Alteration of the ADP/ATP translocase isoform pattern improves ATP expenditure in developing rat liver mitochondria

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Abstract The expression of adenine nucleotide translocase isoforms (AAC) during perinatal development of the rat was studied by measuring mRNA transcript levels of AAC1 and AAC2 genes in liver, heart and brain tissue. In contrast to heart and brain, AAC1 mRNA is not present in adult liver tissue, but is transiently expressed around birth. AAC1 expression in liver did not respond to cold stress (examined with adult rats), therefore a possible involvement of AAC1 in the liver thermogenesis of newborns is excluded. Measurement of the [^{3}H]ADP uptake by liver mitochondria revealed that the molecular activity of the AAC protein was significantly higher in mitochondria from 4-day-old neonates compared with adults. We suggest that the transient AAC1 gene expression in the perinatal liver helps to accommodate the mitochondrial ATP supply to the increased cytosolic ATP consumption initiated at birth.

Key words: Perinatal development; Adenine nucleotide translocator; Isoform; Liver mitochondrion

1. Introduction

Newborn mammals are under strong 'energy stress' in the early postnatal period. Due to the onset of a variety of ATP-consuming reactions at birth, such as gluconeogenesis, ureogenesis and proliferation of mitochondria, the liver of newborns has a high energy demand. For that reason, the postnatal development of pre-mature mitochondria to fully developed active phosphorylating organelles is of major importance for the accommodation of the energy metabolism to the cellular energy demand (for review, see [1,2]). Functional maturation results from an enrichment of mitochondria with proteins assembling the energy transduction system [2], the reduction of the passive proton permeability of the inner mitochondrial membrane [3], and the import of adenine nucleotides from the cytosol into the matrix compartment (for review, see [4]).

The enrichment of the nuclear encoded adenine nucleotide translocase (AAC) contributes mainly to the maturation of mitochondria [3,5–8]. This is illustrated by the decline of the control of the AAC over phosphorylating mitochondrial respiration concomitant with postnatal enrichment of the AAC in the inner mitochondrial membrane [5–8]. In addition, this fact indicates that the AAC strongly limits the export of mitochondrial generated ATP to the cytosolic compartment during the postnatal period.

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Abbreviations: AAC, adenine nucleotide translocase

The AAC is expressed in different isoforms in a tissue-specific manner [9,10]. In rat, heart/muscle-type (AAC1) and fibroblast-type (AAC2) isoforms have been identified. Both isoforms were expressed in heart, muscle, brain, kidney, but not in the adult liver, where only the AAC2 isoform was found [11–14]. Surprisingly, AAC1 is transiently expressed in liver tissue of newborn rats [14]. However, whether this developmental change in the AAC isoform expression pattern in the liver of newborns is of physiological importance is not known. We therefore examined a possible involvement of AAC1 in (i) liver thermogenesis by cold exposure of adult rats and (ii) the kinetics of [^{3}H]ADP uptake by liver mitochondria from 4-day-old neonates and adults. We show in this study that in the newborn rat liver AAC1 is predominantly expressed and that the expression of AAC1 is in line with an increased molecular activity of AAC.

2. Materials and methods

2.1. Animals and isolation of mitochondria

Rats were maintained at 22°C with a 12-h light/dark cycle. Adult females (mean mass 180 g) were caged with males overnight. Fetuses were obtained from pregnant anesthetized rats by cesarean section. Tissue samples were from liver, heart and brain of fetal, newborn and adult rats. For investigation of cold stress on AAC isoform expression, adult rats were kept at 4°C for 1 week before liver tissue was removed. Liver mitochondria were isolated from 4-day-old pups and adult rats as in [15]. Protein concentrations in mitochondrial preparations were determined with the biuret method using bovine serum albumin as standard.

2.2. Assays

Oxygen uptake by intact mitochondria was measured at 25°C in a water-jacketed chamber equipped with a Clark-type oxygen electrode. Incubation medium was composed of 110 mM mannitol, 60 mM KCl, 60 mM Tris, 10 mM potassium phosphate, 5 mM glutamate, 5 mM malate and 0.5 mM Na_2EDTA, pH 7.4. The determination of AAC protein content in samples of mitochondria and tissue homogenates was based on the high affinity binding of [^{3}H]atractylate to the AAC protein as described in [15]. The activity of AAC in mitochondria was measured at 4°C by the atractyloside-stop method as in [7]. From the [^{3}H]ADP uptake during 10-s incubation periods, and the amount of the mitochondrial AAC protein, the molecular translocation activity was calculated.

2.3. Northern blots

Total RNA was prepared by homogenization of frozen tissue in the presence of 4 M LiCl/8 M urea. RNA was precipitated selectively during overnight incubation at 4°C. RNA was resuspended in 10 mM Tris/0.5% SDS buffer (pH 7.5) and incubated for 30 min at 37°C with 50 μg proteinase K/ml. After treatment of this incubation mixture with phenol/chloroform, RNA was precipitated by ethanol. The concentration of RNA was determined spectrophotometrically. After separation of 10 μg RNA on a 1% (w/v) agarose/formaldehyde gel containing ethidium bromide, RNA was transferred onto a nylon membrane (Hybond N, Amersham) and fixed by cross-linking. Membranes were hybridized with [α-^{32}P]dCTP-labeled cDNA probes. Prehybridization and hybridization were carried out in Rapid-hyb buffer

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(Amersham) at 65°C. Membranes were washed successively in 2×SSC, 0.5×SSC and 0.1×SSC each with 0.1% SDS at 65°C for 20 min. Autoradiography was done at −70°C using intensifying screens. The signals were quantified by densitometric scanning (Bio-1D software, Vilber Lourmat). Sample loading was referred to ethidium bromide-stained ribosomal RNA.

2.4. Cloning of AAC1 and AAC2 cDNA fragments

For PCR amplification of fragments of both genes (AAC1 and AAC2) degenerated primers AntF (5′-ACTGGATCCGCTGCTGAYGTKGGYAAA) and AntR (5′-GCTGGTACCCTYTGKGCWATCATCCA) were used. These primers were defined from the published sequences of rat (GenBank accession numbers D12770 and D12771). After first strand reverse transcriptase reaction with AntR using total RNA of liver or heart, a 239-bp fragment was amplified by PCR. These fragments were purified and blunt-end cloned in pBluescript according to standard protocols. The isoforms of the corresponding cDNAs were identified by sequencing.

3. Results and discussion

To study the perinatal expression of AAC isoforms, total mRNA was extracted from samples of heart, brain and liver tissue obtained from rats at various developmental ages and quantified as described in Section 2. Representative blots and densitometric quantifications reflecting the developmental changes in the transcript levels of AAC1 mRNA and AAC2 mRNA are shown in Figs. 1 and 2. In the adult rat liver, the AAC1 transcript is absent, a finding which is in line with recent reports [11–14]. However, in the perinatal period (up to about 2 weeks after birth) AAC1 transcript was found in liver at a high level. Such a transient expression of AAC1 in the neonate rat liver confirms a recent report by Grado et al. [14]. These investigators further demonstrated that the immunoreactivity of the AAC proteins obtained from neonate and adult liver is different, probably by a prevalent expression of the AAC1 isoform in the neonatal liver. We found overexpression of AAC at the transcript and protein level in newborn liver (Figs. 2 and 3), but not in heart and brain (Fig. 3). The transcript level of AAC2 gene is already high in the late

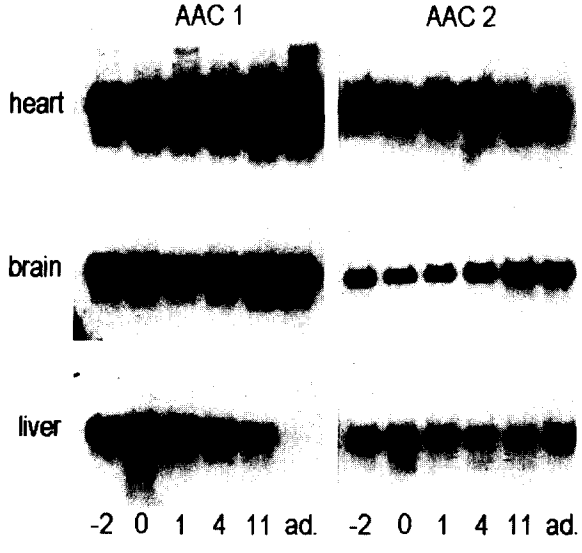

Fig. 1. Northern blots of adenine nucleotide translocase isoforms in heart, brain and liver tissues of developing rats. Total RNA was extracted from tissues of fetal (−2), newborn (0), 1-day-old, 4-day-old, 11-day-old and adult rats. Northern blots were probed with cDNA for AAC1 and AAC2 as described in Section 2. Specific activity of ^{32}P-labeled probes was comparable.

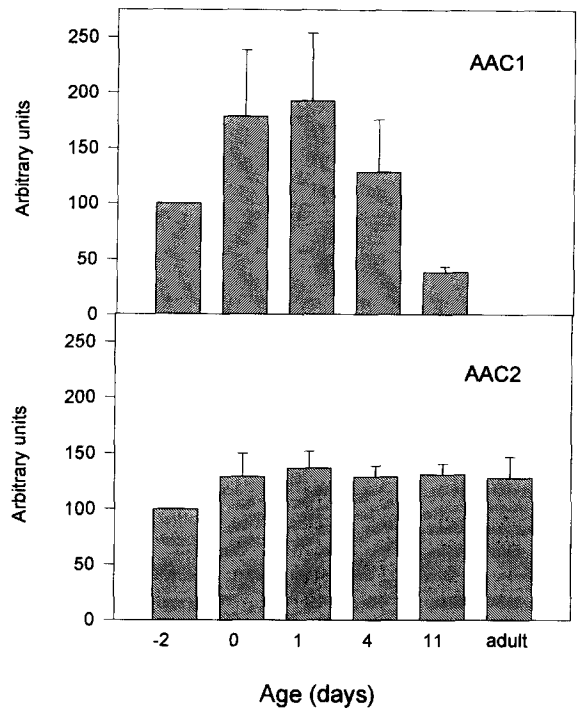

Fig. 2. Transcript levels of AAC1 mRNA and AAC2 mRNA liver tissue of various developmental stages. Quantification was performed as described in Section 2. The data shown are means ± S.E.M. of three different preparations.

fetal liver, and did not significantly increase during postnatal development. In addition, the transcript level of the AAC2 gene is much higher in heart than in liver or brain (Fig. 1). This suggests that, despite the prevalent expression of AAC1 in heart (Fig. 1), the AAC2 isoform is functionally important in this tissue. Indeed, the state 3 respiration of heart mitochondria isolated from 'knockout' mice deficient in the AAC1 isoform is not much lower than that obtained with control animals [16], indicating that the AAC2 isoform is involved in the adenine nucleotide translocation in heart.

What could be the physiological role of a transient expression of the AAC1 gene in the newborn rat liver? Firstly, newborn rats are stressed by cold. Therefore, it could be that the liver has a particular thermogenic function in newborns. Such a possible physiological role is supported by the expression of thermogenin (uncoupling protein 1) in the newborn rat liver, and in addition, by expression of thermogenin in the adult rat liver after cold exposure [17]. Thermogenin, an intrinsic uncoupler of oxidative phosphorylation, converts the electrochemical proton gradient into heat (for review, see [18,19]). AAC might also contribute to the cellular thermogenesis under certain conditions [18,20]. Its thermogenic function is switched on by low concentrations of free fatty acids, which induce the AAC-mediated dissipation of the electrochemical proton gradient [18]. Based on this framework, it might be hypothesized that an expression of AAC1 around birth improves the thermogenic capacity of rat liver. An increase of AAC gene expression (probably that of AAC1 gene) was found in the liver of wood frogs, when they were exposed to freezing stress [21]. Therefore, we examined the effect of cold stress on AAC1 gene expression in the adult rat liver. However, no increase in the mRNA transcript level of AAC1 or AAC2 was seen in liver tissue after 1-week cold exposure

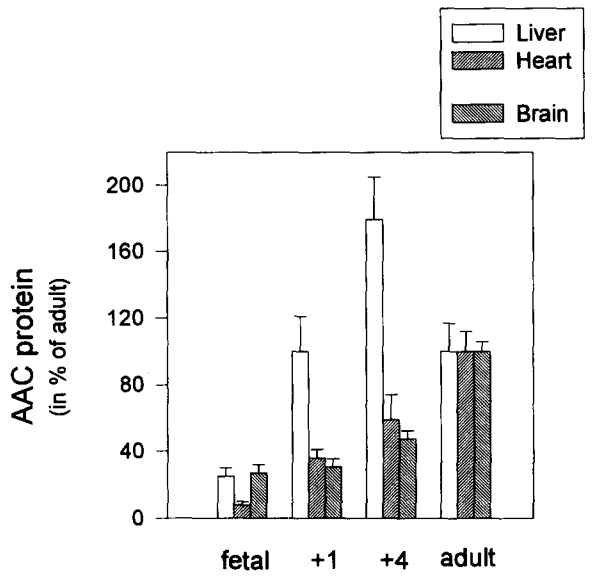

Fig. 3. Developmental changes in the adenine nucleotide translocase protein in rat liver, heart and brain tissue. The AAC protein content was estimated by measuring the [^{3}H]atractyloside binding sites in tissue homogenates as described in Section 2. The data are given in % of the AAC tissue content of adult rats (3–5 preparations). 100% AAC content in adult liver, heart, brain tissue corresponds to 19 ± 3, 244 ± 29 and 20 ± 2 pmol/mg tissue protein.

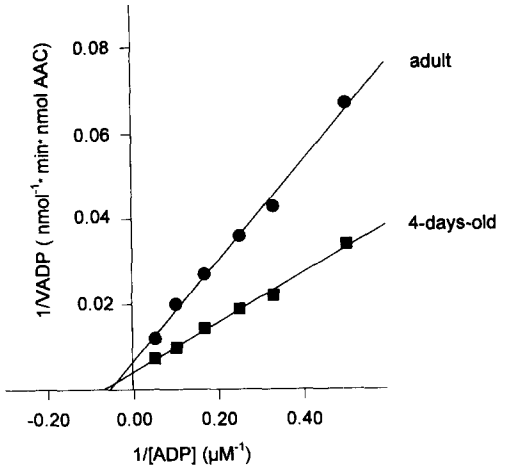

Fig. 4. Lineweaver-Burk plots of the [^{3}H]ADP uptake by liver mitochondria from 4-day-old and adult rats. The uptake of [^{3}H]ADP by suspensions of mitochondria (triplicate incubations) was measured as described in Section 2.

(not shown). It may be assumed that cold stress in a newborn rat is much stronger than in an adult animal exposed to 4°C.

Secondly, the energy metabolism of fetal liver tissue and fast growing tumors is similar in ATP production, which is predominantly generated glycolytically [2,22]. Moreover, increased levels of oxidative phosphorylation gene transcripts have been found in certain neoplastically transformed tissues when compared with the normal tissue [23–25]. Thus, an overexpression of AAC1 and AAC2 genes was found in tumor fibroblast cell lines [23]. In addition, in patients suffering from Kearns-Sayre syndrome (a myopathy caused by deletions in the mitochondrial DNA), overexpression of AAC1 and AAC2 genes was found in various tissues, including liver [26]. All these findings support the hypothesis that the AAC1 gene is expressed in physiological states where the mitochondrial ATP production is low, but the cell has a high cytosolic ATP demand. Furthermore, no transient increase of the β-F_1 ATPase subunit protein (which is considered a marker of mitochondrial biogenesis [2]) or respiratory chain proteins was found in the developing liver [27,28]. Thus, the expression of the AAC protein seems to be a major factor in the supply of oxidative generated ATP to the cytosolic compartment in the developing rat liver. In addition, it has been discussed that the AAC isoforms might differ in their kinetic properties, and that AAC1 is the isoform which is probably most suitable to catalyze rapid exchange of matrix ATP for cytosolic ADP in tissues [9]. Therefore, we measured the molecular activity of AAC as a function of external ADP concentration with liver mitochondria prepared from 4-day-old and adult rats. The kinetic data derived from Lineweaver-Burk plots (Fig. 4) reveal that in liver mitochondria from 4-day-old rats the molecular activity of AAC is twofold higher (Table 1), a finding which is in line with [9]. On the other hand, no change in the K_m for the ADP uptake was found with mitochondria from both ages (Table 1).

In summary, in the liver of newborn mammals the AAC exerts a high control over the supply of mitochondrial generated ATP to the cytosolic compartment [5]. The transient appearance of AAC1 indicates an active energy metabolism in the neonatal liver, which results from the onset of various ATP-consuming processes at birth (e.g. mitochondrial biogenesis, gluconeogenesis, ureogenesis). We suggest that the transient expression of the AAC1 isoform helps to accommodate the mitochondrial ATP supply to the increased cytosolic ATP consumption in the liver of newborns. With proceeding postnatal maturation of mitochondria, the control of other steps over mitochondrial ATP generation like cytochrome *c* oxidation and the hydrogen supply to the respiratory chain increases [29], and consequently the expression of AAC1 declines.

Table 1
Respiration (state 3), adenine nucleotide translocase content and kinetic parameter of the ADP uptake of liver mitochondria from 4-day-old and adult rats

	Respiration (ng atom O/min/mg protein)	AAC content (pmol/mg protein)	V_{max} (nmol ADP/min/nmol AAC)	K_m (μM)
4 days old	78 ± 23	46 ± 9	222 ± 42	13 ± 2
	(n = 4)	(n = 4)	(n = 3)	(n = 3)
Adult	136 ± 15	68 ± 2	115 ± 15	11 ± 4
	(n = 5)	(n = 5)	(n = 3)	(n = 3)

Rat liver mitochondria were treated as described in Section 2. State 3 respiration was measured with 5 mM glutamate plus 5 mM malate as respiratory substrate. The content of AAC protein is given as pmol atractyloside binding sites per mg mitochondrial protein. V_{max} and K_m were calculated from experiments as shown in Fig. 4. The numbers of preparations are in parentheses.

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References

[1] Aprille, J.R. (1986) in: Mitochondrial Physiology and Pathology (Fiskum, E., Ed.), pp. 66–69, Van Nostrand Reinhold, New York.
[2] Cuezva, J.M., Ostronoff, L.K., Ricart, J., Deheredia, M.L., Dilieggro, C.M. and Izquierdo, J.M. (1997) J. Bioenerg. Biomembr. 29, 365–377.
[3] Valcarce, C. and Cuezva, J.M. (1991) FEBS Lett. 294, 225–228.
[4] Aprille, J.R. (1988) FASEB J. 2, 2547–2556.
[5] Hale, D.E. and Williamson, J.R. (1984) J. Biol. Chem. 259, 8737–8742.
[6] Schönfeld, P. and Bohnensack, R. (1995) Biochim. Biophys. Acta 1232, 75–80.
[7] Schönfeld, P., Schild, L. and Bohnensack, R. (1996) Eur. J. Biochem. 241, 895–900.
[8] Portman, M.A., Xiao, Y., Song, Y. and Ning, X.H. (1997) Am. J. Physiol. 273, H1977–H1983.
[9] Stepien, G., Torroni, A., Chung, A.B., Hodge, J.A. and Wallace, D.C. (1992) J. Biol. Chem. 267, 14592–14597.
[10] Doerner, A., Pauschinger, M., Badorff, A., Noutsias, M., Giessen, S., Schulze, K., Bilger, J., Rauch, U. and Schultheiss, H.-P. (1997) FEBS Lett. 414, 258–262.
[11] Luciakova, K. and Nelson, B.D. (1992) Eur. J. Biochem. 207, 247–251.
[12] Shinohara, Y., Kamida, M., Yamazaki, N. and Terada, H. (1993) Biochim. Biophys. Acta 1152, 192–196.
[13] Dümmler, K., Müller, S. and Seitz, H.J. (1996) Biochem. J. 317, 913–918.
[14] Grado, A., Manchado, C., Iglesias, R., Giralt, M., Villarroya, F., Mampel, T. and Vinas, O. (1998) FEBS Lett. 421, 213–216.
[15] Schönfeld, P., Fritz, S., Halangk, W. and Bohnensack, R. (1993) Biochim. Biophys. Acta 1144, 353–358.
[16] Graham, B.H., Waymire, K.G., Cottrell, B., Trounce, I.A., Gregor, G.R., MacGregor, I. and Wallace, D.C. (1997) Nature Genet. 16, 226–234.
[17] Shinohara, Y., Shima, A., Kamida, M. and Terada, H. (1991) FEBS Lett. 293, 173–174.
[18] Skulachev, V.P. (1991) FEBS Lett. 294, 158–162.
[19] Skulachev, V.P. (1998) Biochim. Biophys. Acta 1363, 100–124.
[20] Brustovetsky, N.N., Egorova, M.V., Gnutov, D.Yu., Gogvadez, V.G., Mokhova, E.N. and Skulachev, V.P. (1992) FEBS Lett. 305, 15–17.
[21] Cai, Q., Greenway, S.C. and Storey, K.B. (1997) Biochim. Biophys. Acta 1353, 69–78.
[22] Pedersen, P.L. (1978) Prog. Exp. Tumor Res. 22, 190–274.
[23] Torrini, A., Stepien, G., Hodge, J.A. and Wallace, D.C. (1990) J. Biol. Chem. 265, 20589–20593.
[24] Luciakova, K. and Kuzela, S. (1992) Eur. J. Biochem. 205, 1187–1193.
[25] Faury-Vigny, H., Heddi, A., Giraud, S., Chautard, D. and Stepien, G. (1996) Mol. Carcinogenesis 16, 165–172.
[26] Heddi, A., Lestienne, P., Wallace, D. and Stepien, G. (1994) Biochim. Biophys. Acta 1226, 206–212.
[27] Izquierdo, J.M., Ricart, J., Brandt, U. and von Jagow, G. (1995) Eur. J. Biochem. 230, 235–241.
[28] Schägger, H., Noack, H., Brandt, U. and von Jagow, G. (1995) Eur. J. Biochem. 230, 235–241.
[29] Groen, A.K., Wanders, R.J.A., van den Meer, H.V. and Tager, M. (1982) J. Biol. Chem. 257, 2754–2757.