COMPETITIVE EFFECT OF SUCCINATE ON GLYCEROL-1-PHOSPHATE OXIDATION IN LIVER MITOCHONDRIA FROM THYROXINE-TREATED RATS

P.SWANLJUNG* and N.-E.L.SARIS
Department of Clinical Chemistry, University of Helsinki, Helsinki 29, Finland

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Treating rats with thyroid hormones causes a considerable increase in the activity of mitochondrial glycerol-1-phosphate oxidase (EC 1.1.2.1.) in the liver [1,2]. The physiological significance of this increase is not quite clear. A glycerol-1-phosphate cycle has been proposed as a mechanism for the transport of reducing equivalents into mitochondria [3]. The quantitative importance of this cycle in mammalian tissues has, however, been questioned [4]. Although the rate of glycerol-1-phosphate oxidation by isolated liver mitochondria from thyroxine-treated rats is greatly enhanced [1,2], it is open to some doubt whether the oxidation of glycerol-1-phosphate at *in vivo* concentrations and in competition with other substrates can explain, to any larger extent, the well-known increase in oxygen uptake in tissues from thyroxine-treated animals. In this work the Michaelis constant for the oxidation of glycerol-1-phosphate in a reconstructed glycerol-1-phosphate cycle [5] has been determined together with the inhibitor constant for succinate on this oxidation.

The results are presented in table 1. NADH-linked substrates such as malate and glutamate, alone and together, were found to have no effect on the glycerol-1-phosphate oxidation. The inhibitive effect of succinate is almost completely reversed by malonate. Malonate should only add to the interanion compe-

* Present address: Biokemiska Institutionen, Uppsala Universitet, Uppsala, Sweden.

Table 1
Succinate and the oxidation of glycerol-1-phosphate in liver mitochondria from thyroxine-treated rats.

Expt.	Substrate	K_m (mM)	O_2 uptake at K_m (nmoles ml^{-1} min^{-1})	K_i for succinate (mM)
1	Glycerol-1-phosphate	1.5	20	0.78
1	Succinate	0.76	42	
2	Glycerol-1-phosphate	1.4	34	0.80
2	Succinate	0.84	75	

Male Sprague-Dawley rats were treated 4 days with 150 μg L-thyroxine (Fluka) *per diem*. The weights at sacrifice were 258 g (Expt. 1) and 231 g (Expt. 2). Mitochondria were prepared as described in [9]. The activity of the glycerol-1-phosphate cycle was measured at 345–374 nm, using an Aminco-Chance dual wavelength spectrophotometer. Each cuvette contained 50 mM tris buffer (pH 7.4), 110 mM KCl, 8 mM NaCl, 2 mM $MgCl_2$, 8 mM KH_2PO_4, 0.67 mM ADP, 0.33 mM NADH, 0.5 μM rotenone, 16.7 μg/ml glycerol-1-phosphate dehydrogenase (Boehringer) and 0.57 mg/ml (Expt. 1) – 1.30 mg/ml (Expt. 2) mitochondrial protein. Temperature 25°C. Glycerol-1-phosphate and succinate were added in varying amounts. Oxygen uptake was measured polarographically in a similar system using a GME Oxygraph. K_m and K_i values were calculated graphically using double reciprocal plots.

tition for positive charges at the level of penetration [6]. Both succinate and glycerol-1-phosphate are oxidized by flavoproteins that enter the respiratory chain in close proximity to each other [7]. Thus the competition between succinate and glycerol-1-phosphate, at low and moderate concentrations, is located in the respiratory chain, probably where the flavoproteins feed reducing equivalents to a common carrier (ubiquinone, cytochrome b) [8].

From the data in table 1 it is apparent that the succinate dehydrogenase is much more efficient in channelling electrons into the respiratory chain than the glycerol-1-phosphate enzyme. The K_m for succinate oxidation and the K_i for succinate on glycerol-1-phosphate oxidation are practically identical. Although the concentrations of metabolites in the compartmented mitochondrion and in the cytosol are poorly known, the values for these constants, together with the higher K_m value for glycerol-1-phosphate oxidation, would indicate that the enhanced glycerol-1-phosphate activity can contribute only very little to the overall increase in respiration in thyroxine-treated animals.

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References

[1] Y.P.Lee, A.E.Takemori and H.Lardy, J. Biol. Chem. 234 (1959) 3051.
[2] B.Kadenbach, Biochem. Z. 344 (1966) 49.
[3] T.Bucher and M.Klingenberg, Angew. Chem. 70 (1958) 552.
[4] I.Hassinen, Ann. Med. Exptl. Biol. Fenniae (Helsinki) 45 (1967) 35.
[5] E.M.Suranyi, R.Hedman, R.Luft and L.Ernster, Acta Chem. Scand. 17 (1963) 877.
[6] K.Van Dam and C.S.Tsou, Biochim. Biophys. Acta 162 (1968) 301.
[7] R.L.Ringler and T.P.Singer, J. Biol. Chem. 234 (1959) 2211.
[8] L.Ernster and C.-P.Lee, Ann. Rev. Biochem. 33 (1964) 729.
[9] M.K.F.Wikström and N.-E.L.Saris, European J. Biochem. 9 (1969) 160.