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EFFECT OF INTRACELLULAR pH ON FORCE AND HEAT PRODUCTION IN ISOMETRIC CONTRACTION OF FROG MUSCLE FIBRES

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SUMMARY

1. The intracellular pH (pH_i) of live fibres from the anterior tibialis of the frog *Rana temporaria* was measured at 10 °C (using pH-sensitive microelectrodes) in Ringer solutions containing a fixed bicarbonate concentration (20 mM) and varying P_{CO_2} concentrations of 0·5–54 %. As extracellular pH was changed from 7·99 to 6·00, mean pH_i changed from 7·24 to 5·97. Similar results were obtained at 20 °C.

2. In parallel experiments force and rate of heat production in 4 s isometric tetani at 10 °C were measured, and compared to control observations (5% CO_2, pH_i 6·80).

3. As the fibres became more acid (to pH_i 5·95), force and heat rate were progressively reduced (to 0·75 and 0·71 of the control values, respectively).

4. As the fibres became more alkaline (to pH_i 7·26), force increased slightly (by a maximum of 0·03 of the control value) but heat rate did not increase.

5. When the dependence on pH of the molar enthalpy change for phosphocreatine splitting is taken into account, these results indicate that the force–time integral per cross-bridge cycle increases with pH_i over this range.

INTRODUCTION

Exposing frog muscle fibres to CO_2 causes an acid shift of the intracellular pH (pH_i) to a new, steady value (Bolton & Vaughan-Jones, 1977; Abercrombie, Putman & Roos, 1983; Curtin, 1986*c*). Studies of both single fibres and whole muscles show that such a change in pH_i influences the mechanical aspects of contraction. Less force is produced under isometric conditions (Edman & Mattiazzi, 1981; Curtin, 1986*a*,*b*) and during shortening (Renaud & Stevens, 1984; Curtin & Rawlinson, 1984). In addition, there is a reduction in the maximum velocity of shortening (Edman & Mattiazzi, 1981) and in the rate of relaxation (Curtin, 1986*b*). Are these changes accompanied by changes in the turnover of cross-bridges? It could be that the number of active cross-bridges has decreased, causing a proportional change in force;

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alternatively the cross-bridge cycle itself could have changed so that a smaller impulse (force–time integral) is produced during each cycle.

The stable heat production during isometric contraction is largely due to the ATP usage by the cross-bridges and the resynthesis of the ATP from phosphocreatine by the creatine kinase reaction (Curtin & Woledge, 1979; Homsher, Kean, Wallner & Garibian-Sarian, 1979). Using the molar enthalpy change (ΔH_{obs}) for these reactions, the stable heat production can give a measure of the number and rate of cross-bridge turnover (Woledge, Curtin & Homsher, 1985). To gain some insight into how pH_i may be acting in its effects on force, we have measured the stable heat rate as well as the force during isometric contraction with a range of different CO_2 levels. In parallel experiments the intracellular pH was measured. Single fibres were used, so that a loss of excitability could easily be identified, and distinguished from other causes of force reduction. This distinction is difficult to make in whole-muscle experiments.

METHODS

Fibres or fibre bundles were dissected from the anterior tibialis muscles of the frog, *Rana temporaria*. The Ringer solution contained (mM): NaCl, 96·6; KCl, 2·2; $MgCl_2$, 1·0; $CaCl_2$, 1·8; $NaHCO_3$, 20; Ca–EGTA, 1·0. The pH of the Ringer solution was changed by varying the percentage of CO_2 (0·5–54%) in the O_2+CO_2 gas mixture, while bicarbonate concentration was kept constant.

Measurement of intracellular pH

The intracellular pH was measured using liquid-membrane pH-sensitive microelectrodes (Schulthess, Shijo, Pham, Pretsch, Ammann & Simon, 1981). Measurements were made on single fibres and bundles of fibres at 10 and 20 °C using methods similar to those described by Curtin (1986*c*). Preparations were slightly stretched over a fine glass rod and pinned by the tendons to a layer of silicone rubber in the experimental chamber. A pH-sensitive microelectrode and a conventional microelectrode (containing 3 mM-KCl) were inserted into the same fibre. The pH-sensitive microelectrode was connected to a Varactor Bridge operational amplifier (311J, Analog Devices Inc., Norwood, MA, U.S.A.). After amplification, the outputs of the two microelectrodes were subtracted and sent to a digital oscilloscope to give a record of intracellular pH. A flowing KCl electrode in the bath near the outlet was the reference. The membrane potential was recorded on the second channel of the oscilloscope. The pH microelectrodes were calibrated before and after each experiment. Ringer solution flowed through the experimental chamber continuously and could be changed without interrupting the flow. Intracellular pH was measured continuously whilst the solution bathing the preparation was changed.

Heat and force measurements

All the heat measurements were made on single fibres during isometric tetani. The methods were similar to those described by Curtin, Howarth, Rall, Wilson & Woledge (1986). Only a brief summary is given here.

A section (4 mm long) of a Hill–Downing type thermopile was used which contained thirty-two constantan–chromel thermocouples; each thermocouple produced 62 μV per degree at 10 °C. Thermopile output was sent to a chopper amplifier (Ancom C3A modified to chop at 1 kHz) and then recorded on a microcomputer, along with measurements of force. The thermopile frame was fixed on the floor of an anodized aluminium chamber which was mounted on a metal plate in contact with the water in a large Thermos flask. Fluid from the Thermos flask was circulated through channels in the aluminium lid of the chamber. A humidified mixture of O_2 and CO_2 was introduced near the bottom of the chamber at 50 ml min^{-1}.

The tendons of the fibre were held by T-shaped clips of platinum which were used as stimulus electrodes and as connections to the transducer and motor (see below). The fibre was mounted on the thermopile in Ringer solution equilibrated with 95% O_2+5% CO_2. One end of the fibre was

connected to an ergometer and the other to a force transducer (Model 300H and 401 respectively, Cambridge Technology Inc., Cambridge, MA, U.S.A.). The sarcomere length of the resting fibre was set to 2·25 μm using a He–Ne laser. After about 1 h of equilibration, the Ringer solution was drained from the chamber and the stimulus parameters were determined. Fibres were stimulated by square-wave pulses of 0·6–1·2 V and 2 ms duration at 20–30 Hz. Stimulus parameters were constant for each fibre. After recording three or four successive contractions in 5% CO_2 at 15 min intervals, the CO_2 content of the gas flowing through the chamber was increased or decreased. After various CO_2 concentrations had been used, records were generally again made for 5% CO_2. Heat loss was observed by Peltier heating at each CO_2 concentration. The time course of cooling could be described adequately as a sum of two exponential terms, and heat loss correction was carried out by the method of Curtin, Howarth & Woledge (1983). Total heat capacity of the fibre, thermopile and adhering Ringer solution was estimated from the initial slope of the record of Peltier heating (Kretzschmar & Wilkie, 1975; Curtin *et al.* 1986). No correction was made for stimulus heat which was probably about 5% of the total heat (Curtin *et al.* 1986). No correction was necessary for lag in the heat recording system.

At the end of each experiment the fibre was cut free of its tendons and clips. Adhering solution was removed and the dry weight of the fibre was measured on a Cahn 26 automatic electrobalance. Results for heat production were normalized by the dry weight, and force was normalized by fibre length at 2·25 μm sarcomere length and by dry weight (force × length ÷ weight).

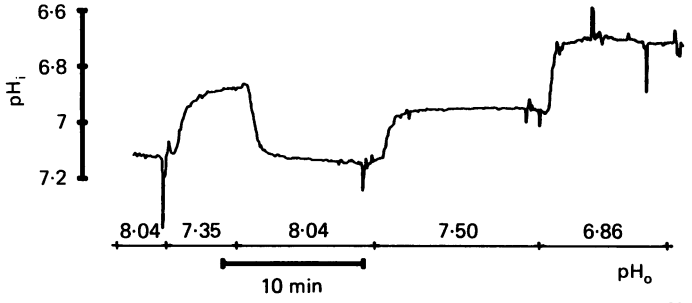

Fig. 1. Digital oscilloscope recording of intracellular pH (pH_i) of a muscle fibre at 20 °C. Ringer solutions of various pH (pH_o) values were used as indicated below the record.

RESULTS

Intracellular pH

Intracellular pH (pH_i) was measured in fibres in Ringer solution at pH values (extracellular pH; pH_o) between 6·00 and 7·99. The concentration of bicarbonate in the Ringer solution was constant (20 mM); differences in pH_o were due to differences in the CO_2 content of the gas mixture in equilibrium with the solution. Figure 1 is part of a recording from one fibre showing changes in pH_i as the extracellular solution was changed. The rate of change of pH_i was determined largely by the time required to change the solution in the experimental bath. The record shows that after pH_i reached each new level it remained relatively stable. The pH_i did not systematically overshoot the new value, nor did it return to its initial value on the time scale of these experiments. Measurements were made on three fibres at 10 °C and on three other fibres at 20 °C. The individual pH_i values for each extracellular pH are shown in Fig. 2*A* and the mean values in Fig. 2*B*. The strong dependence of pH_i on pH_o reflects the fact that CO_2 is a highly permeant acid.

Force and heat production in 5% CO_2

Typical records of force and of temperature change in a 4 s tetanus of a single fibre in 5% CO_2 are shown in Fig. 3*A*. The mean decrease in force during stimulation was

4·5% (S.D. 2·3% from nineteen observations on nine fibres). Heat production and its rate, calculated from the thermopile output, are shown in Fig. 3*B*. The time course of the heat production in this fibre was similar to that observed in whole muscle (see for example Curtin & Woledge, 1979). The rate of heat production is highest at the start of the tetanus, decreasing exponentially during the first few seconds to a slower,

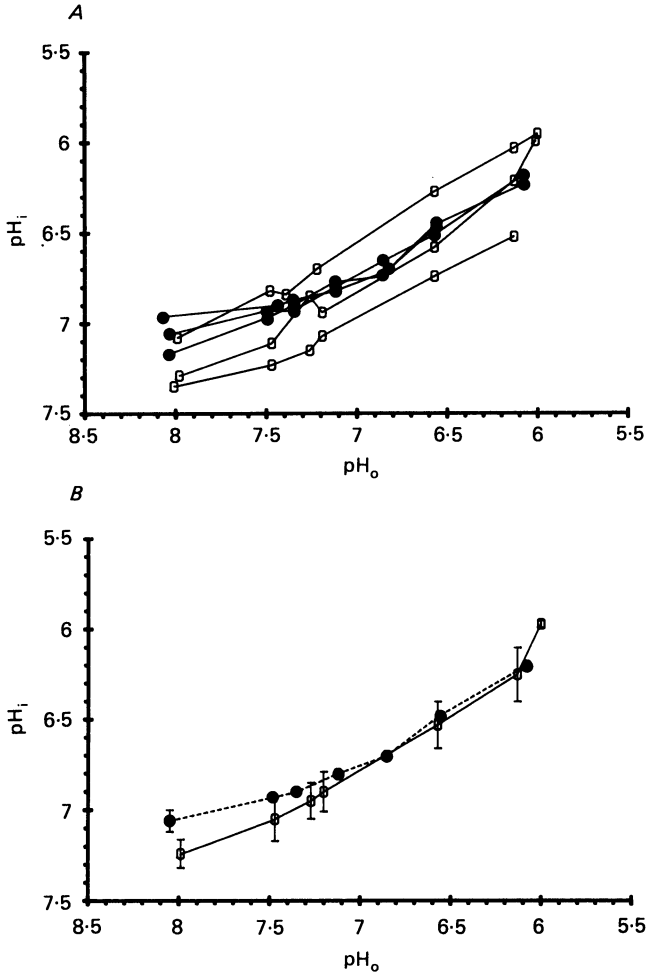

Fig. 2. *A*, measured values of pH_i for muscle fibres in Ringer solutions having various pH values (pH_o). Lines join points for the same fibre. ●, 20 °C; ○, 10 °C. *B*, mean values of pH_i at each value of pH_o. ●---●, 20 °C; ○——○, 10 °C. Bars show ±1 S.E.M.

constant rate which was taken as the stable maintenance heat rate (h_b). However, in other fibres these two phases in the rate of heat production were not clear and heat was produced at an almost constant rate throughout the contraction; this rate was therefore taken as h_b. The maximum force varied from 0·42 to 0·97 N m (g dry wt)$^{-1}$ for forty-one observations of nine fibres in 5% CO_2. The value of h_b varied from 0·210 to 0·442 watts (g dry wt)$^{-1}$. The stable heat rate is known to be very dependent on temperature (Aubert, 1956; Curtin *et al.* 1986). As the temperature

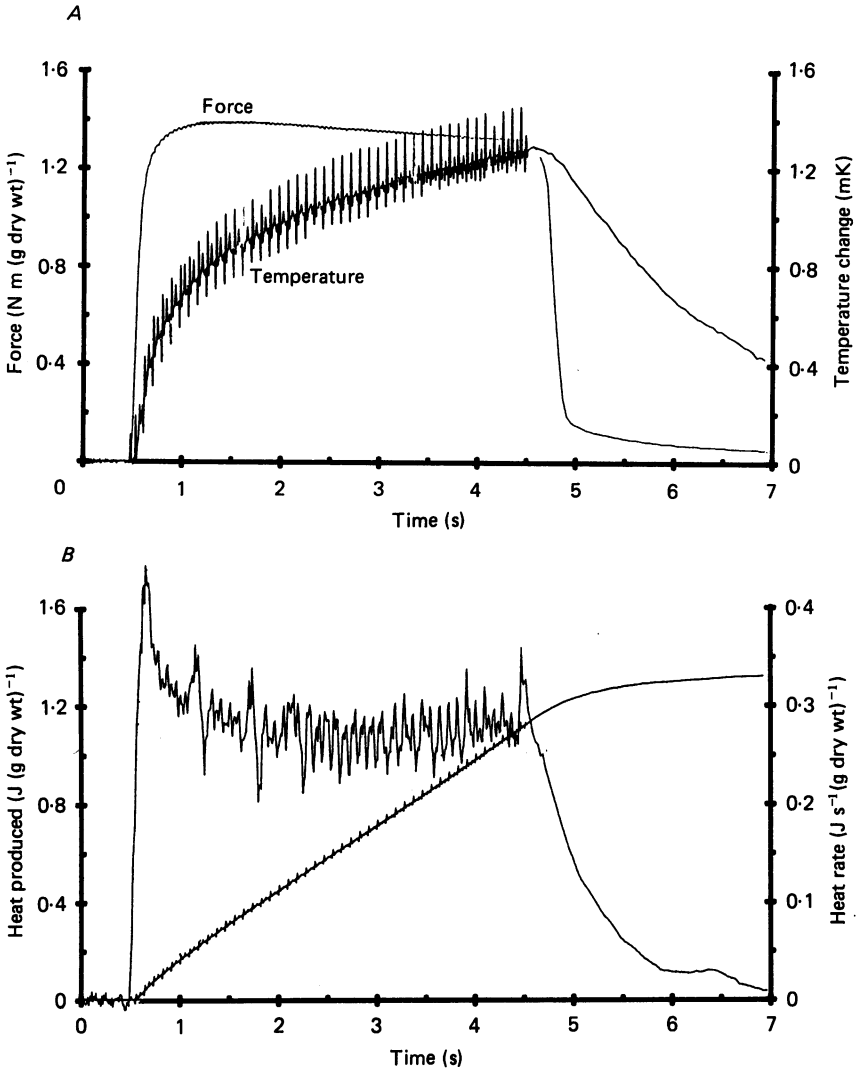

Fig. 3. *A*, records of force and of temperature change in an isometric contraction in 5% CO_2. The artifacts on the temperature record are caused by the stimulation (23 Hz) which started at 0·5 s and ended at 4·5 s. *B*, heat produced during the contraction and rate of heat production (the more noisy trace) calculated from the record of temperature change.

varied between experiments by up to 0·7 °C, a significant part of the variance in h_b could be due to this. We therefore calculated for each observation the value of h_b for 10 °C using a Q_{10} of 4·06 (Curtin *et al.* 1986). These corrected values of h_b had a variance 24% less than that of the uncorrected values, and were therefore used in the study of the effects of changing CO_2 concentrations. The temperature dependence of maximum force is much less (Q_{10} = 1·24; Edman, 1979) and so the 0·7 °C variation in temperature would change it by no more than ±1·5%. This was considered negligible and so no corrections were made. The correlation between heat rate and

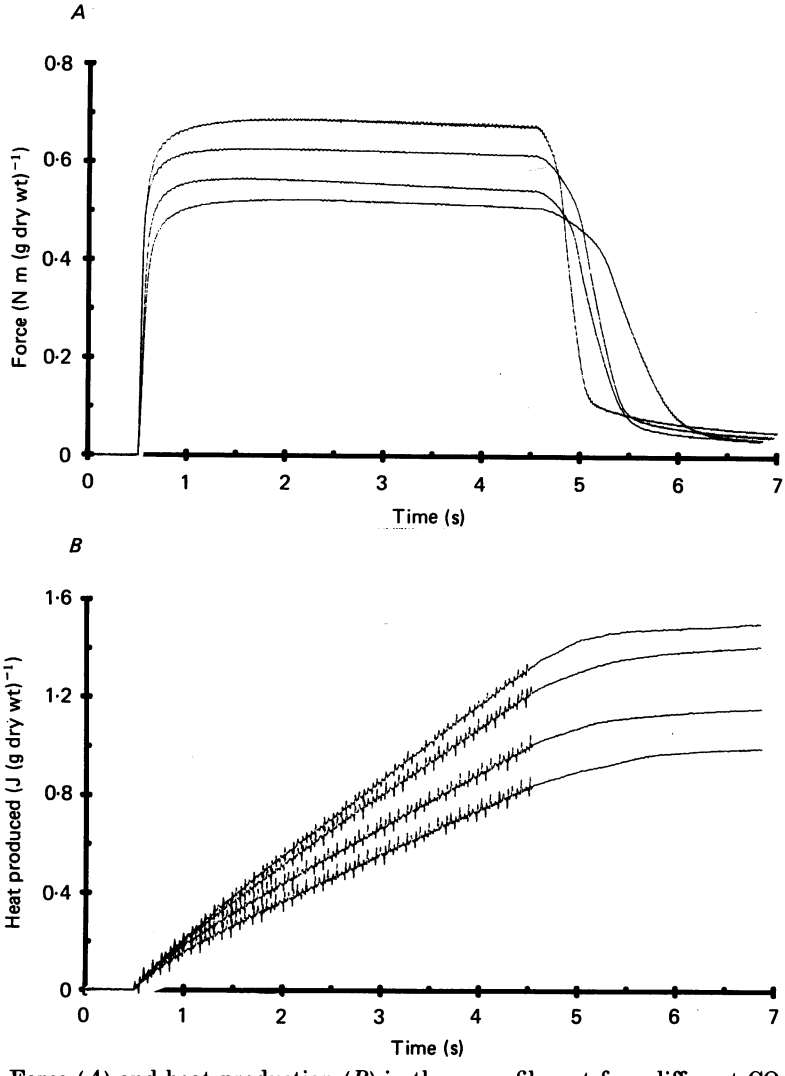

Fig. 4. Force (*A*) and heat production (*B*) in the same fibre at four different CO_2 levels, which were, from the top 5, 12, 24 and 54%. From experiment with fibre (see Fig. 5) a14.

maximum force for the nine fibres used was statistically significant ($r = 0{\cdot}714$, $P = 0{\cdot}031$). We note that this correlation coefficient is similar to the value found by Elzinga, Howarth, Wilson & Woledge (1985) ($r = 0{\cdot}675$) in a larger sample.

Responses to changes in the CO_2 concentration

Some examples of records of heat and force in different CO_2 concentrations are shown in Fig. 4. For CO_2 less than 24%, force and heat rate showed no consistent upward or downward trend during the set of three or four repeated tetani at fixed CO_2 concentration. However, in seven of the eight sets of observations with 24 and 54% CO_2, progressive decreases in force occurred. The mean extent of this decrease

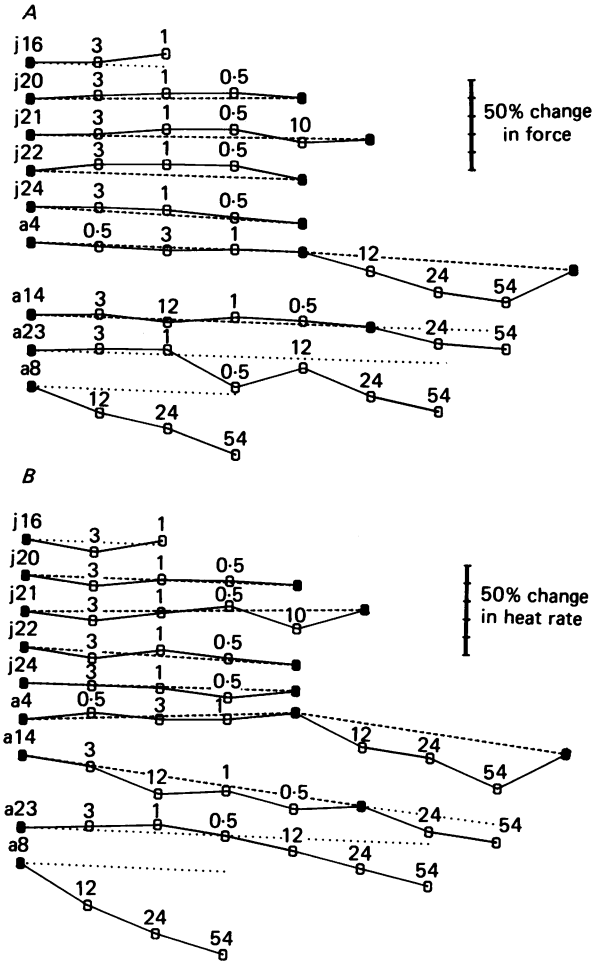

Fig. 5. Changes in maximum force (*A*) and changes in the stable heat rate (*B*) during the progress of experiments in which the percentage of CO_2 was changed. Results for nine individual fibres (labelled j16 etc.) are shown in the order the records were made. Each point is the mean value for 2–4 tetani at the percentage of CO_2 indicated above the point. Filled symbols are for 5% CO_2, which was taken as the control value (see text). Change in force or heat rate is expressed as a fraction of the initial control value. Continuous lines join the experimental points. Dashed lines join control values. Dotted lines were drawn with a slope equal to the mean slope of the dashed lines.

was 7·8% of control values. Heat rate did not show a similar decline. The mean values for the set of three observations at each CO_2 concentration will be used in further descriptions of the results.

Figure 5 shows the measurements of force (*A*) and of heat rate (*B*) in the sequence in which they were made. Each point is the mean of two to four observations. The dashed lines join the control observations (those in 5% CO_2) and show that force generally declined during the experiments; the mean rate of decline was 0·3% for each tetanus (S.E.M. 0·07%, $n = 7$). The decline in the heat rate had a similar mean

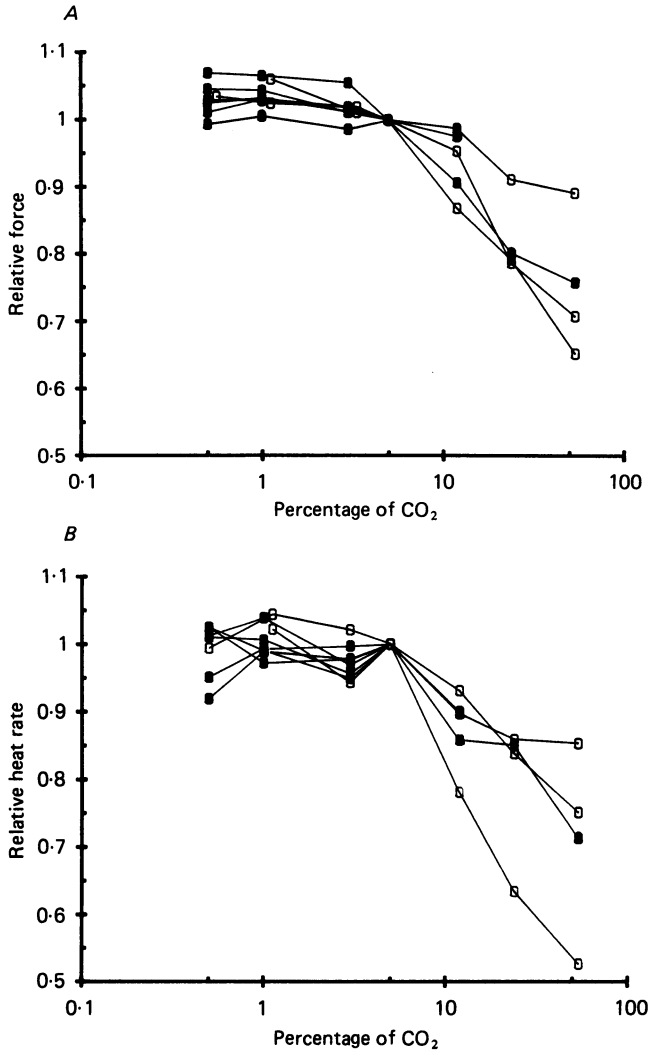

Fig. 6. Maximum force (*A*) and stable heat rate (*B*) during tetani in various percentages of CO_2. The values are expressed as a fraction of the interpolated (filled symbols) or extrapolated (open symbols) control values (see text). Each point is the mean of two to four tetani. The lines join points for the same fibre. When values superimpose, the open symbols have been displaced slightly to the right for clarity.

value but was much more variable ($0{\cdot}28 \pm 0{\cdot}19\%$ S.E.M. per tetanus). Because of these changes in the control values it seems best to analyse the effects of changing CO_2 concentrations by comparing the observed values to interpolated control values, that is to the dashed lines in Fig. 5. Figure 6 shows comparisons based on such controls (filled symbols). In experiments in which final control observations could not be made, the extrapolated lines (dotted) in Fig. 5 have been used to estimate an appropriate control value. These results, obviously less reliable are shown by the open symbols in Fig. 6.

Figure 7 shows the mean values of force and heat rate plotted against mean pH_i, based on the parallel measurements described above. Raising pH_i by lowering the CO_2 concentration below 5% has only small effects, but some of these are statistically significant (* in Fig. 7). It seems clear that force is increased when pH_i increases. However, the heat rate is not similarly increased; indeed for 3%

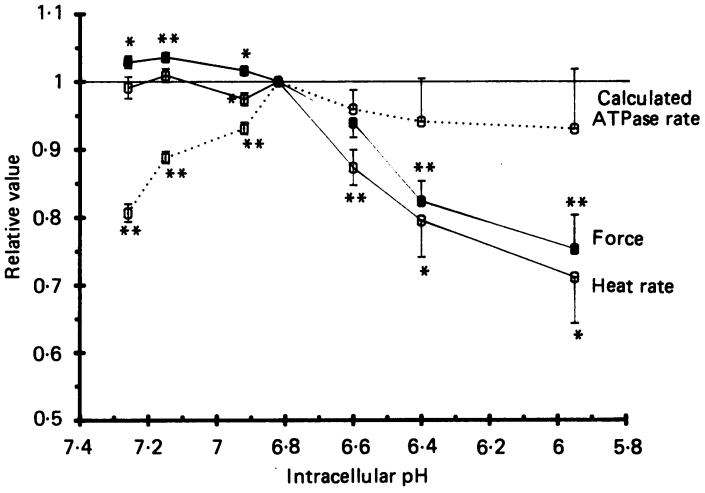

Fig. 7. Mean values of maximum force (filled symbols) and stable heat rate (open symbols) plotted against intracellular pH. The intracellular pH corresponding to extracellular pH values used when observing the heat and force were obtained from the mean line for 10 °C in Fig. 2*B*. Force and heat rate are expressed as a fraction of control values in 5% CO_2. The bars show ±1 S.E.M. where this is greater than the diameter of the symbols. Results for nine fibres; asterisks indicate that the mean is significantly different from 1·0 (*$P < 0.05$; ** $P < 0.01$).

CO_2 the heat rate is significantly *less* than the control value. While these changes are probably genuinely consequences of the change in CO_2 level, it should be remembered that they are of the same order of size as the progressive changes occurring during these experiments (see Fig. 5). The experiments reported here were not specifically designed to separate these small changes from one another.

When pH_i is made more acid by raising the CO_2 concentration both force and heat rate are reduced, and to a similar extent. These changes (illustrated in Fig. 4) are much larger than those seen with CO_2 levels below 5%. In three out of four fibres used in 24 and 54% CO_2, the rate of rise of force became slower than in the control observations, the maximum force was reached much later, and force hardly declined at all during the tetanus. The stable heat rate was constant during the period between 2 and 4 s of stimulation in all concentrations of CO_2.

DISCUSSION

The effects on the mechanics of muscle contraction of changing CO_2 concentration have been studied previously by many investigators (for example Edman & Mattiazzi, 1981; Curtin, 1986*a*), who have noted the lower maximum tetanic force

and slower relaxation that are characteristic of increasing CO_2 concentrations. The energetic consequences of changing CO_2 have been studied previously in whole muscle by Kitano, Kometani, Tanokura & Yamada (1986). They investigated CO_2 concentrations between 5 and 45% and found that the stable heat rate was decreased as CO_2 concentration was raised. Our observations in this paper confirm this general trend, and show that the effect is not solely due to the failure of activation, which could have caused the effects found by Kitano *et al.* (1986) in whole sartorius muscles. As the decrease in heat rate they observed (to 0·37 of the control value in 50% CO_2) was almost twice as great as that reported here (to 0·71 for 54% CO_2), part of their effect may have been due to activation failure in some of the fibres in whole sartorius.

We can now consider the question why force declines as pH_i becomes more acid; two extreme possibilities are: (1) there are fewer cross-bridge cycles each producing the same force–time integral, and (2) each cycle produces a lower force–time integral. Possibility (2) is supported by the finding that lowering pH_i reduces the maximum velocity of shortening (Edman & Mattiazzi, 1981) which shows that the kinetics of the cross-bridge cycles, not just their number, is altered. To use our experimental evidence to address this question we have to consider the chemical origin of the stable heat rate. It was shown by Curtin & Woledge (1979) and by Homsher *et al.* (1979) that this component of the heat production can be entirely explained (in frog sartorius muscle at 0 °C) by the heat produced by the splitting of ATP and its almost immediate resynthesis from phosphocreatine by the creatine kinase reaction.

We shall therefore assume that in anterior tibialis fibres at 10 °C it is also the case that the net reaction producing the stable heat is phosphocreatine splitting. The amount of heat produced by each mole of phosphocreatine split (ΔH_{obs}, molar enthalpy change) is a function of pH because the number of moles of H^+ ions absorbed in the reaction is a function of pH, and because part of the heat is evolved by the shift of these protons from the intracellular buffers to the HPO_4^{2-} ions formed by phosphocreatine splitting. Thus heat rate during contraction measures the rate of phosphocreatine splitting (and hence ATP splitting) with a scale factor (ΔH_{obs}) which is pH dependent.

Consider first what happens to ΔH_{obs} as pH_i is lowered from 6·82 (the value in the control conditions, 5% CO_2) to 5·95. The value of ΔH_{obs} declines over this range of pH_i from -33 to -26 kJ mol^{-1} (Woledge & Reilly, 1988), assuming that intracellular buffering is due mainly to the histidine and carnosine which are known to be present in frog muscle. This assumption about the identity of the buffers is probably correct because the observed buffer power in this pH range is not significantly different from that expected for these buffers (Curtin, 1986*c*). Using these values for ΔH_{obs} we can calculate from the observed heat rate the rate of phosphocreatine splitting at each pH. As shown in Fig. 7, the value at pH_i 5·95 is 0·91 (±S.E.M. 0·09) of that at pH_i 6·82; this change is not statistically significant. In contrast, the force declines significantly to 0·75 (±S.E.M. 0·05) of the control value. Thus it seems likely that as pH_i becomes more acid in this range, there is a decline in the mechanical impulse (tension–time integral) for each cross-bridge cycle.

As pH is increased from 6·82, the control value, ΔH_{obs} will also increase (Woledge & Reilly, 1988). There is some uncertainty in quantifying this increase because in this

pH region the measured buffer power in muscle exceeds that predicted from the known intracellular buffers (Curtin, 1986*c*). Thus, part of the buffering is due to other processes or buffers different from carnosine and histidine. But the contribution of heat from these reactions decreases as pH becomes more alkaline because fewer H^+ ions are absorbed by phosphocreatine splitting. Even making extreme assumptions about the nature of the buffering processes operating at pH 7·26 (the highest pH_i observed), the value of ΔH_{obs} for phosphocreatine splitting is unlikely to be outside the range -37 to -43 kJ mol^{-1}, an increase of 15–30% over the control value. The observed heat rates do not increase in this way (indeed one of the observations shows a significant reduction). Therefore it is necessary to suppose that the rate of ATP splitting is reduced (by about 20%) as pH_i is increased from 6·82 to 7·26. By contrast tension is increased.

About a quarter of the total ATP split in muscle contraction is used by the sarcoplasmic reticulum calcium pump (Curtin & Woledge, 1981). This ATPase activity is lowered by increased acidity *in vitro* (MacLennan, 1970), and *in vivo* it is probably this effect that causes the obvious slowing of relaxation produced by increasing CO_2 concentration (for example Fig. 4, and Curtin, 1986*b*). This contrasts with the total ATP splitting, which, as we have just seen, is increased by increased acidity in the range pH 7·26–6·82. Thus the activity of the actomyosin ATPase, which is about three-quarters of the total in 5% CO_2, must increase with acidity rather more than does the total ATPase.

Thus, in summary, the mechanical impulse per molecule of ATP split increases with pH_i over the whole of the pH_i range investigated. In quantitative terms, the tension–time integral produced by each cross-bridge cycle is at least 40% greater when the intracellular pH is 7·26 than when it is 5·95.

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