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Differences in Heart Phospholipids in Two Inbred Rat Strains Differing in Sensitivity to the Development of Heart Lesions

E. KMONÍČKOVÁ, O. NOVÁKOVÁ[1], E. TVRZICKÁ[2], M. MRÁZ, S. HYNIE

Institute of Pharmacology, First Faculty of Medicine, Charles University, [1]Institute of Physiology, Faculty of Natural Sciences, Charles University, and [2]Fourth Internal Clinic, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

The content of phospholipids and their fatty acid composition were followed in the hearts of two inbred strains of rats: IR, resistant against the development of isoprenaline-induced myocardial lesions and IS, sensitive to their development. In the hearts of rats of the resistant strain, a lower content of phosphatidylcholine and its plasmalogen fraction was found compared to IS rats. The total amount of phospholipids was only insignificantly lower in IR rats. Greater differences were found in individual fatty acids. The most important finding concerned lower arachidonic acid and higher linoleic acid content in heart phospholipids of IR rats. These differences were exactly opposite to changes reported in the literature in animals known to have a higher resistance against myocardial damage due to various interventions. Our results do not support the hypothesis claiming the importance of changes in phospholipids and their FA composition for the resistance of the heart against the development of necrotic lesions.

Key words

Heart – Rat – Phospholipids – Fatty acids – Catecholamine-induced heart lesions

Introduction

In experimental studies addressing cardiovascular diseases, special strains of animals are often used which spontaneously develop pathological states corresponding to certain diseases or which are predisposed to be afflicted by them. Spontaneously hypertensive rats (SHR) (Okamoto and Aoki 1963) are used for studies following the pathogenesis or therapeutic possibilities in hypertension, hypertriglyceridaemic (HTG) rats (Vrána *et al.* 1993) are suitable for the study of analogical metabolic disorders in man, etc.

For studies investigating cardiac necrosis, two inbred rat strains were developed which differ in their sensitivity to isoprenaline-induced myocardial lesions (IML) (Mráz *et al.* 1986, 1995). The isoprenaline-resistant (IR) strain is relatively resistant against the development of IML, isoprenaline-sensitive (IS) strain, on the contrary, is more sensitive to their development.

In previous studies, we also found several other differences between these two strains. When compared to IS rats, IR rats have a higher glycogen content in the heart, higher adipose tissue weight and lower muscle weight (Mráz *et al.* 1986). They also differ in their behaviour (IR rats are more aggressive), immunological reactivity (more extensive adjuvant arthritis in IR rats) and their reaction to stress (more gastric ulcers are produced in IR rats) (Mráz *et al.* 1995, Starec *et al.* 1994).

Fax +420 2 293 894
E-mail physres@sun1.biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

In an attempt to explain the differences in biological characteristics between these two strains we decided to study their membrane phospholipids.

In animal cells, a variety of physiological functions are related to plasma membranes. It has been well documented (Van der Vusse *et al.* 1991) that membrane phospholipids play an essential role in maintaining cell integrity and function. Their composition can be altered by diet, pharmacological interventions and other factors (Benediktsdottir and Gudbjarnason 1988, Gudbjarnason and Hallgrimsson 1975). Characteristic changes in phospholipid composition were found after the administration of various catecholamines (CA), e.g. norepinephrine or isoprenaline, during the development of myocardial damage and in animals with altered sensitivity to myocardial damage development (Gudbjarnason and Hallgrimsson 1975, Emilsson and Gudbjarnason 1983, Faltová *et al.* 1983).

We therefore tried to ascertain whether genetically determined differences in the resistance against IML were also accompanied by differences in membrane phospholipid composition.

Material and Methods

Male IR and IS rats of our breed were used in this study for phospholipid analysis. The animals were 60 days old, weighed 250 to 300 g, and were fed *ad libitum* a pellet diet (ST1, Velaz, Prague).

Phospholipids were estimated in left cardiac ventricles of IR and IS rats. The hearts were rapidly excised, the left ventricles separated, weighed and kept frozen in liquid nitrogen until further manipulation. They were pulverized in liquid nitrogen. Lipids were extracted by a slightly modified method of Folch *et al.* (1957) and the extracts evaporated under nitrogen.

Separation of phospholipids by two-dimensional thin layer chromatography and phosphorus analysis were carried out according to the method of Rouser *et al.* (1970). The method of Horrock (1968) was used for the determination of plasmalogens of phosphatidylcholine and of phosphatidylethanolamine.

For fatty acid analysis, total phospholipids were separated from other lipids by thin layer chromatography. The developing system was a mixture of hexan:diethylether:acetic acid (85:15:1, v/v/v). Separated phospholipids were transmethylated. Methylesters were extracted into hexan, dried under nitrogen and analysed by gas chromatography (Tvrzická *et al.* 1994).

Student's t-test was used for statistical evaluation.

Results

Table 1 shows the concentration of individual phospholipids in left heart ventricles of IR (isoprenaline-resistant) and IS (isoprenaline-sensitive) rats. The only significant differences between these two strains concerned lower concentrations of phosphatidylcholine and its plasmalogen fraction in IR rats. The total amount of phospholipids was lower in IR rats than in IS ones, the difference being not significant.

Table 1. The content of phospholipids in left heart ventricles of isoprenaline-resistant (IR) rats and isoprenaline-sensitive (IS) rats

	IR rats n=6	IS rats n=5
	μmol P.g^{-1} wet weight	
PC	9.80±0.10	10.61±0.24*
PE	9.24±0.30	9.19±0.44
PS	0.59±0.05	0.53±0.06
SM	0.60±0.05	0.57±0.07
DPG	3.81±0.19	3.79±0.26
PI	1.04±0.10	1.16±0.07
PCP	0.77±0.03	0.90±0.04*
PEP	2.57±0.08	2.79±0.18
Total	25.08±0.18	25.83±0.94

*Each value represents the mean±S.E.M. * $p<0.05$, significantly higher than the appropriate value in the other strain, PC – phosphatidylcholine, PE – phosphatidylethanolamine, PS – phosphatidylserine, SM – sfingomyelin, DPG – diphosphatidylglycerol, PI – phosphatidylinositol, PCP – phosphatidylcholine plasmalogen, PEP – phosphatidylethanolamine plasmalogen. Both plasmalogens are included in the PC and PE values.*

The relative amount of various fatty acids (FA) in total phospholipids in left heart ventricles of IR and IS rats is shown in Table 2. We found no difference in the total amount of saturated FA, however, the relative concentration of palmitic acid (C-16:0) was higher and stearic acid (C-18:0) lower in IR animals. The relative proportion of monounsaturated FA was higher in IR rats. The content of polyunsaturated FA accounts for 53 % of FA residues in phospholipids in both strains. The main difference was particularly observed in the distribution of n-6 polyunsaturated FA, e.g. linoleic acid (C-18:2) and arachidonic (C-20:4) acid. The proportion of linoleic acid was higher by 15%

and that of arachidonic acid lower by 14 % in the hearts of IR rats than in those of IS rats. The ratio of linoleic to arachidonic acid is, therefore, higher in IR rats (1.23 as compared to 0.93 in IS rats). We did not observe any difference in the saturated to unsaturated FA ratio between IR and IS strains.

Table 2. The proportion of individual fatty acids in membrane phospholipids in left heart ventricles of IR and IS rats (n=7)

Type of fatty acid	IR rats %	IS rats %
14:0	0.17±0.01	0.17±0.03
16:0	13.72±0.11*	13.04±0.23
16:1	0.59±0.04***	0.36±0.02
18:0	23.35±0.61	24.98±0.34*
18:1 n-9	4.22±0.16**	3.59±0.07
18:1 n-7	5.37±0.05***	4.88±0.05
18:2 n-6	23.81±0.91*	20.65±0.59
18:3 n-6	1.17±0.06	1.21±0.09
18:3 n-3	0.11±0.00	0.11±0.01
20:3 n-6	0.55±0.02**	0.46±0.01
20:4 n-6	19.42±0.22	22.17±0.03***
20:5 n-3	0.11±0.01	0.09±0.00
22:4 n-6	0.73±0.01	0.79±0.10*
22:5 n-3	1.24±0.05	1.39±0.04*
22:6 n-3	5.41±0.39	6.11±0.23

*Each value represents the mean ± S.E.M. * <0.05, ** p<0.01), *** p<0.001 significantly higher than the appropriate values in the other strain.*

Discussion

Under physiological conditions, there is very small variability in the composition of membrane phospholipids and in the proportions of various FA in heart phospholipids. Characteristic changes, however, are produced in rats or mice by various interventions, e.g. diet, catecholamine (CA) administration or during myocardial damage (Gudbjarnason and Oskarsdottir 1975, Montfoort *et al.* 1986, Drnková *et al.* 1990).

After the administration of a single dose of ISO, no changes of phospholipid composition were found in the heart in spite of the fact that a very marked increase of the turnover rate of phospholipids was observed in phosphate incorporation (Nováková *et al.* 1994).

On the other hand, repeated administration of CA to animals decreased the content of most individual phospholipids including phosphatidylcholine (Montfoort *et al.* 1986, Drnková *et al.* 1990) and, therefore, reduced the total phospholipid content in the heart.

It is well known that repeated CA administration increased the resistance against myocardial damage (Faltová *et al.* 1983). In an attempt to find possible factors important for the genetically determined resistance to IML in our IR and IS rats, we examined the possible involvement of differences in phospholipid content between IR and IS rats.

Genetically determined resistance in our resistant strain of rats (IR) was also accompanied by lower content of phosphatidylcholine and its plasmalogen fraction in the heart as compared to the IS strain, but the difference was small and the total amount of heart phospholipids did not differ significantly (Table 1). We did not find any difference between IR and IS rats in other phospholipid classes. This finding is in contrast with the lowered phospholipid concentration mentioned above (Montfoort *et al.* 1986, Drnková *et al.* 1990).

In our opinion, the small difference between IR and IS rats was not sufficient to explain the large difference in resistance between our two rat strains. Moreover, rats fed a cod liver oil diet had also a lower content of heart phospholipids, including phosphatidylcholine (and thus resembled our IR rats), but their resistance against heart damage was decreased (Gudbjarnason and Hallgrimsson 1975).

Fatty acid composition of heart phospholipids and their changes have been studied extensively. Large changes were described after various interventions mainly by dietary factors and pharmacological interventions (after CA administration and during the development of isoprenaline-induced myocardial necrosis).

Several authors studied the changes of FA composition of heart phospholipids and suggested their possible relation to the resistance against myocardial damage. The changes of three important FA, linoleic (C-18:2), arachidonic (C-20:4) and dodecahexaenoic (C-22:6) are given in Table 3.

Table 3. The changes of fatty acid composition in heart phospholipids and resistance against heart damage under various conditions

Experimental conditions	Type of FA 18:2	20:4	22:6	Reference	Change of resistance
1 h after ISO treatment	−	(±)	+	Gudbjarnason and Oskarsdottir (1975)	
48 h after ISO treatment	(+)	(−)	(−)	Gudbjarnason and Hallgrimsson (1975)	
Repeated CA treatment	−	(+)	+	Montfoort *et al.* (1986)	increased
Cod liver oil feeding	−	−	+	Gudbjarnason and Hallgrimsson (1975)	decreased
Tocoferol feeding	−	+	+	Gudbjarnason and Hallgrimsson (1975)	increased
Recovery after repeated CA treatment	+	−	−	Emilsson and Gudbjarnason (1983)	
IR strain untreated	+	−	(−)	this paper	increased

+ increase, − decrease, ± variable changes, symbols in parentheses denote changes found only in individual phospholipids or not significant differences. CA − catecholamines, ISO − isoprenaline, FA − fatty acid.

Gudbjarnason and Hallgrimsson (1975) showed that feeding animals a cod liver oil diet resulted in the replacement of less unsaturated FA acids by polyunsaturated ones (mainly dodecahexaenoic acid). These changes were accompanied by enhanced sensitivity of the heart to damage. Tocoferol administration, on the other hand, increased the resistance against myocardial damage but the content of docosahexaenoic acid was also increased. Furthermore, both interventions (cod liver oil feeding as well as tocoferol administration) simultaneously reduced the amount of linoleic acid in the heart. The dependence of changes in resistance against myocardial damage on these two FA does not therefore seem to be probable.

On the other hand, the resistance against myocardial damage and arachidonic acid concentration showed close parallelism. Both were decreased in animals fed a cod liver oil diet and augmented after tocoferol administration. Moreover, long-term treatment of animals by CA (mainly norepinephrine or isoprenaline), known to increase the myocardial resistance against damage, was also accompanied by an increased proportion of arachidonic acid in heart phospholipids (at least in phosphatidylcholine). The changes in other FA resembled those after tocoferol administration. Arachidonic acid concentration is therefore most closely related to changes of resistance against heart damage.

Taking into account the above mentioned situations known to increase the resistance against heart damage, they were characterized by the rise in arachidonic and docosahexaenoic acids and a decrease in linoleic acid. The ratio of arachidonic to linoleic acid therefore increased. In contrast to these findings, the genetically determined resistance in our two rat strains was accompanied by reverse differences and by a decrease of the arachidonic to linoleic acid ratio. The

differences of other estimated FA in our resistant rats were also opposite to changes induced by repeated CA administration in experiments where they were estimated (Emilsson and Gudbjarnason 1983).

The only situation corresponding closely to our IR rats from the aspect of FA composition is the recovery period after CA administration (Emilsson and Gudbjarnason 1983). In this situation, however, we have no information about the resistance of these animals against heart damage.

The changes in FA composition of heart phospholipids present no clear evidence for a relation between FA composition of phospholipids and the resistance against heart damage development. As this problem is of considerable importance, it is worthwhile of further study. At present, we feel that the genetically determined resistance in our rat strains is primarily caused by other factors than FA composition of heart phospholipids and might be more dependent on other factors, e.g. on different levels of glycogen in the heart (Mráz and Hynie 1996).

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Reprint requests
Prof. S. Hynie, Department of Pharmacology, First Faculty of Medicine, Charles University, Albertov 4, 128 00 Prague 2, Czech Republic