Sarcolemmal Phosphatidylethanolamine N-Methylation in Diabetic Cardiomyopathy

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SUMMARY. Phosphatidylethanolamine N-methylation was studied in cardiac sarcolemma 8 weeks after the induction of chronic experimental diabetes in rats by a streptozotocin injection (65 mg/kg, iv). Incorporation of radiolabeled methyl groups from S-adenosyl-L-methionine into intramembranal phosphatidylethanolamine, assayed under optimal conditions, confirmed the existence of three catalytic sites involved in the sequential methyl transfer reactions. Total methyl group incorporation at all three sites was significantly depressed in diabetic myocardium, but this change was reversible by a 14-day insulin therapy to the diabetic animals. Measurements of phospholipid N-methylation activity at different times indicated that the depression was evident at 6 weeks after the induction of diabetes. This defect was also seen for the individual methylated lipid products (monomethyl-, dimethylphosphatidylethanolamine, and phosphatidylcholine) specifically formed at each catalytic site. Experiments with different concentrations of S-adenosyl-L-methionine revealed that, for all three sites, the apparent affinity for the methyl donor did not change, whereas the apparent Vₘₐₓ values were significantly lowered in diabetes. The results of this study identify a defect in the sarcolemmal phosphatidylethanolamine N-methylation in diabetic cardiomyopathy. (Circ Res 55: 504-512, 1984)

RECENT studies have suggested that phosphatidylethanolamine N-methylation in the plasma membrane is an important process in the regulation of several membrane-related events (cf Hirata and Axelrod, 1980; Mato and Alemany, 1983, for reviews). The N-methylation reaction consists of the sequential addition of three methyl groups from the physiological donor S-adenosyl-L-methionine (AdoMet) to the amino moiety of an intramembranal phosphatidylethanolamine molecule. The final synthesis of phosphatidylcholine is preceded by the formation of intermediates, mono- and dimethyl derivatives, in the presence of two membrane-bound enzymes, methyltransferase I and methyltransferase II. Methyltransferase I catalyzes the methylation of phosphatidylethanolamine to form phosphatidyl-N-monomethylethanolamine, which is then converted into phosphatidylcholine via phosphatidyl-N,N-dimethylcalamine by methyltransferase II (Hirata and Axelrod, 1978; Yamashita et al., 1982). The presence of phospholipid N-methylation has been demonstrated in myocardium (Limas, 1980a; Mogelson and Sobel, 1981; Okumura et al., 1983), and a defect in this process has been shown to occur in alcoholic cardiomyopathy (Prasad and Edwards, 1983). Recently, methyltransferase activities that synthesize phosphatidylcholine formation in cardiac sarcolemmal membrane have been identified and characterized by us (Panagia et al., 1984; Ganguly et al., in press). Since microsomal phosphatidylethanolamine N-methylation has been shown to be increased in cardiac hypertrophy due to pressure overload and hyperthyroidism (Limas, 1980a, 1980b), it is likely that an alteration in this process under various pathological conditions could modify the lipid microenvironment and biophysical properties of the cardiac membranes and, thus, may influence their functional activities. Although abnormalities in sarcolemmal Na⁺,K⁺-ATPase, ATP-independent Ca⁺⁺ binding, and Ca⁺⁺-ATPase have been identified in diabetic myocardium (Pierce and Dhalla, 1983; Pierce et al., 1983; Dhalla et al., in press), no information concerning the phospholipid N-methylation activity in heart sarcolemma from animals with diabetes is available in the literature. It should be noted that various studies have demonstrated that chronic diabetes results in cardiomyopathy which is associated with defects in cardiac ultrastructure, function, and metabolism (Tarach, 1976; Penpargkul et al., 1980; Ganguly et al., 1983; Regan, 1983). The present study therefore was undertaken to examine the phosphatidylethanolamine N-methylation process in heart sarcolemmal membrane from animals with experimentally induced diabetes.

Methods

Animal Model
Male Sprague-Dawley rats (150–175 g) of the same age were used in this study. Diabetes was induced under ether anesthesia by a single intrafemoral injection of streptozotocin (65 mg/kg body weight) dissolved in a citrate-buff-
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Isolation and Characterization of Sarcolemmal Membrane

Purified sarcolemmal vesicles were isolated from pools of two to three hearts by hypotonic shock-LiBr treatment method (Dhalla et al., 1981). We have previously demonstrated that the sarcolemmal preparation containing basement membrane obtained by this procedure is of cell membrane origin and possesses minimal contamination by other subcellular organelles, as well as myocardial ultrastructural damage (Tarach, 1976; Penparkkul et al., 1980; Ganguly et al., 1983; Pierce et al., 1983).

Assay for Phospholipid Methyltransferase

Phospholipid methyltransferase was assayed by measuring the incorporation of [3H]methyl groups into membrane phospholipids in the presence of S-adenosyl-L-[methyl-3H]methionine ([3H]-AdoMet) essentially as described by Hirata et al. (1979). Assays were performed with 0.5 mg sarcolemmal protein in 0.5 ml reaction mixture by using the incorporation of approximately 3 μmol of phospholipid into methylated phospholipids, a 1-ml aliquot of the chloroform phase was transferred to a counting vial and evaporated to dryness in an oven at 80°C. Finally, 10 ml of scintillation fluid (Ready-Solv HP, Beckman) were added to the residue for counting the radioactivity. Blanks were also done under identical conditions, as except that boiled membrane proteins were added to the reaction mixture.

Separation of Methylated Phospholipids

To fractionate and quantify the various [3H]methyl-labeled phospholipids of the membrane, the remaining chloroform phase was evaporated almost to dryness under N₂ stream at 37°C. The residue was immediately dissolved in 50 μl of chloroform:methanol (2:1, by volume), and quantitatively applied to silica gel 60 F-254 thin layer plates under a light N₂ stream. The chloroform-containing test tubes were washed twice with 50 μl of chloroform:methanol mixture, and each washing was again applied to the layer. The chromatogram was run at room temperature in a solvent system containing propionic acid:n-propyl alcohol:chloroform:water (2:2:1:1, by volume). Appropriate standards (phosphatidylcholine, monomethyl-, dimethyl-, and phosphatidylethanolamine) were run concomitantly. After the solvent front had been migrated approximately 15 cm, the plates were air dried at room temperature. The lipid spots were visualized by exposure to iodine vapors, scraped, and radioactivity was counted in 10 ml Ready-Solv HP. Corrections were made for the quenching due to silica gel, in all the data expressed. Radioactivity recovered from the thin layer plate accounted for 70–80% of the total radioactivity present in the chloroform phase.

Materials

S-Adenosyl-L-[methyl-3H]methionine (specific activity, 80.6 Ci/mmol) was purchased from New England Nuclear. Streptozotocin, phosphatidylethanolamine, phosphatidylcholine, S-adenosyl-L-methionine and S-adenosyl-L-homocysteine were purchased from Sigma Chemical Co. Phosphatidyl-N,N-dimethyllethanolamine and phosphatidyl-N,N-dimethyllethanolamine were obtained from Calbiochem-Behring. Silica gel 60 F-254 thin layer chromatography plates were obtained from E. Merck. Chromatographic analysis indicated that the radiochemical purity of S-adenosyl-L-[methyl-3H]methionine was 99.9%. S-Adenosyl-L-methionine was purified by ion-exchange chromatography according to the method of Glazer and Peale (1978). All other reagents were of analytical grade.

Statistics

Results were expressed as the mean ± SE. Statistical analysis was carried out by Student’s t-test, and a P level less than 0.05 was taken to reflect a significant difference between control and experimental values.
Results

Characteristics of Diabetic Animals

Body and ventricular weights of the experimental rats, 8 weeks after streptozotocin injection, were significantly lower in relation to the control group, whereas the ventricular-to-body weight ratio was significantly elevated (Table 1). Severe hyperglycemia in the presence of significantly depressed plasma insulin levels was also observed. These characteristics are similar to those reported earlier, in studies in which analogous experimental protocols were employed (Penpargkul et al., 1980; Ganguly et al., 1983; Pierce and Dhalla, 1983). Daily injection for 2 weeks with 3 U insulin to the 6 week diabetic animals completely reversed the observed changes in plasma glucose and insulin levels (data not shown). The results in Table 1 also indicate that the yield of sarcolemmal membrane proteins was similar in control and diabetic hearts.

Characterization of Sarcolemmal Preparation

The specific activities of putative marker enzymes were examined in homogenate and sarcolemmal fractions from control and diabetic hearts to determine the possible extent of cross-contamination from other subcellular organelles (Table 2). The activities of enzymes in homogenates from control and diabetic groups were not statistically different. Ouabain-sensitive Na⁺,K⁺-ATPase and adenylyl cyclase activities, which are well-recognized markers of the plasma membrane, were similar in sarcolemmal preparations from both groups, and exhibited an almost identical increase in purity (8- to 9-fold) over homogenate values. It should be pointed out that the activity of latent Na⁺,K⁺-ATPase, as measured after the membranes had been treated with detergents, was decreased while the latent Na⁺,K⁺ ATPase activity in untreated membranes did not change in diabetic myocardium (Pierce and Dhalla, 1983). Furthermore, extensive studies in our laboratory have shown no change in the basal adenylyl cyclase activity in diabetic myocardium (Smith et al., 1984). K⁺-EDTA-stimulated ATPase activity was not detectable in control and diabetic preparations, and this seems to exclude the existence of myofilibrillar contamination in these membranes. Similar levels of cytochrome c oxidase and rotenone-insensitive NADPH cytochrome c reductase with purification factors in the range of 0.2–0.4 were found in both sarcolemmal fractions. These observations appear to indicate that mitochondrial and sarcoplasmic reticular contaminations were minimal, but similar, in control and diabetic samples.

Table 1

<table>
<thead>
<tr>
<th>General Characteristics of Experimental Animals</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>450 ± 12</td>
<td>273 ± 15*</td>
</tr>
<tr>
<td>(13)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Ventricular wt (g)</td>
<td>1.11 ± 0.06</td>
<td>0.82 ± 0.07*</td>
</tr>
<tr>
<td>(13)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Ventricular wt/body wt ratio (mg/g)</td>
<td>2.47 ± 0.09</td>
<td>3.07 ± 0.08*</td>
</tr>
<tr>
<td>(13)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>150 ± 15</td>
<td>480 ± 18*</td>
</tr>
<tr>
<td>(10)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>32 ± 3.2</td>
<td>14 ± 2.0*</td>
</tr>
<tr>
<td>(5)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Sarcolemmal yield (mg protein/g tissue)</td>
<td>3.32 ± 0.38</td>
<td>3.51 ± 0.29</td>
</tr>
<tr>
<td>(5)</td>
<td>(5)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se. Numbers of animals are given in parentheses.
* Significantly (P < 0.05) different from control.

Characteristics of Phosphatidylethanolamine N-Methylation in Sarcolemma

In one series of experiments, phosphatidylethanolamine N-methylation activity in rat heart sarcolemma was studied in the presence of different concentrations of [³H]-AdoMet (0.01–250 µM) at pH 8.0 (data not shown). As reported earlier (Panagia et al., 1984), we observed that the activity curve had three saturation phases, indicating the presence of three catalytic sites, I, II, and III, with apparent Kₘ values for AdoMet of 0.1, 3.6, and 119 µM, respectively. The optimal conditions for the N-methylation reaction for site I were found to be 1 mM MgCl₂, 0.055 µM [³H]-AdoMet (80.6 Ci/mmol) at pH 8.0 (50 mM Tris-glycylglycine buffer). On the other hand, sites II and III did not require MgCl₂ as a cofactor, and their optimal assay conditions were 10 µM [³H]-AdoMet (200 µCi/µmol) at pH 7.0 (50 mM phosphate buffer) and 150 µM [³H]-AdoMet (200 µCi/µmol) at pH 10.0 (50 mM sodium hydroxide-glycine buffer), respectively (Panagia et al., 1984). These assay conditions were typical for the synthesis of phosphatidyl-N-monomethylethanolamine, phosphatidyl-N,N-dimethylethanolamine, and phosphatidylcholine as major methylated lipid products at sites I, II, and III, respectively, and, unless otherwise indicated, were used in subsequent experiments for determining the phospholipid N-methylation process of heart sarcolemma at sites I, II, and III. It should be noted that, on the basis of biochemical properties, site I seems to correspond to methyltransferase I, whereas the latter two catalytic sites may be responsible for the overall activity of methyltransferase II (Panagia et al., 1984).

Phosphatidylethanolamine N-Methylation during Diabetes

In an effort to characterize the enzymatic N-methylation of phosphatidylethanolamine, sarcolemmal membranes from diabetic (8 weeks after streptozotocin treatment) and paired control groups were incubated for different time intervals, and the incor-
TABLE 2
Specific Activities of Marker Enzymes in Homogenate and Sarcolemmal Fractions from Control and Diabetic Hearts

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control Homogenate</th>
<th>Control Sarcolemma</th>
<th>Purity factor</th>
<th>Diabetic Homogenate</th>
<th>Diabetic Sarcolemma</th>
<th>Purity factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain-sensitive Na⁺,K⁺-ATPase*</td>
<td>1.6 ± 0.2</td>
<td>13.9 ± 0.7</td>
<td>8.6</td>
<td>1.5 ± 0.2</td>
<td>13.2 ± 0.9</td>
<td>8.8</td>
</tr>
<tr>
<td>Adenylate cyclase†</td>
<td>43 ± 3</td>
<td>369 ± 21</td>
<td>8.5</td>
<td>41 ± 4</td>
<td>330 ± 17</td>
<td>8.0</td>
</tr>
<tr>
<td>Cytochrome c oxidase‡</td>
<td>106 ± 8</td>
<td>23 ± 5</td>
<td>0.2</td>
<td>88 ± 11</td>
<td>18 ± 4</td>
<td>0.2</td>
</tr>
<tr>
<td>K⁺-EDTA ATPase*</td>
<td>16.2 ± 1.7</td>
<td>ND</td>
<td>0.0</td>
<td>14.9 ± 1.2</td>
<td>ND</td>
<td>0.0</td>
</tr>
<tr>
<td>Rotenone-insensitive NADPH cytochrome c reductase§</td>
<td>5.3 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>0.4</td>
<td>4.9 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

These values represent mean ± se of three different experiments. ND = not detectable. In symboled footnotes that follow, specific activities of enzymes are expressed as:
* μmol Pi/mg per hr.
† pmol cAMP/mg per min.
‡ nmol cytochrome c/mg per min.
§ nmole cytochrome c reduced/mg per min.

Portion of [³H]methyl groups into total as well as individual methylated phospholipids was examined at each catalytic site. The activity curves for the three sites indicate that the methylation rates were reduced in diabetic animals (Figs. 1–3). Furthermore, the results show that total incorporation of radiomethyl groups into membranes was always linear in both control and diabetic groups for at least 30 minutes of incubation for the amount of sarcolemmal protein employed. The concomitant formation of single methylated phospholipids at each site was also linear for the same period of time in control membranes. On the other hand, in the diabetic group, the rates of phosphatidylcholine synthesis at sites I and II and phosphatidyl-N-monomethylethanolamine synthesis at site II were not linear (Figs. 1 and 2). The reasons for these findings are not clear at present, but such a change in diabetic myocardium may be a consequence of differential alterations at sites I and II. However, in view of these complex changes in methylated products, subsequent experiments were carried out with total methyl group incorporation.

The catalytic activity of the three sites involved in sarcolemmal phospholipid N-methylation was examined at 3, 7, 14, 28, 42, and 56 days after streptozotocin injection (Table 3). Although the blood glucose level was raised and plasma insulin declined within 3 days (data not shown), a decrease in the specific activities of all three sites was observed only at 42 and 56 days after induction of diabetes. By 42 days postinjection, sarcolemmal phospholipid N-methylation was significantly depressed by approximately 20–30%, and by 56 days it decreased by about 25–50% of the paired control values. A 14-day insulin therapy to the diabetic animals reversed and normalized the activities of the three sites, indicating that these were diabetes-specific alterations. From Table 3, it can also be seen that at 3 and 7 days after inducing diabetes, the N-methylation activity for site I was higher, compared with that of preparations from age-matched control animals. Thus, unlike sites II and III, changes at site I appear to be biphasic in nature. Furthermore, the developmental changes at site I, unlike sites II and III, seen in this study, are similar to those reported by Ganguly et al. (in press). It is evident that the observed depression in N-methylation activity for all three sites was gradual in onset and is consistent with the gradual nature of the cardiomyopathic process in this experimental model (Vadlamudi et al., 1982; Ganguly et al., 1983). It is also pointed out that the
TABLE 3
Phospholipid N-Methylation of Cardiac Sarcolemma after Induction of Diabetes

<table>
<thead>
<tr>
<th>Days after streptozotocin injection</th>
<th>Experimental group</th>
<th>Catalytic site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>0</td>
<td>Control</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>0.35 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>0.36 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>28</td>
<td>Control</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td>42</td>
<td>Control</td>
<td>0.43 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td>56</td>
<td>Control</td>
<td>0.32 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>42 + 56</td>
<td>Control</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.61 ± 0.05</td>
</tr>
</tbody>
</table>

*Significantly (P < 0.05) different from paired control.

Assays at catalytic sites I, II, and III were performed in the presence of 0.055, 10, and 150 μM [3H]-AdoMet respectively, as described in Methods. Results are the means ± SE of three different experiments and are expressed as pmol total [3H] methyl group incorporated/mg per 30 min.

Specific activities for catalytic sites I, II, and III involved in sarcolemmal phospholipid N-methylation were enriched 5- to 8-fold with respect to the corresponding values in the heart homogenate from control animals (0.13, 1.2, and 16.9 pmol/mg per 30 min, respectively). Similar enrichments of these sarcolemmal activities were noted in diabetic animals.

Kinetics of Phospholipid N-Methylation

Using [3H]-AdoMet as radiolabeled methyl donor, we studied the kinetic properties of sarcolemmal phosphatidylethanolamine N-methylation in hearts from normal and diabetic animals (8 weeks after streptozotocin administration). The concentration of [3H]-AdoMet was varied from 0.02 to 0.33 μM (site...
I), 1 to 25 μM (site II), and 50 to 250 μM (site III).

The data obtained are illustrated in Figures 4-6. A kinetic analysis of these data indicates that, in diabetes, at all three catalytic sites, the apparent affinity for AdoMet was unaltered. On the other hand, apparent V_max values for the diabetic preparations were significantly lower than control values (Figs. 4-6). Quantitatively, this loss of catalytic capacity was in the order of 30–40% for all three sites.

Inhibition of S-Adenosyl-L-Homocysteine

S-Adenosyl-L-homocysteine (AdoHcy), one of the products of AdoMet-dependent methyl transfer reactions, has been shown to be a potent and specific inhibitor of phospholipid methyltransferases in cell-free systems (Schanche et al., 1982). The specificity of total [3H]methyl incorporation into phospholipids of sarcolemmal membranes from both control and experimental groups was therefore tested by examining the inhibitory action of AdoHcy (Fig. 7). As reported earlier (Panagia et al., 1984), a different AdoHcy-inhibitory curve was obtained for each catalytic site involved in the transmethylation process in normal sarcolemma. Similar patterns of inhibition were observed for the diabetic group.

Discussion

Conversion of phosphatidylethanolamine to phosphatidylcholine in the plasma membrane is considered to occur through the S-adenosylmethionine-dependent N-methylation catalyzed by membrane-bound methyltransferases. Several studies have indicated that phosphatidylethanolamine N-methylation may be involved in cellular functions, such as regulation of biological signal transmission, control of Ca⁺⁺ influx, and activity of membrane-bound enzymes (cf Hirata and Axelrod, 1980; Mato and Alemany, 1983, for reviews). The present study, using sarcolemmal membranes isolated from ventricular muscle of the control and streptozotocin-induced diabetic rats, has demonstrated the existence of a depressed phospholipid N-methylation in sarcolemma from diabetic myocardium. Similar abnormality has been described in liver microsomes from alloxan-diabetic rats (Hoffman et al., 1981). The depression in N-methylating activity of cardiac sarcolemma appears to be of gradual onset during the disease process and is evident at all the three catalytic sites involved in the transfer reactions. Although complete reversal of this enzymatic alteration was obtained after 14 days of insulin therapy to diabetic animals, the possibility that hypothyroidism associated with the diabetic model (Penpargkul et al., 1980; Malhotra et al., 1981; Ganguly et al., 1983) could contribute to this sarcolemmal lesion cannot be excluded on the basis of the present results. However, it should be noted that experimental protocols which normalize plasma thy-
Effects of different concentrations of [3H]-AdoMet (50–250 μM) on total [3H]methyl groups incorporation into sarcolemmal phospholipids at catalytic site III. The double reciprocal plots (inset) indicate that the apparent Kₘ and Vₘₐₓ values are, control (○): Kₘ = 114 ± 6 μM, Vₘₐₓ = 250 ± 12 pmol/mg per 30 min; diabetic (●): Kₘ = 123 ± 9 μM, Vₘₐₓ = 175 ± 13* pmol/mg per 30 min. Assay conditions were as in Figure 3. Each point represents mean ± SE of three different experiments. *Significantly (P < 0.05) different from control.

We have observed an initial increase of the methylating activity at catalytic site I, followed by a depression at all sites involved in the process of phosphatidylethanolamine N-methylation in diabetic animals. Since plasma catecholamines have been shown to be elevated in diabetes (Christensen, 1974) and these hormones have been demonstrated to enhance the N-methylation activity in normal myocardium (Okumura et al., 1983), it is likely that the observed initial increase may be due to high level of circulating catecholamines in diabetic animals. Stimulation of β-adrenergic receptor in rat reticulocytes and hepatocytes has also been shown to increase phospholipid N-methylation (Hirata et al., 1979; Marin Cao et al., 1983). On the other hand, the observed depression in the phospholipid N-methylation activity at late stages of diabetes may be due to a defect in the adrenergic receptor mechanism in diabetic heart. Such a view is based on the fact that the number of β-adrenergic receptors in cardiac muscle has been shown to be reduced in chronic diabetes (Heyliger et al., 1982; Williams et al., 1983). It should also be pointed out that an increase in the number of β-adrenergic receptors has been shown to be associated with an increased phospholipid N-methylation in hypertrophied myocardium (Limas, 1980a). Since an increase in N-methylation activity by incubating cardiac membranes with S-adenosyl-L-methionine was shown to...
increase the number of adrenergic receptors, it was suggested that phospholipid N-methylation may be an important mechanism for regulation of β-adrenergic receptors (Limas, 1980a). In light of such observations, it appears that there is a close relationship between changes in phospholipid N-methylation and β-adrenergic mechanisms in myocardium, and these two processes influence each other. Thus, it is difficult to state whether the observed changes in phospholipid N-methylation in diabetic sarcolemma are due to an alteration in the β-adrenergic receptor system or vice versa.

The possibility that depressed phospholipid N-methylation in diabetic myocardium may be due to a diminished rate of biosynthesis of methyltransferases is suggested by the decrease in apparent $V_{\text{max}}$ values of the three catalytic sites. A similar explanation has also been offered for other sarcolemmal enzymes in aged myocardium (Awad and Chattopadhyay, 1983). It should also be noted that the sarcolemmal latent Na+,K+-ATPase activity was decreased (Pierce and Dhalla, 1983), whereas sarcolemmal Ca++-ATPase activity was increased (Dhalla et al., 1984) in chronic diabetic myocardium. Since the effect of phospholipid methyltransferase reaction on Na+,K+-ATPase activity has not yet been demonstrated, the significance of observed alterations in N-methylation cannot be appreciated with respect to changes in Na-K pump mechanism. On the other hand, experiments in our laboratory have revealed a depression in sarcolemmal Ca++-ATPase activity upon phospholipid N-methylation under in vitro conditions (Panagia et al., 1983). Therefore, it is possible that increased sarcolemmal Ca++-ATPase activity in chronic diabetes may be a consequence of the depression in phospholipid N-methylation observed in this study. In this regard, it may be noted that sarcolemmal Ca++-ATPase referred to here is activated by mM concentrations of Ca++ (Dhalla et al., 1981), and should not be confused with Ca++-stimulated Mg++-ATPase which requires $\mu$M concentrations of Ca++ in the presence of Mg++. It is considered to serve as a Ca++-pump mechanism. Sarcolemmal ATP-independent Ca++ binding has also been reported to decrease in chronic diabetic myocardium (Pierce et al., 1983), but this change is unlikely to be due to depressed phospholipid N-methylation, because Ca++ binding has been observed to decrease upon N-methylating the heart sarcolemma (Panagia et al., 1983). Thus, it appears that alteration in phosphatidylethanolamine N-methylation may be one of the mechanisms leading to abnormalities in heart sarcolemmal function in chronic diabetes.

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