BIOCHEMICAL JOURNAL LETTERS

The role of adenosine kinase in regulating adenosine concentration

Fisher & Newsholme (1984) recently reported the kinetic properties of the adenosine kinase purified from rat heart. The enzyme was similar to that isolated from a number of other species and organs, had a K_m for adenosine in the range 0.2–0.4 μM and was uncompetitively inhibited by Mg^{2+}. Based on these experiments *in vitro* and on the total tissue content of adenosine, the authors repeated their conclusion (Arch & Newsholme, 1978) that the adenosine kinase is saturated with substrate in the normoxic heart and also in other tissues subjected to basal metabolic loads. They further speculate that the activity of adenosine kinase might be reduced during ATP catabolism and thus enhance the elevation of adenosine concentration seen during perfusion of the heart with hypoxic buffer.

Both these conclusions are at variance with our experiments directly testing these proposals and reported in Newby *et al.* (1983). In our experiments, the activity of adenosine kinase was determined *in situ* by measuring the rate of incorporation of radiolabelled adenosine into cellular nucleotides. The validity of this measurement, which is a minimum estimate of the activity of the enzyme under conditions of endogenous regulation, was established by inhibiting nucleoside incorporation with two specific inhibitors of adenosine kinase, 5'-amino-5'-deoxyadenosine and 5-iodotubercidin (Newby, 1981). When the rate of adenosine formation was determined in polymorphonuclear leucocytes or in cultured heart cells it was only a small proportion of the activity of adenosine kinase determined *in situ*. Thus at the steady state, the rates of adenosine formation and metabolism being equal, the kinase reaction must be far from saturation. Using similar methodology, Bontemps *et al.* (1983) reached a similar conclusion for isolated hepatocytes.

In the perfused rat heart the maximal rate of incorporation of adenosine into cellular nucleotides was 33 nmol/min per g wet wt. (Newby *et al.*, 1983). Infusion of adenosine into the heart at 20 nmol/min per g led to a readily detectable efflux of adenosine and inosine. However, no efflux of either nucleoside was detectable when adenosine kinase was inhibited by 90% with 5-iodotubercidin. Again we concluded that adenosine kinase was not rephosphorylating adenosine at a significant rate in the normoxic heart. In agreement with this, Achterburg & DeJong (1984) estimated the flux through adenosine kinase to be 3 nmol/min per g in the normoxic rat heart.

Hypoxia reduced ATP concentration to 65% of that in normoxic hearts over 10 min, but did not detectably diminish the incorporation of radiolabelled adenosine into cellular nucleotides. Thus it is unlikely that inhibition of adenosine kinase enhances the rise in adenosine concentration seen in the hypoxic heart. On the contrary, when purine formation was stimulated by acetate infusion into normoxic rat hearts, flux through adenosine kinase was increased to 10 nmol/min per g (Achterburg & DeJong, 1984). Polymorphonuclear leucocytes and cultured heart cells subjected to more extreme ATP depletion did show a decrease in adenosine kinase activity *in situ*.

These results illustrate the danger of basing hypotheses solely on the properties of enzymes determined in cell-free systems. The free cytosolic concentration of adenosine is, at present, unknown but is certainly less than the total tissue content owing to the presence of well-characterized adenosine-binding proteins (Ueland & Saebo, 1979). Similarly the K_m of the adenosine kinase *in situ* is not known, nor has its distribution amongst the various cell types in the heart been determined. Currently, the experiments with intact cell systems suggest that the role of adenosine kinase is solely to recover nucleosides lost during ATP depletion.

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Adenosine kinase and the control of adenosine concentration in the heart

The qualitative approach in metabolic control is to study the properties of enzymes that catalyse non-equilibrium reactions in order to identify possible external regulators of this enzyme and then test the resulting hypothesis of control in the intact system (see Newsholme & Leech, 1983). Adenosine kinase catalyses a non-equilibrium reaction in all tissues investigated, and if both 5′-nucleotidase and adenosine kinase are simultaneously catalytically active a substrate ('futile') cycle between AMP and adenosine will occur and this might be of considerable importance in control of the adenosine concentration in various tissues including the heart (Arch & Newsholme, 1978). As part of a systematic study of the enzymes involved in the control of adenosine concentration, the properties of the kinase were investigated and a hypothesis for control of the adenosine concentration put forward (Fisher & Newsholme, 1984). The work of Newby *et al.* (1983) tested this hypothesis and, as pointed out by Newby (1984), the results provided little or no support for the hypothesis. This suggests either that adenosine kinase plays no significant role in control of the adenosine concentration (Newby, 1984) or that there are other as yet undiscovered properties of this enzyme that are important in control. We wish to put forward a speculative proposal.

The improvement in sensitivity for controlling fluxes or concentrations of metabolites via a substrate cycle depends upon the ratio, cycling rate/flux (Newsholme & Crabtree, 1976; Newsholme *et al.*, 1984). Consequently the sensitivity provided by such cycles is variable depending on the cycling rate. Recently it has been shown that catecholamines can dramatically increase the cycling rate in the triacylglycerol/fatty acid cycle (Brooks *et al.*, 1983) and the fructose/fructose 6-phosphate cycle (Challiss *et al.*, 1984). Such increases in cycling might be achieved by covalent modification of one or both enzymes in the cycle. Hence it is suggested that catecholamines or other hormones increase the activity of adenosine kinase (and possibly 5′-nucleotidase) which increases the cycling rate between AMP and adenosine in such a way that the properties reported by Fisher & Newsholme (1984) would *now* be important in the regulation of the adenosine concentration. Thus, we expect that the rate of cycling would increase dramatically in heart when an increase in heart work was anticipated so that a sensitive mechanism for control of adenosine concentration would be available to adjust precisely coronary flow to increased metabolic demand by the heart.

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