Changes in cardiac protein kinase C activities and isozymes in streptozotocin-induced diabetes

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Liu, Xueiang, Jingwei Wang, Nobuakira Takeda, Luciano Binaglia, Vincenzo Panagia, and Naranjan S. Dhalla. Changes in cardiac protein kinase C activities and isozymes in streptozotocin-induced diabetes. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E798–E804, 1999.—To understand cardiac dysfunction in diabetes, the activity of protein kinase C (PKC) and protein contents of its isozymes (PKC-α, β, ε, and ζ) were examined in diabetic rats upon injection of streptozotocin (65 mg/kg iv). The hearts were removed at 1, 2, 4, and 8 wk, and some of the 6-wk diabetic animals had been injected with insulin (3 U/day) for 2 wk. The Ca2+-dependent PKC activity was increased by 43 and 51% in the homogenate fraction and 31 and 70% in the cytosolic fraction from the 4- and 8-wk diabetic hearts, respectively, in comparison with control values. The Ca2+-independent PKC activity was increased by 24 and 32% in the homogenate fraction and 52 and 89% in the cytosolic fraction from the 4- and 8-wk diabetic hearts, respectively, in comparison with control values. The relative protein contents of PKC-α, β, ε, and ζ isozymes were increased by 43, 31, 48, and 38%, respectively, in the homogenate fraction and by 126, 119, 148, and 129%, respectively, in the cytosolic fraction of the 8-wk diabetic heart. The observed changes in heart homogenate and cytosolic fractions were partially reversible upon treatment of the diabetic rats with insulin. The results suggest that the increased myocardial PKC activity and increased protein contents of the cytosolic PKC isozymes are associated with subcellular alterations and cardiac dysfunction in the diabetic heart.

myofibrillar ATPase (24) and sarcoplasmic reticular Ca2+ pump (28) activities, it is possible that subcellular changes in the diabetic heart may be due to alterations in the PKC activity and/or PKC-mediated signal transduction mechanism. This view is consistent with observations that diabetes is associated with translocation of the ε-isoformal PKC from cytosolic to particulate fraction of cardiomyocytes, and this change was prevented by the blockade of angiotensin II receptors, which are known to stimulate the PKC activity (21). Furthermore, increased phosphorylation of troponin-I in the diabetic heart has been considered to be due to the activation of PKC (18, 21). In fact, the concentration of diacylglycerol, which is known to activate PKC, was increased in the diabetic heart (25). However, the results with respect to changes in the PKC activity in the diabetic heart are conflicting. In this regard, Xiang and McNeill (32) have reported an increase and a decrease in cardiac PKC activities in particulate and cytosolic fractions, respectively, from diabetic rats. On the other hand, an increase of PKC activity in both particulate and cytosolic fractions from the diabetic heart was observed by Tanaka et al. (30). In view of such contradictory findings and no information regarding changes in the PKC activity in heart homogenate, this study was undertaken to examine PKC activities in the homogenate, cytosolic, and particulate fractions from the 1-, 2-, 4-, and 8-wk diabetic hearts. Because PKC is expressed in rat heart in different isoforms such as α, β, ε, and ζ isoforms (2, 13), it was also the purpose of this investigation to examine changes in the contents of different PKC isozymes in cardiac homogenate, cytosolic, and particulate fractions in diabetes induced by streptozotocin in rats for a period of 8 wk. The effects of insulin on changes in cardiac PKC activities and PKC isozyme contents were studied by treating the 6-wk diabetic rats with insulin for a period of 2 wk.

MATERIALS AND METHODS

Experimental model. Male Sprague-Dawley rats weighing ~175–200 g were randomly separated into control and experimental groups. The experimental animals received an intravenous injection of 0.1 M citrate-buffered streptozotocin (pH 4.5) at a dosage of 65 mg/kg body wt. Control animals received a similar injection of the vehicle alone. These animals were maintained on normal rat chow and water ad libitum and then killed by decapitation at 1, 2, 4, and 8 wk. In some experiments, randomly selected diabetic animals at 6 wk after streptozotocin injection were given subcutaneous injec-
sections of 3 U Humulin U/day for 2 wk and were labeled as the insulin-treated group. It is pointed out that Humulin U (Eli Lilly Canada, Toronto, ON) is a long-acting insulin with a slower onset of action in comparison with the regular insulin. To assess the control of diabetic state by insulin, the blood samples were obtained from the tail vein twice a week in the morning before insulin was injected, and the glucose concentration was measured with a blood-glucose meter (Lifescan Canada, Burnaby, BC, Canada). The blood from the decapitated animals was collected in heparinized tubes and centrifuged at 1,000 g for 10 min in order to obtain plasma. The heart was dissected out immediately and perfused for 10 min with ice-cold Krebs-Ringer phosphate buffer containing 5 mM glucose to remove blood. The atria and large vessels were minced in 1 ml of 50 mM Tris·HCl (pH 7.5). Each of these columns was washed twice with 5 ml of buffer B containing 50 mM Tris-HCl, 10 mM EGTA, 5 mM EDTA, 0.3% 2-mercaptoethanol (wt/vol), and 200 U/ml aprotinin (pH 7.5). The enzyme was finally eluted with 1 ml of buffer B containing 200 mM NaCl.

Assay of PKC activity. Unless otherwise mentioned in the text, okadaic acid was used in PKC activity assay in small samples of nonpurified homogenate, cytosolic, and particulate fractions from ventricular tissue (3). It should be mentioned that okadaic acid is a specific inhibitor of type 1 and type 2A phosphatases and is thus valuable in measuring protein phosphorylation due to protein kinases (1). The Ca2+-dependent PKC activity was determined with a PKC assay kit (Upstate Biotechnology, Lake Placid, NY) in the reaction buffer containing 20 mM MOPS, pH 7.2, 25 mM β-glycerol phosphate, 1 mM sodium orthovanadate, 1 mM dithiothreitol, and 4 mM CaCl2. Substrate cocktail containing 500 μM PKC substrate peptide in buffer C, inhibitor cocktail containing 2 μM protein kinase A inhibitor peptide in buffer C, and lipid activator containing 0.5 mg/ml phosphatidylserine and 0.05 mg/ml diglyceride in buffer C was used. Each assay tube contained substrate (10 μl), inhibitor (10 μl), lipid activator (10 μl), enzyme preparation (10 μl), and 26 μM okadaic acid (1 μl). The reactions for both Ca2+-dependent and Ca2+-independent PKC activities were initiated by the addition of [γ-32P]ATP (10 μl) and allowed to proceed at 30°C for 10 min. [γ-32P]ATP was prepared by mixing one part of ~3,000 Ci/mmol [γ-32P]ATP and nine parts of MgATP (75 mM MgCl2 and 500 μM MgATP either in buffer C or in buffer D). The reaction mixture (25 μl) was placed on the P81 phosphocellulose paper for 30 s, and this paper was then washed three times (5 min each time) with 0.75% phosphoric acid and once with acetone. The papers were placed in scintillation vials, and the radioactivity was counted in a scintillation counter.

The incorporation of 32P from γ-32P into the synthesized substrates, which are specific substrates with respect to Ca2+-dependent and -independent PKC, was measured (17, 23). Ca2+-dependent PKC activities in the partially purified homogenate and cytosolic and particulate samples were measured in the absence of okadaic acid in the assay medium.

Analysis of PKC isozyme contents. The relative contents of α-, β-, ε-, and ζ-isozymes in the homogenate, cytosolic, and particulate fractions were measured by 8% mini-SDS-PAGE and Western blot analysis. The concentration of protein in each fraction was adjusted to 1 mg/ml with the homogenizing buffer (buffer A or buffer A with 1% Triton-100). The sample loads for control, experimental, and insulin-treated groups were of the same volumes (10 μl in each well). The proteins thus separated by SDS-PAGE were electroblotted to the Immobilon-P transfer membrane (Millipore, Bedford, MA) for the determination of relative protein contents by immunoblotting. Primary binding of PKC isozyme-specific antibody was detected by with anti-rabbit IgG (1:1,000 for PKC-α, β, ε, and ζ-isozymes; Life Technologies, Burlington, ON) conjugated with horseradish peroxidase (1: 5,000, Amersham). For
chemiluminescent detection, the electrochemiluminescence system (Amersham) was employed according to the instructions of the manufacturer. The relative contents of PKC isozymes were determined by the model GS-670 imaging densitometer (Bio-Rad, Hercules, CA) with the image analysis software version 1.0. The contents of PKC isozymes were also measured by the employment of the partially purified homogenate, cytosolic, and particulate samples.

Data analysis. Data are expressed as means ± SE. The differences among different groups were evaluated statistically by one-way ANOVA followed by the Newman-Keuls test. A P value < 0.05 was taken to represent a significant difference.

RESULTS

General characteristics of diabetic and insulin-treated animals. In comparison with the control animals, the body weight and ventricular growth were significantly decreased, whereas the ventricular-to-body weight ratio was increased in rats 8 wk after the streptozotocin injection (Table 1). The plasma glucose concentration was increased markedly, and the plasma insulin level in diabetic animals was depressed compared with control values. Daily injections of insulin to the 6-wk diabetic animals for 2 wk normalized the plasma glucose and insulin concentrations as well as the ventricular weight and the ratio of ventricular to body weight; however, the insulin-treated diabetic rats still had lower body weight. Diabetic rats exhibited a lower body weight; however, the insulin-treated diabetic rats at different times of diabetes induction. A: Ca\(^{2+}\)-dependent PKC activity. B: Ca\(^{2+}\)-independent PKC activity. D, diabetes; I, insulin-treated; w, week; Pi, inorganic phosphate. Values are means ± SE of 6 animals in each group. *Significantly different from control (P < 0.05). #Significantly different from diabetic (P < 0.05).

Cardiac PKC activities. PKC activities were measured in the homogenate, cytosolic, and particulate fractions of control, diabetic, and insulin-treated diabetic hearts (Fig. 1). As shown in Fig. 1A, the Ca\(^{2+}\)-dependent PKC activities were increased by 43 and 51% in the homogenate fraction and 31 and 70% in the cytosolic fraction from the 4- and 8-wk diabetic hearts, respectively, in comparison with control values. There were no significant changes in the Ca\(^{2+}\)-dependent PKC activity in all fractions from the 1- and 2-wk diabetic hearts and particulate fractions from all diabetic hearts. The Ca\(^{2+}\)-independent PKC activity was increased by 32% in homogenate and 89% in the cytosolic fraction from the 4- and 8-wk diabetic hearts in comparison with control values, respectively (Fig. 1B). No significant changes in Ca\(^{2+}\)-independent PKC activity were found in all fractions from 1- and 2-wk diabetic hearts and the particulate fractions from all diabetic hearts (Fig. 1B). Compared with 8-wk diabetic hearts, the Ca\(^{2+}\)-dependent PKC activity in the insulin-treated group was decreased by 19 and 18%, whereas the Ca\(^{2+}\)-independent PKC activity in the insulin-treated group was decreased by 15 and 33%, in the homogenate and cytosolic fractions, respectively. However, these values were still higher (by 23% in the homogenate fraction and 39% in the cytosolic fraction for the Ca\(^{2+}\)-dependent PKC activity and by 14% in the homogenate fraction and 28% in cytosolic fraction for the Ca\(^{2+}\)-independent PKC activity) than the control values (Fig. 1). To examine if the changes in PKC activities observed in the diabetic homogenate and cytosolic fractions are due to any alterations in the protein concentrations of these fractions, the yield of proteins in different fractions was determined. The results shown in Table 2 indicate no difference in

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic+Insulin</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>554±22.5</td>
<td>307±16(^a)</td>
<td>369±25(^a)</td>
</tr>
<tr>
<td>Ventricular wt, mg</td>
<td>1,081±95</td>
<td>852±37(^#)</td>
<td>922±26(^#)</td>
</tr>
<tr>
<td>Ventricular/body wt, mg/g</td>
<td>2.01±0.06</td>
<td>2.78±0.005(^*)</td>
<td>2.55±0.006(^*)</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>161±9.5</td>
<td>496±5.3(^*)</td>
<td>183±6.2(^*)</td>
</tr>
<tr>
<td>Plasma insulin, µU/ml</td>
<td>29.2±3</td>
<td>125±0.9(^*)</td>
<td>35±1.4(^*)</td>
</tr>
<tr>
<td>+dP/dt, mmHg</td>
<td>5,620±250</td>
<td>3,575±214(^*)</td>
<td>4,792±258(^*)</td>
</tr>
<tr>
<td>-dP/dt, mmHg</td>
<td>5,496±124</td>
<td>3,254±218(^*)</td>
<td>4,583±130(^*)</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 animals in each group. Ventricular/body wt, ventricular-to-body wt ratio; +dP/dt, rate of pressure development; -dP/dt, rate of pressure fall. *Significantly different from control (P < 0.05); †significantly different from diabetic (P < 0.05).
Protein concentrations in the homogenate, particulate, or cytosolic fractions obtained from control, diabetic, and insulin-treated diabetic hearts.

Relative protein content of PKC isozymes. The relative protein contents of PKC-α, β, ε, and ζ isozymes in homogenate, cytosolic, and particulate fractions of the cardiac muscle from control, 8-wk diabetic, and insulin-treated diabetic rats were identified by Western blotting. The typical bands representing of PKC-α, β, ε, and ζ isozymes in these fractions of rat hearts are shown in Figs. 2 and 3. Polyclonal antibodies to PKC isozymes detected proteins at 76 kDa for α-isozyme, 77 kDa for β-isozyme, 83 kDa for ε-isozyme, and 67 kDa for ζ-isozyme. There was a nonspecific band at ~80 kDa below the bands for ε-isozyme in Figs. 2 and 3; however, in view of the fact that its identity is unknown, this band was not included in the densitometric analysis. The densitometric analysis of bands for PKC-α, β, ε, and ζ isozymes revealed a significant increase in relative protein contents in the diabetic homogenate fraction by 43, 31, 48, and 38%, respectively, and in the cytosolic fraction by 126, 119, 148, and 129%, respectively, in comparison with the control values, respectively (Figs. 4 and 5). Insulin administration to diabetic rats decreased protein contents for PKC-α, β, ε, and ζ isozymes by 19, 23, 14, and 19%, respectively, in the homogenate fraction (Fig. 4) and by 29, 35, 48, and 51%, respectively, in the cytosolic fraction when compared with diabetic hearts, respectively (Fig. 5). There were no significant alterations in PKC isozyme protein contents of the particulate fractions from the 8-wk diabetic rat heart compared with values from the control or insulin-treated diabetic hearts (Figs. 4 and 5).

Table 2. Protein concentration per unit of heart tissue in homogenate, particulate and cytosolic fractions isolated from control, diabetic, and insulin-treated diabetic rat hearts

<table>
<thead>
<tr>
<th>Protein Concentration, µg/mg heart tissue</th>
<th>Homogenate</th>
<th>Cytosolic</th>
<th>Particulate</th>
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<tbody>
<tr>
<td>Control</td>
<td>52.11 ± 4.30</td>
<td>26.11 ± 1.32</td>
<td>19.67 ± 1.84</td>
</tr>
<tr>
<td>Diabetic</td>
<td>53.28 ± 1.98</td>
<td>24.39 ± 3.49</td>
<td>19.48 ± 1.65</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>51.63 ± 4.71</td>
<td>25.21 ± 0.76</td>
<td>19.39 ± 0.75</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 animals in each group.

Fig. 2. Typical immunoblots for PKC-α, β, ε, and ζ isozymes in homogenate fractions from control, diabetic, and insulin-treated diabetic rat hearts at 8 wk after induction of diabetes.

Fig. 3. Typical immunoblots for PKC-α, β, ε, and ζ isozymes in cytosolic (C) and particulate fractions (P) from control, diabetic, and insulin-treated diabetic rat hearts at 8 wk after induction of diabetes.

Fig. 4. Densitometric analysis of results for relative protein contents of protein kinase C-α (A), β (B), ε (C), and ζ (D) isozymes in homogenate fraction of control, diabetic, and insulin-treated diabetic rat hearts at 8 wk after induction of diabetes. Values are means ± SE of 6 animals in each group. *Significantly different from control (P < 0.05). #Significantly different from diabetic (P < 0.05).
preliminary experiments with two preparations from 4-wk diabetic animals, PKC-\(a\), \(-b\), \(-e\), and \(-\zeta\) isozymes in both homogenate and cytosolic fractions, unlike the particulate fraction, were increased by 15–50% of the respective control values.

PKC activities and isozyme contents in partially purified preparations. \(Ca^{2+}\)-dependent PKC activities (in the absence of okadaic acid) and isozyme contents were also measured in the partially purified homogenate, cytosolic, and particulate preparations. The results in Fig. 6 show an increase in PKC activities in the homogenate and cytosolic fractions from the 8-wk diabetic heart without any significant change in the particulate fraction. Furthermore, treatment of diabetic animals with insulin significantly reduced the PKC activities in both homogenate and cytosolic fractions toward the control levels. Increases in protein contents of PKC-\(a\), \(-b\), \(-e\), and \(-\zeta\) isozymes in the purified homogenate and cytosolic fractions, unlike the particulate fraction, were significantly attenuated upon treatment of the animals with insulin (Table 3). The pattern of changes in PKC isozymes observed in partially purified fractions from the diabetic heart was essentially similar to that observed with unpurified fractions (Figs. 4 and 5).

DISCUSSION

In this study, we observed an increase in the \(Ca^{2+}\)-dependent and -independent PKC activities in the ventricular homogenate fraction obtained from the 4- and 8-wk diabetic rats. Such an increase in the enzyme activity seems to depend on the duration of diabetes because no change in the \(Ca^{2+}\)-dependent and \(Ca^{2+}\)-independent PKC activities was evident in hearts from 1- and 2-wk diabetic rats. Because the observed increase in the PKC activities in the diabetic heart homogenate fraction was associated with an increase in the PKC activity in the cytosolic fraction without any change in the particulate fraction, it is unlikely that these changes in cardiac PKC activities are due to translocation of the enzyme from the particulate to the cytosolic fractions. This view is supported by the observation that the protein concentrations of cytosolic fraction or particulate fraction from the diabetic heart were not different from those from the control hearts. Although an increase in cardiac PKC activity in both

Table 3. Relative protein content of PKC isozymes in partially purified homogenate, cytosolic, and particulate fractions of the 8-wk diabetic and insulin-treated diabetic hearts

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Homogenate</th>
<th>Cytosolic</th>
<th>Particulate</th>
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<tbody>
<tr>
<td>PKC-(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>142 ± 13.1</td>
<td>227 ± 13.9</td>
<td>85 ± 8.2</td>
</tr>
<tr>
<td>Diabetes + insulin</td>
<td>115 ± 8.0</td>
<td>160 ± 4.2</td>
<td>102 ± 8.3</td>
</tr>
<tr>
<td>PKC-(\beta)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>130 ± 11.5</td>
<td>239 ± 15.6</td>
<td>127 ± 27.5</td>
</tr>
<tr>
<td>Diabetes + insulin</td>
<td>101 ± 5.8</td>
<td>184 ± 7.6</td>
<td>91 ± 10.8</td>
</tr>
<tr>
<td>PKC-(e)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>148 ± 12.1</td>
<td>268 ± 13.3</td>
<td>118 ± 13.1</td>
</tr>
<tr>
<td>Diabetes + insulin</td>
<td>128 ± 8.2</td>
<td>194 ± 11.5</td>
<td>85 ± 11.9</td>
</tr>
<tr>
<td>PKC-(\zeta)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>159 ± 11.8</td>
<td>252 ± 10.5</td>
<td>88 ± 9.1</td>
</tr>
<tr>
<td>Diabetes + insulin</td>
<td>122 ± 8.8</td>
<td>185 ± 149</td>
<td>114 ± 5.2</td>
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Values are means ± SE of 6 animals in each group. *Significantly different from control (P < 0.05); †significantly different from diabetic (P < 0.05).
cytosolic and particulate fractions in 10-wk diabetic rats has been observed by some investigators (30), others (32) have reported a decrease and an increase in the PKC activity in cardiac cytosolic and particulate fractions, respectively, from 6-wk diabetic animals. These differences in the results for the PKC activities in the cytosolic and particulate fractions of the diabetic heart as seen in the present and previous studies may be due to differences in the intensity and duration of diabetes, methods for the preparation of cytosolic and particulate fractions, methods for the extraction of the enzyme, and procedures employed for the assay of PKC activities. In this regard, it should be noted that Xiang and McNeill (32), unlike the present and other studies (30), employed histone H-1 protein, a nonspecific substrate for assaying the PKC activity. Because previous investigators (30, 32) did not measure the PKC activity in the homogenate fraction, it is difficult to evaluate their results in terms of the status of PKC activities in the diabetic heart. Furthermore, a decrease in the PKC activity in the cytosolic fraction without any significant alterations in the particulate fraction and an impaired translocation of PKC were observed in mononuclear cells from poorly controlled diabetic patients (22). Nonetheless, the observed changes in cardiac PKC activities in diabetes were not due to any artifact because a similar pattern of changes in the enzyme activity was seen in a separate set of experiments in which partially purified samples were employed. Furthermore, treatment of 6-wk diabetic animals for 2 wk with insulin was found to partially reverse the elevated levels of PKC activity in both homogenate and cytosolic fractions without any change in the particulate fraction of the diabetic heart.

The increased PKC activities in the homogenate and cytosolic fractions were associated with corresponding increases in the contents of PKC-α, β, ε, and ζ isoforms, whereas neither the PKC activities nor the content of PKC isozymes was altered in the particulate fraction of the diabetic heart. It thus appears that the observed increase in cardiac PKC activities may be due to an increase in protein contents of different PKC isozymes in the cytosolic fraction. Whether such an increase in different PKC isozymes in the diabetic heart is a consequence of an increase in gene expression or some other molecular mechanism remains to be investigated. Because the increases in different PKC isozyme contents varied from 30 to 59% in homogenate fraction and from 127 to 168% in the cytosolic fraction from the diabetic heart, it is possible that insulin deficiency may affect the genes specific for these isozymes in a differential manner. It should be noted that PKC-α, β, ε, and ζ isoforms have also been reported to increase in the diabetic liver (4), whereas PKC-α, β, ε, and ζ isoforms have been shown to increase in both the aorta and heart from diabetic rats (12, 15, 21). Although Malhotra et al. (21) observed translocation of ε-isozyme of PKC from the cytosolic to the particulate fraction without any change in the β-isozyme in the diabetic cardiomyocytes, these findings should not be compared with the observations reported in this study. The reasons for this view are based on the fact that these investigators (21) employed 3- to 4-wk diabetic rats, whereas we have used rats at 4 and 8 wk after the induction of diabetes. Furthermore, Malhotra et al. separated the cytosolic fraction and particulate fractions by centrifugation at 25,000 g for 30 min, whereas the present experiments were carried out by employing centrifugation at 105,000 g for 60 min.

Because the increase in the contents of cardiac PKC isozymes as well as the elevated plasma levels of glucose were partially or fully reversible upon the treatment of diabetic animals with insulin, the observed increase in PKC isozymes may be due to an increase in the plasma glucose level. In this regard, it should be noted that glucose has been shown to modulate the PKC isozymes and PKC activities in different types of cells (14, 15, 31); however, it is unlikely that the increased PKC activity and contents of PKC isozymes in the diabetic heart are entirely due to alterations in plasma glucose in diabetes. This contention is substantiated by the fact that treatments of diabetic animals with verapamil or an angiotensin II receptor blocker, L-158,809, which does not affect the plasma level of glucose, reversed the increased cardiac PKC activities in both cytosolic and particulate fractions (30) and changes in the contents of cardiac PKC ε-isozyme in the particulate and cytosolic fractions (21), respectively. Although a number of receptor systems including angiotensin II and α1-adrenergic receptors have been reported to be coupled with PKC enzyme (9), the exact role of these receptor mechanisms in increasing the contents of PKC isozymes in the cytosolic fraction of the diabetic heart remains to be investigated.

It has been reported that the activated PKC exerts a negative inotropic effect in the heart at the level of the contractile apparatus by phosphorylation of troponin I and troponin T and subsequent inhibition of the myofibrillar ATPase activities (24). Another mechanism, which has been suggested to account for this cardiodepression, concerns the phosphorylation of phospholamban by PKC and subsequent inhibition of Ca2+ transport by the sarcoplasmic reticulum (28). In view of the inhibitory effects of PKC on myofibrillar ATPase and sarcoplasmic reticular Ca2+ transport, the increased cardiac PKC activity as well as contents of different PKC isozymes in the cytosolic fraction can be seen to contribute toward the depression in the rate of contraction as well as the rate of relaxation of the diabetic heart. It should be noted that heart dysfunction in this experimental model has been reported to occur at 4 and 8 wk of induction of diabetes (11); this time coincides with the time of PKC changes observed here. PKC ε-isozyme, a predominant isoform in cardiomyocytes (2, 29), has been shown to be associated with sarcomeres upon activation (7) and to be responsible for the phosphorylation of troponin I (21, 24). On the other hand, PKC β-isof orm has been shown to stimulate the promoter of β-myosin heavy chain in the myocardium (16). However, further evidence is needed to demonstrate whether increased PKC isoforms are associated with increased phosphorylation of troponin I and marked
changes in the myosin isozyme composition in the diabetic heart (6, 20). Because the role of α- and ζ isoforms of PKC in changing the characteristics of any specific subcellular or metabolic site has not yet been established, it is difficult to speculate the exact functional significance of the increased contents of these PKC isoforms in the diabetic heart. However, sustained increase in the contents of different PKC isoforms in the cytosolic fraction and subsequent increase in PKC activity in the diabetic heart may reflect signal transduction abnormalities and Ca²⁺ handling defects in cardiomyocytes during the development of heart dysfunction in chronic diabetes (5, 10).

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