# Determination of the rate of ATP hydrolysis during the development of fatigue in single muscle fibres

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Experiments on skinned muscle fibres indicate that loss of mechanical performance of fatigued muscle is due to increased inorganic phosphate concentration and acidification (Chase & Kushmerick, 1988; Cooke et al., 1988; Godt & Nosek, 1989), whereas experiments on intact fibres indicate that reduced calcium efflux from the sarcoplasmic reticulum is the most important force-reducing mechanism (Allen et al., 1989). To investigate the relative importance of the various mechanisms responsible for reduced mechanical performance, which may all relate to energetic properties of muscle fibres, we have determined metabolite contents (phosphocreatine, creatine, ATP, IMP), lactate production, and oxygen uptake of single, intact muscle fibres. These data are used here to determine the relationship between force production and the rate of ATP hydrolysis during the development of fatigue induced by intermittent tetanic stimulation.

## Methods

Single, intact, fast-twitch, low oxidative (type 1) muscle fibres were dissected from the iliofibularis muscle of *Xenopus laevis* (Lännergren and Smith, 1966). They were stimulated intermittently at 40 Hz to produce one 250 ms tetanus every 5 s in oxygenated, phosphate buffered Ringer solution, pH 7.2, at 20 °C. Oxygen uptake of three fibres was determined as described by Elzinga and van der Laarse (1988). Nineteen fibres were freeze-clamped after different stimulation times (0, 1.5, 4, 5 and 10 min, n = 3 to 5) and used to determine metabolite contents as described by Nagesser et al. (1992). Lactate efflux was also determined. From these data the amount of energy-rich phosphate (~P) utilized was calculated using ~P/ATP = 2 (because ATP is converted to IMP), ~P/lactate = 1.5 and ~P/$O_2$ = 6.3.

## Results and Discussion

Fig. 1 shows that the rate of ATP hydrolysis is not proportional to force production (contrary to results of Dawson et al., 1978): after a fairly proportional decrease during the first 5 min of intermittent stimulation (coinciding with full reduction of the phosphocreatine store and an increase of lactate content to 145 $\mu mol.g^{-1}$ dry weight, results not shown), the rate of ATP hydrolysis decreases faster than force. The rate of ATP hydrolysis must fall because ATP regeneration in the sarcoplasm (from phosphocreatine and by glycolysis) is almost fully reduced and the rate of oxygen consumption cannot increase further: during this phase and beyond virtually all ATP is regenerated by oxidative phosphorylation. Mitochondrial capacity is too low to maintain the high rate of ATP hydrolysis. ATP is not depleted to compensate for the reduced rate of ATP production, but is converted slowly to IMP. When the low, steady rate of ATP hydrolysis is reached, force decreases rapidly. This major force reduction is probably due to decreased calcium efflux from the sarcoplasmic reticulum (Allen et al., 1989).

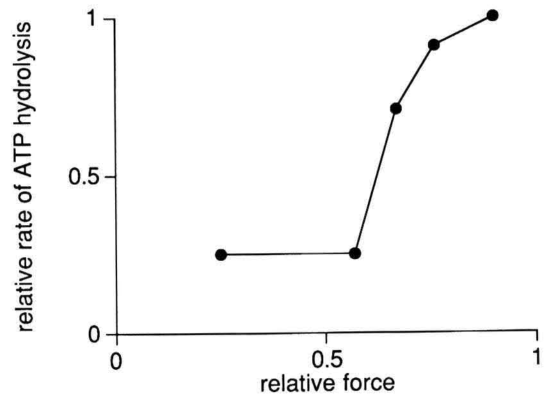


**Figure 1.** The rate of ATP hydrolysis during five time intervals of fatiguing intermittent stimulation (see Methods) plotted against the mean force production during the time intervals. The initial rate of ATP hydrolysis during contraction is 9 $nmol.mm^{-3}.s^{-1}$, corresponding to about 7 mM per 250 ms tetanus in the overlap zone of actin and myosin filaments.

The results are compatible with the idea that the rate of ATP hydrolysis is inhibited first by inorganic phosphate and protons (Cooke et al., 1988) and subsequently by ADP, which is expected to rise during contraction when ATP regeneration in the sarcoplasm is reduced.

## References


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