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Tricarboxylic acid cycle flux and enzyme activities in the isolated working rat heart

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Flux through the tricarboxylic acid cycle was calculated from oxygen consumption in hearts perfused near the physiological work load. Activities of citrate synthase, 2-oxoglutarate dehydrogenase and succinate dehydrogenase were measured in the same hearts. Only the activities of 2-oxoglutarate dehydrogenase correlated with calculated fluxes through the cycle.

Evidence that the maximum catalytic activities of certain key enzymes measured *in vitro* provide a quantitative indication of maximal flux through metabolic pathways *in vivo* has been presented previously (Crabtree & Newsholme, 1975; Newsholme *et al.*, 1980). For example, the activities of 6-phosphofructokinase (EC 2.7.1.11) in different muscles were similar to the measured maximum glycolytic rates, and the activities of 2-oxoglutarate dehydrogenase (EC 1.2.4.2) were similar to the maximum capacity of the tricarboxylic acid cycle calculated from oxygen-uptake data (Read *et al.*, 1977). However, in all these studies, enzyme activities were measured and compared with oxygen-consumption data obtained from the literature; hence different animals were compared under different conditions. Consequently, it was decided to compare directly the flux and enzyme activities in the same tissue; the activities of 2-oxoglutarate dehydrogenase were measured in extracts of the left ventricle of hearts that had been perfused *in vitro* under conditions that approach maximum cardiac work, during which the rate of oxygen uptake was measured. Hence, from the latter, the flux through the tricarboxylic acid cycle could be calculated and compared with 2-oxoglutarate dehydrogenase activities from the heart. In addition, the maximum activities of citrate synthase (EC 4.1.3.7) and succinate dehydrogenase (EC 1.3.99.1) were measured, since they have also been used as indicators of the flux through the tricarboxylic acid cycle.

Materials and methods

Acetyl-CoA, CoA, NAD^+ and dithiothreitol were obtained from Boehringer Mannheim G.m.b.H., Lewes, Sussex, U.K.; phenazine methosulphate, 2,6-dichlorophenol-indophenol and 5,5′-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma Chemicals, Poole, Dorset, U.K.; Triton X-100 and all inorganic reagents were obtained from B.D.H., Poole, Dorset, U.K.; and Tes (2-{[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]amino}ethanesulphonic acid) was obtained from Hopkin and Williams, Chadwell Heath, Essex, U.K.

Heart perfusions

Hearts were obtained from male Wistar rats and perfused with bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 5mM-glucose by the method previously described (Taegtmeyer *et al.*, 1980). To simulate conditions of maximum cardiac work, the left-ventricular filling pressure was set at 15 cmH_2O and mean aortic pressure at 140 cmH_2O. Perfusions were carried out by recirculation of 150ml of perfusate for 30min. Cardiac performance (aortic pressure and cardiac output) was monitored, and oxygen uptake was measured at intervals of 5min. The oxygen uptake by the heart was measured as described previously (Taegtmeyer *et al.*, 1980). The rate did not change by more than 10% after the first 5min of perfusion. At the end of perfusion, the heart was removed from the cannula quickly and dropped into ice-cold saline (0.9% NaCl). Since almost all of the external work of the preparation was due to left-ventricular contraction, it was assumed that almost all the oxygen uptake could be accounted for by the left ventricle. Hence, this portion of the heart was dissected after completion of perfusion, weighed and the rate of oxygen uptake calculated on the basis of this weight.

Preparation of heart homogenate

A sample (about 300mg) of the left ventricle was homogenized in 10vol. of extraction medium that

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contained 250mM-mannitol, 5mM-Tes (potassium salt) and 1mM-EGTA and pH 7.4 in a Polytron PCU-2 at position 3 for 10s. This crude homogenate was used without further treatment for the assay of 2-oxoglutarate dehydrogenase and citrate synthase. A portion of homogenate was sonicated in a MSE 150W sonic disintegrator for 15s at an amplitude of 6μm for the assay of succinate dehydrogenase.

Enzyme assays

All enzyme assays were performed at 25°C with a Gilford 240 recording spectrophotometer. For the assay of the maximal activity of 2-oxoglutarate dehydrogenase, the reaction mixture contained 250mM-mannitol, 10mM-potassium phosphate, 100mM-Tris/HCl, 10mM-KCl, 5mM-$MgCl_2$, 1mM-dithiothreitol, 0.05% (v/v) Triton X-100, 2mM-NAD^+, 0.63mM-CoA and 10mM-2-oxoglutarate at pH 7.4, to which 20μl of homogenate was added to start the reaction. The final volume in the cuvette was 2.0ml. Controls from which 2-oxoglutarate was omitted were run concurrently, and the rate of change of A_{340} was followed. (Addition of ADP or Ca^{2+} to the reaction was found to have no effect on activity.) Citrate synthase was assayed as described previously (Alp *et al.*, 1976). The reaction medium consisted of 50mM-Tris/HCl, 0.2mM-5,5'-dithiobis-(2-nitrobenzoic acid), 0.05% (v/v) Triton X-100, 0.1mM-acetyl-CoA and 0.5mM-oxaloacetate at pH 8.1, to which 20μl of 1:40 dilution of heart homogenate was added. The reaction was started by the addition of the oxaloacetate and the increase in A_{412} followed. The final volume in the cuvette was 2.0ml. Succinate dehydrogenase was also measured spectrophotometrically with the artificial electron acceptors phenazine methosulphate and 2,6-dichlorophenol-indophenol (Cooney & Dawson, 1979). The reaction mixture contained 50mM-potassium phosphate, 1mM-KCN, 0.36mM-phenazine methosulphate, 0.12mM-2,6-dichlorophenol-indophenol, 10mM-succinate and 20μl of sonicated heart homogenate in a final volume of 2.0ml at pH 7.4. The reaction was started by the addition of succinate and the decrease in A_{600} was followed.

Presentation of results

The enzyme activities were measured at 25°C, but are presented at 37°C to aid comparison with the calculated flux through the tricarboxylic acid cycle; activities at 37°C are calculated from the Arrhenius equation, assuming that a rise of 10°C increases the rate by a factor of 2.0 (see Crabtree & Newsholme, 1975). The flux through the tricarboxylic acid cycle was calculated from the rate of oxygen uptake. From the stoichiometry of the pathway of glucose oxidation, the flux through the

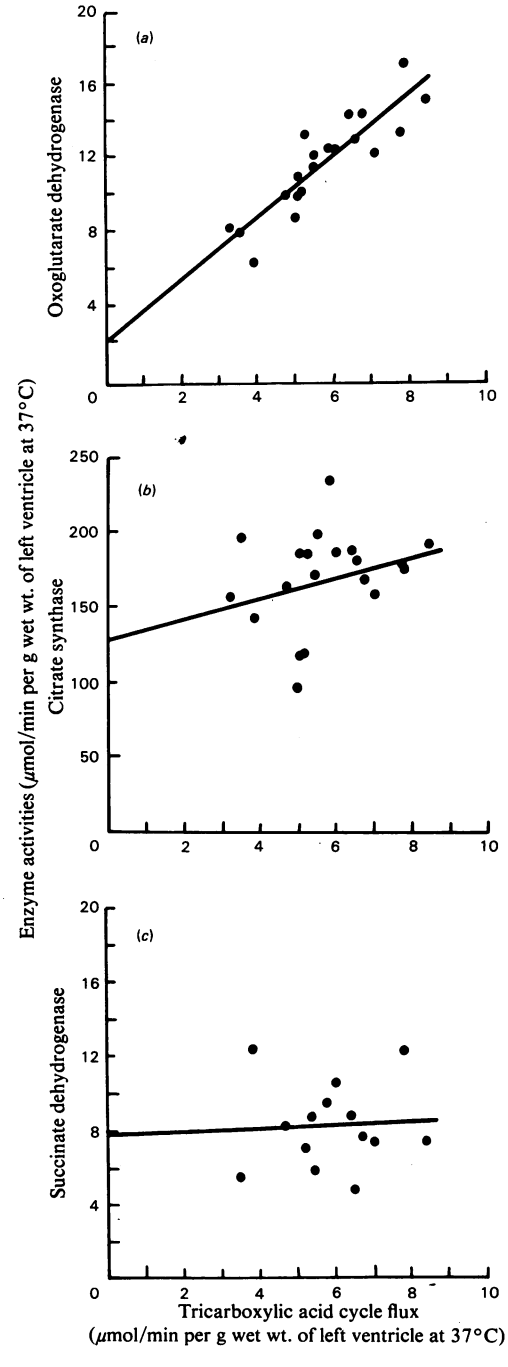

Fig. 1. *Relationship between the maximum activities of 2-oxoglutarate dehydrogenase, citrate synthase or succinate dehydrogenase and the tricarboxylic acid cycle flux*
The tricarboxylic acid cycle flux and maximum enzyme activities at 37°C were calculated as described in the Materials and methods section. A linear regression through the points on each graph is also shown.

tricarboxylic acid cycle is equal to one-third of the oxygen uptake.

Results and discussion

Preliminary observations indicated that the rate of oxygen uptake by a small heart (wet wt. 0.7g) was greater than that of a larger heart (wet wt. 1.5g). Hence, in order to provide a range of rates of oxygen uptake, hearts from different-sized animals were used (the weights of the animals ranged from 200 to 450g). The oxygen uptake in 21 perfusions ranged from 9.7 to 25.3 μmol/min per g wet wt. and the calculated tricarboxylic acid cycle flux from 3.2 to 8.3 μmol/min per g wet wt. of left ventricle.

The maximal activities of the three enzymes, calculated for 37°C are plotted against the calculated flux through the tricarboxylic acid cycle in Fig. 1. There was a good correlation between the activity of 2-oxoglutarate dehydrogenase and the cycle flux ($r = 0.880$, $P < 0.001$ for 19 degrees of freedom), whereas there was no correlation between the activities of citrate synthase or succinate dehydrogenase and the flux ($r = 0.281$ for 19 degrees of freedom and 0.051 for 12 degrees of freedom, respectively). This indicates that of the enzymes investigated only 2-oxoglutarate dehydrogenase provides a quantitative index of the maximum flux through the tricarboxylic acid cycle. The mean activities at 37°C were 30.9 ($\pm$ 2.0), 1.48 ($\pm$ 1.5) and 2.1 ($\pm$0.5)-fold greater than the calculated flux through the cycle for citrate synthase, succinate dehydrogenase and 2-oxoglutarate dehydrogenase, respectively. Although the mean activity of succinate dehydrogenase was closer to the cycle flux, the lack of correlation suggests that the activity of the enzyme is not useful as an index of maximum flux through the cycle. Estimation of the maximum activity of succinate dehydrogenase as measured *in vitro* may be low, owing to the necessary use of artificial electron acceptors in the assay (see Singer, 1976; Klingenberg, 1970). The 2-fold greater activity of 2-oxoglutarate dehydrogenase compared with the cycle flux could be explained by the assumption that the perfused heart was not working close to its maximum capacity. This assumption is supported by the following considerations. First, in the present experiments the rate of contraction was only 70% of that observed for the same heart *in vivo* (results not shown). Secondly, at high work loads, the low oxygen-carrying capacity of saline in comparison with blood or saline containing stroma-free haemoglobin (Taegtmeyer, 1980) could limit the performance of the left ventricle. Thirdly, a greater work output may occur *in vivo* when the heart is under the influence of inotropic hormones and the left ventricle pumps blood, which has a greater viscosity than saline. Hence it is concluded that the maximum activity of 2-oxoglutarate dehydrogenase probably provides a quantitative index of the maximum flux through the tricarboxylic acid cycle in heart muscle *in vivo*. Nonetheless, this reaction is unlikely to be the only important one in the regulation of the flux through the cycle (see Safer & Williamson, 1973; Ottaway *et al.*, 1981).

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References

Alp, P. R., Newsholme, E. A. & Zammit, V. A. (1976) *Biochem. J.* **154**, 689–700

Cooney, G. J. & Dawson, A. G. (1979) *Biochem. Pharmacol.* **28**, 1067–1070

Crabtree, B. & Newsholme, E. A. (1975) in *Insect Muscle* (Usherwood, P. N. R., ed.), pp. 405–500, Academic Press, London

Klingenberg, M. (1970) *Eur. J. Biochem.* **13**, 247–252

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Newsholme, E. A., Crabtree, B. & Zammit, V. A. (1980) *Ciba Found. Symp.* **73**, 245–258

Ottaway, J. H., McClellan, J. A. & Saunderson, C. L. (1981) *Int. J. Biochem.* **13**, 401–410

Read, G., Crabtree, B. & Smith, G. H. (1977) *Biochem. J.* **164**, 349–355

Safer, B. & Williamson, J. R. (1973) *J. Biol. Chem.* **248**, 2570–2579

Singer, T. P. (1976) *Methods Biochem. Anal.* **22**, 133–137

Taegtmeyer, H. (1980) D. Phil. Thesis, University of Oxford, pp. 59–60

Taegtmeyer, H., Hems, R. & Krebs, H. A. (1980) *Biochem. J.* **186**, 701–711