Reconstruction of the Glycerol-1-phosphate Cycle with Subcellular Fractions from Rat Skeletal Muscle

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Isolated mitochondria from rat skeletal muscle catalyze the aerobic oxidation of added reduced diphosphopyridine nucleotide (DPNH) only at a slow rate [1]. This rate can be enhanced considerably by the addition of glycerol-1-phosphate and a purified preparation of rabbit skeletal muscle glycerol-1-phosphate dehydrogenase (Baranowski enzyme) (Table 1). Nearly maximal rate of DPNH oxidation occurs when the concentration of added glycerol-1-phosphate is 1 mM. Like the aerobic oxidation of glycerol-1-phosphate[1], the reaction is inhibited by antimycin A, but not by amytal or rotenone. Hence it is concluded that the reaction involves the glycerol-1-phosphate cycle [2-4], *i.e.*, a cyclic oxidation and reduction of glycerol-1-phosphate *via* the mitochondrial glycerol-1-phosphate oxidase and the soluble glycerol-1-phosphate dehydrogenase, respectively. These results are similar to those reported by Zebe *et al.* [4] and Sacktor and Dick [5] with insect flight muscle and by Borst [6] with Ehrlich ascites tumor.

Table 1. Reconstruction of the glycerol-1-phosphate cycle with subcellular fractions from rat skeletal muscle. Each cuvette contained 25 mM Tris buffer (pH 7.4), 50 mM KCl, 8 mM $MgCl_2$, 5 mM orthophosphate (pH 7.4), 3 mM adenosine diphosphate, 0.1 mM DPNH, rat muscle mitochondria (0.65–0.70 mg protein), and, when indicated, glycerol-1-phosphate (Gl-1-P), Baranowski enzyme (B.E.), cell-sap (C.S.), 2 mM amytal, 3 μg rotenone, and 3 μg antimycin A. Amount of C.S. is expressed in mg protein/mg mitohcondrial protein. Total volume 3.0 ml. Temperature 28°C. Aerobic oxidation of DPNH was followed in a Beckman DK-2 spectrophotometer at 340 mμ.

Expt. No.	Additions	DPNH oxidized, mμmoles min^{-1} mg^{-1} mitochondrial protein
1	None	4.3
	Gl-1-P (20 mM)	2.2
	B.E. (50 μg)	3.6
	Gl-1-P (20 mM) + B.E. (50 μg)	39.6
	» » + » » + antimycin A	3.6
	» » + » » + amytal	38.2
	» » + » » + rotenone	40.0
2	None	3.4
	Gl-1-P (1 mM) + B.E. (50 μg)	49.7
	» (3 mM) + » »	60.0
	» (5 mM) + » »	61.4
	» (10 mM) + » »	58.6
	» (15 mM) + » »	60.7
3	None	7.4
	C.S. (3.4 mg prot.)	12.6
	» » + Gl-1-P (20 mM)	51.9
	» » + » » + antimycin A	1.5
	» » + » » + amytal	53.8
	» » + » » + rotenone	48.3
4	Gl-1-P (20 mM)	5.1
	» » + B.E. (50 μg)	68.5
	» » + » (100 μg)	68.5
	» » + C.S. (1.1 mg prot.)	32.5
	» » + » (2.1 mg prot.)	49.6
	» » + » (3.7 mg prot.)	66.2

Replacement of the purified Baranowski enzyme in the above system by cell-sap, obtained from the preparation of the rat skeletal muscle mitochondria, also resulted in an enhanced DPNH oxidation, which again was sensitive to antimycin A and insensitive to amytal and rotenone. 1.1 mg cell-sap protein per mg mitochondrial protein gave rise to a glycerol-1-phosphate cycle activity of about 50 %, and 3.7 mg cell-sap protein to an activity of almost 100 %, of that obtained with an excess of purified Baranowski enzyme.

These results demonstrate the operation of the glycerol-1-phosphate cycle in a reconstructed subcellular system from rat skeletal muscle. The contribution of this cycle to the oxidation of extramitochondrial DPNH by the mitochondrial respiratory chain in the intact muscle may be ascertained in the future by investigating the effects of antimycin A, amytal and rotenone on the respiration of whole muscle under appropriate experimental conditions.

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1. Hedman, R., Suranyi, E. M., Luft, R. and Ernster, L. *Biochem. Biophys Res Commun.* **8** (1962) 314.
2. Bücher, T. and Klingenberg, M. *Angew. Chem.* **70** (1958) 552.
3. Estabrook, R. W. and Sacktor, B. *J. Biol. Chem.* **233** (1958) 1014.
4. Zebe, E., Delbrück, A. and Bücher, T. *Biochem. Z.* **331** (1959) 254.
5. Sacktor, B. and Dick, A. *J. Biol. Chem.* **237** (1962) 3259.
6. Borst, P. *Biochim. Biophys. Acta* **57** (1962) 270.

Steroid Effects on Antimycin Inhibited NADH and Succinate Oxidation

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In experiments where spectral changes in the respiratory chain components of heart sarcosomal fragments were recorded during oxidation of NADH, it was observed that the time required for the suspension to expend dissolved oxygen in the presence of antimycin was longer than when deoxycorticosterone was added together with antimycin. Deoxycorticosterone and other steroids are potent inhibitors of NADH oxidation[1,2] and although Yielding *et al.*[3] found that the steroids apparently lost their inhibitory effect in the presence of antimycin, a partial reversal of antimycin inhibition as indicated here was unexpected.

A change in the rate of oxygen consumption could be demonstrated directly in experiments where oxygen concentration was recorded by means of a Clark oxygen electrode. The combined addition of antimycin and deoxycorticosterone led to a rate of oxygen consumption lower than that obtained with steroid alone, but higher than that with antimycin added alone. It appeared as if the steroid had prevented antimycin from excerting its full inhibitory effect. Preincubation of ETP with antimycin prior to the start of the reaction and addition of steroid did not abolish this effect of the steroid.

Neither steroids nor amytal normally inhibit succinate oxidation, and this reaction should therefore provide a more simple system for investigation of steriod and amytal effects on antimycin inhibition. When NADH was replaced with succinate and oxygen concentration recorded, the results showed, however, that steroid had an effect on antimycin inhibited succinate oxidation opposite to what it had on antimycin inhibited NADH oxidation. Amytal in 20 times higher concentrations had no effect at all. Again preincubation of ETP with antimycin did not change this picture. The results with succinate were, however, less reproducible than with NADH. Ageing both at $-15°$ and at 0°C appeared to influence the response to steroid, and also the initial succinate concentration in the reaction medium played a role.

These observations show that steroid and amytal can react with the respiratory chain in a more complex manner than previously assumed, and that steroid and amytal need not necessarily have identical effects.

1. Jensen, P. K. *Nature* **184** (1959) 451.
2. Yielding, K. L. and Tomkins, G. M. *Proc. Natl. Acad. Sci. U.S.* **45** (1959) 1730
3. Yielding, K. L., Tomkins, G. M., Munday, J. S. and Cowley, I. J. *J. Biol. Chem.* **235** (1960) 3413.