Hereditary Cardiomyopathy in W/SSM Rats: Biochemical Mechanisms of Development

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One mechanism shown to be responsible for the occurrence of hypertrophic cardiomyopathy in rats of the W/SSM strain, in which this disease is genetically determined, is impairment of cellular membrane integrity resulting from increased hexose transport to cells, generation of hydroxyl radicals, and intensified lipid peroxidation.

Key Words: *W/SSM rats; cardiomyopathy; myocardium; leukocytes; lysosomal hydrolases; lipid peroxidation*

The etiology and pathogenesis of cardiomyopathy and, in particular, of its hypertrophic form remain unclear [7]. The availability of an animal model of hypertrophic cardiomyopathy (HCMP) may therefore offer good opportunities for studying the mechanisms by which this disease evolves and facilitate the development of methods for its prevention and treatment.

We produced a strain of W/SSM rats with manifestations of hereditary HCMP [3]. Rats of this strain have hypertrophic changes in their cardiac muscle very similar to those seen in human HCMP without showing any anatomical or physiological evidence that might shed light on the cause(s) of the myocardial hypertrophy [9].

It has been established that elevated activity of glucose carriers within the plasma membranes of W/SSM rats results in enhanced transport of hexoses to the cells, leading to excessive accumulation of these monosaccharides therein [10]. The intensified hexose transport to the cells of W/SSM rats and the many attendant pathological phenomena have been shown to be controlled by a single gene and to be inherited as a dominant trait [3]. The elevated hexose concentrations in the cells raise the intracellular osmotic pressure, which damages their membranes [5].

The purpose of the present study was to measure biochemical variables (activity of acid glycosidases and acid hydrolases, protein, ^{3}H-2-deoxyglucose transport, formation of hydroxyl radicals in homogenates and mitochondrial fraction, and lipid peroxidation) in W/SSM rats with genetically determined HCMP.

MATERIALS AND METHODS

The study was conducted on 40 young (1 to 3 months old) and adult (10 to 12 months old) male W/SSM rats with hereditary HCMP; healthy rats of the strain W/SSM-R of the same age ranges served as controls. The rats were killed by decapitation.

The activity of acid glycosidases was measured in the myocardium, skeletal muscle, and blood leukocytes. From the heart and somatic muscle 10% homogenates were prepared in 0.01 M Tris-HCl buffer, pH 7.2, which was then centrifuged at 10,000 g for 60 min in the cold (at 2-4°C) and the activity of acid glycosidases was determined in the supernatants [13]. Leukocytes were isolated from the blood containing added heparin (100 U/ml), mixed with 0.83% NH_4Cl in a 1:5 ratio,

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homogenized, and centrifuged at 600 g. Acid glycosidase activity was also determined in the supernatants [13]. Enzyme activity was estimated by the amount of liberated 4-methylumbelliferone (its fluorescence was measured in a Hitachi MPF-2a spectrofluorimeter at an excitation wavelength of 366 nm and an emission wavelength of 466 nm) and expressed in nmol of 4-methylumbelliferone per mg protein per hour [13]. Protein was determined by Lowry's method [16].

^{3}H-2-deoxyglucose transport to erythrocytes was evaluated as described by Okuno *et al.* [17]. The formation of hydroxyl radicals in the homogenate and mitochondrial fraction of the myocardium was determined by EPR spectroscopy [14]. The myocardial mitochondrial fraction was isolated by Schneider's method [18].

The formation of hydroxyl radicals was studied using 2-dimethylamino-3-chloro-1,4-naphthoquinone (synthesized at the Institute of Chemical Kinetics and Combustion, Siberian Division of the Russian Academy of Sciences). The reaction mixture contained 5,5-dimethyl-1-pyrroline N-oxide (DMPO) (Sigma) and 0.5 mM sodium azide. After a 10-min incubation, 1 mM NADPH, 0.05 mM quinone, 0.5 mM hydrogen peroxide in 100 mM Tris-HCl buffer (pH 7.4), and 100 mM KCl were added to the reaction mixture. The formation of hydroxyl radicals was followed by recording changes in the EPR spectrum of the spin adduct DMPO-OH [14].

Lipid peroxidation (LPO) in the myocardial mitochondrial fraction was evaluated by measuring the accumulation of malonic dialdehyde produced in the reaction with 2-thiobarbituric acid (TBA) [12].

The results were statistically analyzed with Student's *t* test.

RESULTS

Glucose is used to study the transport of hexoses in view of the possibility that their transport may be increased because they undergo intensified oxidation in cells. Accordingly, we utilized the nonmetabolizable glucose analog ^{3}H-2-deoxyglucose to examine hexose transport to rat erythrocytes. The intensive ^{3}H-2-deoxyglucose absorption from the incubation medium (240±29.9 Bq $\times 10^2$/ml in W/SSM rats and 74.1±8.1 Bq$\times 10^2$/ml in W/SSM-R rats) indicates that sugars are transported at higher rates in W/SSM than in W/SSM-R rats (Fig. 1) and that this is not associated with enhanced sugar metabolism in the cells. As a consequence of increased hexose transport in W/SSM rats, monosaccharides and their metabolic products accumulate in

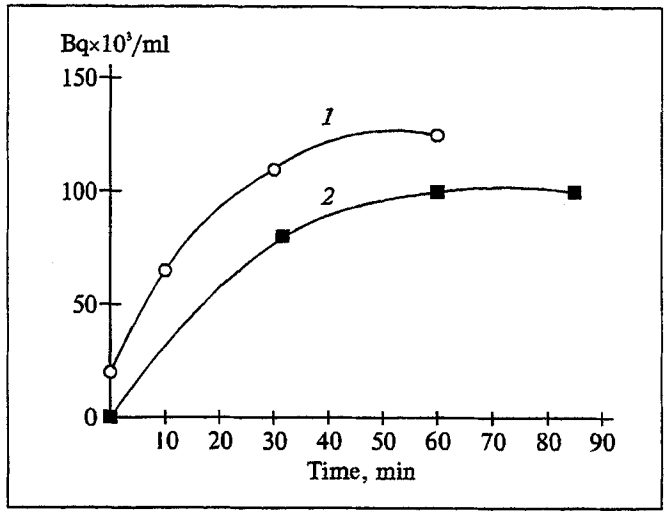

Fig. 1. Time–courses of ^{3}H–2–deoxyglucose accumulation in erythrocytes of diseased W/SSM (*1*) and healthy W/SSM–R (*2*) rats.

their cells. Intracellular hexose accumulation results in a rise of osmotic pressure in the cells, which damages the cell membranes [5]. Hexose accumulation in cells may also lead to intensified formation of hydroxyl radicals therein [15].

Hydroxyl radicals are highly reactive electrophilic particles interacting with unsaturated fatty acids of membrane phospholipids and thus initiating LPO [1,4,14]. A quantitative indicator of the latter process is the accumulation in the body of TBA-reactive products, including malonic dialdehyde [1,2].

We examined hydroxyl radical generation in the myocardium of diseased W/SSM and healthy W/SSM-R rats using homogenates and the mito-

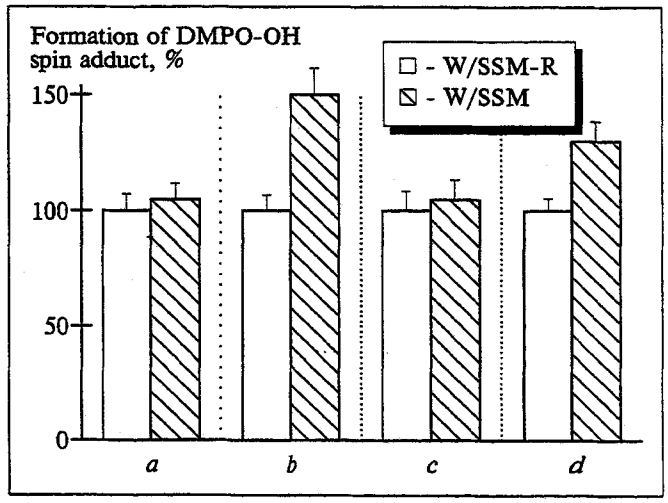

Fig. 2. Generation of hydroxyl radicals in the myocardia of diseased W/SSM and healthy W/SSM–R rats. *a*) myocardial homogenates from rats aged 1.5 months ($n=15$; $p>0.05$); *b*) myocardial homogenates from rats aged 10–12 months ($n=7$; $p<0.001$); *c*) myocardial mitochondrial fraction from rats aged 1.5 months ($n=15$; $p>0.05$); *d*) myocardial mitochondrial fraction from rats aged 10–12 months ($n=7$; $p<0.01$).

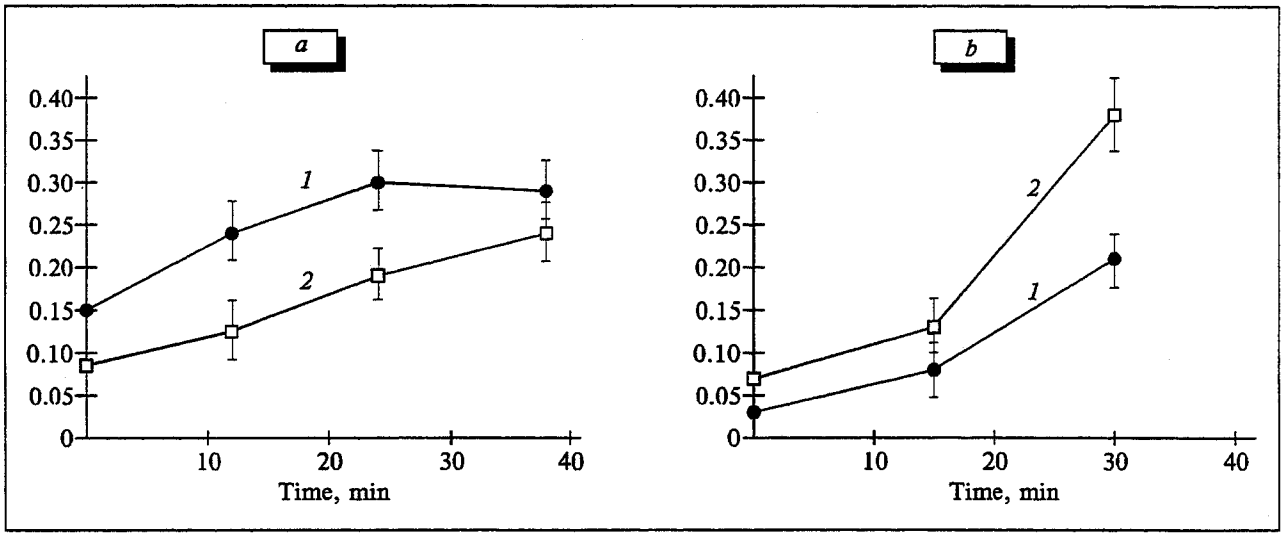

Fig. 3. Formation of TBA-reactive products in myocardial mitochondria (in the presence of 10 mM Fe-ADP in the medium) from diseased W/SSM (*1*) and healthy W/SSM-R (*2*) rats. *a*) rats aged 3 months; *b*) rats aged 10 months. *Ordinate*: levels of TBA-reactive products relative to 1.

chondrial fraction from myocardia of rats aged 1-1.5 and 10-12 months. No differences in hydroxyl radical generation were found between the younger W/SSM and W/SSM-R (control) rats (Fig 2). In contrast, hydroxyl radical production in the homogenates and mitochondria from W/SSM rats exceeded the control level by 50% (Fig. 2).

LPO was evaluated by measuring spontaneous and Fe-ADP-stimulated [14] generation of TBA-reactive products in myocardial mitochondria of W/SSM and W/SSM-R rats. The mitochondria of W/SSM rats aged 3 months were found to have significantly higher spontaneous and stimulated levels of these products than the mitochondria of W/SSM-R rats (Fig. 3, *a*). Conversely, the mitochondria of W/SSM rats aged 10-12 months contained much smaller amounts of TBA-reactive products than did those of W/SSM-R controls (Fig. 3, *b*). These findings agree with the observations indicating that the rapid development of LPO processes as a result of exposure to radiation, toxic chemicals, or other adverse factors involves a similar inversion in the levels of TBA-reactive products [1]. Such inversion can be explained by the fact that intensive and long-lasting oxidation of unsaturated fatty acids results in a tapering off of LPO because the oxidation substrate is depleted [1,2].

LPO activation is now thought to play a key role in the pathogenesis of cardiogenic stress, being directly responsible for the damage suffered by cellular membranes, including lysosomal membranes [2]. One consequence of this appears to be the elevated activity of lysosomal hydrolases in the blood plasma and leukocytes [6] of W/SSM rats compared to the W/SSM-R strain.

As shown by this study, the activity of lysosomal enzymes in the myocardium and cross-striated muscle of W/SSM rats is much higher than in the W/SSM-R controls (Table 1). We found that the intensive transport of sugars to the cells of W/SSM rats is determined by one mutant gene which produces pleiotropic effects and is responsible for a number of inherited pathological phenomena in this strain [3,10,11].

Hereditary factors are presumed to play an important role in the etiology of human HCMP

TABLE 1. Lysosomal Hydrolase Activity (nmol 4-methylumbelliferone/mg protein/h) in the Myocardium and Skeletal Muscle of Diseased W/SSM and Healthy W/SSM-R Rats ($M \pm m$)

Enzyme	Myocardium		Skeletal muscle	
	W/SSM-R	W/SSM	W/SSM-R	W/SSM
β-Galactosidase	145.2±8.07 (18)	217.8±19.3* (17)	105.6±13.8 (10)	155.0±12.2* (9)
N-acetyl-β-D-glucosaminidase	400.0±5.3 (19)	391.8±48.9 (19)	154.5±10.0 (18)	273.2±33.4* (10)
N-acetyl-β-D-galactosaminidase	176.0±22.4 (19)	248.1±17.7* (19)	86.3±16.3 (8)	124.7±1.34* (10)

Note. *$p<0.05$. Figures in parentheses indicate the number of rats.

[7,9]. There is evidence that the disease has multiple causes [7]. One of the common mechanisms of human HCMP is likely to be impairment of cellular membrane integrity (similar to that we recorded for W/SSM rats) resulting from increased hexose transport, excessive production of free hydroxyl radicals, and intensified LPO processes [8].

The findings presented above open up prospects for developing methods that permit hereditary forms of HCMP to be diagnosed, prevented, and even treated by normalizing hexose transport, eliminating free hydroxyl radicals, and correcting LPO processes and other metabolic disorders underlying this disease.

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