Biochimica et Biophysica Acta, 1026 (1990) 113–116
Elsevier

BBAMEM 74917

Membrane specific carbonic anhydrase (CAIV) expression in human tissues

N.D Carter [1], A Fryer [2], A.G. Grant [3], R. Hume [4], R.G. Strange [2] and P.J. Wistrand [5]

[1] *Department of Child Health, St George's Hospital Medical School, London (U K)*, [2] *Clinical Biochemistry Research Laboratory School of Postgraduate Medicine, University of Keele, Staffs (U K)*, [3] *Department of Surgery, St George's Hospital Medical School, London (U K)*, [4] *Department of Child Life and Health, University of Edinburgh, Edinburgh (U K)* and [5] *Institute of Medical Pharmacology, Uppsala (Sweden)*

(Received 21 December 1989)

Key words Carbonic anhydrase, Enzyme expression, (Human)

Membrane-bound carbonic anhydrase IV (CAIV) expression has been evaluated in a range of fetal and adult human tissues and in cell culture. All tissues tested showed expression of CAIV, assessed by Western blotting, with a single immunodetected band at 55 kDa. The levels varied in fetal lung and liver during development and in various zones of the fetal brain. CAIV was clearly expressed in lung, pancreatic tumour and skin cell cultures.

Introduction

Carbonic anhydrase (EC 4 2 1 1), an efficient catalyst of the reaction, $H_2O + CO_2 \rightarrow H^+ + HCO_3^-$, is present at high levels in erythrocytes and electrolyte-transporting epithelia where it is believed to mediate the transfer of CO_2, H^+, HCO_3^- and Cl^- [1]

A variety of loci encode the isoenzymes identified in mammalian tissues These include CAII which is ubiquitously expressed in mammalian cells and CAI and CAIII where expression is largely restricted to erythrocytes and skeletal muscle, respectively [2] More recently, mitochondrial (CAV) and salivary (CAVI) isoforms have been identified as well as the membrane-associated enzyme, CAIV, which is the subject of this communication [2]

In man, CAIV has been identified in the microvilli and basal infoldings of renal tubular cells and this isoform has recently been purified and characterised [3] An apparently homologous enzyme [4] has also been purified from bovine and adult human lung It is proposed that the CAIV isoenzyme is primarily responsible for the reabsorption of HCO_3^- in the proximal tubule and is involved in the formation of lung liquid during fetal life

We now report, for the first time, the demonstration of CAIV expression in a wide range of fetal, adult, normal and malignant human cells The ubiquitous expression of the isoenzyme has enabled us to make new proposals regarding it's function

Material and Methods

Source of specimens

Samples of lung, liver, kidney and brain were obtained within 4 h of death from aborted fetuses (10–22 weeks gestation) following termination of pregnancy, premature and term infants (24–42 weeks gestation) who died in the neonatal period and infants who suffered sudden infant death syndrome

Red blood cells were also taken from fetuses, neonates and infants Blood specimens were obtained from abortuses (10–22 weeks gestation) by cardiac puncture within 2 h of delivery and from neonates (24–42 weeks gestation) by venipuncture within 24 h of birth as part of an infection screen in infants without overt neurological problems Blood samples were centrifuged at 3000 rpm at room temperature for 5 min and the supernatant plasma and buffy coat removed The red cells were resuspended in 154 mM NaCl and recentrifuged The supernatant was discarded and this wash procedure repeated twice more The pellet was then stored at $-70\,^{\circ}C$ until required

A careful estimate of developmental age was made in each fetus, based on size (including crown-heel, crown-rump and heel-toe measurements), menstrual history and ultrasound In neonates, gestational age was assessed by the Dubowitz score

Correspondence N D Carter, Department of Child Health, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE, U K

Approval to take samples was obtained from the Reproductive Medicine Ethics Committee of the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh

Samples from adult pancreas, colon, kidney were obtained at post mortem or surgical biopsy Samples are stored at −70 °C before use

Preparation of tissue homogenates

Tissues, stored at −70 °C, were allowed to thaw slightly and a sample was removed and washed in ice-cold homogenisation buffer to remove superficial blood contamination and then chopped into small pieces using scissors Sample size varied (0 25 to 2 g) with the tissue and for fetal samples, gestational age

Tris-HCl buffer (20 mM, pH 7 20) containing sucrose (250 mM), EDTA (0 1 mM) and reduced glutathione (1 mM) were added to the tissue pieces which were homogenised on ice and centrifuged (2000 × *g* 4 °C, 10 mm) The supernatant provided the membrane fraction for analysis by Western blotting

Lung and pancreatic carcinoma cell culture

Lung tissue was obtained under sterile conditions from 15 fetuses between 12 and 18 weeks gestation Cubes of lung tissue of 1–2 mm were cultured at the air/medium interface on lens paper supported by steel grids in organ culture dishes The cultures were maintained for 2–14 days in serum-free Glasgow Modified Eagle Medium (MEM) or in MEM supplemented with 20% fetal calf serum Cultures were maintained at 37 °C in 5% CO_2/95% air and media were changed daily All media contained penicillin and streptomycin (both 100 I U /ml)

Pancreatic tumour cells were grown and harvested as previously described [5] Cultured skin fibroblasts were kindly supplied by the S W Thames Regional Cytogenetics Unit

Production of CAIV antiserum

CAIV (molecular mass 35 kDa) purified as described [3] was used to raise a rabbit polyclonal antiserum This antiserum failed to detect any band at 28 kDa (the molecular mass of CAII) on Western blots However, restaining of immunoblots with anti CAII serum gave a striking band at 28 kDa This experiment therefore excludes cross reaction of CAIV antibodies with CAII isoenzyme under the conditions used

Immunoblotting

SDS-discontinuous polyacrylamide gel electrophoresis was performed in 10% gels Before loading, the protein content of samples was equalised by appropriate dilution with 154 mM NaCl Proteins were blotted onto Nitrocellulose membranes (Hybond C, Amersham International) using the Novablot system (Pharmacia LKB Biotechnology) CAIV was visualised using an alkaline phosphatase system

TABLE I

Summary of relative CAIV expression in adult and fetal tissues and in tissue culture

+/− signs represent relative level compared with a standard kidney membrane preparation

1 Adult	Colon (normal and malignant)		+ + +
	Pancreas (normal and malignant)		+ + +
	Kidney membrane vesicles		+ + +
2 Tissue culture	Fetal pneumatocytes		+ + +
	Skin fibroblasts		+ + +
	Ger pancreatic tumour line		+ + +
3 Fetal tissues	(a) Liver	15 weeks	+
		40 weeks	+ + +
		40 + 9 weeks	+ + + +
	(b) Lung	15 weeks	+ + + +
		28 weeks	+
		40 weeks	+/−
		40 + 15 weeks	+/−
	(c) Kidney	15 weeks	+ + +
		28 weeks	+ + +
		40 weeks	+ + +
		40 + 84 weeks	+ + +
	(d) Brain	(single 19 week fetus)	
		Cerebellum	+ + +
		Lateral choroid plexus	+
		Cerebrum Lateral inner	+/−
		and outer wall	+/−
		Medial thin cerebral wall	+/+
		Germinal emminence	+/−
		Thalamus	+

Results

CAIV was detected by immunoblotting in a number of fetal, normal and malignant adult human tissues and in cultured cells The enzyme was visualised as a single protein band with a monomer molecular mass of 55 kDa The 'overall' comparative distribution is summarised in Table I Since samples were first equalised for protein content, the intensity of bands during development can be compared

In fetal life CAIV was detected in all tissues tested except red cells Levels of lung CAIV were observed to decrease through fetal life, whereas levels in liver increased (Fig 1) CAIV levels in fetal kidney appeared to remain constant through gestation In brain samples obtained from a single individual (19 weeks gestation), expression was strongest in cerebellum

CAIV was clearly expressed in cultured skin, pneumatocytes and Ger tumour cells (Fig 1) Fig 1 also shows CAIV expression in normal and malignant pan-

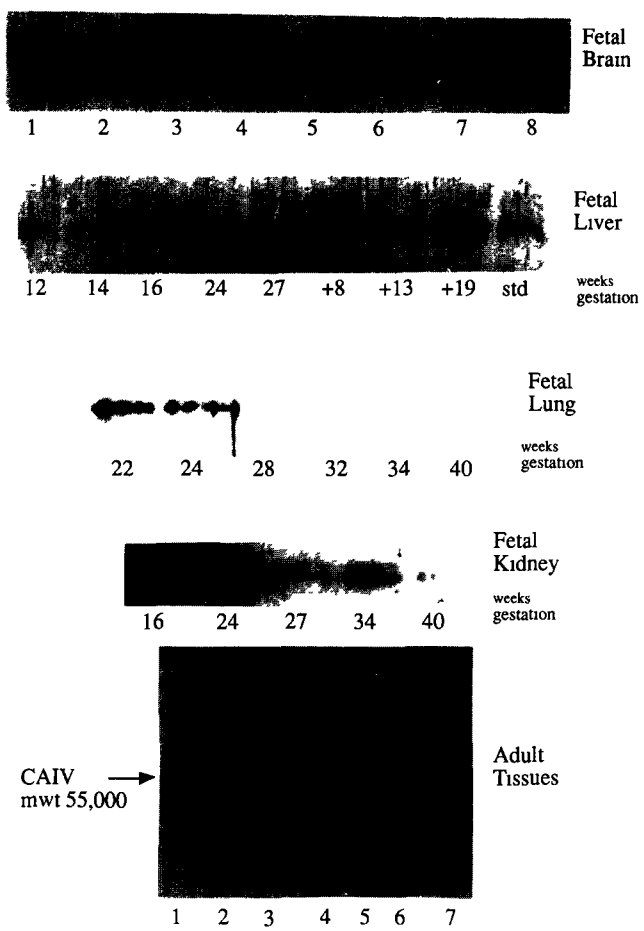

Fig 1 Western blots of whole homogenates from human tissue sand cell extracts Protein concentrations have been normalised to enable comparison of relative CAIV concentration 'Adult tissue lanes' were 1, kidney membrane vesicles (standard), 2, colon carcinoma, 3, pancreatic carcinoma, 4, pancreatic carcinoma, 5, normal pancreas, 6, normal colon, 7, Ger pancreatic tumour line 'Fetal brain lanes' 1, kidney membrane vesicles (standard), 2, thalamus, 3, germinal emminence, 4, medial thin cerebral wall, 5, cerebrum (lateral outer wall), 6, cerebrum (lateral inner wall), 7, lateral choroid plexus, 8, cerebellum

creas and colon, showing equal expression in these tissues

Discussion

Carbonic anhydrase IV was detected, by Western blotting, in the particulate fraction of all tissues examined, except red blood cells In each case a band with a monomer molecular mass of about 55 kDa was detected Other workers have found the bovine enzyme to have a similar molecular mass However, estimates of molecular mass based on amino acid composition suggest that a 20 kDa component represents carbohydrate content [4] The consistency of molecular weight values of human CAIV in diverse tissues would suggest that the carbohydrate (or other ligand) content is constant but this will need to be confirmed by sequence analysis If this extra 20 kDa component (added to the native 35 kDa enzyme) is involved in anchoring the CAIV isozyme to the cell membrane, the fact that it is similar in all tissues tested indicates a common structure for the enzyme-membrane complex

It has been previously suggested that CA may contribute to lung liquid formation in fetal life [6] and the precipitate decrease in expression of CAIV at 28 weeks (Fig 1) suggests a possible role for the enzyme in lung during the first two trimesters, but this does not necessarily point to a role in lung liquid formation

Fetal pneumatocytes expressed consistent levels of CAIV through 9 days in culture with only minor fluctuations The enzyme appeared to be more strongly expressed if fetal calf serum was present in the culture medium, but this will need to be confirmed by more quantitative assay Skin fibroblasts expressed constant levels of CAIV during culture

Only minor differences of CAIV expression in other fetal tissues was observed, for example fetal liver showed a small but consistent increase in level through development Kidney showed more or less consistent expression through fetal life Different areas of the fetal brain varied in CAIV expression (Fig 1) CAIV bands in immunoblots of fetal liver were consistently 'fuzzy' (Fig 1) and we speculate that this is because of residual binding of the enzyme to membrane fragments during electrophoresis Blotting of purified kidney membrane vesicles gave clearer bands (Fig 1) and restaining of blots with antiserum to cytosolic CAII produced very sharp zones of immunoreaction at 28 kDa This result suggests that CAIV membrane associating properties may indeed cause the lack of immunoblot definition

The true function of carbonic anhydrases I–III has been the subject of much conjecture (for review, see Ref 2) and the most likely (major) role of CAIV would seem to be as a membrane-bound enzyme controlling H^+ and HCO_3^- transport This has been assumed to be the function of CAIV in lung and kidney epithelial membranes [3] CAIV has been found in both membrane and endoplasmic reticulum fractions of the kidney in man and in the rat, both brush border and basolateral membranes of tubular cells contain a CAIV like enzyme However, the unexpectedly ubiquitous distribution of this enzyme found in the present study as well as previous data showing particulate carbonic anhydrase activity in brain, muscle and liver (which seems likely to represent at least in part CAIV [7,8]) may indicate a 'housekeeping role' in the cell, possibly in the endoplasmic reticulum It has been proposed that CAIV can function as an ion channel [9] and the associated membrane bound CO_2 hydrase activity in muscle, for example, related to calcium flux in the sarcoplasmic reticulum [10] may provide a unique role for the enzyme in channeling calcium into the sarco-

plasmic reticulum during the phases of muscle contraction Thus CAIV may fulfil different roles in different tissues

The likely availability of cDNA clones for CAIV in the near future, coupled with expression in tissue culture will make a useful system for studying control and also defining the function of CAIV in relation to the other soluble carbonic anhydrases in the same cell

Acknowledgements

We gratefully acknowledge grant support as follows National Kidney Research Fund (N D C), Swedish Medical Research Council (Grant No 2874, P J W), British Lung Foundation (R S) and Chest, Heart and Stroke Association (R S)

References

1 Carter, N and Jeffery, S (1985) Biochem Soc Trans 13, 531–533
2 Tashian, R (1989) BioEssays 10, 186–192
3 Wistrand, P J and Knuttila, K -G (1989) Kidney Int 35, 851–859
4 Whitney, P L and Briggle, T V (1982) J Biol Chem 257, 12056–12059
5 Grant, A G , Duke, D and Hermon-Taylor, J (1979) Br J Cancer 39, 143–151
6 Lonnerholm, G and Wistrand, P (1982) Pediatr Res 16, 407–411
7 Sapirstein, V S , Strocchi, P and Gilbert, J M (1984) Am N Y Acad Sci 429, 481–493
8 Garcia-Mann, J J , Perez-Barriocanal, F , Garcia, A , Serrano, M A , Regueiro, P and Esteller, A (1988) Biochim Biophys Acta 945, 17–22
9 Diaz, E , Sandblom, J P and Wistrand, P J (1982) Acta Physiol Scand 116, 461–463
10 Gros, G and Dodgson, S (1988) Annu Rev Physiol 50, 669–694