Mitochondrial and cytosolic ATP/ADP ratios in rat liver *in vivo*

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The ratio of ATP content/ADP content in livers from unanaesthetized fed rats was 0.9 in the mitochondrial matrix and 6.9 in the cytosol; the values for starved (48 h) animals were 1.0 and 5.9 respectively. The mitochondrial ratios observed in unanaesthetized animals were higher than in haemoglobin-free perfused liver and lower than in isolated hepatocytes. Possible reasons for these differences may be related to oxygen supply and/or other factors. Further, data from anaesthetized rats with the liver exposed are given: mitochondrial ATP/ADP ratios were decreased with pentobarbital, but less so with ketamine as narcotic agent.

Significant differences in mitochondrial ratios of ATP content/ADP content were found between haemoglobin-free perfused rat liver (Soboll *et al.*, 1976, 1978) and isolated hepatocytes (Siess & Wieland, 1976; Akerboom *et al.*, 1978; Soboll *et al.*, 1980) when measured by either the fractionation technique in non-aqueous solvents and/or the digitonin technique for fractionation of hepatocytes (Elbers *et al.*, 1974; Zuurendonk & Tager, 1974). In a search for the reason for these differences, it appeared worthwhile to examine first the subcellular distribution of ATP and ADP in the intact rat, since both isolated perfused liver and isolated hepatocytes are experimental systems in which hepatic metabolism might be perturbed to different extents. Therefore we modified the fractionation technique for tissue in non-aqueous solvents for application to livers *in vivo*. The livers were sampled from the rat with the double-hatchet method (Faupel *et al.*, 1972). This technique essentially excludes perturbations such as stress and the influence of narcosis during withdrawal of the liver and minimizes the problem of anoxia. In such liver samples mitochondrial and cytosolic contents of ATP and ADP were measured and compared with values from perfused livers and isolated hepatocytes.

Experimental

Extraction of the liver in vivo

Male Wistar rats weighing 200–250 g, fed on stock diet (Altromin; Lage, Lippe, Germany) or starved for 48 h, were used for rapid liver sampling by the double-hatchet method (Faupel *et al.*, 1972).

With this method the animals are placed on a wooden block and cut into three pieces with the double-hatchet between the neck and the abdominal space so that the liver can be removed quickly to be freeze-clamped between aluminium blocks pre-cooled in liquid nitrogen. The frozen liver sample is maximally 2 mm thick, so that the whole sample will be frozen in less than 0.8 s (Hohorst *et al.*, 1959; Wollenberger *et al.*, 1960). Thus within 3 s after application of the double-hatchet the liver samples were deep-frozen (Faupel *et al.*, 1972). Within the 3 s interval no changes were detectable in the content of metabolites such as adenine nucleotides, glycolytic intermediates and cyclic AMP, which are known to respond rapidly to external perturbations (Faupel *et al.*, 1972; Seitz *et al.*, 1977). The first signs of metabolic alteration in unanaesthetized rats were found to occur at 4 s (H. J. Seitz, unpublished work). The frozen tissue was then freeze-dried at 0.26 Pa at -40°C . For comparison, rats were anaesthetized with pentobarbital (50 mg/kg body wt.) or ketamine [2-(*o*-chlorophenyl)-2-methylaminocyclohexan-1-one hydrochloride] (120 mg/kg body wt.), both intraperitoneally. In these experiments the abdomen was opened and the liver transferred to aluminium tongs pre-cooled in liquid nitrogen and freeze-dried as described above.

Fractionation of tissue in non-aqueous solvents

The fractionation procedure as developed for the isolated perfused liver (Elbers *et al.*, 1974) was modified for application to livers *in situ* as follows. About 0.3 g of freeze-dried liver powder was

sonicated in a mixture of heptane/carbon tetrachloride [densities, determined accurately, of 1.290 g/ml (approx. 3.35:1.65, v/v) for liver from fed rats, and 1.276 g/ml (approx. 3.23:1.78, v/v) for starved] for 3–4 min with 5 s periods of sonication (150 W; B12 instrument; Branson, Danbury, CT, U.S.A.) and cooling alternately. The homogenate was then filtered through a column (10 cm × 2 cm) containing glass beads 0.5 mm in diameter and was then centrifuged at 10 500 *g* for 5 min. The supernatant contained most of the erythrocytes (about 70%) and was discarded. The sediment was layered on top of a density gradient of the same solvents (density 1.29–1.38 g/ml), centrifuged for 4 h at 16 000 *g* and fractionated into seven to nine fractions (1.2–1.6 ml). Most of the remaining erythrocytes appeared in the first fraction, which was also discarded. The amount of haemoglobin found in fractions 2–9 was less than 40 pmol/mg of protein.

Assays

Marker enzymes, protein, ATP and ADP were measured as described previously (Soboll *et al.*, 1978). Metabolite assays were performed with a Sigma ZWS-11 dual-wavelength spectrophotometer (Biochem, München, Germany) at 340–400 nm.

Materials

Enzymes and coenzymes were purchased from Boehringer, Mannheim, Germany. Pentobarbital sodium (Nembutal) was purchased from Abbott, Neuilly-sur-Seine, France, and ketamine hydrochloride (Ketanest) from Parke-Davis, München,

Germany. All other chemicals were from E. Merck, Darmstadt, Germany, and were of the highest purity available.

Results

Mitochondrial and cytosolic ATP/ADP ratios in livers from unanaesthetized rats

Table 1 shows the overall contents and subcellular concentrations of ATP and ADP and the ATP/ADP ratios in livers from unanaesthetized rats fed *ad libitum* or starved for 48 h. The mitochondrial ATP/ADP ratios are about 6–12-fold lower than the cytosolic ratios, in agreement with earlier measurements in isolated mitochondria and perfused liver (Heldt *et al.*, 1972; Soboll *et al.*, 1978). Interestingly, the mitochondrial ATP/ADP ratios are higher *in vivo* than in perfused liver, but they are lower than in isolated hepatocytes (Table 2). The overall ATP/ADP ratios were similar to those obtained previously with the double-hatchet method (Faupel *et al.*, 1972). The subcellular ATP/ADP ratios were not significantly different in the starved state as compared with the fed state. It may be noted, however, that differences were observed in perfused liver, but not with isolated hepatocytes (Table 2).

Effect of anaesthesia on hepatic subcellular ATP/ADP ratios

Subcellular ATP/ADP ratios were also measured during anaesthesia by the standard freeze-stop method (Wollenberger *et al.*, 1960) as applied to the exposed liver with surgically opened abdominal

Table 1. Subcellular ratios of ATP and ADP content of liver in unanaesthetized and anaesthetized rats
Subcellular concentrations were calculated from contents by assuming 0.8 ml of water/g of mitochondrial and 3.8 ml/g of cytosolic protein respectively. Abbreviation: n.m., not measured. Data are given as means ± S.E.M. (*n* = four different livers).

	Unanaesthetized		Anaesthetized	
	Fed	Starved	Pentobarbital Fed	Ketamine Fed
Total content (μmol/g dry wt.)				
ATP	12.4 ± 0.65	9.97 ± 0.39	9.88 ± 0.28	11.3 ± 0.56
ADP	3.19 ± 0.16	3.04 ± 0.12	3.14 ± 0.11	2.67 ± 0.25
AMP	0.58 ± 0.10	0.67 ± 0.11	n.m.	n.m.
ATP/ADP	3.90 ± 0.21	3.29 ± 0.16	3.06 ± 0.22	4.30 ± 0.39
Mitochondrial matrix (μmol/ml of mitochondrial water)				
ATP	7.49 ± 0.73	7.92 ± 0.93	4.21 ± 0.82	6.42 ± 0.60
ADP	8.98 ± 1.84	7.62 ± 0.84	7.43 ± 0.73	7.55 ± 0.97
ATP/ADP	0.92 ± 0.18	1.04 ± 0.03	0.55 ± 0.06	0.86 ± 0.04
Cytosol (μmol/ml of cytosolic water)				
ATP	6.18 ± 0.62	5.33 ± 0.25	5.62 ± 0.36	5.91 ± 0.41
ADP	0.86 ± 0.23	0.93 ± 0.08	0.99 ± 0.12	0.69 ± 0.19
ATP/ADP	6.94 ± 0.59	5.91 ± 0.65	5.83 ± 0.40	10.3 ± 2.10

Table 2. Mitochondrial and cytosolic ATP/ADP ratios in rat livers (unanaesthetized), perfused livers and isolated hepatocytes

Liver <i>in situ</i>	Fed	Starved	Reference
ATP/ADP mitochondrial	0.9	1.0	The present paper
ATP/ADP cytosolic	6.9	5.9	
Perfused liver			
ATP/ADP mitochondrial	0.2	0.7	Soboll <i>et al.</i> (1978)
ATP/ADP cytosolic	10.3	2.6	
Isolated hepatocytes			
ATP/ADP mitochondrial	2.3	1.5	Akerboom <i>et al.</i> (1978); Siess & Wieland (1976); Soboll <i>et al.</i> (1980)
ATP/ADP cytosolic	5–12	9.1	

cavity (Table 1). The overall as well as mitochondrial-matrix and cytosolic ATP/ADP ratios are slightly lower with pentobarbital-treated than in unanaesthetized rats, whereas with ketamine anaesthesia no significant difference from the values *in vivo* was observed.

Discussion

Subcellular ATP/ADP ratios in vivo compared with perfused liver and isolated hepatocytes

With slight modifications of the non-aqueous fractionation procedure it now became possible to determine hepatic mitochondrial and cytosolic ATP/ADP ratios in samples obtained with the double-hatchet method. The samples can be considered to approach closely the condition *in vivo* (Faupel *et al.*, 1972; Seitz *et al.*, 1977), thus permitting a comparison of data *in vivo* with those found in the two widely used metabolic systems, i.e. the isolated perfused liver and isolated hepatocytes. The mitochondrial ATP/ADP ratio *in vivo*, 0.9, is in between the two extremes, i.e. the low value of 0.2 in perfused liver and the high value of 2.3 in isolated hepatocytes (Table 2). The cause of this difference is unknown. It may be related to oxygen gradients that exist in intact liver (Sies, 1977; Matschinsky *et al.*, 1978), whereas in suspensions of isolated liver cells oxygen diffusion gradients may be negligible (Sies, 1977; Jones & Mason, 1978). Thus it is possible that in the intact liver oxygen may leave the organ in the venous blood or perfusate while at the same time perivenous cell areas may be close to a hypoxic corner because of diffusion in the Krogh cylinder (Lübbbers, 1968). However, it may be noted that in haemoglobin-free perfused liver there is no sign of reduction of cytochrome oxidase (Sies, 1977). Therefore this possibility requires further testing; in preliminary experiments we found that mitochondrial ATP/ADP ratios can be raised from 0.2 up to 1.0 in perfused livers by infusion of H_2O_2 (0.8 mM) in the perfusate to raise the O_2 supply via action of hepatic catalase (S. Soboll, P. Graf & H. Sies, unpublished work).

Effect of anaesthesia on subcellular ATP/ADP ratios in liver

It is well known that drugs used as narcotic agents may inhibit the mitochondrial respiratory chain (Chance & Hollunger, 1963; Ernster *et al.*, 1963). This may become particularly critical with respect to the subcellular ATP/ADP ratios, as may be assumed also from previous studies with whole liver on overall ATP/ADP ratios (Reinauer & Hollmann, 1966). The measurements show (Table 1) that pentobarbital has a detectable effect on the mitochondrial and cytosolic ATP systems of the liver in the dose range for anaesthesia of rats, whereas with ketamine, the other narcotic agent employed, no significant alterations were found. This is in agreement with observations on isolated rat liver mitochondria (results not shown) demonstrating that half-maximal inhibition of O_2 uptake by the respiratory chain is achieved with ketamine at a concentration 50 times higher than with pentobarbital.

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