have been shown to neutralise the capacity of viruses or viral antigens to induce interferon α .¹¹ Although in newborn infants with herpes encephalitis the peripheral blood mononuclear cells have been shown to produce lower amounts of interferon than those from adults in vitro,¹² our infants had concentrations of interferon γ in cerebrospinal fluid equivalent to those in the adult and child patients. The presence of interferon γ in cerebrospinal fluid has already been reported in tuberculous meningitis,¹³ but no interferon α was detected.¹⁴ Interferon γ was shown to be produced in vitro by T lymphocytes in the presence of herpes virus antigens and accessory cells¹⁵; its synthesis therefore needs interleukins 1 and 2, which are probably also secreted in the central nervous system during the course of herpes encephalitis. In addition to its property of inducing histocompatibility antigens in different cells,¹⁶ interferon γ enhanced the production of antibodies by B cells.¹⁷ It may consequently participate in the sequence of events leading to the synthesis of virus antibodies to herpes in the central nervous system. In this study we could not make a precise correlation between the evolution of the disease and the concentrations of interferon γ in the cerebrospinal fluid as antiviral treatment was introduced at different stages of the disease. High concentrations of interferon γ and interferon α , as seen in cases 10 and 11, however, were not an aggravating factor of the disease as these patients recovered with only minor sequelae after antiviral treatment.

Despite the presence of viral antigens in the brain tissue patients with subacute sclerosing panencephalitis had no interferon γ detectable in the cerebrospinal fluid or serum; the important local production of specific antibodies in this disease probably masks the viral antigens and switches off the induction of both interferon γ and interferon α .⁸

We thank Marie-Claire Rufino and Linda Sergent for their help in preparing the manuscript. This study was supported by a grant from the Association pour la Recherche sur la Sclérose en Plaques.

References

- Johnson RT, Griffin DE, Hirsch RL. Measles encephalomyelitis: clinical and immunological studies. *N Engl J Med* 1984;310:137-41.
- Lebon P, Ponsot G, Aicardi J, Goutières F, Arthus M. Early intrathecal synthesis of interferon in herpes encephalitis. *Biomedicine* 1979;31:267-71.
- Dussaix E, Lebon P, Ponsot G, Huault G, Tardieu M. Intrathecal synthesis of different alpha-interferons in patients with various neurological diseases. *Acta Neurol Scand* 1985;71:504-9.
- Fierwz W, Endler B, Reske K, Wekerle H, Fontana A. Astrocytes as antigen presenting cells. I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effect on antigen presentation. *J Immunol* 1985;134:3785-91.
- Tucker C, Korman AJ, Wake CT, et al. Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. *Proc Natl Acad Sci USA* 1984;81:4917-21.
- Vesikari T, Vahery A. Rubella: a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. *Br Med J* 1968;i:221-2.
- Denoyel GA, Gaspart A, Peyramond D. Diagnosis of recent rubella virus infection by demonstration of specific immunoglobulin M antibodies: comparisons of solid-phase reverse immunosorbent test with sucrose density gradient centrifugation. *J Clin Microbiol* 1981;13:698-704.
- Gresser I, Bandu MT, Brouty-Boye D, Tovey M. Pronounced antiviral activity of human interferon on bovine and porcine cells. *Nature* 1974;251:543-5.
- Chang TW, McKinney S, Liu V, Kung PC, Vilcek J, Le J. Use of monoclonal antibodies as sensitive and specific probes for biologically active human gamma interferon. *Proc Natl Acad Sci USA* 1984;81:5219-22.
- Sedmak JJ, Siebenlist R, Grossberg SE. HuIFN antiviral activity correlates with immunological reactivity in a double-monoclonal antibody radiometric assay. *J Interferon Res* 1985;5:397-402.
- Lebon P. Inhibition of herpes simplex virus type 1-induced interferon synthesis by monoclonal antibodies against virus glycoprotein D and by lysomotropic drugs. *J Gen Virol* 1985;66:2781-6.
- Wilson CB, Westall J, Johnston L, Lewis DB, Dower SK, Alpert AR. Decreased production of interferon-gamma by human neonatal cells. *J Clin Invest* 1986;77:860-7.
- Hirsh RL, Panitch HS, Johnson KP. Lymphocytes from multiple sclerosis patients produce elevated levels of gamma interferon in vitro. *J Clin Immunol* 1985;5:386-9.
- Vezinet F, Lebon P, Amoury C, Gilbert C. Synthèse d'interféron au cours des encéphalites herpétiques de l'adulte. *Nouv Presse Med* 1981;10:1135-8.
- Rasmussen LE, Jordan GW, Stevens DA, Merigan TC. Lymphocyte interferon production and transformation after herpes simplex infections in humans. *J Immunol* 1974;112:728-34.
- Rosa FM, Cochet MM, Fellous M. Interferon and major histocompatibility complex genes. *Interferon* 1986;7:47-87.
- Sidman CL, Marshall JD, Shultz LD, Gray PW, Johnson HM. Gamma interferon is one of several direct B-cell maturing lymphokines. *Nature* 1984;309:801-4.

(Accepted 30 September 1987)

Acylcoenzyme A dehydrogenase deficiency in heart tissue from infants who died unexpectedly with fatty change in the liver

FIONA ALLISON, M J BENNETT, S VARIEND, P C ENGEL

Abstract

Heart muscle from infants who died unexpectedly and who showed fatty changes in the liver at necropsy was analysed for long chain and medium chain acylcoenzyme A dehydrogenase activities by using the natural electron acceptor. In two of the seven cases investigated a deficiency in acylcoenzyme A dehydrogenase activity was found. In one case the deficiency was in medium chain acylcoenzyme A dehydrogenase activity and in the other long chain acylcoenzyme A dehydrogenase activity.

These findings emphasise the importance of investigating fatty acid oxidation in infants who have died unexpectedly.

Introduction

It has recently been shown that some cases of unexpected death in infancy and which previously would have been classified as the sudden infant death syndrome are attributable to various inherited defects of fatty acid beta oxidation.^{1,2} Three fatty acylcoenzyme A dehydrogenases are concerned in the mitochondrial degradation of straight chain fatty acids: these are long chain, medium chain, and short chain acylcoenzyme A dehydrogenases.³ When fatty acylcoenzyme A's are oxidised by the acylcoenzyme A dehydrogenases the electrons generated reduce electron transfer flavoprotein, which in turn is reoxidised by electron transfer flavoprotein dehydrogenase, and are then passed along the mitochondrial respiratory chain to oxygen, the terminal electron acceptor. Defects of either long chain or medium chain acylcoenzyme A dehydrogenase often result in periodic episodes of hypoglycaemia accompanied by a hypoketotic dicarboxylic aciduria.⁴

Defects of electron transfer flavoprotein and electron transfer

Children's Hospital, Sheffield S10 2TH

FIONA ALLISON, BSC, basic grade biochemist
M J BENNETT, PHD, MRCPATH, principal biochemist
S VARIEND, MD, MRCPATH, consultant pathologist

Department of Biochemistry, University of Sheffield, Sheffield S10 2TN
P C ENGEL, MA, DPHIL, reader in biochemistry

Correspondence to: Mrs Fiona Allison, Department of Chemical Pathology, Children's Hospital, Western Bank, Sheffield S10 2TH.

flavoprotein dehydrogenase have also been recognised. These defects, which affect all the dehydrogenases concerned in beta oxidation, are known as multiple acylcoenzyme A dehydrogenase deficiency.⁵ Fasting hypoglycaemia is a feature of multiple acylcoenzyme A dehydrogenase deficiency. Acylcoenzyme A dehydrogenase deficiencies are associated with pronounced fatty changes in liver and other tissues.

In a retrospective study of 200 sudden infant deaths in Sheffield 14 of the infants were found at necropsy to have pronounced panlobular microvesicular fatty infiltration of the liver. Two were subsequently shown to have deficient medium chain acylcoenzyme A dehydrogenase in the liver by an assay using an artificial electron acceptor.¹ Insufficient liver was available for assay in the other 12 cases. In seven of these 12 cases frozen heart tissue was still available for study and we therefore decided to examine this for medium chain and long chain acylcoenzyme A dehydrogenase activities using the natural electron acceptor electron transfer flavoprotein (cases 1-7). Control heart tissue from infants without fatty livers was also examined (cases 8-10). The cause of death in these cases was also unexplained. There was no histological evidence of fatty infiltration of the liver.

Materials and methods

Heart tissue was stored for up to five years at -70°C until required for assay. Medium chain and long chain acylcoenzyme A dehydrogenases were assayed by a fluorescence electron transfer flavoprotein linked assay as described by Coates *et al.*⁶ These workers used this assay for liver and skin fibroblasts; we have now employed it for heart tissue. Both the sensitivity and specificity of this assay are greater than those of the assay using artificial electron acceptor. All medium chain acylcoenzyme A dehydrogenase assays were performed in duplicate. Long chain acylcoenzyme A dehydrogenase assays were done in duplicate in cases 1 and 2 and singly in cases 3-10. Acylcoenzyme A dehydrogenase activities were expressed in arbitrary fluorescence units/mg protein. Protein concentrations were measured by the method of Lowry *et al.*⁷

Results

The table gives the results. Mean activity of medium chain acylcoenzyme A dehydrogenase with the use of octanoyl coenzyme A as substrate was 1702 units. The mean value was calculated from the results in cases 3-10. Mean

Acylcoenzyme A dehydrogenase activities in heart tissue from infants who died suddenly and unexpectedly. Results expressed as arbitrary fluorescence units/mg protein

Case No	Medium chain acylcoenzyme A dehydrogenase activity	Long chain acylcoenzyme A dehydrogenase activity
1	1460	0
2	0	190
3	2087	114
4	1216	101
5	611	22.7
6	1632	82.0
7	2394	148
8	2928	149.2
9	877	20.2
10	1875	Not done

activity of long chain acylcoenzyme A dehydrogenase with use of palmitoyl coenzyme A as substrate was 91 units, the mean value also being calculated from the results in cases 3-10. In case 1 there was no long chain activity but medium chain acylcoenzyme A dehydrogenase activity was normal. Case 2 showed no medium chain acylcoenzyme A dehydrogenase activity but

normal long chain acylcoenzyme A dehydrogenase activity. As all the samples had been stored for three to five years before analysis medium chain acylcoenzyme A dehydrogenase activity was measured in a case from which tissue had been taken and frozen only a few months before. The activity was within the range of normal found for the rest of the series. Urine was not available for organic acid analysis in any of the cases.

CASE HISTORIES

In case 1 the infant was 2 months of age at death. In the week before death he had had several episodes of diarrhoea and vomiting which were treated by fluid and electrolyte replacement (Dioralyte) but was not ill enough to require admission. He was found dead one morning at home. Necropsy disclosed pronounced panlobular fatty change in the liver but there were no other relevant findings.

In case 2 the child was 16 months of age at death. She was admitted to hospital having been found semiconscious at home after three days of diarrhoea and vomiting. Investigations showed hypoglycaemia (0.9 mmol/l) and a neutrophilic leucocytosis. Atypical Reye's syndrome was considered as a possible diagnosis. She died six days later after a cardiorespiratory arrest and intensive attempts at resuscitation. Severe cerebral oedema and pronounced fatty change in the liver were found at necropsy.

Three other infants (cases 3, 4, and 7) had suffered from diarrhoea and vomiting in the preceding two or three days before death. In case 5 the infant was found grey and unresponsive at home. The remaining infant (case 6) had no symptoms before death. None of the controls (cases 8-10) had diarrhoea immediately before death.

Discussion

In the previous retrospective study of infants who died suddenly and unexpectedly and who were found to have gross panlobular fatty change in the liver samples of liver sufficient for enzyme analysis were available in only two cases. Both showed medium chain acylcoenzyme A dehydrogenase deficiency. Analysis of frozen cardiac tissue from a further seven cases within this group has now shown two further cases with beta oxidation defects. In one there was long chain acylcoenzyme A dehydrogenase deficiency and in the other medium chain acylcoenzyme A dehydrogenase deficiency. Defects in either electron transfer flavoprotein or electron transfer flavoprotein dehydrogenase would not be detected by the assay used.

Finding additional cases of medium chain and long chain acylcoenzyme A dehydrogenase deficiency in subjects from our earlier study emphasises the importance of investigating unexpected infant deaths for defects of fatty acid oxidation. The case histories illustrate the diversity of clinical presentation of beta oxidation defects.

We thank the Foundation for the Study of Infant Deaths for financial support (project No 52) and Professor P M Coates and Professor D E Hale for the gift of electron transfer flavoprotein.

References

- Howat AJ, Bennett MJ, Variend S, Shaw L, Engel PC. Defects in the metabolism of fatty acids in the sudden infant death syndrome. *Br Med J* 1985;209:1771-3.
- Anonymous. Sudden infant death and inherited disorders of fat oxidation [Editorial]. *Lancet* 1986;ii:1073-5.
- Beinert H. Acyl coenzyme A dehydrogenase. In: Boyer PD, Lardy H, Myrback K, eds. *The enzymes*. 2nd ed. New York: Academic Press, 1963:447-66.
- Pollitt RJ. Inherited disorders of straight chain fatty acid oxidation. *Arch Dis Child* 1987;62:6-7.
- Mooy PD, Przyrembel H, Giesberts MAH, Scholte HR, Blom W, van Gelderen HH. Glutaric aciduria type II: treatment with riboflavin, carnitine and insulin. *Eur J Pediatr* 1984;143:92-5.
- Coates PM, Hale DE, Stanley CA, Corkey BE, Cortner JA. Genetic deficiency of medium chain acyl coenzyme A dehydrogenase: studies in cultured skin fibroblasts and peripheral mononuclear leucocytes. *Pediatr Res* 1985;19:671-6.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-72.

(Accepted 9 October 1987)