Recovery after contraction of white muscle fibres from the dogfish Scyliorhinus canicula

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Summary

Recovery after contraction of white muscle fibres of dogfish was investigated using 31P-NMR and measurements of heat production. The muscle fibres were stimulated to perform either a single isometric tetanus or a series of brief isometric tetani; the NMR measurements showed that approximately half of the phosphocreatine (PCr) was used. The period of activity was followed by a recovery period without stimulation.

Both NMR and heat measurements agreed in showing that recovery was very slow, requiring at least 60 min for PCr resynthesis and for the production of recovery heat. The NMR results showed that changes in intracellular pH and in the concentrations of PCr and intracellular phosphate (P_i) had very similar time courses. Intracellular pH moved in the alkaline direction during the period of activity and then returned monotonically during recovery. The non-phosphate buffer power was 13.0±3.1 mmol l⁻¹ intracellular water per pH unit (N=4, mean ± S.E.M.).

The results are consistent with the view that oxidative processes resynthesize PCr during recovery, which is slow because of the low mitochondrial content of these muscle fibres.

Key words: muscle contraction, recovery, energetics, dogfish, Scyliorhinus canicula, 31P-MRS, 31P-NMR, magnetic resonance spectroscopy, nuclear magnetic resonance, heat production, buffer power, buffer capacity, intracellular pH.

Introduction

Dogfish, like most fish, contain two major types of muscle, red and white; the difference in colour is very obvious on visual inspection. As in most fish, the fibre types are well segregated, the white fibres making up the main part of the body and the red fibres being confined to a thin layer just under the skin along the midline. Dogfish use red muscle during slow, sustained swimming, while white fibres remain silent. During vigorous bursts of movement, white muscle is recruited (Bone, 1966; Mos et al. 1990) and presumably powers high-speed swimming.

Studies of isolated fibres from dogfish have shown that the red and white types have very distinct mechanical and energetic properties, although both are twitch fibres (Curtin and Woolledge, 1993b). Compared with red fibres, the white fibres are faster (brief twitch, higher V_{max}) and have higher rates of energy output, both as power and as heat. Red fibres, however, are more efficient; that is, more work is done per unit of energy output (Curtin and Woolledge, 1993a,b, 1996). Thus, mechanical and energetic properties match the requirements of in vivo performance.

Less is known, however, about the metabolic reactions supplying ATP in dogfish fibres. Bone et al. (1986, their Table 1) measured the mitochondrial content and found that it was much lower in the white than the red fibres (percentage mitochondrial volume, 1 and 22%, respectively). Given that this pattern is typical for the white and red muscles of other animals, we might expect the metabolic profiles of the two types of dogfish fibre to be similar to those of other species. For example, it is likely that white fibres have a lower oxidative capacity than red fibres and are more reliant on glycolytic production of ATP with consequent production of lactate + H⁺.

Two reasonable hypotheses can be made about the mechanism of metabolic recovery in white fibres from dogfish. (1) It may be completely oxidative. In this case, recovery would be slow, but behavioural differences mean that quick replenishment of ATP is not important in dogfish. (2) Net glycolysis, forming lactate + H⁺, may be a major source of ATP in these fibres, which might allow more rapid resynthesis of ATP to be achieved.

The aim of the experiments was to observe recovery
metabolism and heat production and compare them with these two extreme hypotheses.

Materials and methods

Dogfish, Scyliorhinus canicula (L.), from the Plymouth Marine Laboratory (Plymouth, UK) were used for all experiments. Fish were killed by decapitation followed by destruction of the brain and spinal cord. Bundles of fibres were dissected under saline from thin slices of the white myotomal muscle taken from the immediate post-anal region. The saline contained (in mmol 1⁻¹): NaCl, 292; KCl, 3.2; CaCl₂, 1.8; MgCl₂, 2.2; Na₂SO₄, 3.5; NaHCO₃, 5.9; urea, 483; and tubocurarine, 1.5 mg 1⁻¹. The composition is based on the standard Plymouth elasmobranch saline.

³¹P nuclear magnetic resonance spectroscopy experiments

Preparation of fibre bundles

Experiments were performed in the Department of Radiology, University of Washington, Seattle, WA, USA. Fish were air-freighted to Seattle and held for less than 4 weeks in isolation tanks at the Seattle Aquarium until use.

Phosphorus metabolite levels were measured with the fibre bundle mounted in a custom-built probe similar to that described by Wiseman et al. (1993) with slight modifications (Wiseman et al. 1996). Briefly, the coil was an eight-turn solenoid of 30 gauge wire, wound around a glass capillary tube (2.2 mm internal diameter). This tube was mounted horizontally between two pots made from Delrin and Teflon. The pots acted as reservoirs for the superfusate and as a mechanically stable foundation for the coil. The force transducer was mounted inside one of the pots and the other contained a fixed hook. The force transducer consisted of wire strain gauges glued to a strip of glass coverslip (0.2 mm thick); the gauges and glass were insulated with silicone coating (Dow 3140). Each pot contained a platinum stimulating electrode.

After dissection of fibres, a piece of myoseptum at each end of the fibre bundle was tied with silk suture. The muscle fibre preparation was placed inside the capillary glass tube and centred with respect to the coil. One end of the fibre bundle was attached to the force transducer and the other to the fixed hook. The bundle was electrically stimulated by passing current between the stimulating electrodes. During the experiment, saline was pumped from an external source into one pot, flowed through the glass tube and was pumped out of the other pot at a rate of approximately 0.5 ml min⁻¹. Temperature within the probe was within the range 12.2–13.3 °C and was maintained constant within each experiment.

The stimulus voltage–twitch tension relationship of each fibre bundle was investigated to establish supramaximal stimulus strength to be used in the experiment.

Cycles of stimulation+recovery

Each cycle consisted of a single, 18 s isometric tetanus or a series of brief isometric tetani, followed by a recovery period. When a series of tetani was given, each tetanus consisted of three stimulus pulses with 30 ms between pulses; one tetanus was given every 0.5 s until a set of 20 or 80 tetani had been completed. A recovery period without stimulation followed. The interval between the start of successive stimulus+recovery cycles was 120 or 117 min (constant within each experiment). Between 2 and 11 stimulation+recovery cycles were given to each muscle preparation. The peak force and the total integral of force and time (tension–time integral) were measured for each cycle of stimulation-recovery.

Maintenance of the ATP and PCr supply

In the longest experiments, the muscle fibres were in the spectrometer for more than 20 h. To assess the metabolic state of the fibres during such long periods, some fibre preparations were kept in saline at 4 °C for up to 4 days after dissection, then frozen, and extracts were analyzed for ATP, PCr and Pᵢ by HPLC. Total creatine was the sum of phosphocreatine (PCr) and creatine (Cr) measured by separate methods. The results showed that ATP content and total creatine content (Cr+PCr) were stable for 2 days and then declined dramatically. The results indicated that, under resting conditions at least, the metabolic energy supplies were maintained for periods as long as the duration of the NMR experiments.

Acquisition and analysis of ³¹P-NMR spectra

Spectra were acquired using a 7T GN300 General Electric Omega Spectrometer. Magnetic field homogeneity was optimized by adjusting the room temperature shims using the available proton signal from the sample (muscle and superfusate water) and was usually less than 0.1 p.p.m. The π/2 pulse duration at a nominal power of 50 W was 8.2 μs. Phosphorus spectra were obtained using a π/4 pulse and a 2.4 s recycle delay, 2048 complex data points and a 5 Hz sweep width. Data were filtered using a 15 Hz exponential and zero-filled once prior to the Fourier transform.

For absolute quantification of spectral areas, each resonance was corrected for partial saturation using the T₁ values. Spin-lattice relaxation times (T₁ values) were obtained from four experiments on resting fibre bundles using a T₁ inversion recovery sequence (180–Tr–90). The recycle delays (Tr values) were 0.05, 0.20, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0 and 15.0 s. T₁ values were estimated using the equation:

\[ y = A[1 - (W + 1)\exp(-x/T₁)], \]  

where \( y \) is the observed peak intensity, \( A \) is a scaling factor, \( W \) accounts for incomplete peak inversion and \( x \) is the recycle time. The resulting T₁ values were 3.0 for PCr, 4.2 for Pᵢ and 1.9 for γ-ATP. Saturation factors were calculated from the T₁ values using the equation:

\[ I = [1 - \exp(-Tr/T₁)\cosθ]/(1 - \exp(-Tr/T₁)\sinθ), \]  

where \( I \) is peak intensity, pulse angle \( θ \) is 45 ° and \( Tr \) is the recycle delay. Saturation factors calculated from the T₁ values were 1.75 for PCr, 1.95 for Pᵢ and 1.58 for γ-ATP.

Preparation size

At the end of each experiment, the fibre bundle was removed from the probe, rapidly blotted of excess water and frozen with
brass Wollenberger tongs precooled to the temperature of liquid nitrogen (−80°C). Non-fibre material was removed under liquid nitrogen and the fibre bundles were weighed while still frozen. The wet masses ranged between 13.1 and 23.5 mg.

**Quantification of PCr, Pi, ATP and intracellular pH**

Muscle extracts were prepared by a perchloric acid extraction method and analyzed by HPLC as described by Wiseman et al. (1992). The NMR spectral peaks were integrated using a commercially available software package (NMRi) assigning an asymmetric Lorentzian line-shape to each resonance. For each spectrum, the integral for each of the three relevant peaks (PCr, Pi and ATP) was multiplied by the appropriate saturation factor. Each of the corrected integrals was then expressed as a fraction of the summed corrected integrals for PCr, Pi and γ-ATP. The resulting ‘corrected fraction’ for γ-ATP was averaged for all the spectra for an individual muscle; this value was taken to be equivalent to integrals for PCr, Pi and the appropriate saturation factor. Each of the corrected integrals was averaged for all the spectra for an individual muscle; this value was taken to be equivalent to integrals for PCr, Pi and the appropriate saturation factor.

Intracellular pH was calculated from the chemical shift of the Pi resonance from titration curves of model solutions at 15 °C as previously described (Wiseman et al. 1996). Solutions contained (in mmol l−1) KCl, 100; ATP, 5; PCr, 15; EGTA, 4; K$_2$HPO$_4$, 1; urea, 483; Mops buffer, 100; potassium acetate, 92; and Tris, 70. Spectra were acquired in a 10 mm commercially available phosphorus NMR probe using an internal reference of dimethyl methyl phosphonate was used.

**Buffer power**

Buffer power is 1/slope of the titration curve of a buffer (the relationship between added acid or base and pH). Our objective was to obtain the titration curve and buffer power of the non-phosphate buffer in the muscle. These can be calculated from the observed pH and Pi concentration ([Pi] = [H$_2$PO$_4$] + [HPO$_4^{2-}$]) using the pK value for the following reaction:

\[
\text{H}_2\text{PO}_4^- \rightarrow \text{HPO}_4^{2-} + \text{H}^+ .
\]  

(3)

We have assumed that this is the only reaction that adds hydrogen ions to the intracellular water during recovery under the conditions used here. In addition, we assume that buffering occurs:

\[
\text{buffer}^- + \text{H}^+ \rightarrow \text{Hbuffer} .
\]  

(4)

As the free hydrogen ion concentration is always very small compared with the concentrations of phosphate and buffer, the extent of reactions 3 and 4 can be taken as equal. The extent of reaction 3 is equal to the change in [H$_2$PO$_4$] but not to the change in [HPO$_4^{2-}$] because this is removed by:

\[
\text{HPO}_4^{2-} + \text{Cr} \rightarrow \text{PCr}^{2-} .
\]  

(5)

Thus, the required titration curve can be made by plotting pH against [H$_2$PO$_4]$ [H$_2$PO$_4$] from the observed Pi and pH and the pK value for reaction 3 (6.7, Bates and Acree, 1945) using the equation:

\[
[\text{H}_2\text{PO}_4^-] = [\text{Pi}]/(1 + 10^{\text{pH}-\text{pK}}) .
\]  

(6)

The non-phosphate buffer power can be converted from units of µmol g$^{-1}$ wet mass of muscle per pH unit to the conventional units (mmol l$^{-1}$ intracellular water per pH unit) using the value of 0.693 for the intracellular water as a fraction of wet muscle mass in the shark (Scyliorhinus stellaris) from Heisler et al. (1980).

**Recovery heat experiments**

Heat production during and after stimulation was measured by conventional myothermal techniques (Hill, 1965; Woledge et al. 1985), and only a brief outline is given here. The muscle fibre preparation was mounted on a vertically oriented thermopile, which consisted of antimony–bismuth thermocouples (four couples per mm) with a thermal electromotive force of 83.2 µV degree$^{-1}$ couple$^{-1}$. Recordings were made from a 2 mm length of thermopile.

The thermopile and muscle fibre preparation were in a chamber partly filled with saline (temperature 9.7–11 °C, constant within each experiment). Before starting to record thermopile output, the thermopile+muscle preparation was moved from the saline to the gas phase above the saline, and only a small volume of saline remained around the muscle preparation. The gas phase above the saline was saturated with water vapour.

The fibre preparation was electrically stimulated end-to-end with 0.2 or 1 ms pulses. In each experiment, the stimulus strength and muscle fibre length were adjusted to achieve maximum isometric force.

**Cycles of stimulation+recovery**

Three muscle fibre preparations (from different fish) were stimulated to produce either a single isometric tetanus (5, 10 or 20 s) or a series of brief isometric tetani. In the experiments on series of tetani, each tetanus consisted of three stimuli at 30 Hz and was repeated at 0.5 s intervals until 20, 40, 60 or 80 tetani had been completed. A recovery period of 67 min followed the single 5 and 10 s tetani; the recovery period was 133 min in all other cases. Two to ten observations with the same stimulus pattern on the same muscle were averaged to give the eight results reported here. The peak force and total integral of force and time were measured for each cycle of stimulation+recovery.

**Quantification of heat**

The thermal baseline was assumed to be a straight line joining the thermopile output before stimulation to that at the end of the recovery period. After subtraction of this baseline, the record was corrected for heat loss and for stimulus heat, which was determined from recordings from inexcitable muscle. The thermopile output was converted to values of heat.
production using characteristics determined for each fibre preparation by passing a known current through the whole thermopile. This produces a known quantity of heat due to the Peltier effect (Woledge *et al.* 1985).

The amount of heat produced from the start of stimulation to the end of relaxation was taken as initial heat. Recovery heat is the heat produced from the end of relaxation to the end of the recovery period.

At the end of each experiment, the length of the fibres was measured under a stereomicroscope and all non-fibre material was carefully removed. The fibres were dried at room temperature and weighed on a Cahn electrobalance. Wet mass is 4.9 times the dry mass (Curtin and Woledge, 1993a). The wet masses ranged between 5.2 and 9.6 mg. The initial and recovery heat are expressed relative to the wet mass of the preparation.

**Results**

**Metabolic changes during stimulation and recovery**

Fig. 1 shows an example $^{31}$P-NMR spectrum from a resting muscle fibre preparation acquired in 5 min. The chemical shift positions of $P_i$, PCr and the $\gamma$, $\alpha$ and $\beta$ resonances of ATP were 2.3, $-2.54$, $-5$, $-10$ and $-18.5$ p.p.m. (relative to phosphoric acid) respectively.

![Fig. 1. $^{31}$P-NMR spectrum from resting muscle fibre preparation. The chemical shift values were 2.3, $-2.54$, $-5.0$, $-10.0$ and $-18.5$ p.p.m. for $P_i$, PCr and the $\gamma$, $\alpha$ and $\beta$ resonances of ATP, respectively. 128 data transients were acquired with a $\pi/4$ pulse width, 2.4 s pre-delay and a 5 kHz sweep width. Total acquisition time was 5.0 min. The data were filtered with a 15 Hz exponential prior to Fourier transform.](image)

Fig. 2 shows the levels of $P_i$ and PCr during one experiment lasting 20 h. Results for nine stimulus+recovery cycles are shown. During each cycle, the muscle was stimulated intermittently (three stimuli at 30 Hz every 0.5 s) for a 40 s period; in the third cycle, the stimulus pattern was different, and the results are therefore not shown. Each period of stimulation was followed by a recovery period without stimulation. This pattern was repeated at 2 h intervals. $[P_i]$ increased and $[PCr]$ decreased during the periods of stimulation. About half of the PCr was broken down during

![Fig. 2. Concentration ($\mu$mol g$^{-1}$ wet mass) of (A) $P_i$ and (B) PCr, and (C) intracellular pH, of a preparation of white muscle fibres measured at 5.4 min intervals during a 20 h experiment which included 10 cycles of stimulation and recovery. The preparation was stimulated intermittently during a 40 s period followed by a recovery period without stimulation; the stimulus+recovery pattern was repeated at 2 h intervals. The third cycle is not included because the stimulation pattern was different from the others.](image)
stimulation (Fig. 2B). Subsequent recovery of these metabolite levels was slow and occupied most of the 2 h intervals.

The results in Fig. 2 show that the sudden changes in P_i (or PCr) which occurred during stimulation were about the same in each of the 9 cycles. In contrast, the concentration reached at the end of each recovery period was not constant. For example, the P_i concentration at the end of recovery increased in successive cycles, and the PCr concentration decreased. One possible explanation is that recovery is progressively less complete in successive cycles. Alternatively, P_i and PCr levels may change owing to a process with a time course much longer than that of a stimulation-recovery cycle.

To quantify the changes in [P_i] and [PCr] during each recovery period, we compared the current value at each time with a baseline joining the end points of successive stimulation-recovery cycles, thus removing the effect of the slow, progressive change described in the paragraph above. The changes for the nine cycles shown in Fig. 2 were averaged and the results are shown in Fig. 3. The time courses with which concentrations of P_i and PCr and intracellular pH change are very similar. The rates of change are greater in the first 60 min than later, and the changes appear to be complete by 100 min. In this muscle fibre preparation, the changes are half complete in 42, 38 and 34 min for P_i, PCr and pH respectively.

Intracellular pH and non-phosphate buffer power

Fig. 2C shows that pH changed with a time course similar to that of P_i and PCr concentrations during the recovery period; Fig. 3B shows pH averaged for the nine stimulus-recovery cycles in one experiment. The pH changed in the alkaline direction during the period of stimulation, as would be expected for PCr splitting, which absorbs hydrogen ions. During the recovery period, pH moves in the acid direction, as expected for reversal of PCr splitting. Note that pH was never more acid than its final value at the end of the recovery period.

The mean pH values at the end of recovery, averaged for all cycle, were 6.55, 6.64, 7.21 and 6.59 in the four preparations measured. In the first three of these preparations, pH was also observed before the stimulation protocol was started and the values were 6.65, 6.40 and 7.07, respectively.

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Fig. 3 shows the changes in pH plotted against the changes in [P_i] during recovery for one of the four experiments in which pH was measured; each value is the average at one time point for all the stimulus-recovery cycles in that experiment. The relationship is linear in this example and in the other three experiments (results not shown). The linearity of these relationships is consistent with the idea that the only metabolic reaction producing hydrogen ions during recovery is the reversal of PCr splitting and that there is a negligible net production or absorption of hydrogen ions by other reactions. If hydrogen ions are involved in other reactions, these reactions must be occurring with a time course similar to that of reversal of PCr splitting.

Assuming that the reversal of PCr splitting is the only reaction causing a net change in levels of hydrogen ions, the results can be used to determine the non-phosphate buffer power, as described in the Materials and methods section. Fig. 4B shows the titration curve of [H_2PO_4^-] plotted against pH for one of the muscle fibre preparations (same preparation as Fig. 2) during its first cycle of stimulation-recovery. There is no evidence of systematic variation from linearity; the non-phosphate buffer power determined from this titration curve is 10.3 mmol l^{-1} pH unit^{-1}.

The same calculation was made with each individual stimulus-recovery cycle and the results are shown in Fig. 4C. As the experiment progressed, the lines became steadily steeper; that is, the buffer power declined (first cycle 10.3 mmol l^{-1} pH unit^{-1} and last cycle 2.05 mmol l^{-1} pH unit^{-1}). The lines cross each other, indicating that there was no major shift, during the experiment, in the range over which pH changed during successive the stimulus-recovery cycles. Thus, the change in buffer power cannot be due to a shift of pH away from the pK of the predominant buffer(s). The buffer power was measured in four muscle fibre preparations. The results for the first stimulus-recovery cycles were 10.8, 22.3, 10.3 and 8.7 mmol l^{-1} pH unit^{-1}; 13.0±3.1 mmol l^{-1} pH unit^{-1} (mean ± S.E.M.).
Heat production during stimulation and recovery

Fig. 5 shows the time course of force and heat production in one experiment with the same stimulus protocol as that used in the NMR experiments. Ten stimulus+recovery cycles were recorded and the mean values are shown. Fig. 5A shows the force during the 40 s period of intermittent stimulation. Peak force is highest in the early part of the series and then declines. Relaxation becomes progressively slower and less complete during the series of contractions. Fig. 5B shows the heat production during stimulation up to the end of relaxation (the initial heat). Heat is produced at a fairly constant rate during the stimulation period, and the heat rate falls to a very much lower value after the end of stimulation. Fig. 5C shows the heat produced during the whole of the recovery period starting from the end of relaxation. It is complete by approximately 90 min.

Comparison of heat and chemistry

Fig. 6A,B shows the progress of recovery for the six NMR experiments and the heat observations which give the best information about the time course of recovery. The recovery curves could not be fitted with a simple function suitable for all of them. That this is so is particularly apparent in the heat results in Fig. 6B, for which the signal-to-noise ratio is excellent. Although two fibre preparations (broken lines) gave approximately exponential time courses, the third fibre preparation (solid lines) consistently had more complex kinetics (see below). Therefore, to quantify the time required for recovery, we measured for each curve the time required for 50% recovery. Both NMR and heat measurements show that recovery is slow, requiring more than 60 min to reverse the changes that had occurred during stimulation. The half-times for recovery overlap, being 15–25 min in the heat experiments and 24–52 min in the NMR experiments. The measurements on the NMR results probably slightly overestimate the true half-time because the first chemical observation in recovery was not made immediately after the end of stimulation; there was a delay of up to 4 min and during this time some undetected recovery would have occurred. As can be seen in Fig. 6, the heat production is complete within 120 min, suggesting that recovery is complete, whereas the NMR results suggest that recovery might continue for longer.

Some features of the variation in the results in Fig. 6 merit comment. Fig. 6B shows that one of the fibres (solid lines) consistently produced heat at a rapid rate for a few minutes after the end of stimulation. However, the other two fibre preparations (broken lines) did not. A brief, rapid phase of recovery could not have been detected in the NMR experiments because the time resolution was insufficient (up to 4 min between the end of stimulation and the first NMR observation). Variability of recovery heat production has been an obvious feature in previous studies, its time course often being non-exponential (Hartree and Hill, 1922; Hill, 1965; Godfraind-de Becker, 1972, 1973, 1989; Phillips et al. 1993). The cause of the variation remains to be identified. The variation among the NMR results for different
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preparations of fibres is also noteworthy, since this feature could not have been detected in studies in which spectra were collected from a group of several muscle in the spectrometer at one time (Dawson et al. 1977; Yamada and Tanokura, 1983; Tanokura and Yamada, 1984; Kawano et al. 1988; Phillips et al. 1993).

If the variation among the different muscle preparations could be reduced, it would be instructive to make a more detailed comparison of the time course of recovery metabolism with that of heat production.

Discussion

Slow recovery

As stated in the Introduction, the low mitochondrial content of white muscle fibres from dogfish has implications for recovery after a period of stimulation. If oxidative metabolism alone rebuilds PCr levels, recovery would progress slowly. Alternatively, the PCr level could recover more quickly if, in addition, some net glycolysis occurs, that is, if pyruvate was produced at a higher rate than it is consumed by oxidation, with the result that lactate + H⁺ is produced.

Both NMR observations and the measurements of heat production reported here show that recovery in dogfish white muscle is very slow. It requires 1–2 h to reverse the breakdown of PCr that occurs during approximately 1 min of activity and uses approximately half of the total PCr. Fig. 7 shows that dogfish white fibres recover considerably more slowly than muscles from other animals. It should be noted that the temperature used in our experiments, 12 °C, is within the normal physiological range for a swimming dogfish. It is notable that the intracellular pH is relatively acid at the end of recovery in three of the four fibre preparations in which pH was measured (pH 6.55, 6.59 and 6.64). However, we do not think that low intracellular pH is responsible for the slowness of recovery, since it was also slow in the one fibre preparation in which pH remained above 7.0.

The slowness of recovery of dogfish white fibres makes them a suitable object for detailed study of the time course of metabolic recovery. On the basis of the results presented here, it would clearly be beneficial to study recovery periods substantially longer than the 2 h used here. This would allow the later stages of recovery to be more clearly distinguished from any gradual baseline shifts due to other processes. It is

Fig. 5. Example results from a recovery heat experiment on one muscle fibre preparation (mean of 10 repeats). (A) Force (mN) produced during 40 s of intermittent stimulation. Eighty tetani were given at 0.5 s intervals; each tetanus consisted of three stimuli at 30 Hz. (B) Heat production (mJ) during and shortly after the period of stimulation. The horizontal bar indicates the period of stimulation. Initial heat is defined as heat produced between the start of stimulation and the complete relaxation of force after the end of stimulation. (C) Recovery heat production (mJ), which is defined as heat produced after the end of relaxation.
possible that recovery may be even slower than described here; the sloping baselines used in quantifying both the NMR and the heat results may have truncated the measurement of recovery, which may actually have continued for longer.

**Oxidative resynthesis of PCR**

The results show that the time course of acidification during recovery matches the progress of reversal of PCR splitting, a process which produces hydrogen ions. There is therefore no evidence of any other source of hydrogen ions, such as glycolytic production of lactate + hydrogen ions, in our dogfish white fibres under the conditions used here. This is in striking contrast to the net acidification observed in frog sartorius (Dawson et al. 1977), cat biceps (Meyer et al. 1991), rat soleus (Phillips et al. 1993) and human muscle (Kemp et al. 1993).

The absence of net acidification of dogfish muscle is reminiscent of the behaviour of myophosphorylase-deficient muscle (McArdle’s disease), which cannot produce lactic acid (Edwards and Wiles, 1981; Kemp et al. 1993).

Thus, the simplest explanation of our results is that the only net reaction occurring in dogfish white fibres during these stimulus+recovery cycles is the splitting of PCR during stimulation and its resynthesis by oxidative phosphorylation, for which muscle glycogen might be the substrate. The following features of the results are explicable by this hypothesis.

1. The 1:1 stoichiometry of the relationship between the change in Pi concentration and change in PCR concentration during recovery (Fig. 3). In some previous experiments (Kawano et al. 1988), convincing evidence for breakdown of PCR without an equivalent rise in Pi concentration has been reported in the very early stages of recovery of amphibian muscles that consist largely of fast white muscle fibres. In the experiments reported here, the time resolution was not so good, and it is unlikely that such a phenomenon could have been detected.

2. The linear relationship between pH change and reversal of PCR splitting, as shown in Fig. 4A and as explained above.

3. The half-times of recovery heat production and of the reversal of PCR splitting are reasonably similar. A perfect match between the time courses cannot be expected because the two types of measurements were made in different laboratories at different times and with different batches of fish (although from the same source). From both sets of measurements, it is apparent that there is variation between preparations in the exact time required for recovery.

4. Both the recovery heat produced and the amount of PCR resynthesized during recovery increase monotonically with
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The mean non-phosphate buffer power found in the four experiments in which pH was measured was 13.0 mmol\(^{-1}\) intracellular water per pH unit. This value, because of the way it was measured, excludes any contribution to the buffering from the inorganic phosphate present in the intracellular water. Total buffer power is often measured by titrating muscle homogenates, which have a buffer power greater than that measured here (Heisler et al. 1980; Abe et al. 1985; Wood et al. 1990; Dickson et al. 1993). This difference arises, not only because of the P\(_i\) originally present in the intracellular water,

Fig. 8. Recovery heat production (mJ) plotted against the total tension–time integral (N s) produced during the period of stimulation. Open symbols are eight observations of recovery heat production by three muscle fibre preparations (different symbols). The solid line was calculated from the equation y=ax/(x+b), where a and b are constants adjusted to give the best fit to the eight observations of recovery heat. The broken lines are the 95% confidence limits for this set of observations. Filled symbols are the recovery heat production expected during the metabolic recovery observed in NMR experiments on five muscle fibres preparations, assuming that the molar enthalpy change for PCr resynthesis is 38 kJ mol\(^{-1}\) PCr. A satisfactory tension recording was not obtained in the other NMR experiment.

Recovery is more complex in rat soleus than in dogfish white fibres.

If recovery metabolism were glycolytic (producing lactate + H\(^+\)), the overall heat produced during resynthesis of PCr would be smaller than that for oxidative recovery described above. The \(\Delta H_{\text{m}}\) for 0.5 glycogen unit forming lactate + H\(^+\) is 67 kJ mol\(^{-1}\) lactate (Meyerhof, 1930; Curtin and Woledge, 1978), so the \(\Delta H_{\text{m}}\) for the coupled process is correspondingly smaller, 30.6 kJ mol\(^{-1}\) PCr resynthesized. Thus, the heat expected for the resynthesis measured by NMR is 30.6/38.3=0.80 of that shown in Fig. 8. However, five of the points based on the NMR results would still be within the 95% confidence limits shown in Fig. 8.

**Buffer power**

The mean non-phosphate buffer power found in the four experiments in which pH was measured was 13.0 mmol\(^{-1}\) intracellular water per pH unit. This value, because of the way it was measured, excludes any contribution to the buffering from the inorganic phosphate present in the intracellular water. Total buffer power is often measured by titrating muscle homogenates, which have a buffer power greater than that measured here (Heisler et al. 1980; Abe et al. 1985; Wood et al. 1990; Dickson et al. 1993). This difference arises, not only because of the P\(_i\) originally present in the intracellular water,
but also because further P_i is formed in the homogenates by breakdown of the PCr and ATP that the muscle contained (Wiseman and Ellington, 1989; Adams et al. 1990). In order to compare our result with those from measurements on homogenates, we have calculated what the buffer power would have been if, in addition to the buffering we measured, there was an amount of P_i present equivalent to that expected from complete splitting of the PCr and ATP in our muscles. The result is 39 mmol l⁻¹ intracellular water per pH unit. Comparable values have been reported by Dickson et al. (1993) for other ectothermic elasmobranch fish of not very active habits. They point out that larger buffer power, as well as larger aerobic and anaerobic metabolic capacities, are characteristic of very active, endothermic elasmobranch fish. Thus, the value for buffer power that we report in the present paper is in line with previous reports using a very different method.

Buffer power decreases with time

In the four experiments in which we measured pH, the buffer power decreased during the course of the experiment. This could happen if pH were progressively changing away from the pK of the buffer, but the evidence presented in Fig. 4B and described in the Results argues against this being the explanation. Other possibilities are (1) that the buffer concentration is decreasing because the buffer is being modified to a non-buffering form; (2) that the buffer is permeant and is moving from the intra- to the extracellular space and is thus washed out in the superfusate; or (3) that some of the buffering effect is due to H⁺ extrusion from the cell, for example by Na⁺/H⁺ exchange, and that this extrusion decreases during the course of the experiment. Further investigation of these possibilities is needed and would be useful in understanding what is required for keeping these preparations viable in vitro for even longer periods.

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References


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