STUDIES ON THE CREATINE KINASE EQUILIBRIUM IN MUSCLE AND THE SIGNIFICANCE OF ATP AND ADP LEVELS

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Though in many cases metabolite levels[+)] seem to reflect a somewhat realistic picture of steady state equilibria in cells and even in defined cellular compartments (cf. the prec. paper), serious errors may in some instances result from uncritical calculations on this basis. So it has been shown in previous communications (Hohorst et al. 1959, 1961) that DPN and DPNH levels as determined in rat liver extracts give no information about the reduction/oxidation state of the cytoplasmatic DPN system, which can be evaluated only indirectly.

In this paper we deal with studies on the equilibrium of ATP-linked reactions in striated muscle, a further example where uncritical calculations on the basis of metabolite levels lead to incorrect conclusions.

Materials and methods: The abdominal wall muscle of rats was freed from skin and cut along the median line. One half of the muscle layer was pressed between two metal blocks previously cooled in liquid air, separated from the body and immersed in liquid air. By using this method the muscles could be frozen within 0.1 sec. in situ. After pulverization of the tissue under liquid air, perchloric acid extracts were made and neutralized to pH 5-6. Creatinephosphate (CP), creatine (Cr), 3-phospho-

+) {A}: level (overall content) of substance A in the tissue
[A]: concentration of substance A in free solution (cf. Hohorst et al. 1959)

glycerate (3-PGA), 1.3-diphosphoglycerate (1.3-di-PGA), ATP and ADP were estimated by means of enzymatic assays (Honorst et al. 1959, 1961; Tanzer et al. 1959).

Results: In table 1 levels of creatinephosphate, creatine, 1.3-diphosphoglycerate, 3-phosphoglycerate, ATP and ADP as found in the resting abdominal muscles are summarized:

Table 1

CP	21.2 ± .8	(11)	3-PGA	.037 ± .003	(11)
Cr	11.3 ± 1.1	(5)	1.3-di-PGA	.0004 ± .00003	(8)
ATP	6.09 ± .12	(19)	ADP	.741 ± .124	(19)

Values in µMol/g fresh weight $\pm \frac{s}{\sqrt{n}}$, n = number of assays in parentheses.

From these data the following "apparent" equilibrium between creatinephosphate/creatine and 1.3-di-PGA/3-PGA may be obtained:

(1) $$\frac{\{CP\}}{\{Cr\}} : \frac{\{1.3\text{-di-PGA}\}}{\{3\text{-PGA}\}} = \frac{1.9}{1.1 \times 10^{-2}} = 1.7 \times 10^{2},$$

which approaches the mass action equilibrium between these reactants[+]:

(2) $$\frac{[CP]}{[Cr]} : \frac{[1.3\text{-di-PGA}]}{[3\text{-PGA}]} = \frac{K_{PGK}}{K_{CK}} = \frac{3.0 \times 10^{3}}{1.0 \times 10^{1}} = 3.0 \times 10^{2}.$$

Hence it may be concluded that the steady state equilibria of the creatine kinase and the phosphoglycerate kinase reactions are very close to the thermodynamic equilibrium in resting muscle. One can calculate therefore the ratio $[ATP]/[ADP]$ [++] from $\{CP\}/\{Cr\}$ and $\{1.3\text{-di-PGA}\}/\{3\text{-PGA}\}$ values on the basis of the corresponding mass action equilibria (see footnote[+]). The values obtained (about 20-30) are in the same order of magnitude as the ratio $\{ATP\}/\{ADP\}$ (=8.2), calculated from table 1.

+) $K_{PGK} = \frac{[3\text{-PGA}] \times [ATP]}{[1.3\text{-di-PGA}] \times [ADP]}$; pH 7, 25°C (Bücher 1947)

$K_{CK} = \frac{[Cr] \times [ATP]}{[CP] \times [ADP]}$; pH 7.4, 30°C, Mg^{++} 20mM (Noda et al. 1954)

++) The ratio of ATP and ADP concentrations in that compartment of the muscle cell, which contains both creatine kinase and phosphoglycerate kinase.

Though one might thus conclude that $\{ATP\}/\{ADP\}$ ratios represent true $[ATP]/[ADP]$ ratios with sufficient accuracy, the situation found in contracting muscle gives an entirely different picture. Fig.1 demonstrates the change of creatinephosphate-, creatine-, ATP- and ADP-levels during a 60 seconds period of tetanic stimulation of the abdominal muscle in situ+):

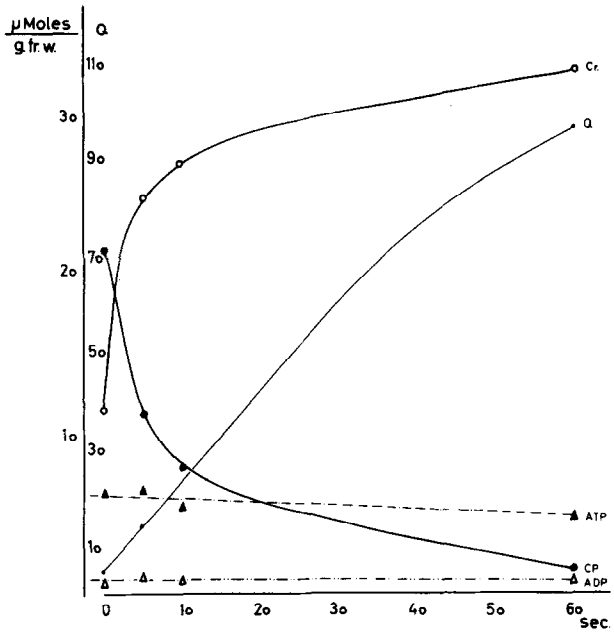

Fig.1. CP-, Cr-, ATP- and ADP-levels in tetanized abdominal muscles.

After stimulation in situ muscles were frozen immediately.

$$Q = \frac{\{Cr\} \times \{ATP\}}{\{CP\} \times \{ADP\}}$$

The rapid and large alteration of CP and Cr levels in contrast to the sluggish and small changes of ATP and ADP levels indicates that an equilibrium $\frac{\{Cr\} \times \{ATP\}}{\{CP\} \times \{ADP\}} = Q \approx K_{CK}$, which might be postulated by the metabolite data in the resting state(table 1), does not actually exist. Thus the constancy of ATP and ADP levels during contraction cannot result from stabilization by the creatinephosphate/creatine system, which would suppose an

+) The change of 1.3-di-PGA and 3-PGA levels may be discussed in a subsequent communication.

equilibrium or at least some approach to it. It also cannot be due to a steady state between dephosphorylating contraction and rephosphorylating metabolism, since the phenomenon has been observed in iodoacetate- and cyanide-poisoned muscles and during the recovery phase too. Thus it must be considered to represent a slow turnover of muscle-ATP.

At first sight it might appear from fig.1 that there is no reaction between creatinephosphate/creatine and ATP/ADP, but this conclusion seems to be very unlikely, since high creatine kinase activities have been found in this tissue (1.4×10^4 µMol/h per gram fresh weight). Moreover, if one excludes the Lohmann-reaction, one has to explain how creatinephosphate could be split without direct or indirect+) participation of ATP/ADP, because creatinephosphate needs at least catalytic amounts of adeninenucleotides in order to be split by contractile proteins (Bozler 1954). The only alternative, which accounts for all these facts, i.e., independence of $\{CP\}/\{Cr\}$ and $\{ATP\}/\{ADP\}$, low reactivity of ATP and ADP levels, and high creatine kinase activity in muscle, seems to be a functional compartmentation++) of ATP and ADP as follows: Most of the ATP and ADP (at least 85 per cent of the total) does not react either with creatine kinase or with other kinases at a rate comparable to the breakdown of creatinephosphate. We may call it the "storage-ATP" system. ATP and ADP levels are regarded as representing mainly this storage-ATP system. In addition to this, the existence of a very reactive "turnover-ATP" system must be claimed being in equilibrium with creatinephosphate/creatine, but amounting only to a few per cent of the total ATP and ADP. Changes in

+) E.g. by action of the CP/3-PGA transphosphorylating enzyme (Cori et al. 1958)

++) The possibility of a functional compartmentation of muscle-ATP has been discussed already by Davies et al. 1959

turnover-ATP and turnover-ADP concentrations therefore are practically without influence on ATP and ADP levels and may be detected only indirectly by means of creatinephosphate and creatine estimations. Accordingly the above experiment demonstrates the reactivity of the turnover-ATP system in contrast to the inactivity of the storage-ATP system.

Both the question of the structural or physical basis of compartmentation and the question of the physiological meaning of the storage-ATP system must remain open for the present. However it should be mentioned that a storage-ATP system has been found in all muscles studied (including heart and smooth muscle) but not in liver, brain and several tumors.

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