PARVALBUMIN, LABILE HEAT AND SLOWING OF RELAXATION IN MOUSE SOLEUS AND EXTENSOR DIGITORUM LONGUS MUSCLES

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SUMMARY

1. Parvalbumin content, heat rate and rate of relaxation were measured in two mouse muscles: the slow-twitch soleus and the fast-twitch extensor digitorum longus (EDL).

2. No trace of parvalbumin was found in the soleus; EDL contained a mean of 4·86 mg of this protein per gram of fresh muscle (s.d. = 1·25).

3. Heat rate during 7 s isometric tetani in isolated soleus muscle at 20 °C can be described by the sum of an exponentially decaying term and a constant term. The exponential term is reduced by 67% in a second tetanus performed 1 s after a first one; its repriming is complete after a resting period of about 1 min. The exponential term has therefore the properties of labile heat.

4. Relaxation rate measured during 15 s of isometric interrupted tetani at 20 °C is nearly constant in the soleus, but decreases continuously with increasing tetanus duration in the EDL. In the latter, isometric tension also decreases continuously.

5. Therefore, parvalbumin can account neither for the labile heat production in mouse soleus nor for the slowing of relaxation associated with muscle fatigue observed after a few seconds of tetanus in EDL. The role of parvalbumin in striated muscles is thus reassessed, and other possible causes of labile heat production and slowing of relaxation are discussed.

INTRODUCTION

Parvalbumin is a soluble calcium–magnesium binding protein present in large amounts in amphibian fast skeletal muscles. In resting muscle, it is mainly saturated with magnesium, but when calcium concentration rises, magnesium is exchanged for calcium. This calcium binding on parvalbumin is thought to be responsible for three properties of amphibian fast muscles: labile heat, unexplained enthalpy and slowing of relaxation with increasing tetanus duration (Woledge, Curtin & Homsher, 1985).

When an isolated frog sartorius muscle contracts isometrically at 0 °C, tension rises to a constant level but heat rate is not constant. It is higher at the beginning of the tetanus and declines exponentially to a steady level. Disregarding the period of tension rise, Aubert (1956) called the constant level of heat rate 'stable heat' and the decreasing exponential term 'labile heat'. If the muscle is allowed to relax and

is rapidly restimulated, stable heat resumes at the same level as during the first tetanus but labile heat is smaller during the second tetanus. In order to recover the same magnitude of labile heat in the second tetanus, a resting period of several minutes is required between the two tetani (Aubert & Maréchal, 1963).

Energy balance studies have shown that at the beginning of an isometric tetanus more heat is evolved than can be accounted for by changes in concentration of identified substrates. The imbalance ('unexplained enthalpy') is strongly reduced in a second tetanus immediately following the first one, but reappears if a resting interval is allowed between tetani (Curtin & Woledge, 1977; Homsher, Kean, Wallner & Garibian-Sarian, 1979). The magnitude of the imbalance is similar to that of labile heat, but its time course during one tetanus is slightly different (Curtin & Woledge, 1979; Homsher et al. 1979).

It was therefore proposed that labile heat was not accounted for by classical chemical reactions, and its source was searched for in other processes. A likely candidate was progressive saturation of parvalbumin with calcium. The in vitro enthalpy of calcium binding (Smith & Woledge, 1985) and the parvalbumin concentration in frog sartorius muscle (see Gillis, 1985 for a review) can satisfactorily account for labile heat (Woledge et al. 1985). The slow repriming of the muscle's ability to produce labile heat is explained by a slow parvalbumin–calcium dissociation during the recovery period.

This 'parvalbumin hypothesis' can also account for the progressive slowing of relaxation with increasing tetanus duration (Abbott, 1951). Peckham & Woledge (1986) found that the rate of the initial phase of relaxation decreased exponentially during the first few seconds of an isometric tetanus, and then became constant. The slowing of relaxation was less pronounced after a tetanus closely following a first conditioning tetanus.

In view of the similarity of these properties with those of labile heat and unexplained enthalpy, these authors proposed that calcium binding to parvalbumin was their common origin. The slowing of relaxation is attributed to a progressive saturation of parvalbumin–calcium binding sites. The stable relaxation rate observed after a few seconds of tetanus is then explained by a stable rate of calcium pumping by the sarcoplasmic reticulum, net calcium binding to parvalbumin being negligible. The slow repriming of the muscle's ability to rapidly relax can be accounted for by the slow parvalbumin–calcium dissociation during the recovery period (Gillis, Thomason, Lefèvre & Kretsinger, 1982; Gillis, 1985; Peckham & Woledge, 1986).

In small mammals like mice, fast-twitch, but not slow-twitch muscles, are known to contain parvalbumin (Klug, Reichmann & Pette, 1983). Heat measurements, however, have not been performed as extensively as in amphibians. Gower & Kretzschmar (1976) have shown that unexplained enthalpy is produced in rat soleus muscle, and the amount is similar to that produced by frog muscle, but the time course of heat rate does not show a typical labile heat (Gibbs & Gibson, 1972; Wendt & Gibbs, 1973).

In order to clarify the role of parvalbumin, heat rate and rate of relaxation have been measured in two mouse muscles. The slow-twitch soleus does not contain parvalbumin and the fast-twitch extensor digitorum longus (EDL) contains parvalbumin at a concentration similar to that of frog muscles. EDL shows a slowing

of relaxation with increasing tetanus duration, but the effect is more complex than expected. Soleus, on the other hand, produces labile heat, contrary to expectations. Accordingly, the possible role of parvalbumin is reassessed.

Parts of this work have been published in abstract form (Berquin & Lebacq, 1989, 1990a).

METHODS

Measurement of parvalbumin content

Sixteen EDL and a few solei (C57BL/10 and NMRI strains), distinct from those used in the mechanical experiments, were analysed for parvalbumin content following the method of Klug *et al.* (1983). Mice were killed with large doses of ether, and muscles were dissected and kept at -18 °C for a few weeks before analysis. Each muscle was homogenized in distilled water (0·2 ml of water per ten milligrams of muscle) at room temperature. The extract was centrifuged for 5 min, and the supernatant was diluted three times in an equal volume of a 50% glycerol solution (i.e. 50% glycerol in distilled water). Two 5 μl samples of a solution containing 25% supernatant and two other 5 μl samples containing 12·5% supernatant underwent electrophoresis. This was performed on a 15% polyacrylamide gel (PAGE) with 0·1% sodium dodecylsulphate, in parallel with standards of purified mouse parvalbumin at four different concentrations. Gels were then stained (Laemmli) and analysed by densitometry; the relationship between concentration of parvalbumin standards and staining was linear in each case. Four measures of parvalbumin content, at two different extract concentrations, were thus obtained for each muscle; final parvalbumin concentration was taken as the mean of the four measures. The parvalbumin standard was kindly supplied to us by Dr C. Gerday. Some gels were transferred onto a nitrocellulose paper, and immunoblotting experiments were performed using rabbit anti-parvalbumin antibodies (immunoblotting system for rabbit polyclonal antibodies using Streptavidin-Alkaline Phosphate; Bethesda Research Laboratories, PO Box 6009, Gaithersburg, MD 20877, USA).

Dissection and mounting for heat and mechanical measurements

Mice of the strain C57BL/10 were used in heat measurements. They were supplied by Dr T. Partridge (Charing Cross Hospital, London W6 8RF). Before dissection, the animals were anaesthetized by percutaneous injection of 0·1 ml (10 g body weight)$^{-1}$ of Thalamonal^R (Janssens Pharmaceutica, Belgium), containing 2·5 mg Droperidol and 0·05 mg Fentanyl per millilitre. Soleus muscles were dissected and mounted immediately on a Hill-Downing type thermopile, one tendon being fixed to a hook and the other connected to a strain gauge through a 30 cm stainless-steel rod. The chamber was filled with 100 ml Krebs solution (mM: NaCl, 118; NaHCO$_3$, 25; KCl, 5; CaCl$_2$, 2·5; MgSO$_4$, 1; KH$_2$PO$_4$, 1 and glucose, 5; maintained at 20 °C and continuously gassed with oxygen, 95% and CO$_2$, 5%. After mounting, the animals were killed with inhalation of large doses of ether.

In mechanical experiments, both C57BL/10 and NMRI strains were used. Since no muscle damage was observed when dissection was performed on animals killed shortly before dissection, mice were killed by ether inhalation, and either the EDL or the soleus was rapidly isolated. The muscle was mounted vertically in a 200 ml chamber filled with Krebs solution (see above) gassed with O$_2$, 95% and CO$_2$, 5% at 20 °C. One muscle end was fixed to a hook and the other was connected to a strain gauge through a short light silver chain. In some cases, two muscles were dissected from the same animal. One muscle was mounted immediately, and measurements were performed as described below, while the other muscle floated freely in the same chamber. The first muscle was then removed and the second one was mounted on the apparatus. No significant differences were observed between muscles mounted immediately after dissection and those mounter later.

In both myothermal and mechanical experiments, 10–15 min equilibration were allowed after mounting. Several fused isometric tetani (0·5 s duration for soleus, 0·3 s for EDL) were then performed at 60 s intervals, and length was finely adjusted for maximal isometric force; the muscle was then allowed to rest again for at least 10 min.

At the end of measurements, the length of the muscle body was measured with calipers, the muscle was then removed, its tendon ends were carefully cut off and the remaining mass was

weighed. This gave the 'drained weight'. The muscle was then blotted gently on soft tissue and the remaining 'blotted weight' was measured. This blotted weight and muscle length were used to calculate cross-sectional area, which was used for normalization of tension ($kN\ m^{-2}$). No correction was made for the difference between muscle length and fibre length, as this would not affect the conclusions of this study.

Heat measurements in soleus

When the temperature baseline was stable, the solution was drained out of the chamber and the soleus muscle was stimulated by supramaximal condenser discharges of alternated polarity at 50 Hz. The pattern of stimulation is described in Results. Joule and Peltier heatings were inserted in the sequence to evaluate heat losses and thermal capacitance of the muscle. The thermopile output signal was amplified using an Ancom 15C-3 amplifier with a 100 Hz bandwidth. Force and temperature signals were digitized and stored on magnetic tape. A simultaneous analog record was taken on a fast pen recorder. All data were analysed at the end of the experiment. Heat records were corrected for heat loss following the method described by Hill (1965). No correction was made for time lag as it was negligible for the stimulation periods used in this study.

The thermopile was calibrated both by the immersion method and by the Peltier method (see Woledge et al. 1985, chap. 4). Identical results were obtained by these two methods.

Measure of relaxation rate in soleus and EDL

Interrupted tetani. Each muscle was given four isometric interrupted tetani at 20 min intervals. Stimulation (60 Hz for soleus and 100 Hz for EDL) was briefly interrupted every second, in order to allow partial relaxation. Interruptions of stimulation periods were long enough to ensure at least a 20 % tension fall, i.e. 0·15 s for soleus and 0·10 s for EDL. Stimulation periods lasted 0·85 and 0.90 s respectively, repeated fifteen times. Total tetanus duration was thus 14·85 and 14·90 s respectively. Force was recorded on a SE UV recorder.

Quantification of relaxation rate. The time course of mechanical relaxation in striated muscles is complex, as relaxation usually occurs in three phases after the end of stimulation; (1) a short period during which force is maintained at a plateau value, (2) a 'linear' phase, where the rate of relaxation is fairly constant (corresponding to ca 25 % of the fall in force in amphibian muscles) and (3) an approximately exponential phase. Therefore, the rate of relaxation cannot be satisfactorily characterized by a single parameter (Gillis, 1985). Since we intended to measure the mechanical effects of changes in rate of intracellular Ca^{2+} ($[Ca^{2+}]_i$) decay due to parvalbumin–calcium interaction, and since this $[Ca^{2+}]_i$ decay is correlated with the rate of tension fall during the linear phase of relaxation only (Cannell, 1986), attention was focused mainly on the second phase.

The rate of relaxation was measured in different ways: (1) the time required for force to fall from the last stimulus (100 % force, called P_{ref}) to 95 % ($t_{5\%}$) was measured. In order to more completely characterize relaxation rate during the 'linear' phase of relaxation only, (2) the time required for a 20 % tension fall (from 100 to 80 % = $t_{20\%}$), and (3) the difference between $t_{20\%}$ and $t_{5\%}$ (time taken by a tension fall from 95 to 80 %, designated $t_{20-5\%}$) were also measured. Finally, as a global index of relaxation rate, (4) the percentage drop in force (P_{fall}) during each relaxation period (i.e. from P_{ref} to the tension reached just before the beginning of the next stimulation period) was calculated.

RESULTS

Parvalbumin content of muscles

Figure 1 shows a polyacrylamide gel of mouse soleus and EDL extracts, run in parallel with standards of purified parvalbumin and myoglobin. No trace of parvalbumin was detected in the soleus, whereas a strong band is clearly seen in the EDL. Immunoblotting experiments confirmed that the band observed in the EDL corresponds to parvalbumin. In some soleus muscles, a faint band was observed with a mobility slightly different from that of parvalbumin. Separate analyses showed that this band had the exact mobility of a myoglobin standard; it was not stained in immunoblotting experiments.
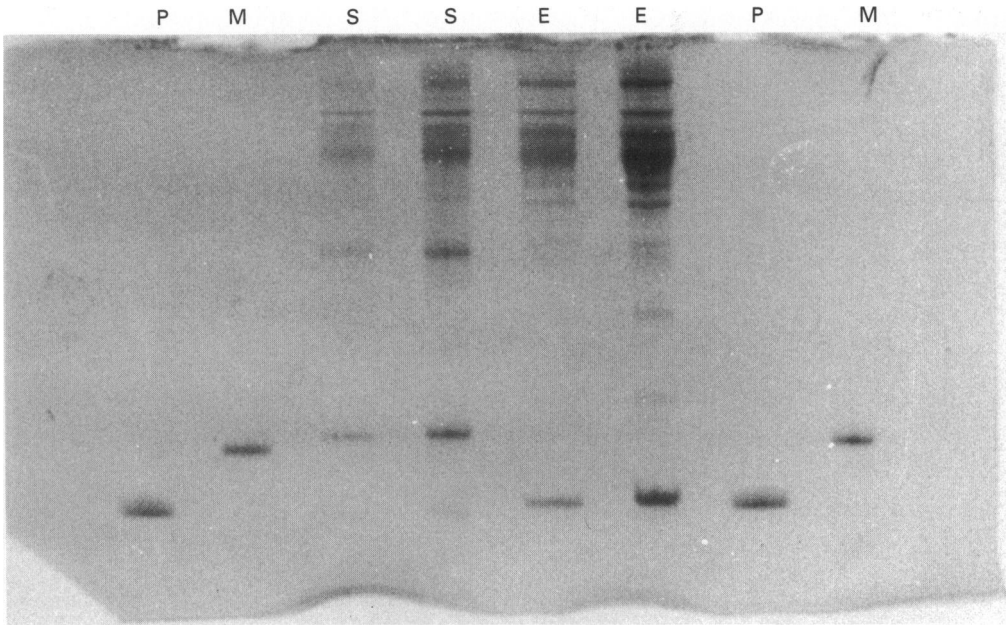
Fig. 1. Electrophoresis of mouse soleus (S) and EDL (E) extracts, containing 62·5 (left) or 125 (right) μg of muscle with standards of 0·25 μg purified parvalbumin (P) and 0·5 μg myoglobin (M).
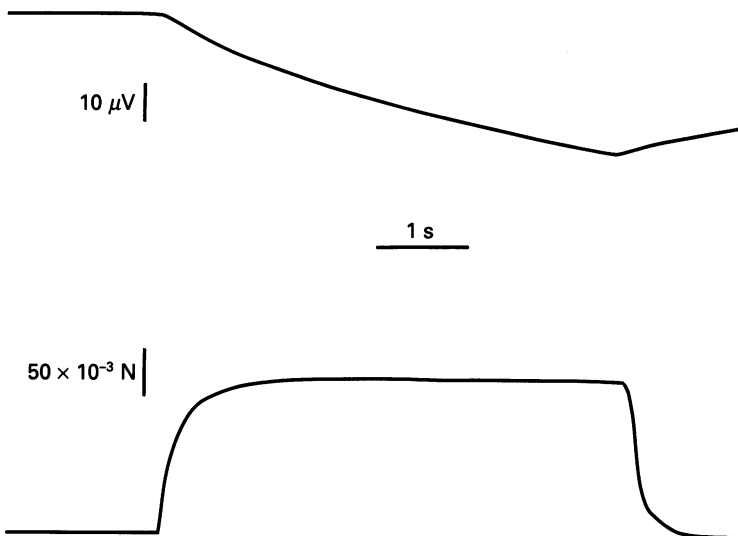

Fig. 2. Analog record of muscle temperature and force during a 7 s tetanus in soleus muscle. Top trace; thermopile output (temperature increases downwards). Bottom trace: strain gauge output.

Parvalbumin content of mouse EDL varies from 3·36 to 7·58 mg parvalbumin per gram of fresh muscle, with a mean of 4·86 mg g^{-1} ($n = 16$ muscles; s.d. = 1·25).

Heat production

Heat rate during a single isometric tetanus in soleus muscle

An example is given in Fig. 2 of the time courses of muscle temperature (top trace) and tension (bottom trace) during a 7 s isometric tetanus in soleus muscle. The
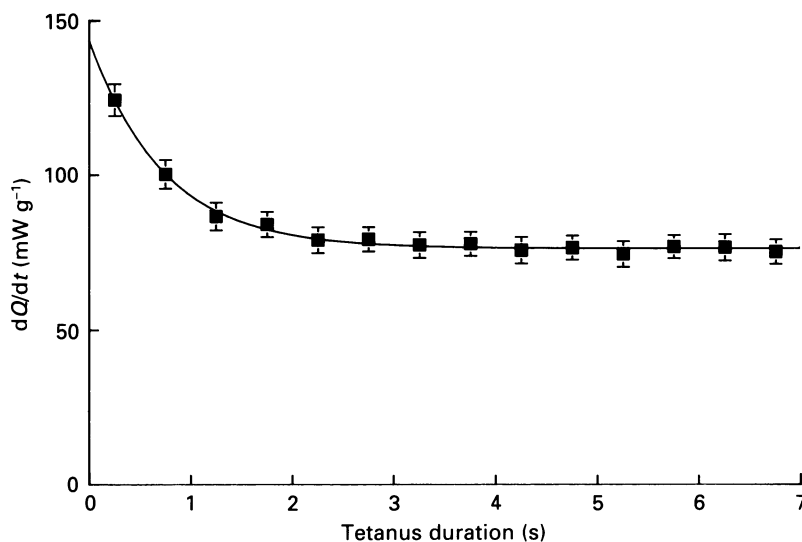

Fig. 3. Rate of heat production during a 7 s tetanus in mouse soleus (mean ± s.e.m.). The curve drawn through the points is computed from eqn (1).

temperature record has not been corrected for heat loss, but it can be seen that the heat rate is highest at the beginning of the tetanus. The tension curve shows that a stable tension level is reached after roughly one second of stimulation.

After correction for heat loss and for thermal capacitance, heat rate has been calculated in blocks of 0·5 s. Figure 3 gives the mean results (± s.e.m) of ten experiments. It can be seen that heat rate (dQ/dt) is maximal in the first time interval, and then decreases to a steady level in about 3·5 s.

These data have been analysed using a non-linear fitting programme. They can be satisfactorily described by the following equation, where the rate constant is expressed in s^{-1} and t is tetanus duration in seconds:

$$dQ/dt \ (\text{mW g}^{-1}) = 67 \cdot 2 \ e^{-1 \cdot 37t} + 76 \cdot 5. \tag{1}$$

The curve representing eqn (1) is given in Fig. 3 superimposed on the experimental data. It can be seen that the curve adequately describes the data points.

The second term of equation (1) represents the stable level of heat production and the exponential term is taken as the labile heat. From the parameters of eqn (1), the total amount of labile heat can be calculated as

$$\text{Labile heat (mJ g}^{-1}) = 67 \cdot 2 / 1 \cdot 37 = 49 \cdot 1 \ \text{mJ g}^{-1}. \tag{2}$$
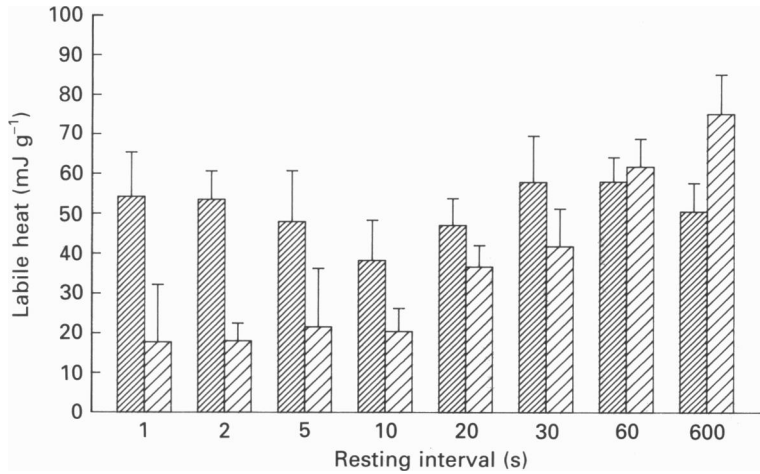
Fig. 4. Total amount of labile heat in two successive tetani, separated by a variable rest interval, in mouse soleus (means + S.E.M.). For each interval, given in the horizontal axis, the left column represents the amount of labile heat in the first tetanus and the right column corresponds to the second tetanus.
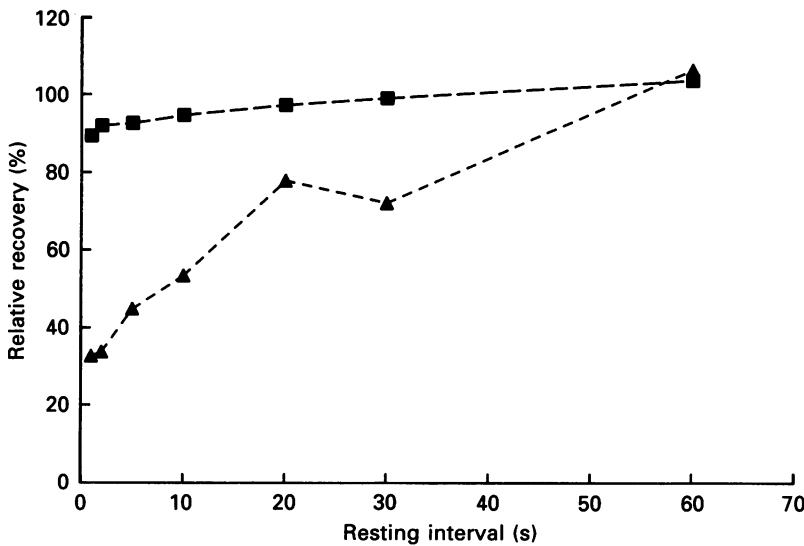

Fig. 5. Recovery of labile heat and peak tension as a function of resting interval in two successive tetani in mouse soleus. ▲, ratio (in %) of amount of labile heat in the second tetanus to amount of labile heat in the first tetanus; ■, ratio of peak tension in the second and the first tetanus.

Heat production during two consecutive tetani in soleus muscle

In order to study the reappearance of the labile term of heat rate, muscles performed pairs of 7 s tetani separated by a variable rest interval. Two ranges of rest intervals (taken from the end of the first to the beginning of the second tetanus of

one pair) were applied; (a) 1, 5, 10 and 30 s, and (b) 2, 20, 60 and 600 s. Each muscle was submitted to one of the protocols, each interval being applied twice, in a mirror order design. The rest interval between the last tetanus of one pair and the first tetanus of the next pair was 30 min. All tetani were analysed in terms of eqn (1) and
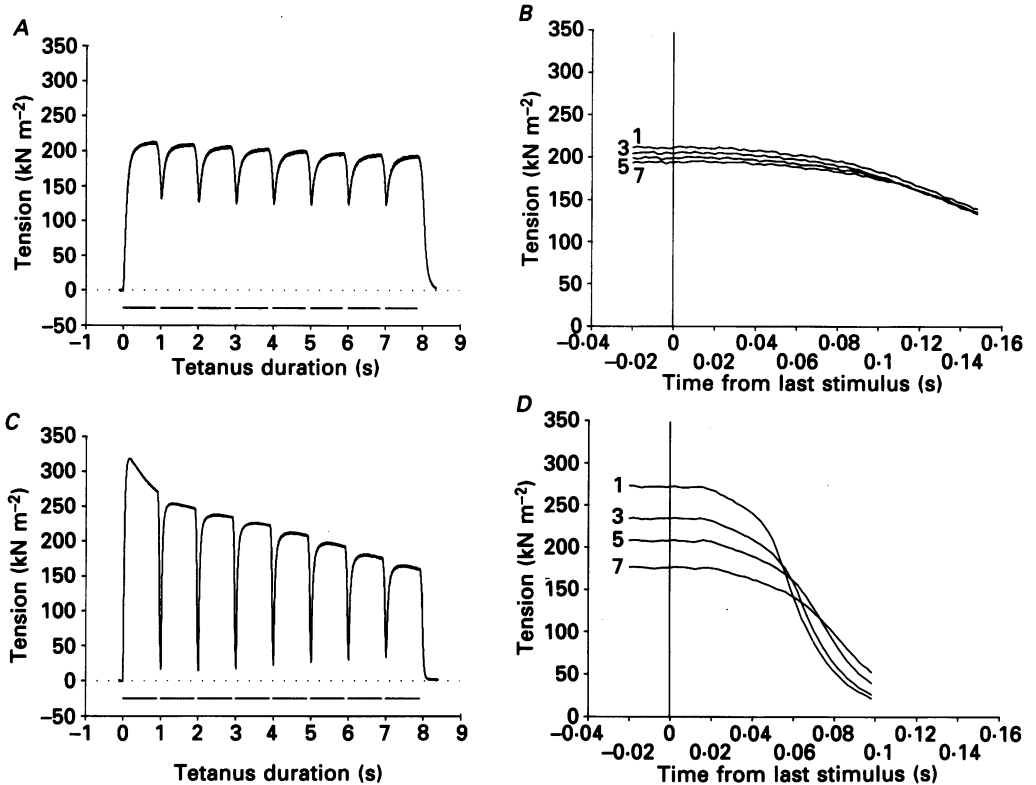

Fig. 6. Tension records during a short (8 s) interrupted tetanus in mouse soleus (*A, B*) and EDL (*C, D*) at 20 °C. *A* and *C*: whole interrupted tetani (stimulation periods are indicated by the horizontal bars. *B* and *D*: superimposed tension curves of 1st, 3rd, 5th and 7th partial relaxations during the same tetanus, on an expanded time scale.

the total amount of labile heat was calculated in each case. The mean results are given in Fig. 4. In this figure, for every rest interval the left-hand column represents the amount of labile heat observed in the first tetanus and the right-hand column gives the labile heat found in the second tetanus. For brief rest intervals, the amount of labile heat is reduced to about one-third of its original value. As the duration of the rest interval is increased, the labile heat progressively recovers its initial value. The total amount of labile heat is recovered within a 60 s interval between the two tetani. It is also clear that for a rest interval of 600 s, labile heat is significantly larger in the second tetanus than in the first one.

The same analysis was applied to the stable part of heat rate (constant term in eqn (1)). No significant difference is found between first and second tetani for any rest interval (results not shown).

Peak tension is only slightly (10%) reduced after a 1 s rest interval, and completely recovers its initial value during a 30 s interval. This is shown on Fig. 5 where the recovery of labile heat and peak tension are compared, and represented on a linear time scale.

Fig. 7. Relaxation rate and tension (means and S.D.) *versus* tetanus duration during 15 s interrupted tetani in mouse soleus at 20 °C. *A*: ▲, $1/t_{5\%}$; ▼, $1/t_{20\%}$; ●, $1/t_{20-5\%}$. *B*: ■, tension at the time of the last stimulus (P_{ref}); □, percentage of tension fall (P_{fall}).

Heat production in EDL

The definition of labile heat (Aubert, 1956) implies that a constant level of tension be maintained throughout the tetanus. This condition is not fulfilled in the case of EDL in which tension shows an initial peak and then decreases continuously during
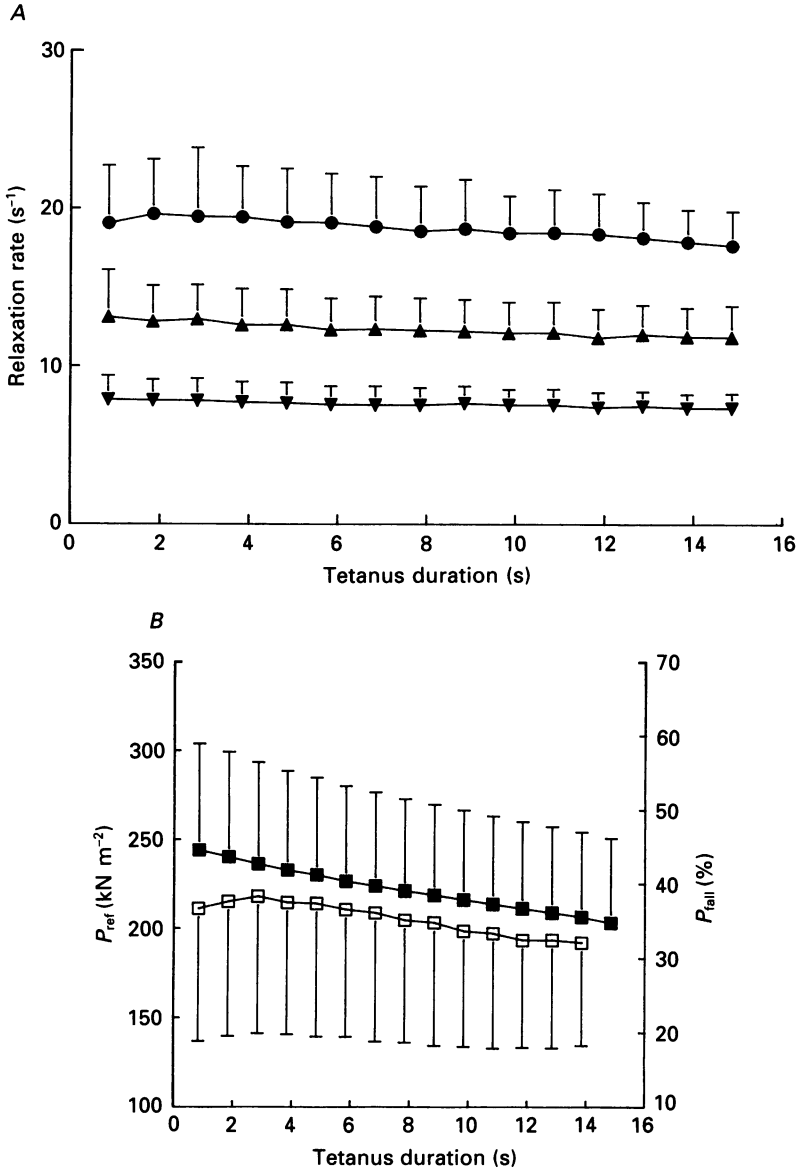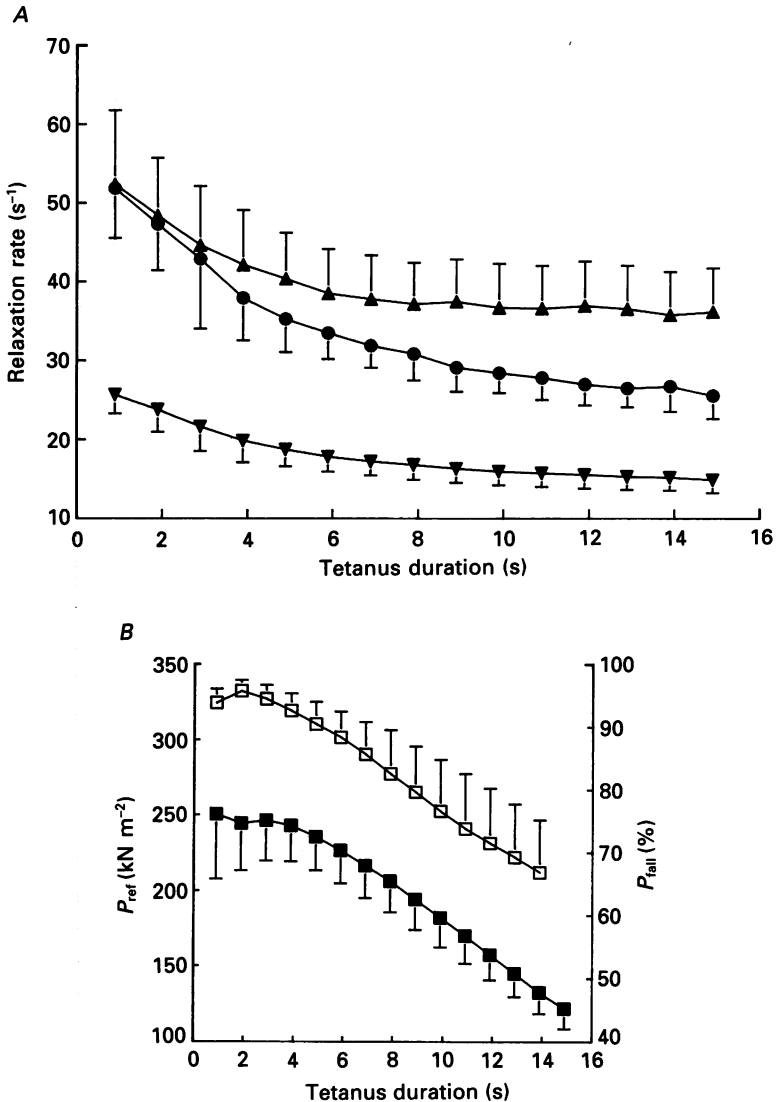

Fig. 8. Relaxation rate and tension (means and S.D.) *versus* tetanus duration during 15 s interrupted tetani in mouse EDL at 20 °C. A: ▲, $1/t_{5\%}$; ▼, $1/t_{20\%}$; ●, $1/t_{20-5\%}$. B: ■, tension at the time of the last stimulus (P_{ref}); □, percentage of tension fall (P_{fall}).

the tetanus (see Fig. 6*C*); heat rate declines accordingly, and thus cannot be described as the sum of an exponential term and a constant term. Therefore, EDL results have not been included in this study.

Relaxation rate

Soleus muscle

Figure 6*A* shows the time course of tension during a short (7·85 s) interrupted tetanus in a soleus muscle. Figure 6*B* gives the superimposed tension curves of the 1st, 3rd, 5th and 7th partial relaxations occurring during the same tetanus on an expanded time scale. It can be seen in Fig. 6*A* that the extent of relaxation is hardly modified with tetanus duration.

The time course of $1/t_{5\%}$, $1/t_{20\%}$, $1/t_{20-5\%}$, P_{ref} and P_{fall} (means and S.E.M.) during 14·85 tetani are shown in Fig. 7. After a slight initial increase in some cases, relaxation rate declines very slowly when tetanus duration increases: all parameters have decreased by a few per cent at the end of a 14·85 s tetanus.

EDL muscle

Tension curves obtained during a 7·9 s tetanus with and EDL muscle are shown in Fig. 6*C* and *D*. It can be seen that the extent of relaxation is largely reduced with increasing tetanus duration and that the shape of the tension curve during relaxation is modified: the shoulder between the 'linear' and 'exponential' phases is delayed and reduced (Fig. 6*D*). The mean results for 14·9 s tetani are given in Fig. 8.

As in amphibian muscles (Peckham & Woledge, 1986), the time course of $1/t_{5\%}$ can be described by an exponential equation (obtained by a non-linear, least-squares fitting procedure):

$$Y = 1/t_{5\%} = 22 \cdot 9 \, e^{-0 \cdot 31t} + 34 \cdot 1, \tag{3}$$

where rate constant is expressed in s^{-1} and t is tetanus duration in seconds.

However, $1/t_{20\%}$ and $1/t_{20-5\%}$ do not follow exponential kinetics and decrease continuously during a 15 s tetanus.

DISCUSSION

Calcium binding to and release from parvalbumin are believed to be the source of labile heat and unexplained enthalpy, and to cause a progressive slowing of relaxation with increasing tetanus duration. Evidence presented in this work (1) confirms that mouse EDL contains large amounts of parvalbumin, while soleus contains none or only trace amounts of this protein, but (2) shows that in mouse muscle, labile heat can be produced in the absence of parvalbumin and (3) that, in the presence of parvalbumin, slowing of relaxation is not fully accounted for by the calcium–parvalbumin interaction.

Parvalbumin in mouse soleus and EDL

Mean parvalbumin content in mouse EDL is 4·86 mg per gram of fresh muscle. In soleus muscle, no trace of this protein was detectable. In view of the relatively high extract dilutions used, this means that parvalbumin concentration is less than 1 mg g^{-1} in this muscle. More precise measurements were not performed since the results agree with those of Heizmann, Berchtold & Rowlerson (1982), who found 4·4 mg g^{-1} in mouse EDL and 0·01 mg g^{-1} in mouse soleus.

Labile heat in mouse soleus muscle

In this muscle, heat rate during an isometric tetanus is adequately described by the sum of an exponentially decreasing term and a constant term. When stimulation is interrupted and then resumed 1 s after relaxation, the exponential term is reduced to about a third of its value during the first tetanus. Full recovery of the exponential term requires a resting period of about one minute. These characteristics of the exponential part of heat rate are typical for labile heat as defined by Aubert (1956) and Aubert & Maréchal (1963). In the case of the EDL, the same analysis could not be applied, since both tension and heat rate decreased continuously during the tetanus.

The rate constant of the exponential term of heat rate in soleus muscle is 1.37 s^{-1} at 20 °C. Values obtained by previous authors in the case of frog muscle at 0 °C range between 0.52 s^{-1} (semitendinosus; Curtin & Woledge, 1981) and 1.35 s^{-1} (sartorius; Curtin & Woledge, 1977). The rate constant of labile heat in frog muscles has a Q_{10} of 1.5 (Curtin, Howarth, Rall, Wilson & Woledge, 1986) to 2.8 (Aubert, 1956), between 0 and 10 °C. At 20 °C, it is therefore expected to range between 1.17 and 10 s^{-1}. Clearly, large differences exist between different animal species.

The total amount of labile heat in our experiments is 49 mJ g^{-1}. Comparing again with the results of Aubert (1956), frog sartorius produces 20–40 mJ g^{-1} of labile heat at 0 °C, with a Q_{10} of 1.5 between 0 and 10 °C. Extrapolating to 20 °C gives 30–60 mJ g^{-1}: mouse soleus and frog sartorius produce similar amounts of labile heat.

Possible origin of labile heat in mouse soleus muscle

In a muscle containing parvalbumin, calcium released from the sarcoplasmic reticulum binds to parvalbumin. Knowing the parvalbumin content of the muscle and the enthalpy change of exchanging magnesium and calcium, it is possible to account for most, but not all, of the labile heat in amphibian muscle (see Berquin & Lebacq, 1990b for a small review). As mouse soleus does not contain parvalbumin, other sources must be found for labile heat in this case.

Thermoelastic and shortening heat

Tension development at the beginning of tetani is much slower in our experiments than in frog sartorius at 0 °C (see for example Aubert, 1956). Slow rise of tension is a typical behaviour of mouse soleus at 20 °C (see for example Crow & Kushmerick, 1983). A tension rise results in two known thermal effects: a thermoelastic heat absorption and a release of heat due to internal shortening of the muscle. Both of these effects have been extensively studied in frog muscle at 0 °C, in which they are small and of opposite sign, so that they roughly cancel each other out (Curtin & Woledge, 1978). The magnitude of these effects in mouse muscles is not known. Assuming a thermoelastic coefficient of 0.01 as in amphibian muscles (Woledge *et al.* 1985), and a mean peak tension of 177 kN m^{-2} in this series, heat absorption by thermoelastic effect during the rise of tension would amount to 1.77 mJ g^{-1}. It is clear that thermoelastic heat is not of the same order of magnitude as labile heat production in mouse soleus.

Calcium binding to troponin

This reaction occurs very early in the tetanus. Estimation of its enthalpy change is not easy in view of the variability of published *in vitro* enthalpy changes and because the *in vivo* enthalpy change (when troponin is bound to actin) is not known. In frog muscles, it could yield 10–16·5 mJ g^{-1} (Yamada, Mashima & Ebashi, 1976; Curtin & Woledge, 1978; Homsher, Lacktis, Yamada & Zohman, 1987).

Exponential breakdown of phosphorylcreatine

Evidence from frog muscles suggests that the rate of phosphorylcreatine breakdown may be larger at the very beginning of the tetanus that later (Homsher *et al.* 1987). This could account for as much as 15 mJ g^{-1}. In the case of mouse soleus, phosphorylcreatine hydrolysis has been measured by Crow & Kushmerick (1982). However, the time resolution of their experiments does not allow detection of exponential term for the rate of phosphorylcreatine hydrolysis, if any exists.

Incomplete cycle of myosin ATPase

Owing to the rate-limiting step being different in resting and in active muscle, redistribution of the ATPase sites among its different states occurs upon activation. The enthalpy change of the redistribution is unknown; it is expected to be an exothermic process, which must take place early in the tetanus, at the same time as the rise of tension (Curtin & Woledge, 1978).

Putting together evidence from frog muscle about the enthalpy changes associated with calcium binding to troponin, phosphorylcreatine breakdown and incomplete myosin ATPase cycle, it is possible to account for a substantial fraction, if not all, of the labile heat produced by mouse soleus muscle.

Relaxation rate in soleus and EDL

The results presented here show that in mouse soleus, which does not contain parvalbumin, the relaxation rate is indeed independent of tetanus duration. But in EDL, which contains large amounts of this protein, there is a progressive slowing of relaxation with increasing tetanus duration. So far, this seems to be well explained by the calcium–parvalbumin interaction.

In amphibian muscles, the rate of relaxation measured either by $1/t_{5\%}$ (Peckham & Woledge, 1986) or by $1/t_{20\%}$ and $1/t_{20-5\%}$ (Hou, Johnson & Rall, 1991) decreases exponentially during the first few seconds of relaxation and then becomes constant. In mouse EDL however, only $1/t_{5\%}$ shows an exponential decay, and $1/t_{20\%}$ and $1/t_{20-5\%}$ do not tend to a stable value.

According to the hypothesis, the rate constant of slowing of relaxation is determined by the slow parvalbumin–magnesium dissociation rate. Hou *et al.* (1991) have shown that these two rate constants (1·18 and 0·93 s^{-1} respectively) are indeed quite similar in the case of frog muscles at 0 °C. The Q_{10} of the magnesium off rate from parvalbumin is 1·9 (Hou, Johnson & Rall, 1990); this rate constant is therefore probably close to 3·1 s^{-1} at 20 °C (while the rate constant of the exponential decay of $1/t_{5\%}$ is only 0·31 s^{-1} in our experiments). Consequently, at 20 °C, the slowing of

relaxation due to the calcium–parvalbumin interaction may be expected to be relevant after short tetani only. This is supported by recent data obtained by Westerblad & Lännergren (1991) on single mouse fibres, showing a slowing of relaxation during 1·5 s interrupted tetani at 25 °C. The slowing of relaxation observed after 2 s of contraction in mouse EDL must be due to other factors.

Possible causes of the late slowing of relaxation in EDL

Both inorganic phosphate (P_i) accumulation and acidosis are believed to slow down relaxation in amphibian muscles, probably via a depression of sarcoplasmic reticulum activity and/or a reduction of cross-bridge cycling rate (see for example Dawson, Gadian & Wilkie, 1980 and Hibberd, Dantzig, Trentham & Goldman, 1985, for the effect of phosphate, and Edman & Mattiazzi, 1981, and Curtin & Edman, 1989, for the effect of acidosis). In fatigued mouse fibres, the slowing of relaxation is also probably due to the combined effects of reduced rate of calcium pumping by sarcoplasmic reticulum and altered cross-bridge kinetics (Westerblad & Lännergren, 1991); the latter possibility is further supported by the results of Crow & Kushmerick (1983), showing a reduction of shortening velocity during isometric tetani in isolated mouse EDL at 20 °C.

In mouse soleus at 20 °C, the increase of P_i concentration is 5·6 mM during a 15 s tetanus (Crow & Kushmerick, 1982). Resting P_i concentration is not known in mouse muscles, but it is less than 2·5 mM in frog muscles (Gilbert, Kretzschmar, Wilkie & Woledge, 1971). As relaxation rate is nearly constant in mouse soleus, this implies that 6–8 mM-P_i does not significantly slow down relaxation. In EDL, this concentration is reached in only 2 s of tetanus, and is over 20 mM after 15 s. On the other hand, lactic acid concentration rises significantly after 6 s of tetanus in the EDL, while there is no lactic acid accumulation in the soleus (Crow & Kushmerick, 1982). Consequently, (1) the slowing of relaxation observed after short tetani in mouse EDL at 20 °C may be explained by calcium saturation of parvalbumin, but (2) sarcoplasmic reticulum depression and reduction of cross-bridge cycling rate, both due to P_i and lactic acid accumulation, are probably more important after 2 s of contraction.

Our results in mouse muscles show that the calcium-parvalbumin interaction is not the unique origin of labile heat and slowing of relaxation in these muscles. Both of these properties of striated muscles are probably due to several effects of different origins.

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