OSPHOLIPIDS AND CHOLESTEROL IN TWO SKELETAL MUSCLES AND ERYTHROCYTES OF THREE FFERENT MALIGNANT HYPERTHERMIA GENOTYPES OF SWINE

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MMARY

The malignant hyperthermia syndrome (MH) in swine is ultimatively triggered by an abnormal intracellular calcium ease as a result of functional disorders in the membranal system of the skeletal muscle.

Five homozygous MH positive, seven heterozygous MH negative and seven homozygous MH negative male castrated man Landrace pigs were examined. Catheters were placed in the vena cava cranialis in order to take multiple and stress free od samples for the preparation of erythrocytes. Post mortem samples of musculus longissimus thoracis and musculus praspinatus were analysed for total lipid content, total cholesterol content and phospholipid pattern.

The stress susceptible MH positive animals showed significantly lower total lipid contents in the musc. long. thorac. and he erythrocytes compared to the more stress resistant homozygous MH negative animals. Significantly higher cholesterol tents in both muscles and lower cholesterol contents in erythrocytes were found in the MH positive and the heterozygous MH tative animals compared to the other genotype. The erythrocytes of these two genotypes contained the highest amounts of rdiolipin, lyso-phosphatidyl-ethanolamine, lyso-phosphatidyl-choline and sphingomyeline and the lowest amounts of sphatidyl-choline compared to the more stress resistant homozygous negative animals. No significant differences in the spholipid patterns in the two muscles of all of the MH genotypes could be detected.

Our results indicate that the MH genotype significantly alters the cholesterol contents in skeletal muscles and erythrocytes, well as the phospholipid pattern of erythrocytes.

TRODUCTION

Phospholipids and cholesterol are the most important structural components of biological membranes. In addition, spholipids are also involved in transmembranal cell signaling (BERRIDGE et al., 1989; RANA et al., 1990). Episodes of ignant hyperthermia are introduced by an abnormal high calcium release from the sarcoplasmatic reticulum. A mutation of porcine ryanodine receptor is supposed to be responsible for this inadequate calcium regulation (FUJII et al., 1991). ferences in the lipid composition of cell membranes from different MH genotypes have been reported (SEEWALD et al., 1991; ETCHER et al., 1988). These combinations allow the postulation of interactions between the lipid composition of cellular mbranes and the function of the calcium release channel. Thus, it was the aim of this study to investigate the cholesterol ents and the phospholipid patterns of two skeletal muscles and of erythrocytes in three different malignant hyperthermia otypes of swine.

TERIAL AND METHODS

Nineteen male castrated German Landrace pigs from different breeding schemes were tested for their sensitivity to thane (barnyard challenge) and for marker genes. Five of these animals were homozygous MH positive (h^+/h^+), seven

animals heterozygous MH negative (H^-/h^+) and seven animals homozygous MH negative (H^-/H^-). When the animals reach body weight of approximately 85 kg a catheter was placed in the vena cava cranialis in order to take stress free blood sam Blood samples were centrifuged and after removal of the plasma the erythrocytes were washed three times with physiolo NaCl-solution. Animals were slaughtered when they reached a body weight of approximately 100 kg. Samples from musc longissimus thoracis (musc. long. thorac.) and musculus supraspinatus (musc. suprasp.) were removed immediately slaughtering and lipids were extracted by chloroform and methanol (HALLERMAYER, 1976). Total lipid content was measu gravimetrically and phospholipids were determined by HPLC and GC techniques (SEEWALD et al., 1989). The total choles content was measured after saponification by potassium hydroxide according to the method of MANN (1961). Data statistically analyzed by ANOVA, using the SAS software package for personal computers. The following two statistical mo were applied, for total lipids and phospholipids: $y = \mu$ + genotype + b x body weight + e; and for total cholesterol: $y =$ genotype + b_1 x body weight + b_2 x total lipid content + e.

RESULTS AND DISCUSSION

The more stress resistant homozygous MH negative animals showed significantly higher total lipid contents in musc. l thorac. and in the erythrocytes compared to the MH positive animals (Tab. 1,3). Differences between genotypes in the m suprasp. were not significant (Tab. 2).

Total cholesterol content varied significantly between all genotypes in all tissues. In both muscles, homozygous M negative animals showed the lowest amounts of cholesterol compared to the two other genotypes (Tab. 1,2). But in erythrocy homozygous MH negative animals showed the highest cholesterol content compared to the other genotypes (Tab. 3).

The erythrocytes of the homozygous MH negative animals contained the highest amounts of phosphatidyl-cho compared to the other genotypes. This difference was compensated by significantly lower contents of cardiolipin, ly phosphatidyl-ethanolamine, lyso-phosphatidyl-choline and sphingomyeline. Therefore, differences in the total phospholi content between the MH genotypes were only small and not significant. In skeletal muscles, no significant differences betwe MH genotypes in all phospholipid fractions could be detected (Tab.1-3).

CONCLUSIONS

Our results show strong modifications of the total cholesterol contents in all examined tissues according to the differe MH genotypes. Significant differences in the phospholipid pattern between the MH genotypes occured only in erythrocytes. He the contents of lyso-phospholipids were significantly increased in heterozygous and more stress prone MH positive animals. Th can be a result of an increased content of alkyl and alkenyl analogues. Our results indicate that MH in swine is associated wi significant but different alterations of the lipid metabolism in skeletal muscle and in erythrocytes.

TABLE 1: Total lipid, total cholesterol and phospholipid contents of swine musculus longissimus thoracis in three different MH genotypes (least square mean values ± standard error)

	MH genotype			
	h^+/h^+	H^-/h^+	H^-/H^-	
	a	b	c	
	n = 5	n = 7	n = 7	Signif.
Total lipid content (% wet muscle weight)	1.44 ± 0.20	1.95 ± 0.15	2.15 ± 0.16	a:c *
Total cholesterol (mg/100 g w.m.w.)	43.9 ± 1.7	41.4 ± 1.2	37.7 ± 1.3	a:c * b:c *
(µmol/g n.f.f. w.m.w.)	n = 5	n = 7	n = 7	
Total phospholipids	3.52 ± 0.58	3.07 ± 0.44	3.26 ± 0.45	n.s.
Cardiolipin	0.15 ± 0.03	0.12 ± 0.03	0.09 ± 0.03	n.s.
p-inositol	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	n.s.
p-serine	0.09 ± 0.03	0.10 ± 0.02	0.08 ± 0.02	n.s.
p-ethanolamine	0.31 ± 0.08	0.18 ± 0.06	0.19 ± 0.06	n.s.
lyso-p-ethanolamine	0.29 ± 0.12	0.19 ± 0.09	0.34 ± 0.09	n.s.
p-choline	2.34 ± 0.46	2.14 ± 0.36	2.21 ± 0.36	n.s.
lyso-p-choline	0.17 ± 0.06	0.17 ± 0.04	0.17 ± 0.04	n.s.
Sphingomyeline	0.11 ± 0.05	0.11 ± 0.04	0.14 ± 0.04	n.s.

h^+/h^+ = homozygous halothane pos.
H^-/h^+ = heterozygous halothane neg.
H^-/H^- = homozygous halothane neg.
w.m.w. = wet muscle weight
n.f.f. = neutral fat free
p- = phosphatidyl-
l-p- = lyso-phosphatidyl-
* = significant difference ($p < 0.05$)
n.s. = no significant difference between any possible group comparison

TABLE 2: Total lipid, total cholesterol and phospholipid contents of swine musculus supraspinatus in three different MH genotypes (least square mean values ± standard error)

	MH genotype			
	h^+/h^+	H^-/h^+	H^-/H^-	
	a	b	c	
	n = 5	n = 7	n = 7	signif.
Total lipid content (% wet muscle weight)	2.84 ± 0.25	3.36 ± 0.20	3.01 ± 0.20	n.s.
Total cholesterol (mg/100 g w.m.w.)	50.2 ± 1.6	51.4 ± 1.3	46.7 ± 1.3	b:c *
(µmol/g n.f.f. w.m.w.)	n = 4	n = 7	n = 6	
Total phospholipids	8.14 ± 2.49	8.68 ± 1.69	5.65 ± 1.89	n.s.
Cardiolipin	0.26 ± 0.06	0.23 ± 0.04	0.12 ± 0.04	n.s.
p-inositol	0.14 ± 0.14	0.35 ± 0.09	0.15 ± 0.10	n.s.
p-serine	0.27 ± 0.18	0.39 ± 0.12	0.20 ± 0.13	n.s.
p-ethanolamine	1.22 ± 0.40	1.22 ± 0.27	0.74 ± 0.31	n.s.
lyso-p-ethanolamine	0.22 ± 0.16	0.43 ± 0.11	0.32 ± 0.13	n.s.
p-choline	4.84 ± 1.48	5.30 ± 1.00	3.76 ± 1.12	n.s.
lyso-p-choline	0.16 ± 0.08	0.31 ± 0.06	0.15 ± 0.06	n.s.
Sphingomyeline	1.04 ± 0.51	0.45 ± 0.34	0.20 ± 0.38	n.s.

h^+/h^+ = homozygous halothane positive
H^-/h^+ = heterozygous halothane negative
H^-/H^- = homozygous halothane negative
w.m.w. = wet muscle weight
n.f.f. = neutral fat free
p- = phosphatidyl-
l-p- = lyso-phosphatidyl-
* = significant difference ($p < 0.05$)
n.s. = no significant difference between any possible group comparison

TABLE 3: Total lipid, total cholesterol and phospholipid content of swine erythrocytes in three different MH genotypes (least square mean values ± standard error)

	MH genotype			
	h^+/h^+	H^-/h^+	H^-/H^-	
	a	b	c	
	n=5	n=7	n=7	signif.
total lipid content (mg/g protein)	13.0 ± 0.5	14.3 ± 0.4	14.5 ± 0.4	a:b * a:c *
total cholesterol (mg/g protein)	3.9 ± 0.1	3.8 ± 0.1	4.5 ± 0.1	a:c * b:c***
(µmol/g protein)	n=5	n=7	n=7	
total phospholipids	4.14 ± 0.45	5.00 ± 0.36	5.01 ± 0.39	n.s.
cardiolipin	0.04 ± 0.02	0.06 ± 0.02	0.01 ± 0.02	b:c *
p-inositol	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	n.s.
p-serine	0.57 ± 0.10	0.71 ± 0.08	0.76 ± 0.09	n.s.
p-ethanolamine	1.45 ± 0.34	1.65 ± 0.27	1.75 ± 0.29	n.s.
lyso-p-ethanolamine	0.26 ± 0.08	0.34 ± 0.06	0.14 ± 0.07	b:c *
p-choline	1.27 ± 0.19	1.47 ± 0.15	1.99 ± 0.16	a:c, b:c *
lyso-p-choline	0.13 ± 0.03	0.11 ± 0.02	0.04 ± 0.02	a:c, b:c *
sphingomyeline	0.37 ± 0.10	0.60 ± 0.08	0.28 ± 0.08	b:c *

h^+/h^+ = homozygous halothane pos.
H^-/h^+ = heterozygous halothane neg.
H^-/H^- = homozygous halothane neg.
p- = phosphatidyl-
l-p- = lyso-phosphatidyl-
* = significant difference ($p < 0.05$)
*** = significant difference ($p < 0.001$)
n.s. = no significant difference between any possible group comparison

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