Journal of Bioenergetics and Biomembranes, Vol. 30, No. 3, 1998

Human Mitochondrial Transmembrane Metabolite Carriers: Tissue Distribution and Its Implication for Mitochondrial Disorders

Marjan Huizing,[1] Wim Ruitenbeek,[1] Lambert P. van den Heuvel,[1] Vincenza Dolce,[2] Vito Iacobazzi,[2] Jan A. M. Smeitink,[1] Ferdinando Palmieri,[2] and J. M. Frans Trijbels[1]

Received December 10, 1997; accepted April 29, 1998

Mitochondrial transmembrane carrier deficiencies are a recently discovered group of disorders, belonging to the so-called mitochondriocytopathies. We examined the human tissue distribution of carriers which are involved in the process of oxidative phosphorylation (adenine nucleotide translocator, phosphate carrier, and voltage-dependent anion channel) and some mitochondrial substrate carriers (2-oxoglutarate carrier, carnitine-acylcarnitine carrier, and citrate carrier). The tissue distribution on mRNA level of mitochondrial transport proteins appears to be roughly in correlation with the dependence of these tissues on mitochondrial energy production capacity. In general the main mRNA expression of carriers involved in mitochondrial energy metabolism occurs in skeletal muscle and heart. Expression in liver and pancreas differs between carriers. Expression in brain, placenta, lung, and kidney is lower than in the other tissues. Western and Northern blotting experiments show a comparable HVDAC1 protein and mRNA distribution for the tested tissues. Patient's studies showed that cultured skin fibroblasts may not be a reliable alternative for skeletal muscle in screening for human mitochondrial carrier defects.

KEY WORDS: Mitochondrial transmembrane carrier; tissue distribution; mitochondriopathy; adenine nucleotide translocator; phosphate carrier; voltage-dependent anion channel; citrate carrier; oxoglutarate carrier; carnitine-acylcarnitine carrier.

INTRODUCTION

When studying mitochondrial disorders it is worthwhile to consider the mitochondrial transmembrane carriers because a defective functioning of such carriers may lead to a disturbed mitochondrial energy generation (Ruitenbeek *et al.*, 1995; Huizing *et al.*, 1996a). Of particular interest are the carriers which are directly involved in the process of oxidative phosphorylation, such as the adenine nucleotide translocator (ANT[3]) and the phosphate carrier (PiC). However, also defects in mitochondrial carriers for transport of specific substrates (such as pyruvate, 2-oxoglutarate, malate, carnitine, and glutamate), directly or indirectly involved in mitochondrial energy metabolism, have to be considered. Many of these carriers have been isolated and some of them have been cloned and sequenced (Palmieri, 1994; Palmieri and Van Ommen, 1998). Defects in transmembrane cation transporters may also induce imperfect energy metabolism probably as a result of osmotic disturbances within the mito-

[1] Department of Pediatrics, University Hospital, Nijmegen, The Netherlands.
[2] Department of Pharmaco-Biology, University of Bari, Bari, Italy.

[3] Abbreviations used: ANT, adenine nucleotide translocator; bp, base pairs; CAC, carnitine-acylcarnitine carrier; CIC, citrate carrier; HVDAC, human voltage-dependent anion channel; Mi-CK, mitochondrial creatine kinase; OGC, 2-oxoglutarate carrier; PiC, phosphate carrier.

0145-479X/98/0600-0277$15.00/0 © 1998 Plenum Publishing Corporation

chondrial matrix. In this respect, the voltage-dependent anion channel (VDAC) and the cation carriers have to be taken into consideration, although so far few cation carriers have been isolated from mammalian tissues, and none has been cloned and sequenced (Li *et al.*, 1992; Garlid, 1994; Jung and Brierly, 1994).

Up to now, defects in some mitochondrial transport systems have been established or suggested, among which are ANT deficiencies (Bakker *et al.*, 1993; Schultheiss *et al.*, 1996; Smeitink *et al.*, 1997), a VDAC deficiency (Huizing *et al.*, 1996b), a defect in the protein import machinery (Schapira *et al.*, 1990), disturbances in the aspartate-malate shuttle (Hayes *et al.*, 1987; Brivet *et al.*, 1997), deficiencies of the pyruvate carrier (Selak *et al.*, 1997), defects in the carnitine-acylcarnitine carrier (Stanley *et al.*, 1992; Pande and Murthy, 1994; Huizing *et al.*, 1997), and defects in the ornithine carrier (Inoue *et. al.*, 1988).

Subjects lacking ANT or VDAC were diagnosed applying immunochemical techniques with specific antibodies (Bakker *et al.*, 1993; Huizing *et al.*, 1996b; Smeitink *et al.*, 1997). Both VDAC and ANT deficiencies were detected in skeletal muscle mitochondria, while the deficiencies were not present in cultured skin fibroblasts of these patients, a finding highly suggestive for tissue-specific expression of deficiencies in these transporters.

In the present communication we report on the steady-state mRNA levels of mitochondrial transmembrane carriers in different human tissues. Also the immunochemically established distribution of the HVDAC1 protein in different tissues is reported, whereas antibodies against other human carriers are not available. The consequences of mitochondrial carrier tissue distribution in the diagnostic evaluation of patients suspected to suffer from a mitochondriocytopathy will be discussed.

MATERIALS AND METHODS

Northern Blot Studies

Multiple poly(A)$^+$ RNA Northern blot (Clontech, Palo Alto, California), loaded with 2 μg poly(A) RNA per tissue, was probed with cDNA probes (100 ng). The probes were labeled by random priming with [α-^{32}P]dCTP, according to the manufacturer's protocol (Boehringer-Mannheim, Germany). The filters were autoradiographed at −70°C.

The human VDAC cDNAs for isoforms HVDAC1 and HVDAC2 were a gift from Dr. M. Forte (Blachly-Dyson *et al.*, 1993). The HVDAC1 and HVDAC2 probes were prepared by cloning the total cDNAs in a BlueScript plasmid vector and isolating a 679 bp *HindIII* restriction fragment from the HVDAC1 plasmid and a 796 bp *HeaIII* restriction fragment from the HVDAC2 plasmid.

We cloned and sequenced the two alternatively spliced human PiC isoforms (Dolce *et al.*, 1994, 1996). Specific parts of both cDNAs (PiC-A and PiC-B) were amplified by PCR: primers for PiC-A corresponded to nucleotides 1719–1743 (forward) and 1785–1809 (reversed) of the human PiC gene sequence, and primers for PiC-B corresponded to nucleotides 2013–2037 (forward) and 2076–2100 (reversed) of the same gene sequence (Dolce *et al.*, 1994). The two products were each cloned into a pGEM-4 vector. A 421 bp *PvuII* restriction fragment for PiC-A and a 418 bp *PvuII* restriction fragment for PiC-B were prepared from these plasmids and used as hybridization probes.

We previously cloned and sequenced the human cDNAs for the 2-oxoglutarate carrier (OGC) (Iacobazzi *et al.*, 1992) and for the citrate carrier (CIC) (Iacobazzi *et al.*, 1997). Parts of these sequences were prepared as hybridization probes for the Northern blot experiments. The OGC probe (330bp long) and the CIC probe (284 bp long) corresponded to the coding sequences of their human genes from nucleotide 1066 to 1646 for OGC (Iacobazzi *et al.*, 1992), and from nucleotide 127 to nucleotide 585 for CIC (Iacobazzi *et al.*, 1997), respectively. For normalization of the hybridization signals on the blot, a probe encoding part of the human actin (Clontech, Palo Alto, California) was employed. Quantification of the hybridization signals was performed by visual (intra- and interblot) comparison.

Western Blot Studies

Western blots were loaded with fibroblasts and biopsy samples of different human tissues (liver, kidney, heart, brain, and skeletal muscle) as previously described for fibroblasts and skeletal muscle tissue, respectively (Huizing *et al.*, 1996b). Each loaded tissue contained 100 mU of the reference enzyme cytochrome *c* oxidase, determined according to the method of Cooperstein and Lazarow (Cooperstein and Lazarow, 1951). The blots were incubated with monoclonal antiserum against the human VDAC1 isoform (anti-Porin31HL,

CalBiochem/Novabiochem, La Jolla, California). Antigen-antibody complexes were detected using the Enhanced Chemiluminescence (ECL) method (Amersham International plc, Buckinghamshire, England) with biotinylated horseradish peroxidase as detection ligand (Dako, Glostrup, Denmark).

RESULTS AND DISCUSSION

At present, knowledge about mitochondrial transmembrane carriers in human physiology and pathophysiology is limited. Few carrier proteins have been isolated from animal tissues and have functionally been tested in reconstituted systems (Palmieri, 1994). DNA of the encoding genes of only six human mitochondrial carriers has been cloned and sequenced (Cozens *et al.*, 1989; Cassard *et al.*, 1990; Iacobazzi *et al.*, 1992; Dolce *et al.*, 1994; Iacobazzi *et al.*, 1997; Huizing *et al.*, 1997). In order to get a rather complete picture of human tissue distribution of transmembrane carriers involved in mitochondrial energy metabolism, we tested the mRNA distribution of OGC, CIC, PiC-A, PiC-B, HVDAC1, and HVDAC2 in eight human tissues (Fig. 1). Their transcript levels together with those of the CAC and of the three ANT isoforms are summarized in Table I.

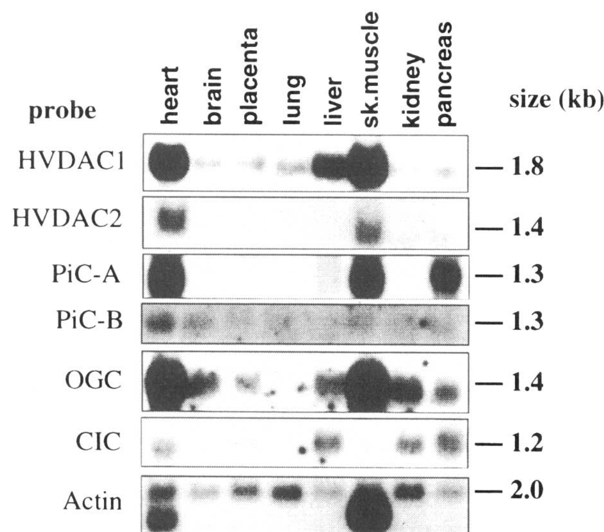

Fig. 1. Multiple tissue Northern blots, incubated with [α-^{32}P]dCTP labeled probes of mitochondrial transmembrane carriers HVDAC1, HVDAC2, PiC-A, PiC-B, OGC, and CIC. Together with an actin probe, mRNA expression is shown for human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

Voltage-Dependent Anion Channel (VDAC)

VDAC is an abundant channel-forming protein present in the outer mitochondrial membrane. Lack of VDAC may cause a mitochondriocytopathy, since this leads to a disturbed transport of ATP and ADP across the outer mitochondrial membrane (Mannella, 1992; Benz, 1994; Huizing *et al.*, 1996b). Also an abnormal ion composition of the mitochondrial matrix can deteriorate the process of oxidative phosphorylation (Mannella, 1992; Benz, 1994; Huizing *et al.*, 1996b). With respect to human VDAC (HVDAC), five genes encoding different isoforms have been reported. The cDNAs of HVDAC1 (on chromosome X) and HVDAC2 (chromosome 21) are completely sequenced (Blachly-Dyson *et al.*, 1993), as well as HVDAC2′ (chromosome 21), which is very closely related to HVDAC2 (Ha *et al.*, 1993). HVDAC3 (chromosome 12) and HVDAC4 (chromosome 1) are only partially sequenced (Blachly-Dyson *et al.*, 1994), and may be pseudogenes. Until now, only HVDAC1 and HVDAC2 have been shown to be expressed at protein level, HVDAC1 being the most abundantly expressed (Winkelbach *et al.*, 1994; Yu *et al.*, 1995). We first examined the tissue distribution of HVDAC1 and HVDAC2 isoforms at the transcript level (Fig. 1). The HVDAC1 mRNA is found to have an ubiquitous distribution, with most pronounced expression in heart, liver, and skeletal muscle. On the contrary, the HVDAC2 isoform appears to be only expressed in heart. The mobility of the HVDAC2 band seems to be greater (mRNA size smaller in Fig. 1) in skeletal muscle than in heart. This might imply cross reactivity of HVDAC2 mRNA with another HVDAC isoform, which means that HVDAC2 is only poorly expressed in skeletal muscle. The tissue distribution of HVDAC1 protein was also investigated by immunodecoration of SDS-lysates of different human tissues with a monoclonal antiserum against HVDAC1 (Fig. 2). The HVDAC1 has the highest expression levels in heart, skeletal muscle, and liver, and lower levels in kidney and brain. The differences in protein levels seem to be smaller than the differences in mRNA levels (Figs. 1 and 2). It is noteworthy that fibroblasts show a high HVDAC1 content as compared to that in skeletal muscle (Fig. 2).

Recently we described the first patient in whom a HVDAC1 deficiency was established (Huizing *et al.*, 1996b). This patient showed psychomotor retardation, macrocephaly, macrosomia, and impaired substrate oxidations in muscle mitochondria. Western blot studies with anti-HVDAC1 antiserum showed a 10-fold

Table I. mRNA Expression Levels of Mitochondrial Membrane Proteins in Different Human Tissues[a]

	Heart	Brain	Placenta	Lung	Liver	Skeletal muscle	Kidney	Pancreas
HVDAC1	+++[b]	+	+	+	++	+++	+	+
HVDAC2	++	−	−	−	−	±	−	−
PiC-A	+++	−	−	−	−	+++	−	++
PiC-B	++	+	+	+	±	+	±	+
OGC	+++	+	+	−	+	+++	+	+
CIC	+	−	±	−	++	±	++	++
CAC	++	+	+	−	++	++	+	++
ANT1	+++	−	U	U	−	++	+	U
ANT2	−	−	U	U	+	−	+	U
ANT3	++	++	U	U	+	+	+++	U

[a] Visual estimation of the transcript levels shown in Fig. 1, and of the described transcript levels of the CAC (Huizing *et al.*, 1997) and the ANT isoforms (Stepien *et al.*, 1992).

[b] −, no detectable expression; ±, low expression level; +, moderate expression level; ++, high expression level; +++, very high expression level; U, unknown.

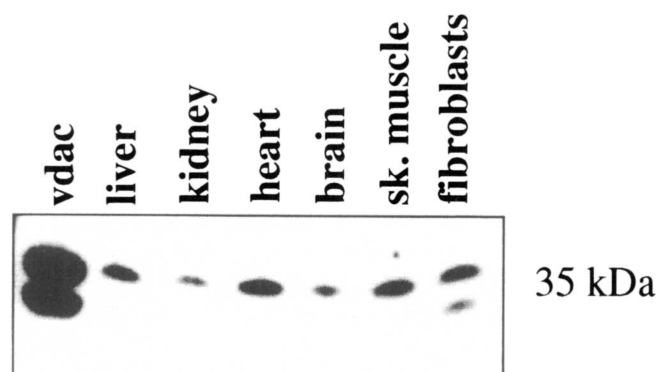

Fig. 2. Multiple tissue immunoblot, incubated with a monoclonal antiserum against HVDAC1. 600 g supernatants of different human tissues, containing 100 mU of cytochrome *c* oxidase, were loaded on each lane. HVDAC1 protein expression is shown for: liver, kidney, heart, brain, skeletal muscle, fibroblasts, and human VDAC (35 kD) isolated from human B lymphocytes (Babel *et al.*, 1991).

decreased amount of HVDAC1 in skeletal muscle, while fibroblasts showed an almost normal VDAC amount. We sequenced the patient's HVDAC1 and HVDAC2 cDNA, but found no mutations. The HVDAC1 defect in the patient's muscle material might be caused by a secondary defect, i.e., a disturbed acetylation of the immature VDAC protein (Huizing *et al.*, 1996b).

Phosphate Carrier (PiC)

The availability of inorganic phosphate for oxidative phosphorylation mainly depends on PiC activity in the inner mitochondrial membrane (Ferreira and Pedersen, 1993). A full-length cDNA encoding the precursor of the human heart PiC has been synthesized and characterized (Dolce *et al.*, 1991). The gene encoding human PiC is located on chromosome 12q23 (Marsh *et al.*, 1995) and is spread over 7.9 kb of DNA, consisting of nine exons (Dolce *et al.*, 1994). Two alternatively spliced mRNAs for PiC (IIIA and IIIB) have been uncovered by comparing the human and bovine genomic sequences with the corresponding cDNAs (Dolce *et al.*, 1994) and have been demonstrated in some bovine tissues (Dolce *et al.*, 1996). The alternative splicing mechanism introduces a different region of 13 amino acids into the human carrier protein, the functional consequences of which are not yet understood (Dolce *et al.*, 1994). In this study the mRNA levels of isoforms A and B of the PiC were investigated in eight human tissues by Northern blot analysis using two probes that are specific for human exon IIIA and exon IIIB, respectively (Fig. 1). These investigations show that PiC-A mRNA is expressed in heart, skeletal muscle, and pancreas in high amounts, while PiC-B mRNA is poorly expressed in all tissues. The observed tissue distribution of the PiC isoforms may be used to modulate the rate of ATP production by oxidative phosphorylation for tissue-specific energy demand. The ubiquitously distributed PiC-B isoform might match the basic energy requirement of the tissues, and isoform PiC-A might become operative in case of energetic stress, i.e., at contraction of striated muscle fibers.

So far, we screened 150 muscle specimens from patients suspected of a mitochondrial carrier defect (patients with decreased substrate oxidations and ATP

production rates, and no detected mitochondrial enzyme deficiencies), but no PiC deficiencies were found.

2-Oxoglutarate Carrier (OGC)

The 2-oxoglutarate carrier (OGC) transports 2-oxoglutarate across the inner mitochondrial membrane in exchange for malate or other dicarboxylic acids (Palmieri *et al.*, 1993). This carrier is part of the aspartate-malate shuttle (Indiveri *et al.*, 1987). The human OGC gene has been cloned and sequenced (Iacobazzi *et al.*, 1992). It consists of six exons and five introns; the chromosomal localization is unknown.

The results of Northern blots performed on total RNA derived from eight human tissues are shown in Fig. 1 The OGC transcript was expressed very strongly in heart and skeletal muscle, less in liver, kidney, pancreas, brain, and placenta. No OGC mRNA was found in lung. Therefore, the observed mRNA level of OGC seems to correlate with the required capacity of the carrier in the various tissues. No patient with a defect in OGC has been described. We studied skeletal muscle tissue of patients with 2-oxoglutaric acidemia, not due to a 2-oxoglutarate dehydrogenase deficiency, for a possible deficiency of the OGC. So far, we did not identify a patient with a deficiency of this carrier using an immunochemical method. But it has to be stressed that identification of such a deficiency may have been hampered by the low intensity of the immunochemical signal observed in control muscle samples.

Citrate Carrier (CIC)

The mitochondrial citrate carrier (CIC), or tricarboxylate carrier, is responsible for the transport of citrate across the inner mitochondrial membrane. CIC plays an important role in fatty acid and sterol synthesis, gluconeogenesis, and the transfer of reducing equivalents across the membrane (Bisaccia *et al.*, 1989; Kaplan *et al.*, 1993). The complete human CIC gene has been sequenced (Iacobazzi *et al.*, 1997) and has been mapped to chromosome 22q11 (Heisterkamp *et al.*, 1995). The CIC mRNA expression pattern is unique in that it differs from the distribution pattern of all other carriers (Fig. 1) Thus, Northern blot experiments (Fig. 1) reveal high steady-state levels of CIC mRNA in liver, kidney, and pancreas, lower levels in heart, skeletal muscle, and placenta, and no detectable mRNA in brain and lung.

High CIC mRNA levels in liver and kidney can be associated with the processes of gluconeogenesis, which mainly occurs in these tissues, and the fatty acid synthesis, which mainly occurs in liver. So far, no clear explanation can be given for the relatively high CIC mRNA level in heart and pancreas. On the other hand, the low CIC mRNA level in skeletal muscle correlates to the very low activity of gluconeogenesis and fatty acid synthesis in this tissue. Because CIC is not very expressed in skeletal muscle tissue, there is no reason to screen muscle biopsies of patients with a mitochondrial myopathy for a defect in CIC. So far, no patients with a defect in CIC have been reported.

Carnitine-Acylcarnitine Carrier (CAC)

The carnitine-acylcarnitine carrier (CAC) shuttles long-chain acylcarnitine esters in exchange for free carnitine across the inner mitochondrial membrane (Pande, 1975; Ramsay and Tubbs, 1975; Indiveri *et al.*, 1990). This transport is an essential step in the process of the mitochondrial long-chain fatty acid β-oxidation (Coates and Tanaka, 1992; Stanley *et al.*, 1992). Very recently we determined the nucleotide sequence of the human CAC cDNA and the corresponding amino acid sequence as well as the distribution of CAC mRNA in human tissues (Huizing *et al.*, 1997). Although the structure of the complete human CAC gene is not yet known, it has been mapped to chromosome 3p21.31 (Viggiano *et al.*, 1997). A high level of CAC mRNA transcripts was found in heart, skeletal muscle, and liver while much lower levels were found in brain, placenta, kidney, pancreas, and especially in lung.

Several cases of CAC deficiency have been reported (see Huizing *et al.*, 1997). The main features in these severely affected patients with onset in the neonatal period are hypoketotic hypoglycemia, mild hyperammonemia, unspecific dicarboxylic aciduria, lethargy/coma, hepatomegaly with abnormal liver functions, cardiomyopathy with or without dysrythmias, and skeletal muscle weakness. The tissue distribution pattern of the CAC is in fair agreement with the clinical involvement of heart, skeletal muscle, and liver in patients with CAC deficiency (Huizing *et al.*, 1997). Recently the first mutations in the CAC cDNA of two patient have been established (Huizing *et al.*, 1997, 1998).

Adenine Nucleotide Translocator (ANT)

ANT catalyzes the transmembrane exchange of cytosolic ADP and matrix ATP, which is essential for the oxidative phosphorylation of ADP inside the mitochondria and for the extramitochondrial utilization of ATP (Klingenberg, 1981; Brandolin *et al.*, 1993). Three human ANT isoforms (ANT1, ANT2, and ANT3) have been identified and their genes have been sequenced. They are expressed in a tissue-specific manner which seems to be related to their function (Battini *et al.*, 1987; Neckelmann *et al.*, 1987; Houldsworth and Attardi, 1988). Human ANT1 may permit for rapid exchange of ADP and ATP to accommodate to the high energy demand associated with contraction of striated muscle fibers. ANT1 has a high mRNA expression level in heart and skeletal muscle and is induced during myoblast differentiation (Neckelmann *et al.*, 1987). The mRNA of the ANT2 isoform is induced in rapidly dividing cells such as fibroblasts, human leukemic cells (Battini *et al.*, 1987), and myoblasts (Stepien *et al.*, 1992), whereas it is either absent or weakly expressed in all other tissues. ANT3 mRNA is expressed in all tissues, mainly in kidney, heart, and brain (Torroni *et al.*, 1990; Stepien *et al.*, 1992).

A deficiency of ANT in human muscle (likely of the ANT1 isoform) has been reported (Bakker *et al.*, 1993). This patient showed severe myopathy with lactic acidosis. The patient's impaired substrate oxidation in muscle mitochondria can readily be explained by a defect in the transport protein. The fibroblasts showed a normal immunochemical signal, probably caused by normal levels of ANT2 and/or ANT3.

Schultheiss *et al.* (1996) demonstrated a decreased ANT transport capacity accompanied by an elevation in total ANT protein content in heart tissue of patients suffering from myocarditis and dilated cardiomyopathy. The alteration in ANT protein amount was due to a mutual shift in ANT isoforms: an increase in ANT1, a decrease in ANT2, the ANT3 content being unchanged. The isoform shift in these patients is not a progressive process but occurs in the early period of illness and becomes permanent. The underlying mechanism and pathognostic significance are still obscure.

Very recently, we examined skeletal muscle specimens from patients with the Sengers syndrome (McKusick 212350), clinically presenting with congenital cataract, hypertrophic cardiomyopathy, mitochondrial myopathy, and lactic acidosis (Sengers *et al.*, 1975; Smeitink *et al.*, 1989). In the muscle specimens a diminished amount of ANT was demonstrated on Western blots (Smeitink *et al.*, 1997). The molecular analysis of the ANT defect(s) in patients with the Sengers syndrome is in progress.

Coordination in Expression

ANT1 mRNA expression pattern is comparable with that of HVDAC2 and PiC-A (Table I). All three are almost exclusively expressed in heart and skeletal muscle. These proteins also have a ubiquitously expressed isoform: ANT3, HVDAC1, and PiC-B. Probably the skeletal muscle and heart isoforms accommodate to the high metabolic turnover rate in these tissues (due to the high energy demand), while the ubiquitous isoforms stand for a continuous basal transport of metabolites in all tissues.

The HVDAC1 immunodecorations of the Western blot (Fig. 2) were all loaded with the same amount of the mitochondrial reference enzyme cytochrome *c* oxidase. The differences detected in HVDAC1 mRNA expression levels are thus due to differences in HVDAC1 levels per unit of cytochrome *c* oxidase. So, in tissues with a high metabolic turnover, the HVDAC1/cytochrome *c* oxidase ratio is higher than in other tissues.

Multi-carrier Structures

Mitochondrial energy metabolism is not only influenced by individually functioning transmembrane carriers but also by complicated structures in which several types of transport proteins and other components are present. In these structures, i.e., contact sites and megachannels, VDAC, ANT, mitochondrial creatine kinase (Mi-CK), hexokinase, glycerol kinase, and the benzodiazepine receptor are involved (McEnery *et al.*, 1993; Benz, 1994; Brdiczka and Walliman, 1994). These structures are thought to facilitate among others the ADP and ATP transport across the inner and outer mitochondrial membranes. The occurrence of these multi-protein structures in various tissues has not yet been established, but a high content of these "megachannels" might be expected in tissues with high energy demand. Therefore, the isoforms ANT1, HVDAC2, and sarcomeric Mi-CK (Wyss *et al.*, 1992) might be components of these megachannels, while the

ubiquitous isoforms ANT2, HVDAC1, and ubiquitous Mi-CK might function in mitochondria independently.

Diagnostic Approach

The Northern blots showed high expression levels of mRNA of all carriers in skeletal muscle and heart (except for CIC). Therefore, and because skeletal muscle tissue is often clinically involved, skeletal muscle is the most suitable tissue to examine in patients suspected to suffer from a mitochondrial carrier defect. In those cases in which it is impossible to obtain skeletal muscle material from patients, measurements in cultured skin fibroblasts may be considered. We showed more or less similar expression levels for HVDAC1 mRNA and protein for the investigated tissues. This points to a comparable mRNA tissue expression and translation for HVDAC1. From the fact that the HVDAC1 signal is comparable in fibroblast and skeletal muscle on Western blot, we assume that HVDAC1 mRNA is also highly expressed in fibroblasts. Therefore, fibroblasts should be suitable for diagnosis of mitochondrial carrier deficiencies. However, immunochemical studies on the recently described ANT-deficient (Bakker *et al.*, 1993) and VDAC-deficient (Huizing *et al.*, 1996b) patients showed the deficiency in skeletal muscle, and not in fibroblasts. For the ANT-deficient patient this tissue-dependent deficiency can be explained by different expression of the isoforms in fibroblasts and muscle tissue. For the VDAC-deficient patient, isoforms may also play a role, but a tissue-specific defect (in muscle tissue) in a post-translational modification step may have caused this phenomenon, too (Huizing *et al.*, 1996b). Whatever the molecular origin, normal contents of transport proteins in fibroblasts do not exclude a deficiency in skeletal muscle. This limitation in using fibroblasts for diagnostic aims is well known for detection of defects in mitochondrial enzymes (Trijbels *et al.*, 1993).

In this paper, the tissue distribution of mitochondrial membrane carriers under physiological conditions is described. Expression might, however, change under pathophysiological conditions, as recently described for patients with myocarditis and dilated cardiomyopathy (Schultheiss *et al.*, 1996). Possible occurrence of such pathophysiological changes should be kept in mind in interpreting diagnostic findings.

CONCLUDING REMARKS

In the present study we showed that human mRNA tissue distribution levels of mitochondrial metabolite carriers are roughly related to the metabolic needs of the tissues. The presence of isoforms of several carriers in man requires a critical selection of the tissue(s) for diagnostic investigation. Based on isoform distribution and on practical availability, skeletal muscle appears to be the most suitable tissue. On the contrary, based on examination of carrier deficiencies with specific antibodies, the use of fibroblasts for diagnostic aims is doubtful.

ACKNOWLEDGMENTS

The financial support of the Prinses Beatrix Fonds (grants Nos. 93-018 and 95-0501 to MH) and of Telethon-Italy (grant No. 985 to FP) is gratefully acknowledged. The Department of Pediatrics Nijmegen is participating in the Institute of Fundamental and Clinical Human Movement Sciences (IFKB).

REFERENCES

Babel, D., Walter, G., Götz, H., Thinnes, F. P., Jürgens, L., Köning, U., and Hilschmann, N. (1991). *Biol. Chem. Hoppe Seyler* **372**, 1027–1034.

Bakker, H. D., Scholte, H. R., Van den Bogert, C., Ruitenbeek, W., Jeneson, J. A. L., Wanders, R. J. A., Abeling, N. G. G. M., Dorland, B., Sengers, R. C. A., and Van Gennip, A. H. (1993). *Pediatr. Res.* **33**, 412–417.

Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S., and Baserga, R. (1987). *J. Biol. Chem.* **9**, 4355–4359.

Benz, R. (1994). *Biochim. Biophys. Acta* **1197**, 167–196.

Bisaccia, F., De Palma, A., and Palmieri, F. (1989). *Biochim. Biophys. Acta* **977**, 171–176.

Blachly-Dyson, E., Zambronicz, E. B., Yu, W. H., Adams, V., McCabe, E. R. B., Adelman, J., Colombini, M., and Forte, M. (1993). *J. Biol. Chem.* **268**, 1835–1841.

Blachly-Dyson, E., Baldini, A., Litt, M., McCabe, E. R. B., and Forte, M. (1994). *Genomics* **20**, 62–67.

Brandolin, G., Le Saux, A., Trezeguet, V., Lauquin, G. J. M., and Vignais, P. V. (1993). *J. Bioenerg. Biomembr.* **25**, 459–472.

Brdiczka, D., and Wallimann, T. (1994). *Mol. Cell. Biochem.* **133/134**, 69–83.

Brivet, M., Slama, A., Rustin, P., Poggi, F., Boutron, A., Rabier, D., Munnich, A., Saudubray, J. M., and Legrand, A. (1997). *International Congress of Inborn Errors of Metabolism*, Vienna, abstract P69.

Cassard, A. M., Bouillaud, F., Mattei, M. G., Hentz, E., Raimbault, S., Thomas, M., and Ricquier, D. (1990). *J. Cell. Biochem.* **43**, 255–264.

Coates, P. M., and Tanaka, K. (1992). *J. Lipid Res.* **33**, 1099–1110.

Cooperstein, S. J., and Lazarow, A. (1951). *J. Biol. Chem.* **189**, 665–670.

Cozens, A. L., Runswick, M. J., and Walker, J. E. (1989). *J. Mol. Biol.* **206**, 261–280.

Dolce, V., Fiermonte, G., Messina, A., and Palmieri, F. (1991). *DNA Sequence* **2**, 133–135.

Dolce, V., Iacobazzi, V., Palmieri, F., and Walker, J. E. (1994). *J. Biol. Chem.* **269**, 10451–10460.

Dolce, V., Fiermonte, G., and Palmieri, F. (1996). *FEBS Lett.* **399**, 95–98.

Ferreira, G. C., and Pedersen, P. L. (1993). *J. Bioenerg. Biomembr.* **25**, 483–492.

Garlid, K. D. (1994). *J. Bioenerg. Biomembr.* **26**, 537–542.

Ha, H., Hajek, P., Bedwell, D. M., and Burrows, P. D. (1993). *J. Biol. Chem.* **268**, 12143–12149.

Hayes, D. J., Taylor, D. J., Bore, P. J., Hilton-Jones, D., Arnold, D. L., Squier, M. V., Gent, A. E., and Radda, G. K. (1987). *J. Neurol. Sci.* **82**, 27–39.

Heisterkamp, N., Mulder, M. P., Langeveld, A., Ten Hoeve, J., Wang, Z., Roe, B. A., and Groffen, J. (1995). *Genomics* **29**, 451–456.

Houldsworth, J., and Attardi, G. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 377–381.

Huizing, M., DePinto, V., Ruitenbeek, W., Trijbels, F. J. M., Van den Heuvel, L. P., and Wendel, U. (1996a). *J. Bioenerg. Biomembr.* **28**, 109–114.

Huizing, M., Ruitenbeek, W., Thinnes, F. P., DePinto, V., Wendel, U., Trijbels, J. M. F., Smit, L. M. E., Ter Laak, H. J., and Van den Heuvel, L. P. (1996b). *Pediatr. Res.* **39**, 760–765.

Huizing, M., Iacobazzi, V., IJlst, L., Savelkoul, P., Ruitenbeek, W., Van den Heuvel, L., Indiveri, C., Smeitink, J., Trijbels, F., Wanders, R., and Palmieri, F. (1997). *Am. J. Hum. Genet.* **61**, 1239–1245.

Huizing, M., Wendel, U., Ruitenbeek, W., Iacobazzi, V., IJlst, L., Veenhuizen, P. T. M., Savelkoul, P., Van den Heuvel, L. P., Smeitink, J. A. M., Wanders, R. J. A., Trijbels, F. J. M., and Palmieri, F. (1998). *J. Inher. Metab. Dis.*, in press.

Iacobazzi, V., Palmieri, F., Runswick, M. J., and Walker, J. E. (1992). *DNA Sequence* **3**, 79–88.

Iacobazzi, V., Lauria, G., and Palmieri, F. (1997). *DNA Sequence* **7**, 127–139.

Indiveri, C., Krämer, R., and Palmieri, F. (1987). *J. Biol. Chem.* **262**, 15979–15983.

Indiveri, C., Tonazzi, A., and Palmieri, F. (1990). *Biochim. Biophys. Acta* **1020**, 81–86.

Inoue, I., Saheki, T., Kayanuma, K., Uono, M., Nakajima, M., Takeshita, K., Koike, R., Yuasa, T., Miyatake, T., and Sakoda, K. (1988). *Biochim. Biophys. Acta* **964**, 90–95.

Jung, D. W., and Brierly, G. P. (1994). *J. Bioenerg. Biomembr.* **26**, 527–535.

Kaplan, R. S., Mayor, J. A., and Wood, D. O. (1993). *J. Biol. Chem.* **268**, 13682–13690.

Klingenberg, M. (1981). *Nature* **290**, 449–454.

Li, W., Shariat-Madar, Z., Powers, M., Sun, X., Lane, R. D., and Garlid, K. D. (1992). *J. Biol. Chem.* **267**, 17983–17989.

Mannella, C. A. (1992). *Trends Biochem. Sci.* **17**, 315–320.

Marsh, S., Carter, N. P., Dolce, V., Iacobazzi, V., and Palmieri, F. (1995). *Genomics* **29**, 814–815.

McEnery, M. W., Dawson, T. M., Verma, A., Gurley, D., Colombini, M., and Snyder, S. H. (1993). *J. Biol. Chem.* **268**, 23289–23296.

Neckelmann, N., Li, K., Wade, R. P., Shuster, R., and Wallace, D. C. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 7580–7584.

Palmieri, F. (1994). *FEBS Lett.* **346**, 48–54.

Palmieri, F., Bisaccia, F., Capobianco, L., Dolce, V., Fiermonte, G., Iacobazzi, V., and Zara, V. (1993). *J. Bioenerg. Biomembr.* **25**, 493–501.

Palmieri, F., and Van Ommen, B. (1998). In *Frontiers of Cellular Bioenergetics: Molecular Biology, Biochemistry and Physiopathology* (Papa, S., *et al.*, eds.), Plenum Press, New York/London, in press.

Pande, S. V. (1975). *Proc. Natl. Acad. Sci. USA* **72**, 883–887.

Pande, S. V., and Murthy, M. S. R. (1994). *Biochim. Biophys. Acta* **1226**, 269–276.

Ramsay, R. R., and Tubbs, P. K. (1975). *FEBS Lett.* **54**, 21–25.

Ruitenbeek, W., Huizing, M., DePinto, V., Thinnes, F. P., Trijbels, J. M. F., Wendel, U., and Sengers, R. C. A. (1995). In *Progress in Cell Research*, Vol. V (Palmieri, F., ed.), Elsevier, Amsterdam, pp. 225–229.

Schapira, A. H. V., Cooper, J. M., Morgan-Hughes, J. A., Landon, D. N., and Clark, J. B. (1990). *N. Engl. J. Med.* **323**, 37–42.

Schultheiss, H. P., Schulze, K., and Dörner, A. (1996). *Mol. Cell. Biochem.* **163/164**, 319–327.

Selak, M. A., Grover, W. D., Foley, C. M., Miles, D. K., and Salganicoff, L. (1997). *International Conference on Mitochondrial Diseases*, Philadelphia, abstract book (Grover, W. D., ed.), abstract 59.

Sengers, R. C. A., Trijbels, J. M. F., Willems, J. L., Daniëls, O., and Stadhouders, A. M. (1975). *J. Pediatr.* **86**, 873–880.

Smeitink, J. A. M., Sengers, R. C. A., Trijbels, J. M. F., Ruitenbeek, W., Daniëls, O., Stadhouders, A. M., and Kock-Jansen, M. J. H. (1989). *Eur. J. Pediatr.* **148**, 656–659.

Smeitink, J., Huizing, M., Ruitenbeek, W., Van den Heuvel, B., Trijbels, F., and Sengers, R. (1997). *J. Inher. Metab. Dis.* **20**, 7 (abstract 013).

Stanley, C. A., Hale, D. E., Berry, G. T., DeLeeuw, S., Boxer, J., and Bonnefont, J. P. (1992). *N. Engl. J. Med.* **327**, 19–22.

Stepien, G., Torroni, A., Chung, A. B., Hodge, J. A., and Wallace, D. C. (1992). *J. Biol. Chem.* **267**, 14592–14597.

Torroni, A., Stepien, G., Hodge, J. A., and Wallace, D. C. (1990). *J. Biol. Chem.* **265**, 20589–20593.

Trijbels, J. M. F., Scholte, H. R., Ruitenbeek, W., Sengers, R. C. A., Janssen, A. J. M., and Busch, H. F. M. (1993). *Eur. J. Pediatr.* **152**, 178–184.

Viggiano, L., Iacobazzi, V., Marzella, R., Cassano, C., Rocchi, M., and Palmieri, F. (1997). *Cytogenet. Cell Genet.* **79**, 62–63.

Winkelbach, H., Walter, G., Morys-Wortmann, C., Paetzold, G., Hesse, D., Zimmermann, B., Flörke, H., Reymann, S., Stadtmüller, U., Thinnes, F. P., and Hilschmann, N. (1994). *Biochem. Med. Metab. Biol.* **52**, 120–127.

Wyss, M., Smeitink, J., Wevers, R. A., and Wallimann, T. (1992). *Biochim. Biophys. Acta* **1102**, 119–166.

Yu, W. H., Wolfgang, W., and Forte, M. (1995). *J. Biol. Chem.* **270**, 13998–14006.