Calcium ions, hormones and mitochondrial metabolism

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Introduction

There is now a massive body of evidence substantiating the role of Ca$^{2+}$ in the regulation of a wide range of cytoplasmic processes. These include not only muscle contraction (see [1, 2]) and glycogen breakdown (see [3]), but also endocrine and exocrine secretion (see [4]), cyclic nucleotide metabolism, especially in the brain (see [5]) and glycerol phosphate oxidation [6]. It is quite clear that many of the effects of hormones and neurotransmitters on cells are brought about by changes in the cytoplasmic Ca$^{2+}$ within the range 0.1–10 μmol/l (see [7, 8]). Specific examples include the inotropic response of the heart after binding of adrenaline to β-type receptors (see [1, 8]) and the increased release of glucose from the liver after binding of agonists to α-type adrenoceptors (see [9, 10]). It has been argued that many of the actions of 5-hydroxytryptamine, histamine, acetylcholine (through muscarinic receptors) and a number of the smaller polypeptide hormones involve an increase in cytoplasmic Ca$^{2+}$ (see [14, 11]) and the possibility that insulin may also cause an elevation in cytoplasmic Ca$^{2+}$ has been raised [12].

The concentration of Ca$^{2+}$ in the extracellular fluids is at least 1000 times that in the cytoplasm. This large gradient is maintained by transport systems in the plasma membrane which pump Ca$^{2+}$ out of all mammalian cells and in addition many mammalian cells appear to contain specific Ca$^{2+}$ ‘channels’, which allow Ca$^{2+}$ into cells down the concentration gradient (see [11, 13]). In the longer term, hormones and other extrinsic agents must influence cytoplasmic Ca$^{2+}$ levels by regulating these transfer systems. However, in the shorter term, transient changes may be brought about by the redistribution of calcium within target cells. Changes in striated muscle contraction brought about by hormones and neurotransmitters certainly involve the uptake and release of Ca$^{2+}$ from the sarcoplasmic reticulum [2]. Endoplasmic reticulum preparations from some non-muscle cells also accumulate Ca$^{2+}$ and it seems reasonable to assume that the reticulum may play a role in determining cytoplasmic Ca$^{2+}$ in these cells also [13–15].

Mitochondria isolated from all mammalian cells are also able to accumulate calcium and, in fact, appear to contain up to 50% and more of the total cell calcium. In recent years, the systems in the inner mitochondrial membrane which are involved in the transfer of calcium have been intensively studied. It has been established that there are separate influx and efflux components, which together determine the distribution of Ca$^{2+}$ across the inner membrane of mitochondria [13, 16]. The principal features of this transport cycle are represented in Fig. 1. It has been rather generally assumed that the principal role of this cycle is in the regulation of cytoplasmic Ca$^{2+}$ [13, 17–19]. For example, Nicholls has demonstrated that isolated mammalian mitochondria can act as buffers of extramitochondrial Ca$^{2+}$ [20]. However, it has now become apparent that mammalian mitochondria contain at least three important dehydrogenases which are activated by Ca$^{2+}$. Therefore increases in the intramitochondrial Ca$^{2+}$ concentration in the range 0.1–10 μmol/l are potentially an important means of enhancing oxidative metabolism and thus, indirectly, ATP production [21]. If this view is correct then the function of the calcium-transport system in the inner membrane of mitochondria should be considered primarily as a means whereby the cell determines intramitochondrial (rather than extramitochondrial) Ca$^{2+}$ in the same way as the system in the plasma.
membrane is usually viewed as a means of determining the cytoplasmic rather than the plasma concentration of Ca\(^{2+}\). In many situations it seems likely that increases in cytoplasmic Ca\(^{2+}\) result in parallel increases in the intramitochondrial concentration of Ca\(^{2+}\). In this article we will briefly outline the properties of the three intramitochondrial dehydrogenases sensitive to Ca\(^{2+}\), and the evidence that increases in the extramitochondrial concentration of Ca\(^{2+}\) within the physiological range result in the parallel activation of all three enzymes. We believe that changes in intramitochondrial Ca\(^{2+}\) may be an important means whereby hormones and neurotransmitters can influence intramitochondrial metabolism, and to illustrate this we will discuss the effects of adrenaline on heart muscle, vasopressin and adrenaline on liver, and insulin on fat cells.

**Intramitochondrial dehydrogenases sensitive to Ca\(^{2+}\)**

To date three dehydrogenases in mammalian mitochondria have been found to be activated by Ca\(^{2+}\) and in each case the concentration of Ca\(^{2+}\) giving half-maximal effects (\(k_{0.5}\) value) was found to be close to 1 \(\mu\)mol/l. The three enzymes are the pyruvate dehydrogenase complex, which converts pyruvate into acetyl-CoA, and two enzymes of the citrate cycle, isocitrate dehydrogenase (NAD\(^{+}\)) (EC 1.1.1.41) and the 2-oxoglutarate dehydrogenase complex (Fig. 2). The effects of Ca\(^{2+}\) on pyruvate dehydrogenase activity are brought about by changes in the proportion of the complex in its active non-phosphorylated form. The phosphatase is activated by Ca\(^{2+}\) [22, 23], whereas pyruvate dehydrogenase kinase may be inhibited by Ca\(^{2+}\) [24]. The effects of Ca\(^{2+}\) on isocitrate dehydrogenase (NAD\(^{+}\)) and oxoglutarate dehydrogenase are more direct. In both cases there are great increases in the affinity of the enzymes for their substrates (isocitrate and oxoglutarate respectively) with little or no change in their maximum activities [21, 25, 26]. In other words, Ca\(^{2+}\) greatly stimulates the activity of these two dehydrogenases at low (and physiological) substrate concentrations but not at high, saturating, concentrations. All three dehydrogenases appear to have very similar properties in all mammalian tissues, including those from pig and human heart and the following rat tissues: white and brown adipose tissue, heart and skeletal muscle, liver, kidney and brain (see [21]). There is one other key intramitochondrial dehydrogenase which may be activated by Ca\(^{2+}\) and that is the branched-chain oxo acid dehydrogenase complex involved in the oxidation of the keto acids derived from leucine, isoleucine and valine. This enzyme is related to the pyruvate dehydro-
FIG. 2. Interaction of Ca\(^{2+}\) with intramitochondrial enzymes. It should be noted that all three dehydrogenases which are stimulated by Ca\(^{2+}\) are also activated by increases in the intramitochondrial ADP/ATP and/or NAD\(^{+}\)/NADH concentration ratios (see [21]). In addition both pyruvate dehydrogenase and oxoglutarate dehydrogenase are also susceptible to end-product inhibition by acetyl-CoA and succinyl-CoA respectively (see [21]). PDH, Pyruvate dehydrogenase; PDHP, pyruvate dehydrogenase phosphate.

genase complex and is also inhibited by phosphorylation [27, 28]. Since these two complexes may share the same phosphatase [27], it follows that an increase in intramitochondrial Ca\(^{2+}\) should result in the activation of the oxidation of the keto acids derived from the branched-chain amino acids, but this important possibility has not been explored in any detail and will not be considered further in this article.

Pyruvate dehydrogenase, isocitrate dehydrogenase (NAD\(^{+}\)) and oxoglutarate dehydrogenase are all exclusively located within the inner membrane of mitochondria and are widely considered to be important sites of regulation of intramitochondrial oxidative metabolism. In particular, the activity of all three dehydrogenases may be elevated by increases in the concentration ratios of ADP/ATP and NAD\(^{+}\)/NADH within mitochondria (see [21]). This can be viewed as the 'intrinsic' means whereby the rate of NADH production in mitochondria is always matched closely to the requirements of the respiratory chain and thus ATP utilization in cells. The exciting possibility which emerges from the activation of the same key dehydrogenases by Ca\(^{2+}\) is that this might provide a mechanism through which control of intramitochondrial metabolism by 'extrinsic' agents such as hormones and neurotransmitters could be superimposed on the 'intrinsic' control mechanisms. In this way, the rates of pyruvate oxidation and of flux through the citrate cycle could be increased without the need to decrease the concentration of ATP and/or NADH. This could well be very advantageous to cells. It has long been something of a puzzle that increases in mitochondrial oxidative metabolism associated with increases in muscular work are not usually associated with clear-cut indications of decreases in the ATP/ADP or NADH/NAD\(^{+}\) concentration ratios (see [29–31]). Activation of mitochondrial oxidative metabolism by increases in Ca\(^{2+}\) could clearly furnish, at least, a partial explanation for these observations.

This hypothesis is greatly strengthened by extensive studies on the activities of the dehydrogenases within intact mitochondria [32–34]. These studies, initially made with uncoupled mitochondria, have shown that the properties of the dehydrogenases when located within mitochondria are extremely similar to those found previously with the separated enzymes. More importantly, with fully coupled mitochondria isolated from both heart and adipose tissue it has been demonstrated that changes in the extramitochondrial Ca\(^{2+}\) concentration within the physiological range (0.1–10 \(\mu\)mol/l) lead to the parallel activation of all three dehydrogenases. In the presence of Na\(^{+}\) and Mg\(^{2+}\), the concentration of extramitochondrial Ca\(^{2+}\) which gives half-maximal activation of the dehydrogenases is close to 0.5 \(\mu\)mol/l. This fits in almost perfectly with the range of cytoplasmic Ca\(^{2+}\) concentrations recently reported to occur in ferret heart cells of 0.13–5 \(\mu\)mol/l, after a small correction is made for the slightly different basis of calculating the
free Ca$^{2+}$ concentration [8]. It also follows from these studies that the concentration of Ca$^{2+}$ within mitochondria may only be about twice the concentration in the cytoplasm despite the large electrical gradient which favours the entry of Ca$^{2+}$ into mitochondria (see Fig. 1).

**Examples of hormone action which may be brought about by an increase in mitochondrial Ca$^{2+}$**

**β-Adrenoceptor stimulation of heart muscle metabolism**

Exposure of heart muscle to adrenaline leads, via a β-receptor interaction, to increased muscle contraction. This is undoubtedly achieved through alterations in the activities of the calcium-transport systems in the plasma membrane and sarcoplasmic reticulum initiated by an increase in cell cyclic AMP (see [1, 2]). This increase in muscle contraction requires an increase in ATP turnover and certainly the rate of oxygen consumption, pyruvate oxidation and the citrate cycle rise markedly. There is some evidence which suggests that there may be transient decreases in mitochondrial ATP/ADP and NADH/NAD$^+$ concentration ratios but these are no longer apparent after 1 min, although the increases in oxidative metabolism are maintained (see [1, 35]). Recently it has been shown that the proportion of pyruvate dehydrogenase in its active non-phosphorylated form increases up to fourfold or more after the perfusion of rat hearts with medium containing adrenaline [36, 37]. We believe that this activation and the persistent increased rate of the citrate cycle is the result, at least in part, of an increase in mitochondrial oxidative metabolism, and this, indeed, is observed (see [39]). Moreover, there are substantial increases in the proportion of pyruvate dehydrogenase in its active non-phosphorylated form without any clear-cut effects on any of the other regulators of the system, including NADH and ATP (O. Oviaasu, unpublished observations). Activation of oxoglutarate dehydrogenase is also indicated as the tissue concentration of oxoglutarate is greatly diminished, although the rate of its oxidation must be markedly enhanced [42]. Further supporting evidence has come from some studies which have reported that α-adrenoceptor stimulation results in an increase in the amount of calcium associated with liver mitochondria [40, 43, 44]. However, there are at least an equal number of reports which conclude that there is a decrease (e.g. [9, 10, 38, 45]).

**Insulin and the stimulation of the conversion of glucose into fat**

Exposure of fat cells to insulin leads to a substantial increase in the proportion of pyruvate dehydrogenase in its active form and this activation is important in the overall stimulation of fat synthesis from glucose (see [46]). Since no appropriate change has been found in the concentration of the other effectors of the pyruvate dehydrogenase system, it has been proposed that insulin may bring about this increase through a rise in the level of Ca$^{2+}$ within fat-cell mitochondria [47, 48]. It has been suggested on the basis of studies on the rate of $^{45}$Ca efflux from fat-cells that insulin may increase the cytoplasmic concentration of Ca$^{2+}$ (see [41]). However, this seems rather unlikely as the spectrum of effects on cytoplasmic processes observed with insulin are, in some cases, the opposite of those usually associated with an increased cytoplasmic Ca$^{2+}$. To take one example, insulin promotes glycogen synthesis, not breakdown as would be expected if cytoplasmic Ca$^{2+}$ were to increase. It is possible to envisage increases in mitochondrial Ca$^{2+}$ occurring without a parallel change in the cytoplasm if one or both components of the calcium-transport system...
in the mitochondria are affected by the hormone. At present we have no firm evidence that insulin regulates the mitochondrial calcium-transport system but we believe that this possibility is worthy of further investigation.

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References


