Mechanisms underlying depressed Na\(^+\)/Ca\(^{2+}\) exchanger activity in the diabetic heart

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Abstract

Objectives: Depression in Na\(^+\)/Ca\(^{2+}\) exchanger activity is an important factor in the development of the diabetic cardiomyopathy. Since the mechanism underlying this depression remains unknown, the aim of this study was to determine the contribution of hyperglycemia and insulinopenia towards the observed impairment in Na\(^+\)/Ca\(^{2+}\) exchanger activity. Methods: Non-insulin-dependent diabetes was induced in neonatal Wistar rats by injection of 90 mg/kg streptozotocin. Na\(^+\)/Ca\(^{2+}\) exchange in sarcolemmal vesicles and isolated cardiomyocytes was determined by Na\(^+\)-dependent 45Ca\(^{2+}\) transport. To assess the role of insulin deficiency and hyperglycemia on Na\(^+\)/Ca\(^{2+}\) exchanger activity, neonatal cardiomyocytes were incubated for 3 days in media containing either 5 mM glucose and 56 \(U/l\) insulin Control, 30 mM glucose and 56 \(U/l\) insulin High glucose or 5 mM glucose and 0 insulin Insulin deficiency. Since hyperglycemia has been shown to affect protein kinase C activity, Ca\(^{2+}\)-dependent isoforms of protein kinase C were examined in non-diabetic and diabetic heart using hydroxylapatite chromatography. Also examined was Na\(^+\)/Ca\(^{2+}\) exchanger mRNA levels in diabetic and non-diabetic hearts using Northern slot blot analysis. Results: Acute insulin produced a dose-dependent increase in Na\(^+\)/Ca\(^{2+}\) exchanger activity, which was dramatically attenuated in diabetic membrane. Myocytes incubated in media containing 30 mM glucose exhibited a 33% reduction in Na\(^+\)/Ca\(^{2+}\) exchanger activity, while insulinopenia reduced activity by 63%. Exchanger mRNA levels of the diabetic heart were normal; however, diabetes was associated with major changes in protein kinase C activity. Conclusions: Reduced Na\(^+\)/Ca\(^{2+}\) exchanger activity resulting from diabetes, hyperglycemia or insulinopenia may be related to changes in protein kinase C activity, but is not caused by altered expression of the transporter.

Keywords: Na\(^+\)/Ca\(^{2+}\) exchange; Diabetes; Protein kinase C; Cardiomyopathy; Rat, ventricular myocytes

1. Introduction

One of the important complications of insulin-dependent and non-insulin-dependent diabetes is the development of a cardiomyopathy characterized by abnormalities in both diastolic and systolic function [1–5]. According to human and experimental diabetes studies, the frequency of diastolic defects exceeds systolic abnormalities, leading some investigators to conclude that diastolic dysfunction precedes systolic dysfunction [3,6]. One of the most important diastolic defects of the diabetic heart is impaired relaxation [1–5]. Although there is some evidence that the relaxation defect may be caused by a change in Ca\(^{2+}\) sensitivity of the myofibrils [7], most investigators have attributed the abnormality to improper handling of Ca\(^{2+}\) by the myocyte [5,8].

In the myocardium the Na\(^+\)/Ca\(^{2+}\) exchanger and the sarcoplasmic reticular Ca\(^{2+}\) pump play dominant roles in the process of relaxation. In the normal, undiseased heart, the Na\(^+\)/Ca\(^{2+}\) exchanger accounts for about 10–30% of diastolic Ca\(^{2+}\) removal, with the rest largely attributed to the sarcoplasmic reticular Ca\(^{2+}\) pump [9]. However, in the diseased heart, an imbalance can develop between the two transporters, resulting in a change in the size of the intracellular Ca\(^{2+}\) pool. This occurs because Ca\(^{2+}\) taken up by the sarcoplasmic reticular Ca\(^{2+}\) pump remains within
the myocardium while Ca\textsuperscript{2+} removed from the cytoplasm by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger is extruded from the cell. Because the activity of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger is reduced while the sarcoplasmic reticular Ca\textsuperscript{2+} pump operates normally in the non-insulin-dependent diabetic heart, an elevation in [Ca\textsuperscript{2+}], occurs [10]. This effect is compounded by diabetes-mediated inhibition of the Na\textsuperscript{+} pump, which increases [Na\textsuperscript{+}], and further elevates [Ca\textsuperscript{2+}], by the actions of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. The net result of the elevation in [Ca\textsuperscript{2+}], is the development of defects in both diastolic and systolic function [5,11,12].

Despite the importance of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger defect in the non-insulin-dependent diabetic heart, the mechanism responsible for reduced exchanger activity has not been examined. As a first step in addressing the causes of the abnormality, the contribution of the two major determinants of diabetes-linked complications, hyperglycemia and insulinopenia, was examined. Glucose toxicity has been commonly attributed to either glycosylation of key proteins or activation of protein kinase C [13,14]. Insulin deficiency, on the other hand, has been linked to a series of phosphorylation–dephosphorylation steps resulting in changes in the activity and expression of several membrane transporters, as well as the modulation of the phospholipid bilayer [15]. In the present study, Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity was evaluated in isolated myocytes exposed chronically to either high glucose or medium lacking insulin. The data suggest that changes in protein phosphorylation may contribute to the decline in Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity.

2. Methods

2.1. Induction of diabetes

Non-insulin-dependent diabetes was produced as described previously [10,11]. Briefly, 2-day-old male Wistar rats were injected with 90 mg/kg streptozotocin while control rats were injected with a citrate buffer. As the diabetic rats aged, they became more glucose-intolerant and by the age of 9–12 months were severely insulin-resistant and glucose-intolerant. At the time of the experiment (ages 12–14 months) the diabetic rats’ fasting and non-fasting glucose levels were 132 ± 5 and 177 ± 18 mg/dl, respectively. One hour after a glucose challenge (2 g/kg i.p.), blood glucose levels rose to 500 mg/dl in the diabetic rats but between 200 and 300 mg/dl in the non-diabetic rats. Plasma insulin levels increased during the challenge from 3.2 to 19.1 ng/ml in the diabetic but only from 2.7 to 9.2 ng/ml in the non-diabetic.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.2. Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity of sarcolemmal vesicles

Enriched sarcolemmal vesicles were prepared from diabetic and age-matched non-diabetic rat hearts using the method of Pitts [16]. Based on standard membrane markers, contamination by mitochondria and sarcoplasmic reticulum was minimal. Ouabain-sensitive Na\textsuperscript{+}/K\textsuperscript{+} ATPase activity was 31.8 ± 2.8 and 16.5 ± 1.9 pmol P\textsubscript{i}/mg/h for non-diabetic and diabetic membrane, respectively. Activity of adenylate cyclase, another sarcolemmal enzyme marker, was identical in the two preparations (236 ± 21 and 255 ± 32 pmol cAMP/mg/min in non-diabetic and diabetic preparations, respectively). These sarcolemmal markers were concentrated approximately 12-fold relative to the homogenate while the purity factor for the mitochondrial marker, cytochrome c oxidase, was 0.3-fold. Oxalate facilitated and p-nitrophenyl phosphate supported Ca\textsuperscript{2+} accumulation, which is a measure of sarcoplasmic reticular function, accounted for about 5% of total 45Ca\textsuperscript{2+} uptake.

The sarcolemmal vesicles were assayed for Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity using a modification of the method of Reeves and Sutko [17]. Briefly, sarcolemmal vesicles were loaded with a sodium buffer containing 160 mM NaCl, 20 mM MOPS, 2 mM ATP, and 1 mM MgCl\textsubscript{2}. After a pre-incubation period of 5 min at 37°C, 10 U/l insulin or sodium buffer was added to the sarcolemmal vesicles. The membranes were then incubated for 10 min at 37°C. The exchange reaction was initiated by the addition of membrane (5–7 μg) to 500 μl of a potassium buffer containing 160 mM KCl, 20 mM MOPS, 30 μM [54CaCl\textsubscript{2}], and 5 μM valinomycin. For the calcium dependency studies, 10–80 μM [54CaCl\textsubscript{2}] was added to the initiation buffer. After 2 s, the reaction was terminated by the addition of 3 ml of ice-cold buffer containing 160 mM KCl, 20 mM MOPS, and 1 mM LaCl\textsubscript{3}. The filters were dried before counting for radioactivity. All data were corrected for non-specific binding, which represents 45Ca\textsuperscript{2+} uptake in the absence of a sodium gradient.

2.3. Northern slot blot analysis

Total RNA was isolated from diabetic and non-diabetic rat ventricles using RNA STAT 60. Poly(A\textsuperscript{+}) RNA was selected using affinity chromatography with oligo(dT) cellulose.

For Northern slot blot analysis, the method described by Boerth and Artman [18] was used. RNA samples were denatured with 1.2 M glyoxal (in phosphate buffer, pH 7.0) and serially diluted with ice-cold 10 × standard saline citrate (150 mM NaCl and 15 mM sodium citrate). After size fractionation on a 1% agarose gel, the samples were transferred to nylon under vacuum with 10 × standard saline citrate washes. After UV cross-linking, glyoxylation was reversed, and the blots were prehybridized. The mem-
branes were then hybridized with the $\text{Na}^+$/Ca$^{2+}$ exchanger probe that was random-prime-labeled with [32P]CTP. The exchanger probe contained a 1.35 kb EcoRI fragment of the guinea-pig cardiac $\text{Na}^+$/Ca$^{2+}$ exchanger (generously provided by Dr. K.D. Philipson, University of California, Los Angeles). After a high-stringency wash with 0.5 standard saline citrate buffer at 55°C, the blots were exposed to film using double-intensifying screens at −70°C. The amount of exchanger probe was normalized to the amount of hybridized oligo(dT).

2.4. $\text{Na}^+$/Ca$^{2+}$ exchanger activity of neonatal cardiomyocytes

Neonatal cardiomyocytes were isolated by enzymatic digestion using the method of McDermott and Morgan [19]. The cells were then plated in tissue culture flasks and incubated for 90 min to remove non-muscle cells. After the incubation period, cell number was determined with a Coulter Counter. The cells were then resuspended in minimum essential media containing 10% newborn calf serum and 0.1 mM 5-bromo-2-deoxyuridine. The cells were plated onto dishes precoated with 0.1% gelatin at a density of 2 $\times$ 10^5 cells/dish. In most studies, the cells were cultured for 3 days in serum-free media containing 56 U/ml transferrin, 30 nM insulin, and 0.25 mM ascorbate. For the insulinopenic study, the cells were incubated in the supplemented serum-free media lacking 56 U/ml insulin, while the cardiomyocytes were incubated in the supplemented, insulin-containing serum-free media containing either 5 or 30 mM glucose in the hyperglycemic study. In all of these study groups, synchronously beating myocytes containing normal morphology were evident. This is in accordance with previous studies [19,20], which showed that supplemented serum-free media supports growth of normal, synchronously beating neonatal cardiomyocytes.

$\text{Na}^+$/Ca$^{2+}$ exchange was assayed in the cardiomyocytes by measuring $\text{Na}^+$-dependent 45Ca$^{2+}$ efflux. This method has the limitation that $\text{Na}^+$-dependent processes, such as the $\text{Na}^+$ pump, may influence the $\text{Na}^+$-dependent 45Ca$^{2+}$ efflux reaction. However, the technique is more physiological than the use of isolated sarcolemmal vesicles. Not only does the technique employ isolated myocytes, but the reaction examined proceeds in the normal forward mode of the $\text{Na}^+$/Ca$^{2+}$ exchanger. In these studies, cardiomyocytes were loaded with 45Ca$^{2+}$ by incubating the cells for 45 min in a loading buffer containing 140 mM choline, 10 mM HEPES, 15 mM dextrose 1.2 mM MgSO_4, 1 mM KH$_2$PO$_4$, 3.8 mM KCl, 2.5 mM sodium pyruvate, 1.8 mM CaCl$_2$, and 40 $\mu$M 45Ca$^{2+}$. In some cases, the cells were incubated with 5 U/l insulin for the last 15 min of the 45 min 45Ca$^{2+}$ loading period. The exchange reaction was initiated by the addition of a loading buffer containing 10 mM NaCl. The low NaCl concentration was used in the reaction buffer to slow the reaction and insure linearity for 15 s. At various time points, the reaction was arrested by the addition of ice-cold buffer containing 20 mM MOPS, 160 mM KCl, and 1 mM LaCl$_3$. The plates were then rinsed 5 times with 2 ml of the arresting buffer. After adding 2 ml of the NaCl-containing loading buffer, the cells were scraped from the bottom of the dish. A 500 $\mu$l aliquot of the cell suspension was then counted for radioactivity. The results were normalized to protein. $\text{Na}^+$/Ca$^{2+}$ exchanger activity was determined from the rate of 45Ca$^{2+}$ efflux.

2.5. Protein kinase C distribution and activity

Individual hearts were minced and placed in 20 mM Tris buffer (pH 7.5) containing 0.25 M sucrose, 5 mM dithiothreitol, 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride and 0.5 mg/ml leupeptin. The samples were homogenized with three 15-s bursts using a Polytron homogenizer at setting 5. Crude sarcolemmal and the cytosolic fraction were prepared according to the method of Heyliger et al. [21]. The resulting pellet was suspended in 20 mM Tris buffer containing 0.25 M sucrose, 5 mM dithiothreitol, 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 0.5 mg/ml leupeptin and 1% Triton X-100. The Triton X-100 extract was centrifuged for 20 min at 20000 $\times$ g. The supernatant of the detergent extract and the cytosolic fraction were separately applied to a DEAE cellulose column (2.5 x 25 cm) pre-equilibrated with 20 mM Tris buffer (pH 7.5) containing 5 mM dithiothreitol, 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 1 $\mu$M cAMP, 20 mM KCl and 0.5 mg/ml leupeptin. The columns were washed with 100 ml of the equilibration buffer before eluting the enzyme with equilibration buffer supplemented with 170 mM KCl. Fractions were collected and assayed for protein kinase C activity and protein content. The protein-kinase-C-containing fractions of the cytosolic and detergent extract samples were separately pooled and dialyzed against 20 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM EGTA, 0.5 mM EDTA, 10% glycerol and 10 mM $\beta$-mercaptoethanol (referred to as ‘buffer I’). The two samples were then loaded onto a hydroxylapatite column (1.5 x 5 cm) equilibrated with buffer 1. After washing the columns overnight with buffer 1, the columns were eluted with 80 ml of buffer 1 containing a linear gradient of potassium phosphate (20-250 mM). Fractions (1.5 ml) were collected and assayed for protein kinase C activity using the method described by Allo and Schaffer [22]. Kosaka et al. [23] have identified elution peaks 1 and 2 from the hydroxylapatite column as PKC$_{\beta}$ and PKC$_{\alpha}$, respectively.

3. Results

Fig. 1 shows that insulin increases $\text{Na}^+$/Ca$^{2+}$ exchanger activity in non-diabetic sarcolemmal vesicles in a
dose-dependent manner, with maximal stimulation occurring at an insulin concentration of 5 U/ml. It also shifted the Ca$^{2+}$ dependence of the Na$^+$/Ca$^{2+}$ exchanger, reducing $K_m$ from 33 to 14 µM without altering $V_{max}$ (Fig. 2). These effects of insulin were dramatically attenuated in membrane obtained from non-insulin-dependent diabetic hearts (Figs. 1 and 2, Table 1). While 5 U/ml insulin reduced the $K_m$ for Ca$^{2+}$, 60% in the non-diabetic, the $K_m$ drop was only 25% in the diabetic. In addition, the diabetic condition was associated with a decrease in $V_{max}$ and an increase in $K_m$ for Ca$^{2+}$ (Table 1). Thus, a greater than 3-fold difference in sarcolemmal Na$^+$/Ca$^{2+}$ exchanger activity exists between the diabetic and non-diabetic at 80 µM Ca$^{2+}$ (6.3 ± 0.5 vs. 1.9 ± 0.3 nmol/mg/s).

Traditionally, hyperglycemia and either insulinopenia or insulin resistance are thought to mediate the effects of diabetes. However, the role of each condition in the development of diabetic complications is difficult to discern because they coexist in the diabetic animal. In order to examine the separate effects of hyperglycemia and insulinopenia on Na$^+$/Ca$^{2+}$ exchanger activity, neonatal cardiomyocytes were incubated with varying concentrations of glucose and insulin. Cardiomyocytes incubated with medium containing 56 U/ml insulin and 5 mM glucose for 3 days exhibited a Na$^+$/Ca$^{2+}$ exchanger rate of 0.42 ± 0.03 nmol/mg/s. By contrast, an elevation in the glucose

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**Table 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol/mg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic</td>
<td>33</td>
<td>7.7</td>
</tr>
<tr>
<td>Non-diabetic + 0 U/l insulin</td>
<td>14</td>
<td>7.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>67</td>
<td>4.4</td>
</tr>
<tr>
<td>Diabetic + 10 U/l insulin</td>
<td>50</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Fig. 2.** Ca$^{2+}$ dependence of the Na$^+$/Ca$^{2+}$ exchanger. Sarcolemma was isolated from nondiabetic and diabetic rat heart according to the procedure described in Section 2. The membrane vesicles were loaded with Na$^+$-containing buffer containing 2 mM ATP. Following a 10 min incubation with either 0 or 10 U/l insulin, the membrane was placed in the Na$^+$/Ca$^{2+}$ exchange assay buffer containing varying concentrations of $^{45}$Ca$^{2+}$. The rate of Na$^+$-dependent $^{45}$Ca$^{2+}$ accumulation was corrected for all Na$^+$-independent reactions. Values shown were obtained from the data in Figure 2.

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**Fig. 3.** Effect of high glucose on Na$^+$/Ca$^{2+}$ exchanger activity of the neonatal cardiomyocyte. Cardiomyocytes isolated from 2-day-old rat neonates were placed in medium containing 56 U/l insulin and either 5 mM (Control group) or 30 mM glucose (High glucose and High glucose + insulin groups). After 3 days, the cells were incubated for 15 min with buffer lacking (High glucose group) or containing 5 U/l insulin (High glucose + insulin group). The cells were then assayed for Na$^+$/Ca$^{2+}$ exchanger activity. Values shown represent the means ± s.e.m. of 4–6 preparations. The High glucose group contained significantly lower Na$^+$/Ca$^{2+}$ exchanger activity than either the Control or High glucose + insulin groups ($P < 0.05$).
The effect of insulinopenia on Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity was evaluated by incubating cardiomyocytes for 3 days with medium containing 5 mM glucose and supplemented with either 0 or 56 U/1 insulin. Cells exposed to buffer lacking insulin exhibited diminished Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity compared to those exposed to insulin. The effect of insulinopenia on Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity was evaluated by incubating cardiomyocytes for 3 days with medium containing 5 mM glucose and supplemented with either 0 or 56 U/1 insulin. Cells exposed to buffer lacking insulin exhibited diminished Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity compared to those exposed to insulin.
exchanger activity (Fig. 5). However, either chronic (56 U/l) or acute (5 U/l) administration of insulin was able to reverse this effect, suggesting that insulin resistance is not present in this insulopenia model.

Since insulin is known to affect the activity of certain transcription factors [24], it is reasonable to predict that insulin deficiency may affect Na\(^{+}/Ca^{2+}\) exchanger activity through a reduction in exchanger expression. To examine this possibility, a Northern slot blot analysis was carried out on RNA isolated from non-diabetic and diabetic hearts. Fig. 6 reveals no difference in exchanger mRNA levels between the non-diabetic and diabetic samples.

4. Discussion

The most significant finding of this study is that sarcolemmal Na\(^{+}/Ca^{2+}\) exchanger activity of isolated myocytes is reduced, not only by diabetes, but also by chronic exposure of the cells to medium containing either high glucose or normal glucose lacking insulin. This finding has particular relevance for the diabetic heart, although neither high glucose exposure nor insulin deficiency exactly duplicates the diabetic condition. While the treated myocytes and the non-insulin-dependent diabetic heart both exhibit reduced basal Na\(^{+}/Ca^{2+}\) exchanger activity [13,25], only the diabetic heart exhibits severe insulin resistance.

The identification of high glucose and insulin as regulators of the Na\(^{+}/Ca^{2+}\) exchanger adds to the growing list of agents affecting this transporter. Recently, we found that a series of effectors, which act through the G protein-coupling protein, serve as activators of the exchanger [26]. Since G\(_{q}\) proteins are coupled to phospholipase C activation, it is likely that the observed activation is caused by the formation of the second messengers, diacylglycerol and inositol triphosphate [27,28]. In addition to the G\(_{q}\)-coupled mechanism, changes in the phospholipid environment of the transporter have been discussed as an important determinant of exchanger activity.

More recently, changes in the expression of the Na\(^{+}/Ca^{2+}\) exchanger have been touted as an important regulator of Na\(^{+}/Ca^{2+}\) exchanger activity [18]. However, the present study argues against this mechanism as a cause of reduced Na\(^{+}/Ca^{2+}\) exchanger activity in the diabetic heart. Not only were mRNA levels of the Na\(^{+}/Ca^{2+}\) exchanger unaffected by diabetes (Fig. 6), but acute insulin treatment reversed the effects of high glucose exposure on Na\(^{+}/Ca^{2+}\) exchanger activity, an effect incompatible with a change in exchanger mRNA levels.

Two other mechanisms have been advanced to explain the effects of diabetes on Na\(^{+}/Ca^{2+}\) exchanger activity. Makino et al. [25] have argued that diabetes-mediated alterations in the phospholipid composition of the sarcolemmal membrane contributes to the decline in Na\(^{+}/Ca^{2+}\) exchanger activity. According to Pierce et al. [29], the major diabetes-induced sarcolemmal changes include a reduction in phosphatidylethanolamine and diphosphatidylglycerol content, an elevation in lyso phosphatidylincholine content and an alteration in the fatty acid profile of the phospholipid bilayer. Three of these changes would be expected to have the greatest effect on exchanger activity. First, the degree of fatty acid unsaturation increases in the diabetic heart, an effect known to depress Na\(^{+}/Ca^{2+}\) exchanger activity [30]. Second, diabetes is associated with an increase in the phosphatidylcholine/phosphatidylethanolamine ratio, which mediates localized changes in the structure of the phospholipid bilayer, thereby reducing Na\(^{+}/Ca^{2+}\) exchanger activity [31]. Third, the diabetes-mediated increase in lyso phosphatidylcholine content should also depress Na\(^{+}/Ca^{2+}\) exchanger activity [32].

The effect of chronic insulin deficiency on Na\(^{+}/Ca^{2+}\) exchanger activity may also be explained by alterations in phospholipid composition of the membrane. Insulin is known to activate several steps in phospholipid metabolism. In isolated sarcolemma, the net effect of insulin action is to increase the number of negatively charged phospholipids in the membrane [33]. This in turn alters the activity of several Ca\(^{2+}\) transporters.

The other putative regulatory mechanism influencing Na\(^{+}/Ca^{2+}\) exchanger activity in the diabetic heart is protein phosphorylation. Recently, Iwamoto et al. [27] have provided compelling evidence that the stimulation of Na\(^{+}/Ca^{2+}\) exchange by protein kinase C activation involves the phosphorylation of the cytoplasmic N-terminal domain of the transporter. The Iwamoto study supported an earlier investigation by our group showing that the activation of the Na\(^{+}/Ca^{2+}\) exchanger by a series of G\(_{q}\)-linked agonists was blocked by the protein kinase C inhibitor, chelerythrine [26]. Yet, the conclusions of these studies remain controversial, perhaps in part because of the existence of multiple protein kinase C isozymes, which differ in substrate specificity and mechanisms of regulation [34]. In the present study, we found that the activity of PKC\(_{\beta}\), is elevated in the non-insulin-dependent diabetic heart while that of PKC\(_{\beta}\) is reduced (Fig. 4). Since PKC\(_{\beta}\) appears to be closely tied to the actions of insulin [35], it is not surprising that insulin-mediated activation of the Na\(^{+}/Ca^{2+}\) exchanger is impaired in the diabetic heart. The reduction in PKC\(_{\beta}\) activity may also explain the reduction in basal Na\(^{+}/Ca^{2+}\) exchanger activity in the diabetic heart. Iwamoto et al. [27] have found that the Na\(^{+}/Ca^{2+}\) exchanger exists in a partially phosphorylated state in unstimulated cells. Therefore, it is logical to assume that a diabetes-mediated reduction in PKC\(_{\beta}\) activity will reduce both the degree of phosphorylation and the activity of the exchanger. On the other hand, an equally plausible conclusion is that changes in Na\(^{+}/Ca^{2+}\) exchanger activity lead to elevations in [Ca\(^{2+}\)]

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The primary defects causing these multiple changes in the diabetic heart have been classically attributed to either hyperglycemia or insulinopenia/insulin resistance. Previously it has been shown that high glucose exposure increases diacylglycerol biosynthesis and total protein kinase C activity in a broad range of cell types [14,36]. In the present study, we were interested in examining the effects of high glucose on Na\(^+\)/Ca\(^{2+}\) exchanger activity because of the link between diabetes, protein kinase C and hyperglycemia. We found that high glucose induces a reduction in Na\(^+\)/Ca\(^{2+}\) exchanger activity (Fig. 3). The most logical explanation for the glucose effect is the activation of a specific protein kinase C isozyme which counteracts the positive effects of insulin on Na\(^+\)/Ca\(^{2+}\) exchanger activity recently, Berti et al. [14] have shown that high glucose appears to activate several protein kinase C isoforms (PKC\(_{\alpha}\), PKC\(_{\beta}\), PKC\(_{\delta}\) and PKC\(_{\zeta}\)) in rat-1 fibroblasts. Since these protein kinase C isoforms also increase the rate of insulin receptor phosphorylation and reduce insulin receptor kinase activity, they serve as negative effectors of insulin action. One of the protein kinase C isoforms elevated by both high glucose and non-insulin-dependent diabetes is PKC\(_{\alpha}\) (Fig. 4a,b). Interestingly, overexpression of PKC\(_{\alpha}\) in Chinese hamster ovary cells decreases insulin responsiveness, suggesting that the \(\alpha\)-isofrom exhibits anti-insulin activity [37].

In conclusion, the reduction in Na\(^+\)/Ca\(^{2+}\) exchanger activity in the diabetic heart appears to be mediated by both hyperglycemia and insulinopenia. These effects may be caused by impaired translocation of PKC\(_{\beta}\) and/ or activation of PKC\(_{\alpha}\) in the diabetic heart, as well as changes in the phospholipid composition of the cell membrane. However, altered expression of the exchanger does not appear to be involved in the defect.

References

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