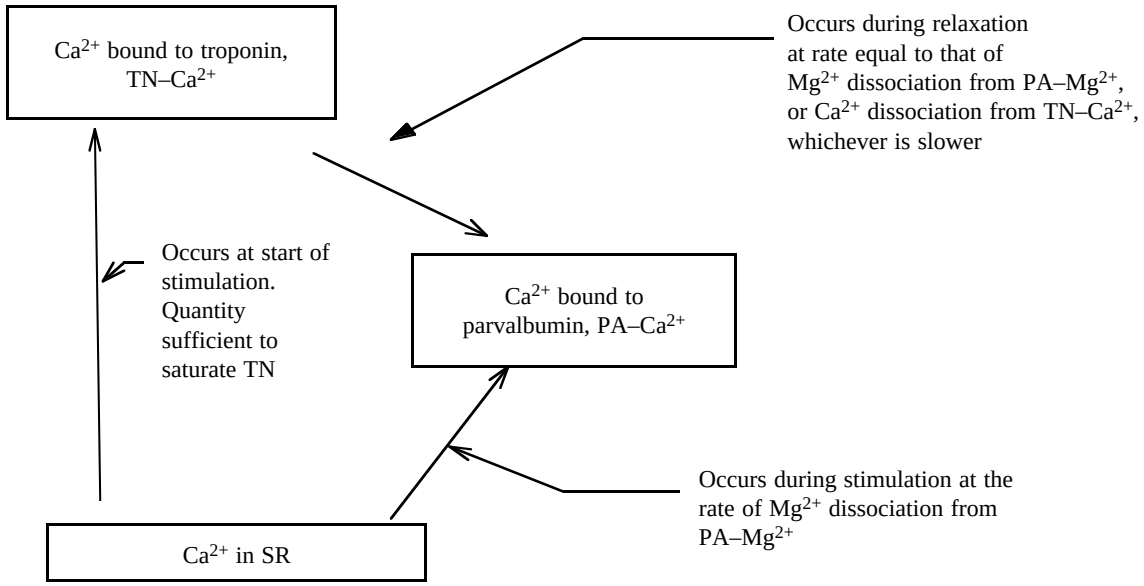

Fig. 5. Schematic diagram of the Ca^{2+} sites (boxes) and transitions between these sites that are included in the model. The rates of the transitions were evaluated as described in the text. SR, sarcoplasmic reticulum. PA–Mg^{2+}, Mg^{2+} bound to parvalbumin.

at 10 °C (Hou *et al.* 1992), we calculated the time course with which Ca^{2+} binds to PA and the reduction in the number of PA sites remaining uncomplexed with Ca^{2+} during stimulation. The total amount of Ca^{2+} that must be pumped into the SR to return to the original resting state is the sum of the Ca^{2+} bound to TN and the Ca^{2+} bound to PA at the end of stimulation. Assuming a stoichiometry of 2 Ca^{2+} pumped per ATP, the energy used for Ca^{2+} removal can be calculated.

The time course of removal of Ca^{2+} from TN after selected durations of stimulation was calculated. The rate of Ca^{2+} binding to PA sites is taken as either the rate of dissociation of Mg^{2+} from PA–Mg^{2+} or the rate of Ca^{2+} dissociation from TN–Ca^{2+}, whichever is less. Assuming that the rate at which the crossbridges use ATP is proportional to the Ca^{2+} occupancy of the TN sites, the amount of ATP used during relaxation by the crossbridges can be found by integrating the occupancy time course. The rate of ATP use by the crossbridges during stimulation was determined from the heat production in Table 1. Assuming a molar enthalpy change for phosphocreatine splitting of 34 kJ mol^{-1} and that the change in the amount of phosphocreatine is equal to the amount of ATP used, the rate of ATP splitting is given by the heat rate divided by 34 kJ mol^{-1}.

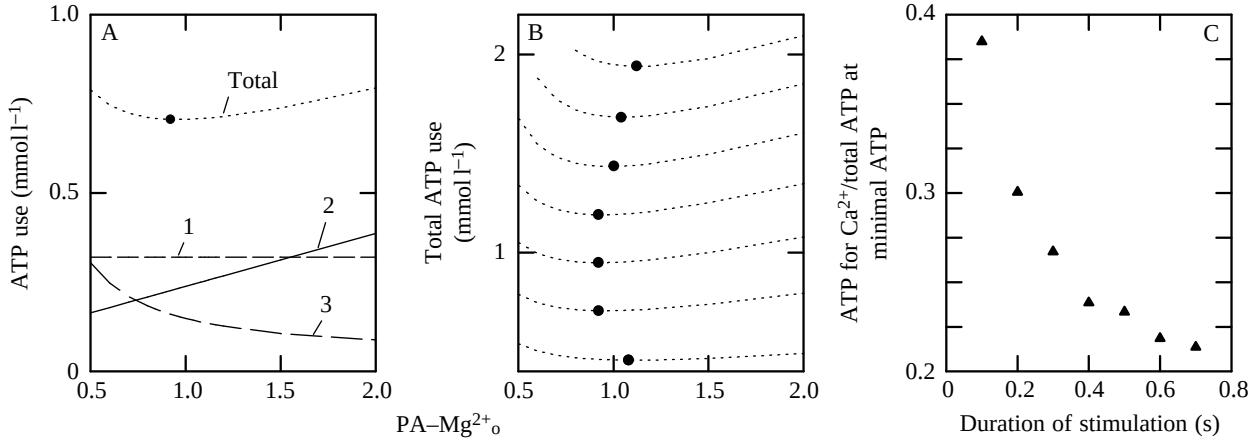

Fig. 6. Values calculated from the model. See text for a detailed explanation. PA–$Mg^{2+}{}_o$ refers to the concentration of parvalbumin (PA) sites with Mg^{2+} bound to them before stimulation; Ca^{2+} can replace Mg^{2+} at these sites during stimulation and relaxation. (A) Dependence of ATP use during a 0.2 s isometric tetanus on PA–$Mg^{2+}{}_o$. (1) ATP use by crossbridges during stimulation, (2) ATP use by the sarcoplasmic reticulum (SR) Ca^{2+} pump to return Ca^{2+} to its pre-stimulation state, (3) ATP use by the crossbridges after the end of stimulation. The total of these three component is also shown. The filled circle refers to the PA–$Mg^{2+}{}_o$ at which the total cost is at a minimum. (B) Dependence of total ATP use during isometric tetani on PA–$Mg^{2+}{}_o$. Each line refers to a particular duration of stimulation: 0.1–0.7 s in 0.1 s steps, increasing from the bottom. The filled circles indicate the minimum ATP use that can be achieved by varying PA–$Mg^{2+}{}_o$. This parvalbumin content is referred to as the 'most economical PA–$Mg^{2+}{}_o$'. (C) ATP used for Ca^{2+}-pumping expressed as a fraction of the total ATP used for complete contraction plus relaxation cycles plotted against the duration of stimulation. These values are for the most economical PA–$Mg^{2+}{}_o$ for each duration of stimulation.

As illustrated by the example in Fig. 6A, the total energy used in a 0.2 s stimulation plus relaxation is the sum of (1) the energy used by the crossbridges during the period of activation, which is independent of the amount of PA present, (2) the amount of energy used for Ca^{2+} turnover, which increases linearly with the amount of PA, and (3) the amount of energy used by the myofibrils during relaxation, which decreases as the amount of PA is increased. At one particular PA concentration, the total cost is at a minimum. The value of this minimum energetic cost and the amount of PA required to achieve it are dependent on the duration of stimulation as shown in Fig. 6B. The measured amount of PA in dogfish white fibres (0.61 mmol l^{-1}) is close to that PA content (1 mmol l^{-1}) giving the minimum ATP use. Fig. 6C shows how the fraction of the energy used for Ca^{2+} uptake varies with the duration of the stimulation for these 'most economical' amounts of PA. For tetani lasting between 0.2 and 0.4 s, the value of this ratio is near 0.3. Thus, the measured value of the activation heat in dogfish white fibres, approximately 35 % of the isometric heat, is close to that giving the minimum energetic cost when both it and ATP use by the crossbridges are taken into account, at least in this simple model.

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