The Lipid Composition of Erythrocyte Ghosts from a Patient with Congenital Paramyotonia

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Abstract. The membrane lipid and fatty acid compositions of red blood cells from a paramyotonia patient were investigated. Cholesterol and total phospholipid contents in paramyotonia were not different from control. Only the sphingomyelin content was lower, and thus the molar ratio of phosphatidylcholine/sphingomyelin was higher than normal. The major abnormality concerned the fatty acid pattern. In all the phospholipid classes saturated fatty acids were increased and unsaturated fatty acids were decreased. The overall ratio of saturated/unsaturated fatty acids was 2.1 vs 1.6 in controls. Similar findings have been reported for the sarcolemma from paramyotonia patients. Thus, the results indicate that the membrane defect in this disease may be generalized.

Key words: Paramyotonia congenita — Membrane composition — Red blood cells — Cholesterol

Introduction

Congenital paramyotonia is a dominantly inherited muscle disease in which the main symptoms — slowed muscle relaxation after voluntary contractions and a long lasting muscle weakness — are brought on by low temperature (Becker 1970). The direct cause of the muscle weakness has been shown to be lasting depolarization of the muscle fiber membranes to about −40 mV which makes the fibers inexcitable (Lehmann-Horn et al. 1981).

Using gas chromatography Kuhn and his co-workers investigated lipids (Fiehn et al. 1974) and fatty acids (Kuhn 1973) of the sarcolemma of two related paramyotonia patients. In the phospholipid composition, there was a decreased (4.6% vs 12.1%) relative content of sphingomyelin (SM), while the content of

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phosphatidyl ethanolamine (PE) was increased. In the fatty acid pattern, the most impressive alterations were an increase of palmitic acid (C 16:0) and a decrease of oleic acid (C 18:1).

It has been demonstrated for several hereditary muscle diseases that membrane abnormalities are not only present in the muscle fibers, but also in the red blood cells (Grey et al. 1980; Richard et al. 1978; Vasiljević and Polić 1983). This might also be the case for paramyotonia congenita. We have, therefore, analyzed the composition of erythrocyte membranes of a paramyotonia patient and found indeed alterations similar to those described for the sarcolemma. This finding raises hopes that the membrane defect can be studied in this much better accessible membrane source.

Materials and Methods

Erythrocytes were obtained from a clinically and electrophysiologically well investigated patient (case "PWM D", Haass et al. 1981), who gave informed consent to the study. Control blood was obtained from an age- and sex-matched healthy volunteer and it was treated under identical conditions. On two occasions, with an interval of one month, 50 milliliters of venous blood were drawn from both the patient and the volunteer. The samples were heparinized and stored at 4 °C for transportation. After 3 h, the blood was centrifuged and washed in a buffered solution (pH 7.5). Ghosts were then prepared according to the method of Steck and Kant (1974). All lipid and fatty acid measurements were related to the protein content of the ghosts which was determined using the method of Lowry et al. (1951), with bovine serum albumin as standard.

Total lipid extraction was accomplished according to Folch et al. (1952). The lipids were then separated into their main classes by thin-layer chromatography following the method of Skipski et al. (1965) and in a few cases by two-dimensional chromatography on silica gel G plates (E. Merck, Darmstadt, West Germany). The latter was performed in $CHCl_3$:CH_3OH:CH_3COOH:H_2O (30:15:4:2) and in $CHCl_3$:CH_3OH:H_2O (60:25:4) for the first and second dimension, respectively. The lipid-containing spots were visualized by spraying the plates with primuline solution (Wright 1971), and scraped into tubes containing pentadecanoic acid as internal standard.

The fatty acid content was measured by gas liquid chromatography (GLC) according to Morrison and Smith (1964). The total cholesterol content was determined by GLC using cholestan as an internal standard (Rose and Oklander 1965), and also by an enzymatic/colorimetric method using the Biochemica test kit (Boehringer, Mannheim, West Germany).

Each ghost sample was divided into 5 specimens which were run simultaneously and the results were averaged. Test and control specimens were treated identically. Since the early and the later samples yielded similar results the results from both analyses were pooled. To assess the significance of differences between mean values, Student's *t*-test was used. Total and free cholesterol in the serum and in the different lipoprotein classes were determined enzymatically using the Biochemica test kit (Boehringer, Mannheim, West Germany). The lipoprotein classes were separated by discontinuous density gradient centrifugation (Havel et al. 1955). The lecithin: cholesterol acyl transferase (LCAT) activity in the total plasma was determined by the method of Dieplinger and Kostner (1980).

Results and Discussion

All control values were in close agreement with published data (Cooper 1970; van

Table 1. Membrane lipids in erythrocyte ghosts from a paramyotonia patient and from a healthy volunteer compared to data from the literature (van Deenen 1979; van Deenen and de Gier 1974; Grey et al. 1980; Skipski 1964).

	Paramyotonia	Control	Literature
Cholesterol/protein (μmol/mg)	0.57*	0.58*	0.34—0.54**
Phospholipid/protein (μmol/mg)	0.51	0.55	0.46—0.67
Cholesterol/phospholipid (molar ratio)	1.1	1.0	0.8—1.1
Phospholipid classes (% of total PL)			
Phosphatidylethanolamine (PE)	26	27	26—33
Phosphatidylserine (PS)	18	16	11—17
Phosphatidylcholine (PC)	36	30	26—31
Sphingomyelin (SM)	20	27	22—28
PC/SM	1.75	1.13	

* enzymatic determination
** gas chromatography

Deenen and de Gier 1974; Dodge and Philipps 1967; Kobayashi et al. 1978; Thomas and Harper 1978). The cholesterol and the phospholipid contents of the membranes of paramyotonia ghosts were normal, and the cholesterol/phospholipid molar ratio was close to unity in both test and control preparations. The distribution of phospholipids in the 4 major classes showed a lower than normal sphingomyelin content. Consequently the molar ratio of phosphatidylcholine-sphingomyelin (PC/SM) was higher than normal. For detailed data see Table 1. The decrease in the SM content and the increase in the PC content were only slight in comparison to variable contents encountered in the red blood cell membranes of different mammalian species (van Deenen and de Gier 1974). Therefore, it seems questionable, whether such small abnormalities can account for the striking symptoms of paramyotonia. Nevertheless, this finding is in concordance with an earlier report on sarcolemma abnormalities in paramyotonia, except for the fact that in the sarcolemma, PE was increased rather than PC (Fiehn et al. 1974). Little is known about possible reasons for the decreased sphingomyelin content. The higher PC/SM ratio in paramyotonia erythrocytes could be due either to an increase in the PC synthesis or to degradation of SM by lipolytic enzymes. The latter possibility seems less likely because in human erythrocytes the lipolytic activity is rather low (van Deenen and de Gier 1974; Paysant et al. 1970) in contrast to the high activity of phospholipase A_2 in sheep erythrocytes (Zwaal et al. 1974). Another possible mechanism is an abnormal phospholipid exchange with the lipoproteins of the plasma (Reed 1968; Renovij and van Golde 1976).

A major abnormality consistently observed concerned the fatty acid pattern. A significant increase of saturated fatty acids was found in the total phospholipids

Table 2. Distribution of fatty acids (means ± standard deviation) in the total phospholipids of erythrocyte ghosts from a paramyotonia patient and a control.

	Paramyotonia ($n=10$)	Control ($n=8$)	P
C16:0	31.40 ± 0.5	23.98 ± 0.7	<0.01
C18:0	36.54 ± 1.5	30.64 ± 0.9	<0.01
C18:1	14.04 ± 0.7	16.98 ± 0.8	<0.03
C18:2	8.72 ± 0.4	11.15 ± 0.9	<0.01
C20:4	8.12 ± 0.9	11.27 ± 0.4	<0.01
others	1.14 ± 0.1	5.62 ± 1.6	<0.03

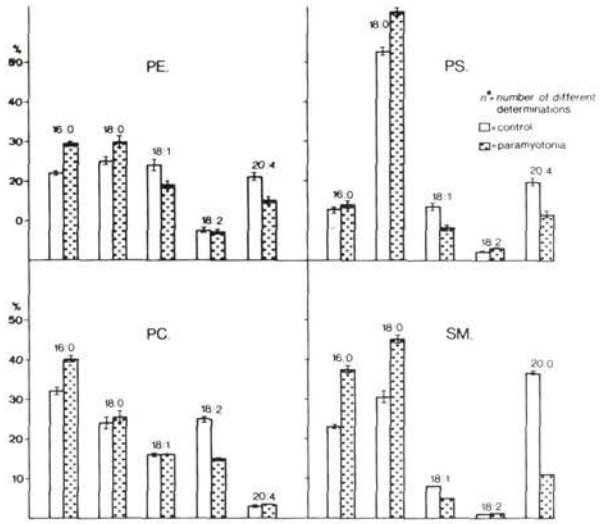

Fig. 1. Distribution of fatty acids in the four main phospholipid classes

(Table 2), and this was true for the sum of saturated fatty acids in each phospholipid class (Fig. 1) as well. At the same time the content of unsaturated fatty acids was decreased in all phospholipid classes. The overall ratio of saturated-/unsaturated fatty acids was 2.1 as compared to 1.6 in the control. Although the patient had been on a normal diet and had normal values (Assmann 1982) of serum cholesterol, LCAT activity and serum lipoproteins (see Table 3), this ratio was greater than what can be induced in man by extreme uptake of saturated fatty acids (van Deenen and de Gier 1974). This ratio was also much greater than that occurring naturally in any of the eight mammalian species investigated so far

Table 3. Total cholesterol (TC) and free cholesterol (FC) in the serum and in the lipoprotein classes, and lecithin : cholesterol acyl transferase (LCAT) activity in the plasma of a paramyotonia patient and of two groups of healthy volunteers. All values means ± S.D.; n.d. = not determined.

		Paramyotonia ($n=4$)[a]	Control ($n=41$)[b]	Control ($n=6$)[c]
Serum cholesterol	TC	4.0 ± 0.1	5.3 ± 1.0	4.3 ± 1.2
(μmol/l)	FC	1.1 ± 0.05	1.6 ± 0.34	1.25 ± 0.31
HDL-cholesterol:	TC	1.9 ± 0.05	n.d.	1.99 ± 0.28
(μmol/l)	FC	0.44 ± 0.03	n.d.	0.57 ± 0.1
LDL-cholesterol:	TC	1.91 ± 0.10	n.d.	1.90 ± 0.35
(μmol/l)	FC	0.57 ± 0.03	n.d.	0.59 ± 0.10
VLDL-cholesterol:	TC	0.23 ± 0.08	n.d.	0.34 ± 0.18
(μmol/l)	FC	0.08 ± 0.02	n.d.	0.13 ± 0.07
LCAT-activity (%/h)[d]		5.6 ± 0.6	5 ± 3	n.d.

[a] number of evaluations,
[b] number of persons (age range 30—80 years),
[c] number of persons (age range 18—30 years),
[d] initial rate of esterification given as % of the initial free cholesterol esterified per hour.

(Wessels and Veerkamp 1973). This high ratio may be associated with the symptoms of paramyotonia. The increased level of the saturated fatty acids could be the result of an altered substrate specificity of the monoacyltransferase (van Deenen 1979; Robertson and Lands 1964) or of the acyl CoA synthetases as was found to be the enzymatic basis for the fatty acid composition of human platelets (McKean et al. 1982; Wilson et al. 1982).

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References

Assmann G. (1982): Lipidstoffwechsel und Arteriosklerose. Schattauer-Verlag, Stuttgart

Becker P. E. (1970): Paramyotonia congenita (Eulenburg). In: Fortschritte der allgemeinen und klinischen Humangenetik (Eds. P. E. Becker, W. Lenz, F. Vogel, G. G. Wendt), Vol. 3, Thieme, Stuttgart

Cooper R. A. (1970): Lipids of human red cell membrane. Normal composition and variability in disease. Seminars Hematol. **7**, 296—322

Deenen van L. L. M. (1979): Structural organization and dynamics of phospholipids in red cell membranes. In: Normal and Abnormal Red Cell Membranes. Progress in Clinical and Biological Research (Eds. S. E. Lux, V. T. Marchesi, C. F. Fox), Vol. 30, pp. 451—456, A. R. Liss, New York

Deenen van L. L. M., de Gier J. (1974): Lipids of the red cell membrane. In: The Red Blood Cell (Ed. D. M. Surgenor), Vol. 1, pp. 147—211, Academic Press, New York

Dieplinger H., Kostner G. H. (1980): The determination of lecithin: cholesterol acyl transferase in the clinical laboratory: a modified enzymatic procedure. Clin. Chim. Acta **106,** 319—324

Dodge J. P., Philipps G. D. (1967): Composition of phospholipids and of phospholipid fatty acids and aldehydes in human red cells. J. Lipid Res. **8,** 667—675

Fiehn W., Peter J. B., Seiler D., Kuhn E. (1974): Abnormalities of the sarcolemma in myopathies. In: Structure and Function of Normal and Diseased Muscles and Peripheral Nerve (Eds. J. Hausmanowa-Petrusewicz, H. Jedrzejowska), pp. 205—209, Polish Medical Publ., Warsaw

Folch P. I., Lees M., Sloane-Stanley S. H. (1952): A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **266,** 479—484

Grey J. A., Gitelman A. J., Roses A. D. (1980): Myotonic muscular dystrophy: defective phospholipid metabolism in the erythrocyte plasma membrane. J. Clin. Invest. **65,** 1478—1482

Haass A., Ricker K., Rüdel R., Lehmann-Horn F., Böhlen R., Dengler R., Mertens H. G. (1981): Clinical study of paramyotonia congenita with and without myotonia in a warm environment. Muscle Nerve **4,** 388—395

Havel R. J., Eder H. A., Bragdon J. H. (1955): The distribution and chemical compositsion of ultracentrifugally separated lipoproteins in human plasma. J. Clin. Invest. **34,** 1345—1353

Kobayashi T., Mawatari S., Kurowa Y. (1978): Lipids and proteins of erythrocyte membrane in Duchenne muscular dystrophy. Clin. Chim. Acta **85,** 259—266

Kuhn E. (1973): Myotonia. In: New Developments in Electromyography and Clinical Neurophysiology (Ed. J. E. Desmedt), Vol 1, pp. 413—419, Karger, Basel

Lehmann-Horn F., Rüdel R., Dengler R., Lorković H., Haass, A., Ricker K. (1981): Membrane defects in paramyotonia congenita with and without myotonia in a warm environment. Muscle Nerve **4,** 396—406

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem. **193,** 265—275

McKean M. L., Smith J. B., Silver M. J. (1982): Phospholipid biosynthesis in human platelets. J. Biol. Chem. **257,** 11278—11283

Morrison W. R., Smith L. M. (1964): Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. J. Lipid Res. **5,** 600—608

Paysant M., Bitran M., Wald R., Polonovski J. (1970): Phospholipase A des globules rouges chez l'homme. Action sur les phospholipides endogenes et exogenes. Bull. Soc. Chim. Biol. **52,** 1257—1269

Pickard N. A., Gruemer A. D., Verrill H. L., Isaacs E. R., Robinow M., Nance W. E., Meyers E. C., Goldsmith B. (1978): Systemic membrane defect in the proximal muscular dystrophies. New Engl. J. Med. **266,** 841—846

Reed C. F. (1968): Phospholipid exchange between plasma and erythrocytes in man and the dog. J. Clin. Invest. **47,** 749—760

Renovij W., Golde van L. M. G. (1976): The exchange of phospholipids between rat erythrocytes and plasma and the translocation of phosphatidylcholine across the red cell membrane are temperature dependent. FEBS Lett. **71,** 321—324

Robertson A. F., Lands W. E. M. (1964): Metabolism of phospholipids in normal and spherocytic human erythrocytes. J. Lipid Res. 5, 88—93

Rose H. G., Oklander M. (1965): Improved procedure for the extraction of lipids from human erythrocytes. J. Lipid Res. **6,** 428—431

Skipski V. P., Peterson R. F., Barday M. (1964): Quantitative analysis of phospholipids by thin-layer chromatography. Biochem. J. **90,** 374—378

Steck T. L., Kant J. A. (1974): Preparation of impermeable ghosts and inside-out vesicles from human erythrocyte membranes. In: Methods in Enzymology (Eds. S. Fleischer, L. Packer), Vol 21., pp. 172—193, Academic Press, New York

Thomas N. S. T., Harper P. S. (1978): Myotonic dystrophy: studies on the lipid composition and metabolism of erythrocytes and skin fibroblasts. Clin. Chim. Acta **83,** 13—23

Vasiljević Z. M., Polić D. D. (1983): Morphological changes of erythrocytes in patients and carriers of Duchenne disease. Acta Neurol. Scand. **67,** 242—244

Wessels J. M. C., Veerkamp J. H. (1973): Some aspects of the osmotic lysis of erythrocytes. Comparison of glycerol permeability and lipid composition of red blood cell membranes from eight mammalian species. Biochim. Biophys. Acta **291,** 190—196

Wilson D. B., Prescott S. M., Majerus P. W. (1982): Discovery of an arachidonyl coenzyme A synthetase in human platelets. J. Biol. Chem. **257,** 3511—3515

Wright R. S. (1971): A reagent for the non-destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms. J. Chromatogr. **59,** 220—221

Zwaal R., Flückiger S., Moser P., Zahler P. (1974): Lecithinase activities at the external surface of ruminant erythrocyte membranes. Biochim. Biophys. Acta **373,** 416—424

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