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5′-Nucleotidase activity and adenosine production in rat liver mitochondria

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The controversial subject of mitochondrial 5′-nucleotidase in the liver was studied employing density gradient fractionation combined with a method for analyzing the distribution profiles of marker enzymes based on multiple regression analysis. Triton WR-1339 was used to improve the separation of mitochondria from lysosomes by the gradient centrifugation technique. Adenosine production was examined further using acetate to increase intramitochondrial AMP, and thus adenosine production, in incubations with gradient centrifugation-purified mitochondria. Distribution analysis of the crude homogenate showed that 5′-nucleotidase activity exists in the mitochondrial fraction. To increase the resolution of this approach with respect to mitochondria, a crude mitochondrial fraction was also studied. In this case the relative mitochondrial activity decreased but 5′-nucleotidase activity was still clearly detectable. The mitochondrial 5′-nucleotidase exhibited a K_m of 94 μM and a V_{max} of 31 nmol/min per mg protein for AMP. The kinetic data for the Mg^{2+}, ATP, ADP and AOPCP sensitivity of the enzyme showed that it differs from the plasma membrane, lysosome and cytosol 5′-nucleotidases. AOPCP was only a moderate inhibitor, and ATP was a more potent inhibitor than ADP at a 1 mM concentration. The enzyme also showed a requirement of Mg^{2+}. Acetate caused the conversion of intramitochondrial adenylates to AMP and the formation of adenosine. Adenosine concentration increased in the extramitochondrial space in a time-dependent manner, but only trace amounts of nucleotides were detected. The data show that 5′-nucleotidase activity producing adenosine exists in rat liver mitochondria and a concentration-dependent adenosine output from mitochondria by diffusion or facilitated diffusion is also suggested.

Introduction

Adenosine is involved in the regulation of a variety of physiological processes in multicellular organisms. The vasoactive properties of adenosine in the heart are well appreciated [1], but recent data indicate that it also acts as a metabolic regulator in the liver, where it has been shown to inhibit glucagon and epinephrine-stimulated glucogenolysis [2,3], gluconeogenesis from lactate and urea synthesis from NH_4Cl [4], although mice injected intraperitoneally with adenosine gradually develop hyperglycaemia. Adenosine has no effect on hepatic cholesterol or de novo synthesis of fatty acids [5], but inhibits the β-oxidation of fatty acids in intact rat liver and liver homogenates [6]. In addition, it has been shown that hypoxia or ethanol administration stimulates adenosine release from hepatocytes and perfused livers [7,8]. It has similarly been suggested that adenosine may play a specific role in antagonizing liver injury after chronic alcohol ingestion by increasing portal blood flow and oxygen delivery [9].

In view of the role of adenosine as a messenger compound in intracellular and intercellular signalling, the distribution of the enzyme system for its production is of great significance. Adenosine is formed by non-specific phosphatases, *S*-adenosylhomocysteine hydrolase and 5′-nucleotidases. Different types of 5′-nucleotidase have been described in variety of subcellular organelles. The plasma membrane-bound ecto-5′-nucleotidase and cytosolic 5′-nucleotidases have been purified from rat heart and liver [10–16]. The existence of a rat heart mitochondrial 5′-nucleotidase was suggested by Bukoski et al. [17], but their later studies have failed to confirm this observation [18]. We have also shown by employing density gradient fractionation of heart muscle combined with a method for

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analysing distribution profiles of marker enzymes for subcellular organelles based on multiple regression analysis that the 5′-nucleotidase activity associated with rat heart mitochondria was totally of lysosomal origin [19]. Cytochemical methods employing Pb-precipitation have shown that distribution of 5′-nucleotidase in rat liver includes the mitochondria [20]. The crude mitochondrial pellet from rat liver was found by Collinson et al. [21] to contain 5′-nucleotidase activity, but the same authors showed by means of sucrose density gradient centrifugation that most of this activity was attributable to lysosomal contamination. Later, however, Henke et al. [22] described a novel 5′-nucleotidase activity in rat liver mitochondria, but the methodology they used does not allow the separation of lysosomes from mitochondria.

In light of the contradictory reports on the existence of mitochondrial 5′-nucleotidase [20–22] in rat liver, we now set out to re-examine the subcellular distribution of hepatic 5′-nucleotidase activity by applying the Nycodenz density gradient fractionation technique to the livers of rats injected with Triton WR-1339, which is known to alter the density of lysosomes. In the absence of ethanol the liver oxidizes acetate [23] and its activation to acetyl-CoA occurs in the mitochondrial matrix leading to increased levels of AMP [23–26]. This allows measurement of mitochondrial adenosine production under conditions of high precursor availability.

Both experimental approaches employed here, namely tissue fractionation combined with regression analysis of the marker enzymes and the experiments on purified mitochondria metabolizing acetate, verified the existence of hepatic mitochondrial 5′-nucleotidase which also in kinetic characteristics differs from the enzyme in other cellular compartments. The results also emphasize that there are tissue-specific differences in the subcellular distribution of this enzyme.

Materials and Methods

Reagents

Routine chemicals were obtained from E. Merck, Darmstadt, Germany. Triton WR-1339 (Tyloxapol^{R}), [α,β-methylene]adenosine 5′-diphosphate (AOPCP), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and oligomycin were purchased from Sigma Chemical Co., St. Louis, MO, USA. (3-[^{125}I]Iodotyrosyl^{54})α-bungarotoxin, [8-^{14}C]adenosine, [U-^{14}]sucrose and tritiated water were obtained from Amersham International, UK. The density gradient medium Nycodenz^{R} was purchased from Nyegaard & Co, Oslo, Norway. Nucleotides and deoxycoformycin (Pentostatin) were obtained from Boehringer-Mannheim, Germany, and the Warner Lambert Company, Pharmaceutical Research Division, Ann Arbor, Michigan, USA, respectively. The reagents for protein measurement were purchased from Bio-Rad Laboratory, Richmond, CA, USA.

Treatment of animals and isolation of mitochondria

Male Sprague-Dawley rats from the stocks of the Department of Medical Biochemistry were allowed free access to food and water until used. 170 mg of Triton WR-1339 dissolved in 1 ml of isotonic saline was injected intravenously under light ether anaesthesia 4 days before the experiments. Triton WR-1339 is taken up by liver, where it accumulates in the lysosomes making them progressively larger and less dense [27]. This treatment makes it possible to separate the lysosomes from the mitochondria by density gradient centrifugation.

Crude mitochondria were isolated in 0.25 M sucrose/0.5 mM EDTA/5 mM Tris-HCl (pH 7.4) [28]. Further purification of mitochondria was performed in a Nycodenz gradient as described below for the tissue fractionation. The respiratory control ratios were 5.5 ± 1.8 and 5.2 ± 1.2 (mean ± S.D.) in the presence of 5 mM glutamate and 5 mM malate for the isolated mitochondrial homogenate and mitochondria collected from density gradient, respectively. The mitochondrial matrix volume was determined in incubations with $^{3}H_2O$ and [U-^{14}C]sucrose as the difference between ^{3}H and ^{14}C accessible spaces [29].

Tissue fractionation for determination of the subcellular distribution of 5′-nucleotidase

To minimize non-specific binding, the liver was labelled with the plasma membrane marker [^{125}I]α-bungarotoxin by perfusion. The portal vein of a sodium pentobarbital anaesthetized rat was cannulated and liver perfusion initiated in situ in the once-through mode with Krebs-Henseleit bicarbonate buffer. After the liver had been totally isolated, recirculating perfusion with [^{125}I]α-bungarotoxin was continued for 30 min, whereafter the perfusion was switched to the once-through mode without [^{125}I]α-bungarotoxin to wash out the non-specific radioactivity. After the 30-min washout period the liver was subsequently homogenized in 75 ml of isolation buffer.

3.5 ml of the homogenate was loaded into a Quick-Seal tube on top of 35 ml of a stepwise gradient of Nycodenz in 10 mM Hepes, 1 mM EDTA (pH 7.0) and KCl to render the solution isosmotic. From bottom to top, the gradient consisted initially of 60% sucrose and 40%, 30%, 25%, 20% and 10% Nycodenz in volumes of 3, 4, 6, 8, 8 and 6 ml, respectively. The tubes were centrifuged in a Beckman VTi 50 vertical rotor for 30 min at 170000 × *g* using the slow acceleration/deceleration mode. The gradients were unloaded from the bottom in 2 ml fractions and the marker enzyme activities analyzed immediately. The same procedure was used to separate the subcellular organelles in a

crude mitochondrial preparation made as described above. The density profiles of the gradients were determined by measuring the refractory index.

Assays of 5′-nucleotidase and the compartment markers

5′-Nucleotidase activity was assayed by two methods, one employing determination of the P_i liberated from the substrate nucleotide and other the measurement of adenosine formation by HPLC. P_i was determined according to Chen et al. [30]. The HPLC method was used when testing the effects of high concentrations of ATP or ADP because there was enzymatic and nonenzymatic liberation of P_i from these nucleotides during the incubation and assay which interfered with the 5′-nucleotidase assay, necessitating separation of the other product, adenosine. The adenosine assay was performed in a tandem column configuration using column switching in order to eliminate of the nucleotides on a Nucleosil 10SB anion exchange column. The nucleosides were separated on a Nucleosil 10 C_{18} reversed-phase column eluted with 10 mM $NH_4H_2PO_4$/5% methanol (pH 5.5). Absorbance of the effluent was monitored at 260 nm and external standards were used to identify and quantify the peaks.

In the 5′-nucleotidase assay the tissue fractions were incubated in 50 mM potassium (Mops), 1 mM AMP and 5 mM $MgCl_2$ (pH 7.0), in a total volume of 0.5 ml. The reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid.

α-Bungarotoxin, which binds to the α subunit of the nicotinic acetylcholine receptor, was used as a plasma membrane marker [31]. ^{125}I radioactivity in the gradient fractions collected was measured in a Wallac-LKB Multigamma Spectrometer.

Cytochrome *c* oxidase, a mitochondrial marker, was assayed essentially according to Cooperstein and Lazarow [32], and oxidation of the reduced cytochrome *c* was measured at 550 nm minus 540 nm. *N*-Acetyl-β-glucosaminidase was used as a lysosomal marker and assayed according to Gressner and Roebruck [33], monitoring the release of *p*-nitrophenol from *p*-nitrophenyl-*N*-acetyl D-glucosaminide by its absorbance in the presence of 5 mM $MgCl_2$. Lactate dehydrogenase, a cytosolic marker, was assayed as described by Bergmeyer and Bernt [34]. Glucose-6-phosphatase was used as a microsomal marker and assayed according to Nordlie and Arion [35].

The protein concentrations were assayed with Bio-Rad protein assay reagent using bovine serum albumin as the standard.

Experiments on mitochondria

Kinetic characteristics of the mitochondrial 5′-nucleotidase. Mitochondria further purified in Nycodenz density gradient were incubated for 45 min in 50 mM potassium Mops (pH 7.0) and the concentrations of AMP, $MgCl_2$, ATP, ADP and AOPCP were adjusted as described in the Results section. In the experiments with high a concentration of ATP or ADP the incubation medium also contained 2 μM CCCP to equalize the intra- and extramitochondrial ATP/ADP ratios and oligomycin (1 μg/mg protein) to inhibit ATPase.

Effects of acetate and pyruvate on mitochondrial adenosine production. Purified mitochondria were incubated in a medium containing 180 mM KCl, 60 mM Hepes, 10 mM potassium phosphate, 2 mM EGTA (pH 7.4) at 30°C with continuous shaking. The preparation may have adenosine deaminase activity, which interferes with the 5′-nucleotidase determination and therefore 5 μM deoxycoformycin was added to the incubation medium before the mitochondria. The inhibition of adenosine deaminase by deoxycoformycin was tested in preliminary experiments using 1–100 μM [^{14}C]adenosine. During a 25-min incubation with deoxycoformycin, 92 ± 2.2% of the radioactivity remained in the adenosine compared to 77 ± 4.2% ($P < 0.001$) in incubations without the inhibitor. The degree of inhibition was not dependent on the adenosine concentration.

To increase adenosine production, 10 mM acetate was added to the incubation medium after the 10 min preincubation period with 1.0 mM malate. In control experiments 10 mM pyruvate was used instead of acetate. At various points in time 800 μl samples from the incubations were added rapidly to an Eppendorf tube on top of 400 μl of silicone oil (AR 200, Serva) above 400 μl of 7% perchloric acid. After immediate centrifugation, a 500 μl sample of the top layer was acidified with perchloric acid. The top and bottom layers were neutralized with 2 M KOH/0.5 M triethanolamine hydrochloride. The nucleotides were analysed by HPLC using a Nucleosil 10SB anion-exchange column under isocratic elution with 150 mM KH_2PO_4/150 mM KCl (pH 6.2). Adenosine was measured on a Nucleosil 10 C_{18} reversed phase column eluded with 10 mM $NH_4H_2PO_4$/5% methanol (pH 5.5). Absorbance of the effluent was monitored at 260 nm and external standards were used to identify and quantify the peaks.

Computation and statistics

The 5′-nucleotidase distribution in the subcellular organelles and compartments was determined by multiple regression analysis. The amount of 5′-nucleotidase (Y_j) in a given sample (j) from the density gradient is given by the equation:

$$Y_j = C_{1,j} \cdot X_1 + C_{2,j} \cdot X_2 + C_{3,j} \cdot X_3 + \cdots + C_{i,j} \cdot X_i$$

where X_i is the 5′-nucleotidase activity in compartment i and C_{ij} is the activity of the marker of compartment i in sample j.

This was resolved by multiple regression analysis and performed on a microcomputer with the Stat-

graphics program package (STSC, Inc., Rockville, MD, USA). Negative proportionality coefficients were not allowed. The goodness of fit to the model is given in terms of a correlation coefficient and the standard errors of the estimates.

Weighted regression of the Michaelis-Menten kinetics was performed according to Wilkinson [36]. The data from the other experiments were expressed as means ± S.D. and the statistical significances of the differences between independent groups were calculated using Student's *t*-test.

Results

Organelle distribution

The distribution profile of 5'-nucleotidase and markers of subcellular organelles in a Nycodenz density gradient of the crude liver homogenate show that mitochondria and lysosomes are well separated after Triton WR-1339 treatment (Fig. 1), whereas without Triton WR-1339 their separation was insufficient to ensure accuracy in analysing the data. The results of the computation show that 5'-nucleotidase activity also exists in the mitochondrial fraction. The fit of the data to the compartmentation model is good ($r = 0.96$). Since this result is different from those of Kiviluoma et al. [19] for the heart and Collinson et al. [21] for the liver but supports the findings of Henke et al. [22] regarding liver, a closer approach was adopted by further purifying the crude mitochondria preparation in the density gradient. This would yield higher grade of purity than use of the gradient method on total homogenate (Fig. 2 and Table I). In this case, the

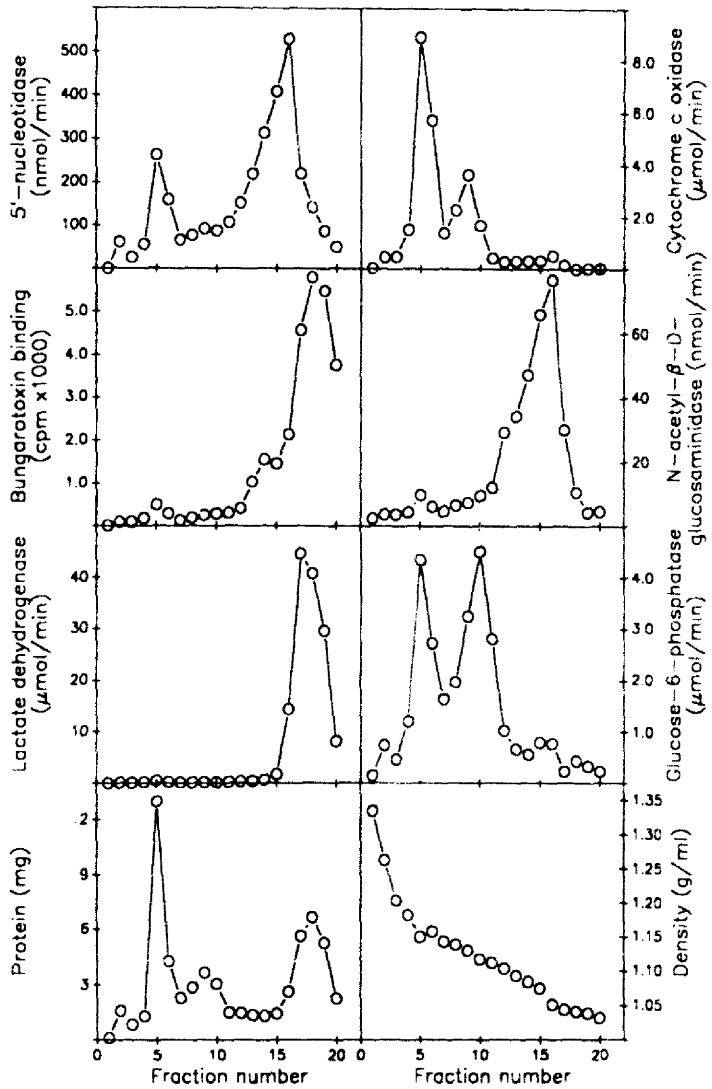

Fig. 1. Distribution profiles for 5'-nucleotidase, markers of subcellular organelles and protein in rat liver homogenate fractioned in a Nycodenz density gradient. Isolated rat livers were labelled with radioactive α-bungarotoxin during perfusion. The experimental conditions were as described under Materials and Methods.

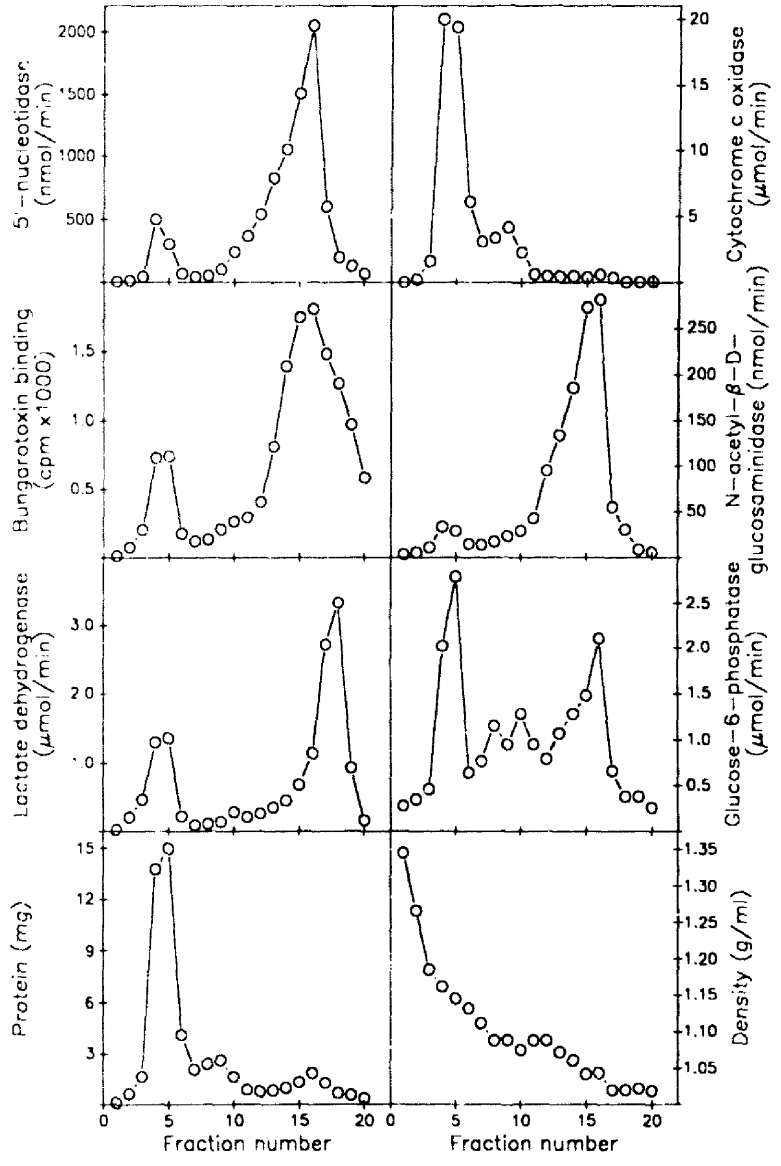

Fig. 2. Distribution profiles for 5'-nucleotidase, markers of subcellular organelles and protein in a crude mitochondrial preparation fractioned in a Nycodenz density gradient. Isolated rat livers were labelled with radioactive α bungarotoxin during perfusion. The experimental conditions were as described under Materials and Methods.

relative mitochondrial 5'-nucleotidase activity decreased, but 4% of the total activity still remained in the mitochondrial fraction ($r = 0.94$), confirming the existence of a mitochondrial 5'-nucleotidase.

Kinetic characterization of the mitochondrial enzyme

Susceptibility to inhibition by the methylene analogue of ADP (AOPCP) has been regarded as a characteristic of the plasma membrane 5'-nucleotidase [37]. This view may have to be tempered in the light of recent observations showing that lysosomes also contain AOPCP sensitive 5'-nucleotidase [19,21]. The effects of AOPCP and Mg^{2+} depletion are shown in Fig. 3. The inhibition by 50 μM AOPCP is only moderate, 10%, and competitive. This low sensitivity to AOPCP suggests that the mitochondrial enzyme is different from the plasma membrane and lysosome enzymes. Also, the mitochondrial enzyme has a requirement of Mg^{2+} (Fig. 3), in contrast to these others [21].

Both ATP and ADP were inhibitory, ATP being more effective at 1 mM concentration (Fig. 4), whereas the plasma membrane enzyme is more strongly inhibited by ADP [10,37]. The lysosomal enzyme shows roughly equal sensitivity to ATP and ADP [21], and both ATP and ADP are activators for the cytosolic enzyme [13].

All these data point out to the existence of a unique mitochondrial 5'-nucleotidase.

TABLE I

Subcellular distribution of 5'-nucleotidase in rat liver homogenate and a crude mitochondrial preparation

The values are representative of a typical Nycodenz density gradient centrifugation and are obtained by processing the data of Figs. 1 and 2 as described under Materials and Methods. The standard errors of the estimates obtained from the linear regression are also shown.

Compartment	Frequency (fraction of total)	
	total homogenate [a]	mitochondrial preparation [b]
Mitochondria	0.19 ± 0.04	0.04 ± 0.06
Plasma membrane	0.07 ± 0.01	0.04 ± 0.20
Lysosomes	0.72 ± 0.03	0.84 ± 0.13
Cytosol	0.02 ± 0.03	0.06 ± 0.10
Microsomes	0.003 ± 0.06	0.02 ± 0.16

[a] $r = 0.96$.
[b] $r = 0.94$.

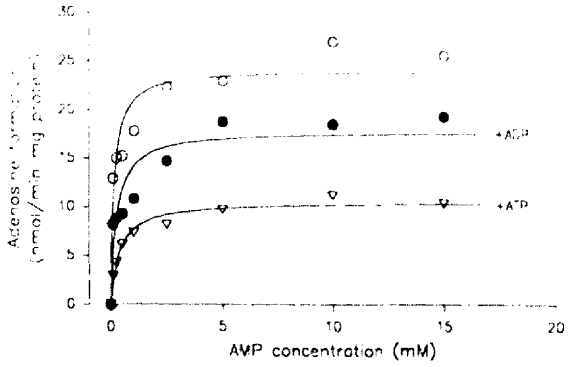

Fig. 4. Effects of 1 mM ATP and ADP on mitochondrial 5'-nucleotidase activity. Incubation conditions and adenosine measurement were as described under Materials and Methods. Weighted regression of the Michaelis-Menten kinetics according to Wilkinson [36] yielded a K_m value of 149 ± 47 (S.E.) μM and V_{max} of 24 ± 1.4 nmol/min per mg mitochondrial protein. ATP and ADP reduced V_{max} to 11 ± 0.4 and 18 ± 1.4 nmol/min per mg protein and altered K_m to 375 ± 68 and 256 ± 7.9 μM, respectively.

Acetate-enhanced production of adenosine in mitochondria

The existence of 5'-nucleotidase activity inside the liver mitochondria was further examined by incubating the gradient-purified mitochondria in the presence of acetate and pyruvate. The results of these experiments are shown in Fig. 5. As an indication of the rapid activation of acetate, the intramitochondrial ATP decreased and AMP increased upon acetate addition. Adenosine increased both intramitochondrially and extramitochondrially. The adenosine increase in the extramitochondrial space was linear with time, in spite of the fact that only trace amounts of nucleotides were detected, while the intramitochondrial concentration reached a plateau. This favours the idea that adenosine is formed intramitochondrially, after which it is transported or diffused out. These results are thus in accordance with the data obtained by distribution analysis and confirm the existence of a hepatic mitochondrial 5'-nucleotidase.

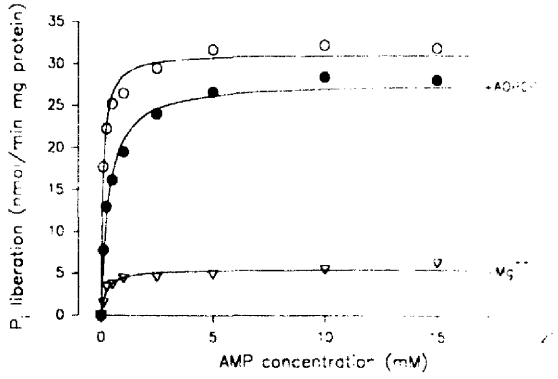

Fig. 3. Effects of 50 μM AOPCP and Mg^{2+} depletion on mitochondrial 5'-nucleotidase activity. Incubation conditions and P_i assay were as described under Materials and Methods. Weighted regression of the Michaelis-Menten kinetics according to Wilkinson [36] yielded a K_m value of 94 ± 15 (S.E.) μM and a V_{max} of 31 ± 0.7 (S.E.) nmol/min per mg mitochondrial protein. AOPCP addition and Mg^{2+} depletion reduced V_{max} to 28 ± 0.7 and 5.5 ± 0.3 nmol/min per mg protein and altered K_m to 345 ± 39 and 245 ± 58 μM, respectively.

Discussion

The intracellular distribution and compartmentation of enzymes is a controversial subject because its demonstration requires (absolute) purification of organelles to exclude contaminating activities. The present approach is much less dependent on the degree of purification because it relies on correlation analysis. Only the accuracy of the knowledge of the distribution of the marker enzymes is needed.

In the light of evidence obtained in this laboratory [19] and elsewhere [20,21], the present results indicate that there are organ-specific differences in the distribution of 5'-nucleotidase, at least in heart and liver. The present data also point out that correlation analysis provides a powerful tool for determining the subcellular distribution of enzymes. As far as 5'-nucleotidase is concerned, much effort has been expended on producing pure preparations of organelles, a task which is seldom achieved. In the present case a greater proportion of this enzyme was found in the lysosomes than has been reported previously [19,38]. This may be due to the formation of phagolysosomes, resulting in an engulfing of the plasma membrane and its 5'-nucleotidase, which has been found to be at least partly identical to the lysosomal enzyme [38]. The AOPCP sensitivity of the rat heart 5'-nucleotidase in the subcellular fraction containing lysosomes also suggests that part of the lysosomal 5'-nucleotidase may indeed originate from the plasma membrane [19].

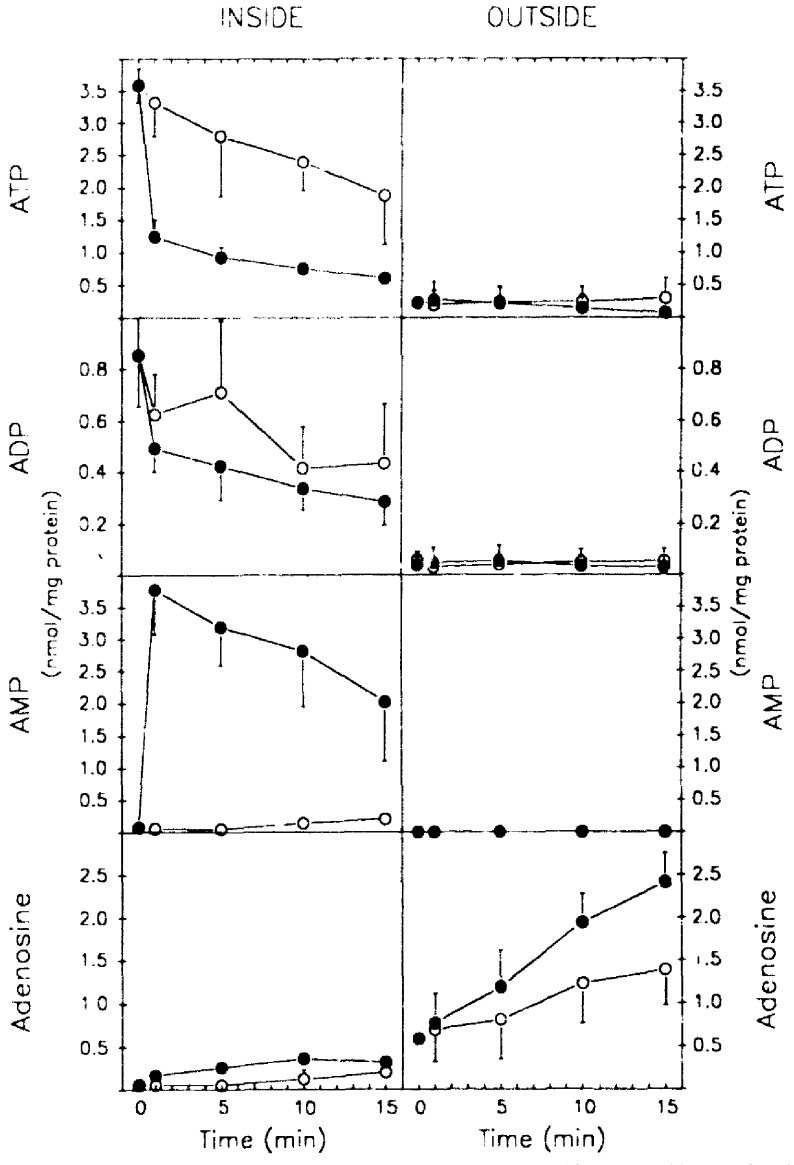

Fig. 5. Effect of pyruvate and acetate on adenine nucleotide and adenosine concentrations inside and outside mitochondria purified by Nycodenz gradient centrifugation. Isolated rat liver mitochondria were preincubated with 1 mM malate for 10 min in an isosmotic medium. Deoxycoformycin was added before the mitochondria. 10 mM pyruvate or acetate was added at the zero time point. The mitochondria were separated from the medium by centrifuging through a layer of silicone oil. Intramitochondrial and extramitochondrial adenine nucleotides and adenosine were analysed as described under Materials and Methods. Values are means ± S.D. from six independent experiments. ○, pyruvate; ●, acetate.

The present correlation analysis results show that mitochondria contain 5′-nucleotidase, and the kinetic data on inhibitor sensitivity show that the enzyme is different from its lysosomal, plasma membrane and cytosolic counterparts. Inhibition by ATP fits with the role of adenosine in signalling a low cellular energy state. The mitochondrial ADP concentration of approx. 3 mM obtained by non-aqueous fractionation of liver [39] would then be sufficient to inhibit mitochondrial 5′-nucleotidase. The physiological importance of this remains to be established.

The maximum activity of the mitochondrial 5′-nucleotidase is two orders of magnitude higher than the rate of acetate-activated adenosine production in isolated mitochondria, and also this points to substrate limitation of the reaction in vivo.

The compartmentation of 5′-nucleotidase and adenosine production in both the heart and the liver poses transport problems. In the heart, where the mitochondrial matrix lacks both adenylate kinase and 5′-nucleotidase, the AMP formed during intramitochondrial fatty acid and acetate activation must be exported, and recent evidence suggests that this is accomplished at least partially by the adenylate translocator [40]. In the liver the mitochondrial matrix produces AMP and contains 5′-nucleotidase, as the

present results demonstrate, which means that adenosine has to be transported from the mitochondria in order to elicit its vasoactive effects, for example. Nucleoside transport in the plasma membrane has been characterized in some detail [41], but mitochondrial nucleoside transport has remained somewhat unclear. Deoxyguanosine uptake, which is insensitive to the common inhibitors of plasma membrane transport, has been shown to occur in rat liver mitochondria [42] and a purine nucleoside transport system mediating at least the efflux of inosine from the mitochondria has been described recently [43]. Adenosine transport by isolated rat liver mitochondria has also been suggested (Peuhkurinen K.J., Collinson A.R. and Lowenstein J.M., unpublished data). The present data show that during acetate metabolism the adenosine concentration in the mitochondrial matrix increases and reaches a plateau after 10 min, concomitantly with a steadily increasing concentration in the extramitochondrial space. During pyruvate metabolism the matrix adenosine concentration is much lower but shows a slowly increasing tendency. When the mitochondrial contents are converted to concentration units by reference to the matrix water space, the adenosine concentration turns out to be quite high (0.2 mM) at the beginning of incubation and increases to 0.4 mM during the incubation of acetate. This means that mitochondrial adenosine is at least partly in a bound form or that an apparent concentration gradient of at least one order of magnitude exists across the mitochondrial membrane. The data are indicative of concentration-dependent export, which may occur by diffusion or facilitated diffusion.

Acetate-enhanced adenosine production in the liver has a bearing on the metabolic effects of ethanol. In fact there is recent evidence that adenosine is involved [44], and some earlier observations [45] point to a vasodilatory effect of ethanol in the liver. It is paradoxical that ethanol is oxidized only to acetate in the liver [46]. Even so, the hepatic AMP concentration increases during ethanol oxidation [23], possibly due to suppression of the inhibition of the tricarboxylic acid cycle [23,47], which leads to a cessation of GTP production and a decrease in GTP-linked rephosphorylation of AMP formed through the activation of acetate [48]. Thus the substrate for mitochondrial 5′-nucleotidase comes to be present in abundance during ethanol oxidation, in spite of the suppression of the activation of acetate, which still serves as an efficient oxidizable hepatic substrate in the absence of ethanol.

In conclusion, the occurrence of a mitochondrial 5′-nucleotidase is organ-specific, and appreciable activities of matrix 5′-nucleotidase activity can be found in the liver even though it was non-existent in heart mitochondria.

Acknowledgements

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References

1 Berne, R.M. (1980) Circ. Res. 47, 807–813.
2 Fain, J.N. and Shepherd, R.E. (1977) J. Biol. Chem. 252, 8066–8070.
3 Oetjen, E., Schwetkhardt, C. Unthan-Fechner, K. and Probst, I. (1990) Biochem. J. 271, 337–344.
4 Lund, P., Cornell, W.W. and Krebs, H.A. (1975) Biochem. J. 152, 593–599.
5 Ismail, N.A. and Hems, D.A. (1978) Biochem. Pharmacol. 27, 1341–1345.
6 De Sanchez, V. and Pina, E. (1977) FEBS Lett. 83, 321–324.
7 Bellomi, F.L., Elkin, P.L. and Giannetto, B. (1985) Br. J. Pharmacol. 85, 441–446.
8 Arnold, S.T. and Cvsyk, R.L. (1986) Am. J. Physiol. 251, G33–G39.
9 Orrego, H.Y., Israel, Y. and Blendis, L.M. (1981) Hepatology 1, 267–283.
10 Lazo, J.P., Bailyes, E.M., Baron, M., Siddle, K., Mullock, B.M., Geuze, H.J. and Stanley, K.K. (1986) in Cellular Biology of Ectoenzymes (Kreutzberg, G.W., ed.) pp. 89–116, Springer, Berlin.
11 Itoh, R. (1981) Biochem. Biophys. Acta 657, 402–410.
12 Itoh, R. (1981) Biochem. Biophys. Acta 659, 31–37.
13 Itoh, R. (1982) Biochim. Biophys. Acta 716, 110–113.
14 Natto, Y. and Lowenstein, J.M. (1981) Biochemistry 20, 5188–5194.
15 Itoh, R., Oka, J. and Ozaka, H. (1986) Biochem. J. 235, 847–851.
16 Truong, V.L., Collinson, A.R. and Lowenstein, J.M. (1988) Biochem. J. 253, 117–121.
17 Bukoski, R.D., Sparks, H.V. and Mela-Riker, L.M. (1986) in Myocardial and Skeletal Muscle Bioenergetics (Bratbar, N., ed.) pp. 157–167, Plenum Press, New York.
18 Bukoski, R.D., Sparks, H.V. and Mela-Riker, L.M. (1986) Biochim. Biophys. Acta 884, 25–30.
19 Kiviluoma, K.T., Hiltunen, J.K., Hassinen, I.E. and Peuhkurinen, K.J. (1990) J. Mol. Cell. Cardiol. 22, 827–835.
20 Widnell, C.C. (1974) Methods Enzymol. 32, 368–374.
21 Collinson, A.R., Peuhkurinen, K.J. and Lowenstein, J.M. (1987) in Topics and Perspectives in Adenosine research (Gerlach, E. and Becker, B.F., eds.) pp. 133–144, Springer, Berlin.
22 Henke, W., Lang, M., Dubiel, W., Holzhatter, H.G. and Gerber, G. (1980) Biochem. Internat. 38, 833–844.
23 Barth, C., Sladek, M. and Decker, K. (1972) Biochim. Biophys. Acta 258, 27–33.
24 Williamson, J.R., Scholz, R., Browning, E.T., Thurman, R.G. and Fukami, M.H. (1969) J. Biol. Chem. 244, 5044–5054.
25 Aas, M. (1971) Biochim. Biophys. Acta 231, 32–47.
26 Scholte, H.R. and Groot, P.H.E. (1975) Biochim. Biophys. Acta 409, 283–296.
27 Wattiaux, R., Wibo, M. and Baudhuin, P. (1963) in Lysosomes (Reuch, A.V.S. and Cameron, M.P., eds.) pp. 177–200, J&A Churchill, London.
28 Johnson, D. and Lardy, H.A. (1967) Methods Enzymol. 10, 94–96.
29 Kauppinen, R.A., Hiltunen, J.K. and Hassinen, I.E. (1980) FEBS Lett. 112, 273–276.

30 Chen, P.S., Jr., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758.
31 Smith, M.M., Linnestrom, J. and Methe, J.P. (1987) J. Biol. Chem. 252, 4367–4376.
32 Cooperstein, S.J. and Lazarow, A. (1951) J. Biol. Chem. 189, 665–670.
33 Gressner, A.M. and Roebruck, P. (1982) Clin. Chim. Acta 124, 315–326.
34 Bergmeyer, H.U. and Bernt, E. (1970) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., ed.), pp. 533–538, Verlag Chemie, Weinheim.
35 Nordlie, R.C. and Arion, W.J. (1966) Methods Enzymol. 9, 619–625.
36 Wilkinson, G.N. (1961) Biochem. J. 80, 323–332.
37 Saito, Y. and Lowenstein, J.M. (1985) Biochem. J. 226, 635–651.
38 Maguire, G.A. and Lazio, P. (1985) FEBS Lett. 180, 122–126.
39 Ellers, R., Heldt, H.W., Schmuckler, P. and Wiese, H. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 378–393.
40 Kivilaoma, K.T., Peuhkurinen, K.J. and Hassinen, I.E. (1989) Biochim. Biophys. Acta 974, 274–281.
41 Plagemann, P.G.W., Wohlhueter, R.M. and Woffendin, C. (1988) Biochim. Biophys. Acta 947, 405–443.
42 Watkins, L. and Lewis, R.A. (1986) Adv. Exp. Med. Biol. 195B, 85–88.
43 Ziegler, M. Dubiel, W. Pimenov, A.M., Tikhonov, Y.V., Toguzov, R.T., Henke, W. and Gerber, G. (1990) Mol. Cell. Biochem. 93, 7–12.
44 Orrego, H., Carmichael, F.J., Saldivia, V., Giles, H.G., Sandrin, S. and Israel, Y. (1988) Am. J. Physiol. 254, G495–G501.
45 Abrams, M.A. and Cooper, C. (1976) Biochem. J. 156, 47–54.
46 Lundquist, F., Tygstrup, N., Winkler, K. Mellemgaard, K. and Munck-Petersen, S. (1962) J. Clin. Invest. 41, 955–961.
47 Forsander, O.A., Raiha, N., Salaspuro, M. and Mäenpää, P. (1965) Biochem. J. 94, 259–265.
48 Lumeng, L. and Davis, E.J. (1973) FEBS Lett. 29, 124–126.