THE OXIDATION OF KETONE BODIES BY RAT BRAIN MITOCHONDRIA

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1. Introduction

There has been considerable interest recently in the possibility that the brain might utilise ketone bodies, particularly in circumstances where the supply of glucose is restricted. The strongest evidence comes from the investigation on obese human subjects deprived of food for five to six weeks [1]. It was calculated that 60 percent of the total cerebral oxygen consumption was accounted for by the oxidation of β-hydroxybutyrate and acetoacetate.

Some studies on D(-)-β-hydroxybutyrate dehydrogenase activity of rat brain have been made [2, 3] and the addition of acetoacetate to rat brain slices has been shown to increase the oxygen uptake [4, 5].

The present communication gives evidence for the oxidation of ketone bodies by isolated rat brain mitochondria and shows that the rate of oxygen uptake and the degree of respiratory control is comparable with that of substrates such as pyruvate plus oxaloacetate measured under the same conditions. These conditions were based on those described recently [6] in a study of acetoacetate oxidation by kidney and heart mitochondria.

2. Experimental

Brain mitochondria were prepared as previously described [7] (with the modification that 0.3 M sucrose [8] was used in place of 0.25 M) from 30 day old male rats of the Porton strain maintained *ad libitum* on M.R.C. diet No. 41B. [9].

Oxygen uptake was measured with a Clark oxygen electrode.

Acetoacetate was prepared from ethylacetoacetate essentially as described previously [10].

3. Results and discussion

The rate of oxygen uptake of brain mitochondria with either DL-β-hydroxybutyrate or acetoacetate plus oxaloacetate was stimulated by 2,4-dinitrophenol (table 1). The stimulated rates approached that attained with pyruvate and oxaloacetate as substrates. The rate of oxygen uptake with oxaloacetate alone was no higher than the rate without added substrates so that the increased observed on addition of acetoacetate, or pyruvate, was indicative of the formation of acetyl CoA.

Table 1
Oxidation of ketone bodies by rat brain mitochondria stimulated with 2,4-dinitrophenol.

Substrate	natom O/mg protein/min before DNP	after DNP	Ratio $\frac{\text{after DNP}}{\text{before DNP}}$
Pyruvate + OAA	71	175	2.46
βOH + OAA	75	156	2.08
AcAc + OAA	57	107	1.87
βOH	63	125	1.98

The incubation medium contained 10 mM tris-HCl buffer (pH 7.4) 6 mM $MgCl_2$, 58 mM KCl and 26 mM NaCl. Temperature 37°C; total volume 1.2 ml, substrate concentrations: pyruvate 4.16 mM, βOH 8.33 mM, acetoacetate 2.08 mM, oxaloacetate 0.104 mM, DNP concentration 22.5 μM.

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Alexandre et al. [6] showed that the oxidation of acetoacetate by kidney mitochondria stimulated by 2,4-dinitrophenol was inhibited by arsenate. They suggested this might be evidence that acetoacetate activation in the presence of 2,4-dinitrophenol was by an ATP-independent process involving the succinyl-CoA: 3 oxoacid CoA transferase reaction. However, the possibility cannot be entirely excluded that ATP formed at the substrate level of 2-oxoglutarate oxidation is utilised in a CoA ligase reaction. In fig. A brain mitochondria are shown to resemble those from kidney and heart [6] inasmuch as the 2,4-dinitrophenol stimulated oxidation of acetoacetate was partially inhibited by arsenate. This inhibition was completely overcome by the addition of oligomycin and ATP. However, the rate of oxygen uptake was dependent upon the concentration of ATP added. As can be seen in fig. A a second addition of 1.75 μmoles of ATP abolished respiration. Presumably this effect was largely due to inhibition of citrate synthase [11, 12]. The results with DL-β-hydroxybutyrate were similar (fig. B) but because of the relatively high rate of oxygen uptake with β-hydroxybutyrate alone (table 1) the effect of arsenate was less distinct.

To confirm that the oxidation of ketone-bodies was coupled to phosphorylation, the rates of oxygen consumption with and without ADP were measured (table 2). The absolute rates of oxygen uptake in the absence and presence of ADP were very dependent on the precise experimental conditions. In particular, the influence of inorganic phosphate was complex in relation to the different substrates tested. The concentrations used in the experiments reported, 0.42 M, did not give optimal rates for pyruvate plus oxaloacetate in the presence of ADP but higher concentrations of inorganic phosphate were inhibitory to the oxidation of ketone-bodies (unpublished observations). The results in table 2 only serve to indicate that oxygen uptake was regulated by ADP.

The data presented show that rat brain mitochondria are capable of activating acetoacetate. However, our results do not allow us to make a quantitative assessment of the relative importance of the succinyl-CoA transferase and the CoA ligase pathways.

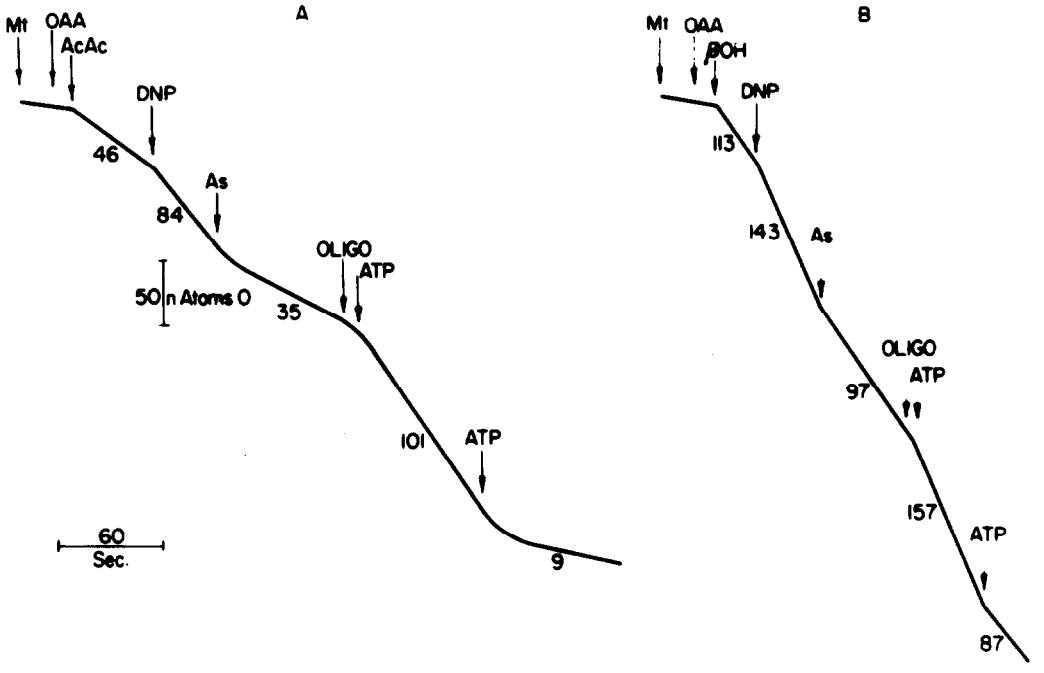

Fig. Incubation conditions were as for table 1. The following were added at the arrows: 1.2 mg brain mitochondrial protein (Mt), 0.125 μmole of oxaloacetate (OAA), 2.5 μmoles of acetoacetate (AcAc), 0.027 μmole of 2,4-dinitrophenol (DNP), 3.5 μmoles of arsenate (As), 5 μg of oligomycin (OLIGO), 1.75 μmoles of ATP. In trace B the additions were the same except that acetoacetate was replaced by 5 μmoles of DL-β-hydroxybutyrate (βOH). Numbers beside the graphs represent natoms O/mg protein/min

Table 2
The respiratory control ratio of rat brain mitochondria oxidizing ketone-bodies.

Substrate	natoms O/mg protein/min. Before ADP	After ADP	Respiratory control ratio
Pyruvate + OAA	87	225	2.58
βOH + OAA	50	220	4.40
AcAc + OAA	66	171	2.53
βOH	66	109	1.65

Incubation conditions as for table 1 except that 0.42 mM P_i was present. Substrate concentrations: pyruvate 4.16 mM, βOH 8.33 mM, acetoacetate 2.08 mM, oxalocetate 1.04 mM. 1 μmole ADP was added.

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References

[1] O.E.Owen, A.P.Morgan, H.G.Kemp, J.M.Sullivan, M.G. Herrera and G.F.Cahill, J. Clin Invest. 46 (1967) 1589.
[2] C.B.Klee and L.Sokoloff, J. Biol. Chem. 242 (1967) 3880.
[3] A.L.Smith, H.S.Satterthwaite and L.Sokoloff, Science 163 (1969) 79.
[4] Z.Drahota, P.Hahn, J.Mourek and M.Trojanová, Physiol. Bohemoslav 14 (1965) 134.
[5] H.Openshaw and W.M.Bortz, Diabetes 17 (1968) 90.
[6] A.Alexandre, D.Siliprandi and N.Siliprandi, Biochim. Biophys. Acta 180 (1969) 237.
[7] J.Somogyi and S.Vince, Acta Physiol. Acad. Sci. Hung. 20 (1961) 325.
[8] W.N.Aldridge, Biochem. J. 67 (1957) 423.
[9] H.M.Bruce and A.S.Parkes, J. Animal Tech. Assoc. 7 (1956) 54.
[10] H.A.Krebs and L.V.Eggleston, Biochem. J. 39 (1945) 408.
[11] G.W.Kosicki and L.P.K.Lee, J. Biol. Chem. 241 (1966) 3571.
[12] D.Shepherd and P.B.Garland, Biochem. J.114 (1969) 597.