Alteration of adenine nucleotide pool in old rat liver and its normalization with ammonium succinate

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Ammonium succinate administration into the old rats increases the sum of adenine nucleotide and normalizes all indices of adenine nucleotide metabolism in the liver to the levels typical for the intact young animals.

Adenine nucleotide *Rat-liver, old* *Ammonium succinate*

1. INTRODUCTION

In spite of the universal role of ATP and other adenine nucleotides (AN) in cellular synthetic, catabolic and regulatory processes, the stability of the sum of AN in animal cells under various conditions has not been widely discussed in biochemical literature.

Investigating energy metabolism in rats we have observed a significant decrease in size AN pool in liver upon aging [1]. The degree of the decrease did not vary in response to fasting, administration of physiological saline or potassium succinate into the animals.

Here, we show that administration of ammonium succinate into old rats lead to normalization of hepatic adenylate metabolic system up to the level typical for the intact young animals.

2. MATERIALS AND METHODS

Young (3–4-month-old) and old (24-month-old) male Wistar rats weighing 250–280 g and 450–600 g, respectively, were used throughout. The animals were adapted to natural light–dark regimen and a balanced diet and water given ad libitum. Some of the old rats were fasted for 24 h. Fed old rats were injected i.p. with either ammonium succinate (50 mg/kg in 0.3 ml water, pH 7.5), ammonium chloride at equivalent dosage (15 mg/kg), or saline daily for 6 days. An additional group received sham administration, and the other was intact. The third additional group was given ammonium succinate (50 mg/kg) by intragastric intubation.

All animals were decapitated 25 h after the last treatment. Portions of livers were immediately (in 10 s) freeze-clamped. ATP, ADP and AMP concentrations in neutralized perchloric acid/ethanol extracts of liver powdered tissue was determined enzymatically [2]. Preparative procedures and chemicals are given elsewhere [3].

Statistical analysis was performed by Student's *t*-test.

Abbreviation: AN, adenine nucleotides

3. RESULTS

The ATP and ADP content of fed old rat liver is less than that in the young animals; the sum of AN decreases by 25–30% (table 1). The decrease in ATP level of old rat liver upon fasting is compensated with corresponding increase in ADP and AMP levels, and the sum of AN remains unusually low.

Potassium succinate administration into old rats did not increase the sum of AN in the liver [1]. In contrast, ammonium succinate administration results in overall quantitative redistribution of AN. As compared to potassium succinate [1], ammonium succinate increases ATP content of the liver by 2-fold, almost to normal level typical for the in-

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Table 1

Indices of adenine nucleotide metabolism in liver of young and old rats under different conditions

Animals and treatment		n	ATP	ADP	AMP	ΣAN	$\frac{ATP}{ADP}$	EC
Young	Intact	26	1880 ± 140	958 ± 60	302 ± 30	3140 ± 220	1.95 ± 0.20	0.76 ± 0.01
	Intact	16	1459 ± 29[b]	617 ± 18[b]	289 ± 21	2373 ± 36[b]	2.34 ± 0.06[a]	0.75 ± 0.01
	24h fasted	6	922 ± 19[d]	1053 ± 22[d]	343 ± 24[d]	2320 ± 45	0.90 ± 0.02[d]	0.63 ± 0.01[d]
	Sham injection i.p.	8	1566 ± 133	537 ± 38[c]	261 ± 19	2363 ± 107	3.06 ± 0.39[d]	0.79 ± 0.01[d]
Old	0.9% NaCl i.p.	14	1609 ± 26[d]	597 ± 16	201 ± 12[d]	2400 ± 28	2.73 ± 0.10[c]	0.80 ± 0.01[d]
	NH_4Cl i.p.	6	1243 ± 92[d]	351 ± 41[d]	185 ± 100	1778 ± 136[d]	3.54 ± 0.16[d]	0.80 ± 0.01[d]
	Succinate NH_4 i.p.	16	1740 ± 28[d]	862 ± 12[d]	307 ± 13	2909 ± 33[d]	2.02 ± 0.04[d]	0.75 ± 0.03
	Succinate NH_4 per os	8	1597 ± 35[d]	488 ± 12[d]	287 ± 22	2372 ± 36	3.26 ± 0.08[d]	0.76 ± 0.01

[a,b] Significant differences from indices of intact young rat liver
[c,d] Significant differences from indices of intact old rat liver
[a,c] $p < 0.05$; [b,d] $p < 0.01$
Content of ATP, ADP, AMP, and the sum of AN (ΣAN) given as nmol/g liver wet mass.
Adenylate energy charge EC = (ATP + ½ADP)/ΣAN.

tact young animals, but results in insignificant change of ADP content and in decreasing AMP level. As to the contents of individual AN and all indices of AN metabolism (e.g., sum of AN, ATP/ADP ratio, adenylate energy charge, phosphorylation potential) liver of old rats given ammonium succinate injections does not differ from that of intact young rats (table 1 and [1]).

Ammonium chloride administration into the old rats leads to a further fall in contents of all individual AN and the sum (table 1).

To determine a therapeutic effect of ammonium succinate, old rats were injected with it intragastrically. However, the results were unexpected, the sum of AN and all the indices of AN metabolism remained nearly the same as in the intact old rats.

4. DISCUSSION

The total content of AN in a cell reflects the functional equilibrium between synthesis and degradation of purine and pirimidine nucleotides as well as numerous synthetic and catabolic processes. However, even in special studies on determination of individual adenyl, guanyl and pyridine nucleotides in rat brain during aging [4] the change in total contents of nucleotides was not discussed. In [4], changes in the sum of AN were explained only by inverse change in the AMP deaminase activity.

Here in [1], the sum of AN in liver of old rats decreased by 25–30%. The extent of the decrease did not vary during fasting or upon the administration of physiological saline or potassium succinate intraperitoneally or upon administration of ammonium succinate per os. Thus, the decrease in the sum of AN is the property of aging tissue; e.g., liver (table 1), brain [4], and skeletal and cardiac muscles [5].

In our experiments, the decrease of AN sum in old rat liver cannot be explained by excessive activity of AMP deaminase, since the content of P_i, a powerful inhibitor of this enzyme [6], increases in rat liver upon aging [1], while the content of AMP, ATP and ADP, the substrate and activators of AMP deaminase [6], decreases (see table 1 and [1]).

The decrease of total AN under steady state of the energy metabolism may be caused by disturbances in both the consumption and synthesis. The content of ribosomal proteins and the RNA polymerase activity in rat-liver nuclei do not change in aging, whereas the total content of nuclear nucleotides gradually decreases [7]. Thus, the decrease in the content of nucleotides in nuclei and, as a result, in cytosol is not associated with their intensified consumption for biosynthetic processes. The inactivation of glucose 6-phosphate dehydrogenase in the liver can lead to a decrease in the steady state concentrations of ribose 5-phosphate and 5-phos-

phorybosyl pyrophosphate, the precursors of all purine nucleotides, and to inactivation of IMP synthesis. However, the literature data obtained from the analysis of this enzyme activity as a function of the age of the animals are contradictory; e.g., hepatic glucose 6-phosphate dehydrogenase activity in old rats and mice reduced [8–10], rose [11,12] or reduced in one strain of rat and rose in another [13].

However, inactivation of aspartate aminotransferase [14] and glutamine synthetase [15] activities, the enzyme not involved directly in AN synthesis, may result in a reduction of cellular glutamine and aspartate, i.e., cosubstrates of enzyme system for purine ring synthesis, and in accumulation of glutamate and fumarate, the product inhibitors of the same system. Such a redistribution of the metabolites can lead to inhibition of IMP biosynthesis at all 3 enzymatic stages at once: phosphoribosylpyrophosphate amidotransferase, phosphoribosylformylglycinamide synthetase, and phosphoribosylaminoimidazol succinocarboxamide synthetase, and of AMP synthesis from IMP at the stages of adenylosuccinate synthetase and lyase.

To compensate for the deficiency in the aspartate aminotransferase activity in old rats we employed the i.p. administration of succinate, and to compensate for the deficiency in the glutamine synthetase activity the injection of ammonium. The reason, first, was that succinate is oxidized in citric acid cycle exclusively and that citrate synthase plus isocitrate dehydrogenase and mitochondrial and cytosolic phosphoenolpyruvate carboxykinase activities are limited as compared to mitochondrial and cytosolic aspartate aminotransferase activities. The result to be expected is accumulation of aspartate (and probably of fumarate). Second, under excess of glutamate in old rat liver (submitted; see [16]) ammonium administered should result in some reactivation of glutamine synthetase activity with concomitant decrease of glutamate (unpublished) and increase of glutamine level.

As it was shown neither succinate (potassium salt) [1] nor ammonium chloride (table 1) increased the sum of AN in old rat liver separately. Upon simultaneous administration of succinate and ammonium as ammonium succinate an overall normalization of the AN sum is observed. This is evidence for ammonium succinate as a biologically active regulator of AN synthesis and proves that our assumption on the mechanism for disturbance in AN synthesis in rat liver upon aging is at least partly true.

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