The Role of Creatine Kinase and Arginine Kinase in Muscle

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Arginine and creatine kinase activities in different muscles are compared with calculated maximum rates of ATP turnover. The magnitude of the kinase activities decreases in the following order: anaerobic muscles and vertebrate skeletal muscles > heart muscle > insect flight muscle. The maximum activity of phosphagen kinases (i.e. creatine kinase and arginine kinase), in the direction of phosphagen formation, is lower than the calculated maximum rate of ATP turnover in insect flight muscle or rat heart.

It is well established that an increase in the work load on a muscle causes a decrease in the concentration of phosphocreatine, whereas that of ATP remains constant. That the constancy of the ATP concentration is due to regeneration of ATP from phosphocreatine breakdown was shown by Cain & Davies (1963). They demonstrated that, provided glycolysis and creatine kinase (EC 2.7.3.2) were inhibited (by iodoacetate and 1-fluoro-2,4-dinitrobenzene respectively), electrical stimulation of frog muscle caused a decrease in the concentration of ATP, whereas that of phosphocreatine remained constant. This experiment demonstrated that ATP was used directly in the contractile process and, in addition, it provided support for the suggested role of phosphocreatine as a reserve of 'energy-rich' phosphate that could be used to maintain the ATP concentration. However, another hypothesis for the role of creatine kinase has been proposed (see Saks *et al.*, 1976). This hypothesis is based partly on the distribution of creatine kinase in heart muscle. This enzyme is found associated with myofibrils, in the intermembrane space of mitochondria and in the cytosolic fraction. The hypothesis proposes that phosphocreatine plus creatine kinase provide a mechanism for transport of 'energy-rich' phosphate from the mitochondria to the myofibrils. Thus ATP that is transported out of the mitochondria via the translocase is used to phosphorylate creatine in the intermembrane space of the mitochondria and the resultant phosphocreatine diffuses to the myofibrils where it regenerates ATP from ADP. The diffusion of creatine from the myofibrils to the mitochondria completes the metabolic loop.

The question arises whether the activity of creatine kinase is sufficient to account for maximum rate of energy-rich-phosphate transfer in heart and indeed in other muscles, since the hypothesis requires that the maximum activity of the phosphagen kinase

should be at least twice the maximum rate of ATP utilization by the myofibrillar adenosine triphosphatase. Indeed, since there is evidence that both creatine kinase and arginine kinase (EC 2.7.3.3) catalyse near-equilibrium reactions in a variety of muscles from different animals throughout the Animal Kingdom (Beis & Newsholme, 1975), it is reasonable that the maximum activity of the phosphagen kinase in any given muscle should be considerably higher than the maximum rate of ATP turnover (for theory see Newsholme & Crabtree, 1976; Crabtree, 1978). The maximum rate of ATP turnover in aerobic muscle can be calculated either from O₂-uptake data (assuming classical P/O ratios) or from the maximum flux through the tricarboxylic acid cycle (calculated from the maximum activity of oxoglutarate dehydrogenase; see Read *et al.*, 1977), or it can be obtained directly from activities of myofibrillar adenosine triphosphatase (see Zammit & Newsholme, 1976). This present paper presents the maximum activities of arginine kinase or creatine kinase in various muscles from different animals and these values are compared with the calculated maximum rates of ATP turnover.

Experimental

Animals

Animals were obtained from the sources given by Newsholme & Taylor (1969) or Sugden & Newsholme (1975) with the exception of scallops, sea-mouse, dogfish, mackerel, bass and flounder, which were obtained from the Marine Biology Association, Citadel Hill, Plymouth, U.K., and pig roundworms, which were obtained from a local slaughterhouse.

Materials

All chemicals and creatine kinase (used for the preparation of phospho[¹⁴C]creatine) were obtained

Table 1. Maximal activities of myofibrillar adenosine triphosphatase, arginine kinase and creatine kinase in the direction of phosphocreatine degradation from vertebrates and invertebrates

Animal	Muscle	Enzyme activities ($\mu\text{mol/min per g}$ of fresh muscle at 25°C)			Creatine kinase calculated in the direction of phosphocreatine formation	Calculated rate of ATP turnover ($\mu\text{mol/min per g at 25°C}$)	Content of phosphagen ($\mu\text{mol/g fresh wt.}$)
		Myofibrillar adenosine triphosphatase	Arginine kinase	Creatine kinase			
Mollusca							
Great scallop (<i>Pecten maximus</i>)	Phasic adductor	71.1*	930*	<0.1 (3)	—	71†	52.1**
	Catch adductor	5.8*	142*	<0.1 (3)	—	6†	—
Arthropoda (Crustaceae)							
Lobster (<i>Homarus vulgaris</i>)	Abdominal flexor	59.1*	1754*	<0.1 (3)	—	59†	33.4**
	Claw adductor	27.7*	714*	<0.1 (3)	—	28†	—
Insecta							
Locust (<i>Schistocerca gregaria</i>)	Flight	—	205 (177–230) (5)	—	—	532‡, 352§	8.8**
	Hind limb femoral	—	723 (540–918) (5)	—	—	—	—
Giant water-bug (<i>Lethocerus cordofanus</i>)	Flight	—	63.1 (43.1–96.2)(11)	<0.1	—	233§	4.4**
Bumble-bee (<i>Bombus</i> sp.)	Flight	—	138 (128–160) (3)	—	—	1017§	—
Yellow-underwing moth (<i>Noctua pronuba</i>)	Flight	—	298 (203–394) (11)	<0.1 (4)	—	637§	—
Cockchafer (<i>Melolontha melolontha</i>)	Flight	—	241 (141–301) (3)	<0.1 (3)	—	—	6.0**
Pisces							
Dogfish (<i>Scylliorhinus canicula</i>)	White	16.4 (13.1–18.7) (3)	—	1031 (859–1312) (3)	258	16†	—
	Red	12.6 (8.8–14.2) (3)	—	401 (357–438) (3)	100	13†	—
Rainbow trout (<i>Salmo gairdneri</i>)	White	—	—	4391 (3943–4506) (4)	1098	—	—
	Red	—	—	2436 (2419–2470) (4)	609	28§	—
	Heart	—	—	646 (568–691) (4)	161	48§	—

Activities of arginine kinase in the direction of phosphoarginine formation and activities of creatine kinase in the direction of phosphocreatine degradation were measured as described in the Experimental section. The activities were measured at 25°C except for the enzymes from the dogfish, bass, flounder and mackerel, which were measured at 10°C (including myofibrillar adenosine triphosphatase and the calculated rate of ATP turnover). The rates of ATP turnover were calculated on the basis of the following: †, myofibrillar ATPase (see Zammit & Newsholme, 1976); ‡, O₂ uptake of the working intact muscle assuming a P/O ratio of 3 (see Crabtree & Newsholme, 1972, for details of O₂-uptake data); §, maximum flux through the tricarboxylic acid cycle indicated by maximum activities of oxoglutarate dehydrogenase (data from J. Paul & E. A. Newsholme, unpublished work) (for evidence for the use of this enzyme see Read *et al.*, 1977); ||, on the basis of maximum rate of glycolysis as indicated by the activities of phosphofructokinase; it is assumed that muscles are anaerobic and that the maximum rate of ATP formation is obtained by multiplying the enzyme activity by 3 (see Crabtree & Newsholme, 1972). The activities of creatine kinase in the direction of phosphocreatine formation were measured by dividing the activities measured in the direction of creatine formation by 4; this value was obtained experimentally (see the text). Activities are presented as means with the ranges and numbers of separate animals used in parentheses. If the data are taken from other papers, ranges are not given.

Bass (<i>Dicentrarchus labrax</i>)	White	34.8 (30.1–41.3) (3)	—	2534 (2010–2947) (3)	633	35†	16.1
	Red	6.2 (4.7–7.2) (3)	—	1126 (894–1329) (3)	281	6†	4.4
	White	40.9 (35.6–47.4) (3)	—	1292 (734–1639) (3)	323	41†	13.7
Flounder (<i>Paralichthys flesus</i>)	Red	10.0 (7.5–11.2) (3)	—	1038 (834–1217) (3)	259	10†	10.2
	White	30.2 (26.2–33.5) (3)	—	1897 (1669–2243) (3)	474	30†	—
	Red	5.7 (4.1–6.6) (3)	—	710 (697–722) (3)	176	6†	—
Amphibia							
Frog (<i>Rana temporaria</i>)	Gastrocnemius	—	—	2839 (1526–4349) (4)	710	66	19.9**
Aves							
Domestic fowl (<i>Gallus gallus</i>)	Pectoral	—	—	1563 (1366–1989) (4)	391	315	27.2**
	Heart	—	—	507 (336–645) (4)	127	91§	—
Pheasant (<i>Phasianus colchicus</i>)	Pectoral	—	—	1259 (1133–1384) (4)	315	429	—
	Heart	—	—	298 (282–314) (4)	74	70§	—
Pigeon (<i>Columba livia</i>)	Pectoral	—	—	1825 (796–3494) (4)	456	63§	14.2**
Mammalia							
Laboratory mouse	Gastrocnemius	—	—	2883 (1919–3314) (4)	721	—	—
	Heart	—	—	540 (496–586) (4)	135	130§	—
Laboratory rat	Gastrocnemius	—	—	3404 (2632–3900) (4)	851	—	—
	Heart	—	—	494 (436–533) (4)	123	166§, 141†	—
Rabbit (<i>Oryctolagus cuniculus</i>)	Semitendinosus	—	—	1184 (1067–1342) (4)	296	15§	—
	Adductor longus	—	—	3460 (2225–4134) (4)	865	78	—

* Data taken from Zammit & Newsholme (1976)
** Data taken from Beis & Newsholme (1975).

from Boehringer Corp. (London), London W5 2TZ, U.K., except for the following: disodium EDTA and all inorganic reagents were obtained from BDH Chemicals, Poole, Dorset; [$1\text{-}^{14}\text{C}$]creatine and L-[$\text{guanido-}^{14}\text{C}$]arginine hydrochloride were obtained from the Radiochemical Centre, Amersham, Bucks., U.K.

The ion-exchange resins AG 50W-X8 and AG1-X8 (200–400 mesh) were from Bio-Rad Laboratories, St. Albans, Herts., U.K.

Preparation of homogenates for enzyme assay

Animals were killed and muscle was removed as quickly as possible. For kinase activities, muscle was homogenized in a ground-glass homogenizer with 10–20 vol. of extraction medium [50 mM-Pipes (1,4-piperazinediethanesulphonic acid), 4 mM-dithiothreitol, 1 mM-EDTA at pH 7.0]. Myofibrils for the assay of myofibrillar adenosine triphosphatase were prepared by the method of Kendrick-Jones *et al.* (1970).

Enzyme assays

The kinase activities were measured radiochemically. Details of the assays, including checks and controls carried out during the development of the assays, are described by Leech & Newsholme (1978) and Leech *et al.* (1978). Creatine kinase was assayed in both directions, but most results were obtained in the direction of creatine formation. For activities measured in both directions under the same conditions in extracts of pheasant and pigeon pectoral muscle and mouse gastrocnemius muscle, the mean ratio of activities of creatine formation/phosphocreatine formation was 4.0. The ratio for the enzyme purified from brain at pH 7.4 is 4.2 (Jacobs & Kuby, 1970).

Myofibrillar adenosine triphosphatase activity was measured as described by Zammit & Newsholme (1976). The calculation of ATP turnover (in $\mu\text{mol}/\text{min}$ per g of muscle) from myofibrillar adenosine triphosphatase activity ($\mu\text{mol}/\text{min}$ per mg of myofibrillar protein) has been described by Zammit (1975). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and Discussion

All activities are presented in $\mu\text{mol}/\text{min}$ per g of fresh muscle at 25°C , except for dogfish, bass, flounder and mackerel, where assays were performed at 10°C .

The highest activities of arginine kinase or creatine kinase are present in muscles that depend on glycolysis rather than the tricarboxylic acid cycle for energy production [see Table 1 for kinase activities, Crabtree & Newsholme (1972) for key glycolytic

enzyme activities and Alp *et al.* (1976) and Read *et al.* (1977) for activities of key enzymes of the tricarboxylic acid cycle]. These muscles include the phasic adductor of the scallop and the abdominal flexor muscles of the lobster (which contain very high concentrations of phosphoarginine; see Table 1) and skeletal muscles of the vertebrates. Although the activities of creatine kinase are, in general, slightly lower in the more aerobic skeletal muscles compared with the anaerobic muscles in the same animal (e.g. fish red and white muscles, semitendinosus and adductor longus of rabbit), heart muscle of vertebrates contains considerably lower activities than skeletal muscle (Table 1).

In the muscles of the marine invertebrates investigated (i.e. lobster and scallop) and in the vertebrate skeletal muscles, the activities of the phosphagen kinase are considerably greater (in most cases more than 10-fold) than the maximum rates of ATP turnover. This suggests that in these muscles the phosphagen is able to regenerate ATP from ADP very rapidly. Since the content of phosphagen in these muscles is high (usually $>20\mu\text{mol}/\text{g}$; see Table 1), these findings support the view that phosphagen functions as an important reserve of 'energy-rich' phosphate. Indeed, in some invertebrate anaerobic muscles (Zammit & Newsholme, 1976) and in the white muscle of some fish (V. A. Zammit & E. A. Newsholme, unpublished work) the maximum capacity of the glycolytic process is not sufficient to provide a rate of ATP generation demanded by the maximum activity of the myofibrillar adenosine triphosphatase. In these muscles, phosphagen may function as an important endogenous energy reserve in a similar manner to that of glycogen.

The activities of arginine kinase in the flight muscles of four insects studied are much lower than the maximum calculated rates of ATP turnover (Table 1). Furthermore, when the creatine kinase activity in the direction of phosphocreatine formation is considered, the maximum activity in rat heart is lower than the calculated maximum rate of ATP turnover, and in hearts from other animals the activities are similar to, or only slightly greater than, the maximum rate of ATP turnover. Since phosphagen kinase appears to catalyse a near-equilibrium reaction in muscle (Beis & Newsholme, 1975), the net flux of 'energy-rich' phosphate through the reaction, either at the myofibrillar or mitochondrial sites in the muscle, must be considerably less than the maximum activity *in vitro* of the enzyme (for theory, see Newsholme & Crabtree, 1973, 1976; Crabtree & Newsholme, 1975; Crabtree, 1978).

These results and discussion suggest that, in insect flight muscles, rat heart and possibly hearts from other animals, phosphagen kinase cannot play a role in the transfer of 'energy-rich' phosphate

from mitochondria to myofibrils, at least under conditions of maximum rates of ATP turnover.

The role of phosphagen and phosphagen kinases in these aerobic muscles may be identical with the role in the more anaerobic muscles as an energy reserve that decreases the extent of transient changes in the ATP concentration when the rate of ATP turnover is modified. However, the distribution of creatine kinase in heart muscle (i.e. in the intermembrane space and associated with the myofibrils) supports the role proposed by Watts (1971) that the phosphagen (and the kinase) prevents large transient changes in the ADP concentration. This action may be important at the myofibrils since a large increase in the concentration of ADP could inhibit myofibrillar adenosine triphosphatase (Maruyama & Pringle, 1967). In addition, the phosphagen kinase present in the intermembrane space could prevent a large increase in the concentration of ATP that could cause inhibition of the adenine nucleotide translocase (see Klingenberg, 1970; Newsholme, 1976). Thus the role of the phosphagen kinase may be to provide stable concentrations of ATP and ADP particularly at certain sites within the muscle cell. This would minimize inhibition of key enzymes or enzyme systems that could result from large changes in the concentration ratio [ATP]/[ADP]. In this way, what has been described as 'kinetic efficiency' of metabolism should be maintained even during high rates of ATP turnover (Newsholme, 1976, 1977).

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