A Study of the Effect of Amytal on the Respiration of Rat Liver Slices

B. Kadenbach*, T. E. Conover** and L. Ernster

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

It has been reported [1] that rat liver slices, incubated for 1 h in a Krebs-Ringer solution containing glucose, exhibit an oxygen uptake which is resistant to amytal (5-ethyl-5-*iso*amylbarbituric acid) to an extent of about 40 %. Closer examination of this amytal-resistant respiration has now revealed that it is characterized by a declining initial phase, lasting about 20−30 min., during which its rate drops from about 60 to 20 % of the control rate, followed by a second stationary phase where the respiration remains constant at about 20 % of the control rate over a period of at least 1 h. Omission of glucose and/or addition of α-ketoglutarate, malate, glutamate, lactate and glycerol-1-phosphate increased the respiration with and without amytal (Table 1). Addition of the glycolytic inhibitors, iodoacetate or fluoride, both of which strongly depressed the control rate, had little effect on the level of the amytal-resistant respiration. Addition of succinate caused, in agreement with earlier literature [2], about a ten fold increase of the respiratory rate, and this respiration was entirely amytal-insensitive. This finding is consistent with the concept [3] that succinate is oxidized by an amytal-insensitive oxidase.

Added DPN or TPN caused about an equal, 50−100 % stimulation of the control respiratory rate both in the absence of added substrate and in the presence of added glucose or the previously mentioned pyridine nucleotide-linked substrates. The two pyridine nucleotides differed, however, with respect to their effect on the amytal-resistant respiration, this being stimulated more by TPN than by DPN. Moreover DPN and TPN added together stimulated the amytal-resistant respiration by more than the sum of the individual stimulations. These effects were apparent only on the stationary, and not the initial, phase of the amytal-resistant respiration. It would therefore seem that while the initial phase of the amytal resistant respiration probably originates from some endogenous substrate which is not continuously formed during incubation, the constant respiration phase is due to substrates which are continuously available, and part of which, at least, appear to be oxidized by way of TPN. The glucose-6-phosphate dehydrogenase shunt would seem to be a likely reaction of this type. That added DPN, in combination with TPN, further increases the amytal-resistant respiratory rate, may be due to the stimulation of a pyridine nucleotide transhydrogenase mechanism, conceivably through the mediation of lactic dehydrogenase [4].

It has been of interest also to establish whether the recently described DT diaphorase [5] is involved in the amytal-resistant respiration. This enzyme can mediate electron transport between both intra- and extramitochondrial [6] DPNH and TPNH and the cytochrome system

Table 1. Effect of added substrates and pyridine nucleotides on the amytal-resistant respiration of rat liver slices. 80 to 120 mg of liver slices (20 mg with succinate) were incubated for 1 h at 37.5°C in Krebs-Ringer-phosphate solution (half Ca^{++} conc.) with 100 % O_2 as gas phase. Final volume, 1 ml; final concentrations of the additions were: glucose, α-ketoglutarate and malate, 0.025 M; glycerol-1-phosphate, lactate, glutamate and succinate, 0.05 M; amytal, 0.002 M.

Expt. No.	Additions	μatoms oxygen/60 min./100 mg wet weight without amytal	with amytal
1	none	4.57	2.39
	glucose	4.11	1.84
2	none	4.51	1.82
	α-ketoglutarate	6.12	4.30
	malate	5.36	2.49
3	none	4.21	1.53
	glutamate	7.15	2.28
	succinate	55.4	60.5
4	none	6.07	7.71
	lactate	8.90	3.71
	glycerol-1-phosph.	7.37	4.48
5	glucose	3.98	2.10
	glucose + DPN	5.58	3.16
	glucose + TPN	5.65	3.70
	gluc. + DPN + TPN	7.85	7.90

* Fellow of the Deutsche Akademie der Wissenschaften zu Berlin, Germany.

** Fellow of the National Foundation, New York, N.Y., U.S.A.

Table 2. Dicoumarol-sensitive respiration of rat liver slices. Conditions as in Table 1. Final concentrations of the additions were: glucose, 0.05 M; iodoacetate, 0.2 mM; amytal 2 mM; vitamin K_3-bisulfite, 0.005 mM; citrate, 0.025 M; TPN, 0.001 M; dicoumarol, 0.005 mM. The system was preincubated for 20 min in the presence of all additions except amytal. The values give the respiration obtained during 48 min following the addition of amytal.

Additions	μatoms oxygen/100 mg wet weight without dicoumarol	with dicoumarol	% inhibition
none	1.79	2.43	−39
vitamin K_3-bisulfite	1.31	1.53	−19
citrate	2.46	1.72	30
citrate + vit. K_3-bis.	4.06	2.46	39
citrate + vit. K_3-bis. + TPN	7.88	4.61	41

of isolated mitochondria in an amytal-insensitive way provided that a catalytic amount of vitamin K_3 is added. To investigate this possibility, the sensitivity of the respiration to dicoumarol was tested, since it has been shown that DT diaphorase is highly sensitive to this agent [7]. It was found that 5×10^{-6}M dicoumarol, which completely inhibits DT diaphorase, had no effect on the respiration under any of the above conditions, either in the absence or in the presence of added vitamin K_3, and occasionally it even caused a slight stimulation probably due to release of the respiratory control. It was found (Table 2), however, that preincubation of the slices for 20 min. in the presence of iodoacetate in order to exhaust glycolytic intermediates, and subsequent incubation with added citrate, resulted in an increased amytal-resistant respiration which now was inhibited by 5×10^{-6}M dicoumarol to about 30 %. Moreover, addition of vitamin K_3 or of vitamin K_3 and TPN both of which further increased the amytal-resistant respiratory rate, also increased its dicoumarol sensitivity in an absolute as well as relative fashion. These findings are preliminarily interpreted to indicate that the DT diaphorase apparently takes no major part in the normal respiration of the liver cell, but may act as an alternative pathway of TPNH (and DPNH) oxidation, which can be made manifest experimentally by suppressing more efficient pathways. The main interest of these findings may lie in the fact that a dicoumarol-sensitive respiration, *i.e.* a DT diaphorase reaction, has been demonstrated in these conditions to function in the intact liver cell even without supplementation with vitamin K_3.

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1. Ernster, L. *Biochem. Soc. Symposia, Cambridge, Engl.* **16** (1959) 54.
2. Kidd, J. G., Winzler, R. J. and Burk, D. *Cancer Research* **4** (1944) 547.
3. Ernster, L., Jalling, O., Löw, H. and Lindberg, O. *Exptl. Cell Research Suppl.* **3** (1955) 124.
4. Navazio, F., Ernster, B. B. and Ernster, L. *Biochim. et Biophys. Acta* **26** (1957) 416.
5. Ernster, L., Ljunggren, M. and Danielson, L. *Biochem. Biophys. Research Comm.* **2** (1960) 88.
6. Conover, T. E. and Ernster, L. *Acta Chem. Scand.* **14** (1960) 1840.
7. Conover, T. E. and Ernster, L. *Biochem. Biophys. Research Comm.* **2** (1960) 26.