Short report

Forearm exercise increases plasma hypoxanthine

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SUMMARY Plasma hypoxanthine was measured in three normal subjects during aerobic forearm exercise. The comparative increase of hypoxanthine greatly exceeded that of ammonia or lactate. It is proposed that hypoxanthine production reflects ATP breakdown in muscle. The test may prove useful in the investigation of patients with metabolic muscle disease.

Much of the metabolism of normal muscle is concerned with maintaining levels of substances with high-energy phosphate bonds, particularly ATP. It is a reasonable assumption that abnormalities of ATP metabolism are associated with some neuromuscular diseases, particularly those such as phosphorylase deficiency and carnitine palmityl transferase deficiency in which there is a primary alteration of muscle biochemistry. Unfortunately, the biochemical measurement of ATP in muscle is complicated by the very short time needed for its regeneration, perhaps shorter than the interval between obtaining the biopsy and freezing it; even *in vivo* studies have not always shown a decrease when expected.¹ There is thus a need to develop new methods of studying metabolism related to ATP.

It has been known for some time that uric acid and its precursor hypoxanthine, substances which are formed by breakdown of ATP and other purines, are increased in urine after strenuous exercise.^{2,3} Recently Sutton *et al*⁴ have demonstrated an increase in urinary and plasma oxypurines with exercise, associated with a decrease in skeletal muscle ATP concentration. We have investigated the effect of aerobic forearm exercise on plasma levels of hypoxanthine and other metabolites of ATP, and report our findings below.

Subjects and methods

Three normal subjects, without neuromuscular symptoms, performed a forearm test under aerobic conditions by squeezing a grip dynamometer at 50% of their maximum

grip strength for 1.5 seconds out of every 2 seconds until they become exhausted; this occurred after 10.5 minutes in two subjects and 8.5 minutes in the other. Blood samples were taken from an indwelling catheter in the basilic vein of the exercising arm before exercise, at 2 and 5 minutes during exercise, and at 2, 5, 10, 30, and 60 minutes following exercise. In one subject, blood was also taken from the non-exercising arm. Hypoxanthine and other purine bases, nucleosides, and nucleotides were measured in plasma by high-pressure liquid chromatography on a μ -Bondapak-C₁₈ column using a phosphate buffer gradient.⁵ Lactate was measured by the method of Lowry and Passonneau⁶ and ammonia by a commercially-available kit method (Sigma no. 170-UV).

Results

All subjects showed a marked rise in plasma hypoxanthine in the exercising arm; mean initial level was 1.58 μ mol/l (SD 1.19) and mean peak level was 25.2 μ mol/l (SD 1.93). This was a comparatively greater increase than for lactate which rose from a mean initial level of 0.95 mmol/l (SD 0.22) to a mean peak level of 4.21 mmol/l (SD 0.89) or for ammonia which rose from a mean initial level of 34.2 μ mol/l (SD 8.2) to a mean peak level of 135.8 μ mol/l (SD 43.5). Data from a subject are given in fig 1. In the non-exercising arm of one subject, the peak plasma hypoxanthine was 7.8 μ mol/l compared with 26.7 μ mol/l in the exercising arm. No significant change was found with exercise in plasma levels of xanthine, adenine, guanine, inosine, adenosine, guanosine, inosine monophosphate (IMP), guanosine monophosphate, AMP or ADP.

Discussion

The results indicate that plasma hypoxanthine increases with aerobic forearm exercise. Moreover,

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Fig 1 A semi-logarithmic plot of comparative rises in plasma hypoxanthine, ammonia, and lactate from the aerobically-exercising forearm of a normal subject.

Fig 2 Proposed scheme for the regeneration of muscle ATP.

the much lower level found in the non-exercising arm suggests that the hypoxanthine is being released from working muscle. The exact mechanism of hypoxanthine production is not clear, but it may arise from breakdown of IMP which itself is formed from AMP by the enzyme adenylate deaminase with concomitant production of ammonia. Removal of AMP in this manner would promote resynthesis of ATP in the reaction catalysed by myokinase in which two molecules of ADP produce one molecule of ATP and one of AMP (fig 2). Although muscle IMP is increased by muscular exercise,⁷ we did not find a rise in plasma levels presumably because the muscle membrane is considerably less permeable to nucleotides than to bases.

Forearm tests are probably better than bicycle ergometry at demonstrating hypoxanthine produc-

tion by muscle because, in the latter test, hypoxanthine produced by the leg muscles will be diluted in the general circulation, partly metabolised by hepatic xanthine oxidase, and partly excreted by the kidneys before being measured in the arm. Since muscle itself contains very little xanthine oxidase,⁸ hypoxanthine produced by a working forearm will be recorded directly in the venous blood of that arm. Measurement of the plasma hypoxanthine response to forearm exercise is therefore a relatively simple method of investigating muscle purine nucleotide breakdown *in vivo*. Whether it proves useful in investigating patients with suspected "metabolic" muscle disease remains to be tested but in this context it is interesting to note that muscle pain was the presenting symptom in two patients with xanthinuria,^{9,10} a condition in which xanthine oxidase is absent and plasma hypoxanthine is raised. In conclusion, hypoxanthine levels are increased in the venous blood from an exercising forearm. The comparative rise in hypoxanthine is greater than that of any other metabolite yet measured.

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