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SARCOLEMMAL PHOSPHOLIPID N-METHYLATION IN GENETICALLY DETERMINED HAMSTER CARDIOMYOPATHY

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The heart sarcolemmal phosphatidylethanolamine N-methylation in UM-X7.1 strain of cardiomyopathic hamsters was examined by using 0.055, 10 and 150 μM S-adenosyl - L -(methyl- ^{3}H) methionine as methyl donor for sites I, II and III, respectively. In comparison with control values, methylation activities at site I was increased in 40, 120 and 250 days old cardiomyopathic hamsters. On the other hand, methylation activities at sites II and III in 120 and 250 days old cardiomyopathic animals were depressed without any change in the 40 days old group. The alterations in N-methylation activities were associated with kinetic changes in apparent Vmax values without any changes in the apparent Km. These results indicate a defect in the phospholipid N-methylation process in heart sarcolemma during the development of genetically determined cardiomyopathy.

The conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) has been shown to be catalyzed by methyltransferase enzyme(s) where phosphatidyl-N-monomethylethanolamine (PMME) and phosphatidyl-N, N-dimethylethanolamine (PDME) are formed as intermediates, and S-adenosyl-L-methionine (AdoMet) serves as the methyl group donor. Recent studies with heart sarcolemma have identified the involvement in this reaction of three catalytic sites with apparent Km values of 0.1, 2.8 and 121 μM AdoMet for sites I, II and III, respectively (1). The phospholipid N-methylation process has been considered to play an important role in membrane-related events including membrane fluidity, β-adrenergic receptor function and Ca^{2+} movements across the plasma membrane (2-4). Although noticeable changes in sarcolemmal Na^{+}, K^{+}ATPase, adenylate cyclase, Na^{+}-Ca^{2+} exchange and Ca^{2+} pump activities have been reported to occur during the development of cardiomyopathy in hamsters (5,6), no information regarding the status of membrane phospholipid N-methylation activities is available in the literature. This study was therefore undertaken to investigate if the sarcolemmal N-methylation activities are altered during the development of cardiomyopathy in the UM-X7.1 strain of hamsters. It may be relevant to note that these animals have been shown to exhibit

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varying degrees of cardiac necrosis beginning at about 40 days of age and die from heart failure at about 250 days (7).

MATERIALS AND METHODS

Cardiomyopathic hamsters (UM-X7.1 strain) of 40, 120 and 250 days of age were employed as experimental animals whereas healthy Syrian hamsters of comparable age served as controls. Purified sarcolemmal membrane preparation was isolated from the ventricular tissue by the hypotonic shock-LiBr treatment method as described previously (8). This preparation has been shown to contain a substantial amount of basement membrane and consist of predominantly right-side out vesicles (5,9). As reported previously (5), routine examination of marker enzyme activities revealed minimal (3-4%) but equal extent of cross contamination in both control and experimental preparations. The phospholipid N-methylation activities at sites I, II and III were determined under optimal conditions (1). For catalytic site I, the incubation was carried out in the present of 1 mM $MgCl_2$, 0.055 μM ^{3}H-AdoMet (75.7 Ci/mmol) in a 50 mM Tris-glycylglycine buffer, pH 8.0. On the other hand, the incubation was performed without $MgCl_2$ in the presence of 10 μM ^{3}H-AdoMet (200 μCi/μmol) in 50 mM phosphate buffer, pH 7.0 for site II and 150 μM ^{3}H-AdoMet (200 μCi/μmol) in 50 mM sodium hydroxide-glycine buffer, pH 10 for site III. After an incubation period of 10 min at 37°C, the reaction was initiated by adding ^{3}H-AdoMet as a methyl donor and terminated 30 min later with the addition of chloroform: methanol: 2N HCl (6:3:1, by vol.). An aliquot of the final lipid extract was taken for the determination of total N-methylation activity whereas another was used to fractionate and quantify radiolabeled phospholipids such as PMME, PDME and PC according to the procedure outlined elsewhere (1). S- Adenosyl-L-methionine was purified by ion-exchange chromatography (10) whereas all other reagents were of analytical grade. The data were expressed as mean ± S.E. and analyzed statistically by using the Student's t-test; a P level less than 0.05 was taken to reflect a significant difference between control and experimental values.

RESULTS

Phospholipid N-methylation activities at sites I, II and III in heart sarcolemma from control and cardiomyopathic hamsters were determined by monitoring the incorporation of methyl groups into total phospholipids as well as into PMME, PDME and PC. Table 1 shows the phospholipid N-methylation activities of control and experimental heart sarcolemmal preparations at site I. Total methyl group incorporation into sarcolemmal phospholipids was significantly higher in 40, 120 and 250 days old cardiomyopathic hamsters than the respective control values. Likewise the formation of radiolabeled PMME, unlike PDME or PC, in the experimental membranes was also increased in comparison with control preparations. The data in Tables 2 and 3 indicate significantly depressed PE N-methylation activities at sites II and III in 120 and 250 days old cardiomyopathic hamsters without any changes in the activities of heart sarcolemma from 40 days old animals. The depression in phospholipid methylation activity at site II was accompanied by a significant depression in the formation of both PDME and PC in experimental preparations (Table 2), whereas the observed decrease at site III was associated with a significant decrease in the formation of PC only (Table 3).

In order to gain further information regarding changes in phospholipid N-methylation activities, kinetic properties of PE N-methylation at sites I, II and

Table 1. Phospholipid N-methylation at catalytic site I of cardiac sarcolemma from control and cardiomyopathic (UM-X7.1) hamsters

Animal Groups	Total	PMME	PDME	PC
	pmol [^{3}H] methyl groups incorporated/mg/30 min			
A. 40 days old				
Control	0.320±0.017	0.152±0.008	0.056±0.004	0.048±0.005
Cardiomyopathic	0.401±0.018*	0.204±0.009*	0.060±0.006	0.052±0.007
B. 120 days old				
Control	0.341±0.022	0.158±0.012	0.064±0.007	0.058±0.009
Cardiomyopathic	0.526±0.042*	0.292±0.035*	0.061±0.006	0.070±0.007
C. 250 days old				
Control	0.382±0.029	0.173±0.014	0.074±0.007	0.064±0.006
Cardiomyopathic	0.488±0.037*	0.234±0.008*	0.067±0.006	0.074±0.004

Results are means ± SE of 4 experiments. Assays were performed in the presence of 0.055 μM [^{3}H]- AdoMet as indicated in Materials and Methods. *Significantly ($P<0.05$) different from control.PMME = phosphatidyl-N-monomethylethanolamine; PDME = phosphatidyl-N, N-dimetylethanolamine; PC = phosphatidylcholine.

Table 2. Phospholipid N-methylation at catalytic site II of cardiac sarcolemma from control and cardiomyopathic (UM-X7.1) hamsters

Animal Groups	Total	PMME	PDME	PC
	pmol [^{3}H] methyl groups incorporated/mg/30 min			
A. 40 days old				
Control	1.52±0.11	0.32±0.01	0.51±0.08	0.34±0.03
Cardiomyopathic	1.46±0.10	0.36±0.04	0.46±0.03	0.28±0.02
B. 120 days old				
Control	1.49±0.07	0.34±0.05	0.53±0.03	0.35±0.02
Cardiomyopathic	1.12±0.10*	0.39±0.04	0.40±0.02*	0.23±0.02*
C. 250 days old				
Control	1.41±0.14	0.34±0.02	0.48±0.04	0.33±0.03
Cardiomyopathic	0.95±0.11*	0.30±0.01	0.32±0.03*	0.16±0.02*

Results are means ± SE of 4 experiments. Assays were performed in the presence of 10 μM [^{3}H]- AdoMet as indicated in Materials and Methods. *Significantly ($P<0.05$) different from control.

Table 3. Phospholipid N-methylation at catalytic site III of cardiac sarcolemma from control and cardiomyopathic (UM-X7.1) hamsters

Animal Groups	Total	PMME	PDME	PC
	pmol [^{3}H] methyl groups incorporated/mg/30 min			
A. 40 days old				
Control	106.8±6.6	21.4±1.0	32.2±1.3	38.6±1.3
Cardiomyopathic	100.1±3.8	23.1±1.8	30.4±2.2	34.9±1.8
B. 120 days old				
Control	114.6±8.6	27.5±2.7	29.9±2.5	35.8±1.4
Cardiomyopathic	88.9±8.1*	28.1±2.4	24.9±2.6	21.7±2.4*
C. 250 days old				
Control	99.1±6.7	24.3±3.1	24.6±3.1	32.4±4.2
Cardiomyopathic	70.3±5.9*	23.0±2.0	21.8±1.8	15.3±1.4*

Results are means ± SE of 4 experiments. Assays were performed in the presence of 150 μM [^{3}H]- AdoMet as indicated in Materials and Methods. *Significantly ($P<0.05$) different from control.

III in heart sarcolemma from 250 days old control and cardiomyopathic hamsters were studied by using different concentrations of ^{3}H-AdoMet. The results in Fig. 1 indicate that the apparent affinity at all three sites for AdoMet (Km values 0.11, 2.4 and 145 μM) was unaltered. On the other hand, apparent Vmax value for the experimental preparations was increased by about 30% at site I and decreased by 30% and 35% at sites II and III respectively. Results in Tables 1,2 and 3 were analyzed in terms of ratios of the predominant N-methylated phospholipids formed under optimal conditions at sites I (PMME, Table 1), II (PDME , Table 2) and III (PC, Table 3). In heart membranes from 40 days old cardiomyopathic hamster, PMME/PDME and PMME/PC ratios were 1.50 fold higher than control values without any change in PMME + PDME/PC ratio. On the other hand, PMME/PDME, PMME/PC and PMME + PDME/PC ratios were increased by 2.44, 3.04 and 1.65 in 120 days old cardiomyopathic preparations and 2,03, 2.86 and 1.80 in 250 days old experimental hearts. This analysis suggests an increase in the intramembranal level of partially N-methylated vs totally N-methylated phospholipids at different stages of the disease and provides further evidence regarding defects in the phospholipid N-methylation activity of heart sarcolemma from cardiomyopathic hamsters.

DISCUSSION

In this study we have observed an activation of the phospholipid N-methylation activity at catalytic site I in 40 to 250 days old cardiomyopathic hearts whereas the methylating activities of catalytic sites II and III were depressed in heart sarcolemma from 120 to 250 days old experimental animals. Since cardiac

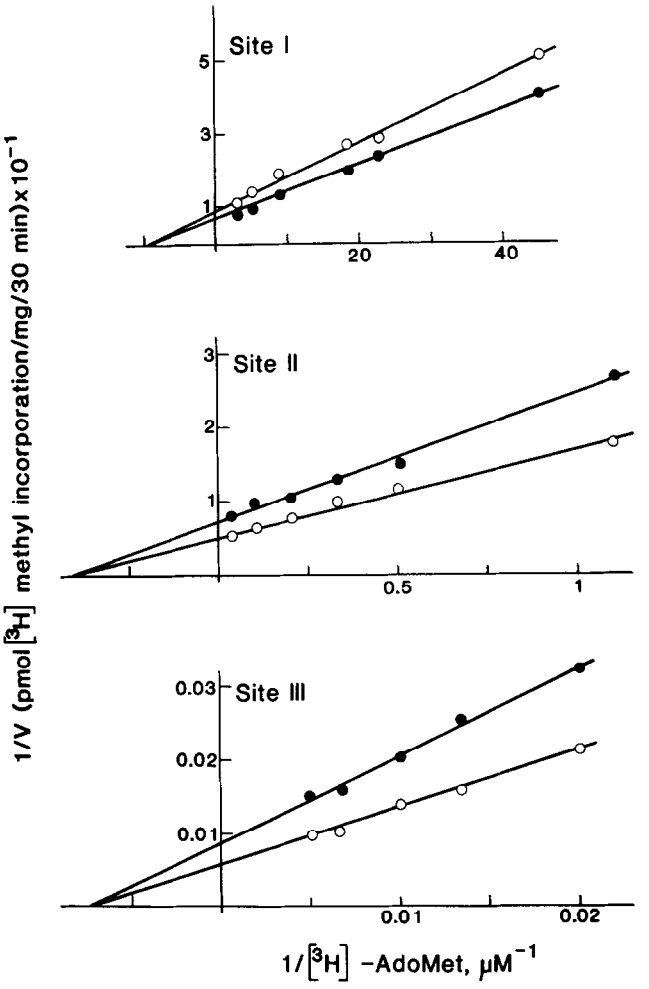

Fig. 1. Lineweaver - Burke plots of total [^{3}H] methyl group incorporation into sarcolemmal phospholipids from control (o) and 250 days old cardiomyopathic (•) hearts. Assay conditions were as described in Materials and Methods. The apparent Km and Vmax values are expressed in μM and pmol/mg/30 min, respectively. Site I control: Km= 0.11, Vmax = 1.05; cardiomyopathic: Km = 0.11, Vmax = 1.32. Site II control: Km= 2.4, Vmax = 2.05; cardiomyopathic: Km = 2.4, Vmax = 1.42. Site III control: Km= 145, Vmax = 181.8; cardiomyopathic: Km = 145, Vmax = 118.4.

sympathetic activity is markedly increased in cardiomyopathic hamsters (11) and since catecholamines have been demonstrated to enhance site I N-methylation activity in normal hearts (12), it is likely that the observed increase at site I may be due to high levels of myocardial catecholamines (11) in these cardiomyopathic animals. On the other hand, the observed depression in the phospholipid N-methylation activities at sites II and III in 120 and 250 days old cardiomyopathic hamster hearts may be due to myopathic changes occurring during the development of cardiomyopathy in these animals. In fact, a similar pattern of changes was also observed during the development of diabetic cardiomyopathy in rats upon injecting streptozotocin (13).

Unlike site I, sites II and III activation of phospholipid N-methylation in cardiac sarcolemma has been shown to stimulate Ca^{2+} pump and inhibit Na^{+} -dependent Ca^{2+} uptake activity of the Na^{+}-Ca^{2+} exchanger (14,15). Previous finding that sarcolemmal Ca^{2+} pump and Na^{+} -dependent Ca^{2+} uptake were not altered in 40 days old cardiomyopathic hamster hearts (6) correlates well with normal site II and III N-methylation as observed at the same stage of disease in the present study. Furthermore, depressed Ca^{2+} pump activity in heart sarcolemma from 120 and 250 days old cardiomyopathic hamsters (6) may be due to the observed depression in phospholipid N-methylation at sites II and III in these animals. However, such changes in the N-methylation activities cannot explain the alterations observed in sarcolemmal Na^{+}-Ca^{2+} exchange because Na^{+}-dependent Ca^{2+} uptake in sarcolemmal vesicles was also decreased in the 120 and 250 days old cardiomyopathic hearts (6). Thus, it appears that changes in the sarcolemmal phospholipid N-methylation may be one of the several mechanisms which may be associated with altering the sarcolemmal Ca^{2+} transport systems during the development of genetic cardiomyopathy in hamsters.

The significance of the increased site I synthesis of PMME observed in 40 to 250 days old experimental animals is not clear at present. However, changes in PMME/PC ratio as those suggested by this study have been shown to be responsible for altering the membrane fluidity (16,17). Therefore, it is possible that an increase in PMME/PC ratio may alter the fluidity of heart sarcolemma and thus may play an important role in the development of cardiomyopathy in these hamsters.

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