Investigation of chronic hindlimb ischaemia in the rat by $^{31}$P-nuclear-magnetic-resonance spectroscopy

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In man, chronic narrowing or occlusion of the arteries causes peripheral arterial insufficiency. Muscle biopsies from such patients have shown an enhanced oxidative capacity, as indicated by increases in the maximal activities of enzymes of $\beta$-oxidation, the tricarboxylic acid cycle and the electron-transfer chain (Bylund et al., 1976). This pattern of metabolic adaptation is similar to that provoked by exercise training (Holloszy & Booth, 1976). Some workers have suggested that the reduction in blood supply to muscle may be the trigger for the induction of mitochondrial enzyme synthesis (Bylund-Fellenius et al., 1981).

Whilst the enzymic adaptations have been characterized (Byland et al., 1976; Byland-Fellenius et al., 1981), the functional role of such changes have not been addressed in vivo. Therefore, we have used $^{31}$P-n. m. r. to investigate the effect of reduced arterial delivery in vivo, both at rest and during periods of isometric contraction.

Under ether anaesthesia, the left femoral artery of male Wistar rats (200–250 g) was exposed, double-ligated and cut proximal to the epigastric branch. Animals were allowed to recover for 7 days before study. Animals were prepared for muscle stimulation (via the sciatic nerve) as described by Shoubridge et al. (1984) for $^{31}$P-n.m.r. experiments, or placed in a rigid animal frame (Hayes, 1983).

$^{31}$P-n.m.r. experiments were carried out at 73.84 MHz in a vertical, widebore 4.3T magnet. Spectral acquisition was carried out as described by Shoubridge & Radda (1984) for $^{31}$P-n.m.r. experiments, or placed in a rigid animal frame (Hayes, 1983).

Seven days after operation, the reduction in bloodflow at rest caused by section of the left femoral artery was about 30\% (as determined by the microsphere technique of Armstrong & Laughlin, 1983). There was also a reduced hyperaemic response to isometric contraction.

At rest, the unilateral reduction in bloodflow to the hindquarter had small but significant effects in gastrocnemius muscle; muscle intracellular pH was lower (Fig. 1c) and lactate and inorganic phosphate were raised. The metabolic demands of muscle contraction produced more striking deviations from the control case. Fatigue
was observed both in terms of a decrease in twitch-tension (Fig. 1a) and an increase in the half-relaxation time (results not shown). Concurrent with these effects, \(^{31}\)P-n.m.r. revealed that creatine phosphate levels dropped more rapidly in the ischemic group and achieved lower steady-state concentrations than in sham-operated controls (Fig. 1b). The isometric contraction-induced acidosis (Fig. 1c) suggests that an accelerated glycolytic flux may be implicated (Sahlin et al., 1976).

The free ADP concentration has been suggested to be an important regulator of oxidative metabolism (Wilson et al., 1981). The concentration of free ADP was calculated from the creatine phosphokinase equilibrium (Veech et al., 1979). The elevated free ADP levels observed in the gastrocnemius muscle of ischemic rats during isometric contraction (Fig. 1d) may be indicative of a reduced rate of ADP re-phosphorylation by mitochondrial oxidative processes.

In recovery, the rates of creatine phosphate re-synthesis and concurrent decreases in ADP and Pi concentrations were slow. This must be a consequence of impaired oxygen delivery. Furthermore, there is an anaerobic component to the recovery process as there is a marked post-exercise acidosis at least in the initial recovery phase (Fig. 1e). Therefore, chronic, mild ischemia only limits muscle function when energy demand is increased above resting levels. Under these conditions, despite an increased glycolytic component, elevated ADP concentrations and an increased utilization of creatine phosphate, there is still a rapid fatigue response.

This work was supported by the British Heart Foundation, the Medical Research Council and the Nuffield Foundation.

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**Fig. 1. Effect of stimulation of the sciatic nerve (1 Hz) of animals 7 days after femoral artery section (●) or sham-operation (●) on twitch-tension (a), creatine phosphate concentration (b), intramuscular pH (c) and free ADP concentration (d) in the gastrocnemius muscle in vivo**

Concentrations of creatine phosphate and free ADP were calculated from \(^{31}\)P-n.m.r. spectral analysis and spectrophotometrically determined ATP and total creatine concentrations and a value of \(K_{eq}\) for creatine phosphokinase taken from Veech et al. (1979). Each point is the mean ± S.E.M. of at least six observations.