Effects of diabetes and hypertension on myocardial Na⁺–Ca²⁺ exchange

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Abstract: Abnormalities in cardiac function have been extensively documented in experimental and clinical diabetes. These aberrations are well known to be exaggerated when hypertension and diabetes co-exist. The objective of the present study was to examine whether alterations in the activity of the myocardial Na⁺–Ca²⁺ exchanger (NCX) can account for the deleterious effects of diabetes and (or) hypertension on the heart. To this aim, the following experimental groups were studied: (i) control; (ii) diabetic; (iii) hypertensive; and (iv) hypertensive–diabetic. Wistar rats served as the control group (C) while Wistar rats injected with streptozotocin (STZ, 55 mg/kg) served as the diabetic (D) group. Spontaneously hypertensive (SH) rats were used as the hypertensive group (H) while SH rats injected with STZ served as the hypertensive–diabetic (HD) group. Sarcolemma was isolated from the ventricles of the C, D, H, and HD groups and NCX activity was examined using rapid quenching techniques to study initial rates over a [Ca²⁺]₀ range of 10–160 µM. The Vₘₐₓ of NCX was lower in the D group when compared with the C group (D, 2.96 ± 0.26 vs. C, 4.0 ± 0.46 nmol·mgprot⁻¹·s⁻¹, P < 0.05), however combined diabetes and hypertension (HD) did not affect the Vₘₐₓ of NCX activity (HD, 3.84 ± 0.88 vs. H, 3.59 ± 0.24 nmol·mgprot⁻¹·s⁻¹, P > 0.05). However, analysis of the Kₘ values for Ca²⁺ indicated that both the D and HD groups exhibited a significantly lower Kₘ when compared with their respective control groups (D, 42 ± 4 vs. C, 56 ± 4 µM, P < 0.05; HD, 33 ± 7 vs. H, 51 ± 8 µM, P < 0.05). Immunoblotting using polyclonal antibodies (against canine cardiac NCX) exhibited the typical banding of 160, 120, and 70 kDa. The 120 kDa band is believed to represent the native exchanger with its post-translational modifications. Examination of the blots revealed a lower intensity of the 120 kDa band in the D group when compared with the C group, however, no significant difference in the HD group was observed. We speculate that the lower Vₘₐₓ in the D group may be due to a reduced concentration of exchanger protein in the membrane. The absence of this defect in the HD group may be a result of compensatory mechanisms to the overall hemodynamic overload, however, this remains to be determined. The increased affinity for Ca²⁺ in both the D and HD groups (determined by the lower Kₘ values) is an interesting finding and may be due to changes in sarcolemmal lipid bilayer composition secondary to diabetes-induced hyperlipidemia.

Key words: diabetes, hypertension, cardiac, Na⁺–Ca²⁺ exchange, contractility.

Résumé : Les anomalies de la fonction cardiaque au cours du diabète clinique et expérimental ont été largement documentées. On sait que ces aberrations sont amplifiées lorsque diabète et hypertension coexistent. La présente étude a eu pour but d’examiner si les altérations de l’activité de l’échangeur Na⁺–Ca²⁺ (NCX) myocardique peuvent expliquer les effets nocifs du diabète et/ou de l’hypertension sur le cœur. Les groupes expérimentaux suivants ont été examinés : (i) témoin, (ii) diabétique, (iii) hypertendu, et (iv) hypertendu–diabétique. Les groupes témoin (T) et diabétique (D) étaient composés de rats Wistar; le groupe diabétique (D) était constitué de rats ayant reçu une injection de streptozotocine (STZ, 55 mg/kg). Des rats spontanément hypertendus (SH) représentaient le groupe hypertendu (H), alors que des rats SH ayant reçu une injection de STZ formaient le groupe hypertendu–diabétique (HD). Le sarcolemme a été isolé des ventricules des groupes C, D, H et HD et l’activité NCX examinée au moyen de techniques d’extinction de fluorescence rapide pour étudier les taux initiaux sur un plage de [Ca²⁺]₀ de 10–160 µM. La Vₘₐₓ de NCX a été plus faible chez le groupe D que chez le groupe C (D : 2.96 ± 0.26 contre C : 4.0 ± 0.46 nmol·mgprot⁻¹·s⁻¹, P < 0.05), alors que le couplage hypertension-diabète (HD) n’a pas influé sur la Vₘₐₓ de l’activité NCX (HD : 3.84 ± 0.88 contre H : 3.59 ± 0.24 nmol·mgprot⁻¹·s⁻¹, P > 0.05). Toutefois, l’analyse des valeurs de Kₘ pour le Ca²⁺ a indiqué que les groupes D et HD ont eu un Kₘ significativement plus faible que celui de leur groupe témoin respectif (D : 42 ± 4 contre C : 56 ± 4 µM, P < 0.05; HD : 33 ± 7 contre H : 51 ± 8 µM, P < 0.05). L’immunotransfert à l’aide d’anticorps polyclonaux (contre le NCX cardiaque canin) a montré le patron de bandes typique de 160, 120 et 70 kDa. On croit que la bande de 120 kDa représente l’échangeur natif et ses modifications post-translationalles. L’examen des immunotransferts a révélé une intensité plus faible de la bande de 120 kDa chez le groupe D que chez...
Introduction

Cardiac complications remain the leading cause of increased mortality and morbidity in diabetic populations. Left ventricular systolic and diastolic dysfunction, lower stroke volume, and abnormalities in left ventricular compliance have been clearly shown to be associated with long-standing diabetes. Hearts isolated from experimental models of diabetes (streptozotocin (STZ) or alloxan induced) exhibit similar defects in cardiac function manifested in an inability of diabetic hearts to respond to increases in preload and afterload. Cardiac output, rate of relaxation, onset of relaxation, and velocity of muscle shortening have all been shown to be depressed in experimentally induced diabetic hearts (such as the STZ-induced diabetic rat) and repeatedly shown in clinical diabetes. The incidence of hypertension is higher in patients with diabetes. Furthermore, states of combined hypertension and diabetes are associated with more pronounced histologic, ultrastructural, and mechanical aberrations on the myocardium than those seen with diabetes alone. Although a variety of theories regarding the etiology of these defects have been proposed, the exact pathogenic and cellular mechanisms remain to be clearly delineated.

It is well established that Ca\(^{2+}\) that enters the cardiac cell via the L-type calcium channels during depolarization triggers the release of additional Ca\(^{2+}\) into the cytosol from the sarcoplasmic reticulum (SR, Fabiato 1983). During myocyte repolarization and relaxation, Ca\(^{2+}\) is pumped back into the SR by the Ca\(^{2+}\)-ATPase (SERCA2a) and also removed from the cell by the Na\(^{+}\)–Ca\(^{2+}\) exchanger (NCX1) and, perhaps, by the sarcolemmal Ca\(^{2+}\)-ATPase (PMCA). The NCX represents the primary mechanism of Ca\(^{2+}\) efflux across the sarcolemma and hence plays a pivotal role in myocyte relaxation (Bridge et al. 1988; Yao et al. 1998). Furthermore, recent studies indicate that the NCX may also contribute towards SR Ca\(^{2+}\) release (Leblanc and Hume 1990; Litwin et al. 1996; Litwin et al. 1998). Given the important role of the NCX in regulating myocyte contractility, it has been suggested that alterations in this exchanger may play a role in the development of diabetes-induced myocardial alterations. Indeed, NCX activity is depressed in hearts from STZ-induced diabetic rats, however, the exact mechanism of this effect remains unknown (Makino et al. 1987). Additionally, NCX activity in states of combined hypertension and diabetes remains to be determined, which was the objective of the present study. To this aim, we examined the effects of combined diabetes and hypertension on NCX activity. Wistar and Spontaneously Hypertensive (SH) rats served as the normotensive and hypertensive groups and diabetes was induced in these groups by a single tail vein injection of STZ. These models have been very well defined previously and closely parallel clinical observations.

Materials and methods

Experimental Design

Male Wistar and spontaneously hypertensive (SH) rats weighing between 200–225 g were purchased from Charles River, Montreal, Canada and were divided into control (C), diabetic (D), hypertensive (H), and hypertensive diabetes (HD) groups. All animals were treated humanely and were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. Diabetes was induced in the D and HD groups by a single tail vein injection of streptozotocin (55 mg/kg dissolved in 0.9% NaCl solution). C and H groups were injected with the vehicle alone. Rats were tested for diabetes three days after injection using the enzymatic test strip, Lily Tes-Tape\(^{6}\) to determine the presence of glycosuria (higher than 2%). Body weight, plasma glucose (Boehringer Mannheim Periodichrom\(^{8}\) GOD–PAP assay kit), and plasma insulin (Amersham RIA kit) were measured in all groups. Although blood pressure was not measured in this study, previous experiments (Rodrigues and McNeill 1986) from our group have indicated that both diabetic and non-diabetic SH rats exhibit marked elevations in systolic blood pressure. Following 6 weeks of diabetes, the rats were sacrificed and the hearts were excised for sarcolemmal isolation.

Sarcolemma (SL) Isolation

Sarcolemma was isolated as described previously (Shi et al. 1989) from pooled rat ventricles to provide a starting wet weight of at least 6.5 g. In C, D, and H groups, 6–7 hearts were required while in the HD group 12–14 hearts were required per isolation. At least four isolations of SL from each group were performed. SL vesicles from all groups were resuspended in a loading medium (LM) which contained 140 mM NaCl and 10 mM MOPS (pH 7.0 at 37°C). Protein concentration was determined in native sarcolemmal vesicles by the Bradford assay (Bradford 1976). The SL marker K\(^+-\)stimulated \(\rho\)-nitrophenylphosphatase (\(\mathrm{K}^+\)-NPPase) was used in order to determine SL yield, purification, and recovery, and was performed as previously described (Shi et al. 1989).

Na\(^+-\)Ca\(^{2+}\) Exchange

Sodium-dependent calcium uptake into SL vesicles was performed as previously described (Tibbits et al. 1989). In brief, 5 µL of Na\(^+\)-loaded sarcolemmal vesicles was suspended on the wall of a polystyrene tube containing 245 µL of uptake medium maintained at 37°C. The medium contained either 140 mM KCl or NaCl, 2 µCi \(^{45}\)Ca\(^{2+}\), 0.5 µM valinomycin, 10–160 µM \(^{40}\)Ca\(^{2+}\) and 10 mM MOPS (3-(N-morpholino) propanesulfonic acid; pH 7.0 at 37°C). The uptake was initiated by vortex mixing and quenched at 2 s by the addition of 30 µL of a solution containing 140 mM KCl and 1 mM LaCl\(_3\). A 220 µL aliquot was filtered (0.45 µm
Millipore) and the filters were washed with 3 mL of a rinsing solution (containing 140 mM KCl and 1 mM LaCl₃) twice. The filters were dried and then counted. The uptake by vesicles diluted into NaCl-represented blanks and was subtracted for all data points. This allowed for the correction of [Ca²⁺]₀ that is bound superficially to the sarcolemma or has permeated the vesicle by some pathway other than Na⁺–Ca²⁺ exchange. This value was not greater than 10% of that obtained with K⁺ dilution in any case.

**SDS–PAGE and Immunoblots**

SDS–PAGE was performed on 4.0 μg of SL protein from each group on a single gel of 7.5% polyacrylamide on a Pharmacia Phast (Baie d’Urfé, Que.) system. The proteins were transferred to nitrocellulose membranes electrophoretically and reacted with antibodies raised against the purified canine exchanger as described previously (Tibbits et al. 1992).

**Chemicals**

[Ca²⁺]₀ was obtained from New England Nuclear. All biochemicals were obtained from Sigma Chemicals Inc., and were of reagent grade.

**Statistics**

Data are expressed as mean ± s.e. Ca²⁺ uptake determinations (as a function of [Ca²⁺]₀) were analyzed by repeated measures ANOVA. All other measurements were analyzed by the Student’s t-test.

**Results**

As seen in Table 1, body weights and heart weights were both significantly lower in the two diabetic (D and HD) groups in comparison to their respective controls. In group D, body and heart weights were 79% and 85% of controls, respectively. In group HD, body and heart weights were 64% and 54% of controls, respectively. The characteristics of the sarcolemma isolated from the four groups are shown in Table 2. The SL fraction K⁺NPPase specific activity, percent recovery, and purification index were not significantly different between both diabetic groups in comparison to their respective controls. However, there was a significantly higher K⁺NPPase specific activity in both the H and HD groups when compared to the C and D groups. This difference was not reflected to the same degree in the crude homogenate: 0.57 ± 0.06, 0.58 ± 0.06, 0.70 ± 0.09, and 0.66 ± 0.07 μmol·mgprot⁻¹·h⁻¹ in the C, D, H, and HD groups, respectively.

Na⁺–Ca²⁺ exchange was measured as Na⁺-dependent [Ca²⁺]₀ uptake (in a 2-s period) in SL vesicles. In previous studies under similar conditions (Tibbits et al. 1989), we have determined that this time is within the period of linearity. The initial rates of uptake as a function of [Ca²⁺]₀ are shown in Fig. 1a for the C and D rats and Fig. 2a for the H and HD rats. As shown in Fig. 1a, the initial rates of Na⁺-dependent Ca²⁺ uptake were significantly lower in the D group when compared to control at 160 μM [Ca²⁺]₀. The Eadie-Hofstee plot of these data is shown in Fig. 1b. Kₘ (Ca²⁺) and Vₘₐₓ values are presented as the means of the derived values from the individual Eadie-Hofstee plots for each of the uptake experiments in Fig. 3 panels A and B, respectively. It can be seen that there are minor discrepancies between the Kₘ (Ca²⁺) and Vₘₐₓ derived from the Eadie-Hofstee plot of the pooled data and the pooled values from the individual plots. Both the Kₘ (Ca²⁺) (Fig. 3a) and Vₘₐₓ (Fig. 3b) of Na⁺–Ca²⁺ exchange are significantly lower in the D group when compared to the C.

The rates of uptake were higher in the HD group when compared to the H group at 30, 40, and 80 μM [Ca²⁺]₀ (Fig. 2a). Neither the initial rate at 160 μM [Ca²⁺]₀ (Fig. 2a) nor the Vₘₐₓ (Fig. 3b) were different in HD compared to H. The Kₘ (Ca²⁺) was significantly (P < 0.05) lower in the HD group (32.6 ± 6.8 μM) compared to H (51.0 ± 7.7 μM) group, as seen in Fig. 3a.

Immunoblots, using antibodies raised against the purified canine Na⁺–Ca²⁺ exchanger are shown in Fig. 4. The pattern of banding was similar in all groups, with bands appearing at 70, 120, and 160 kDa. This is consistent with data from

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**Table 1. Body and Heart Weights.**

<table>
<thead>
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<th>C 33</th>
<th>D 33</th>
<th>H 33</th>
<th>HD 62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>461±7</td>
<td>367±9</td>
<td>298±4</td>
<td>191±6</td>
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<tr>
<td>Heart weight (g)</td>
<td>1.17±0.02</td>
<td>0.99±0.02</td>
<td>1.04±0.03</td>
<td>0.57±0.02</td>
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<tr>
<td>Heart weight/body weight (mg/g)</td>
<td>2.47±0.08</td>
<td>2.69±0.09</td>
<td>3.48±0.09</td>
<td>3.20±0.10</td>
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</table>

**Note:** Values are mean ± s.e. N, number of animals; C, Wistar control; D, Wistar diabetic; H, spontaneously hypertensive control; HD, spontaneously hypertensive diabetic. ¹Significantly (P < 0.05) different from C. ²Significantly (P < 0.05) different from H.

**Table 2. Sarcolemmal characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>C 4</th>
<th>D 4</th>
<th>H 4</th>
<th>HD 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺NPPase (μmol·mgprot⁻¹·h⁻¹)</td>
<td>11.35±2.79</td>
<td>11.24±2.21</td>
<td>17.11±1.81</td>
<td>17.84±1.49</td>
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<tr>
<td>Recovery (%)</td>
<td>10.38±1.67</td>
<td>9.45±1.41</td>
<td>9.78±1.81</td>
<td>10.54±2.54</td>
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<tr>
<td>PI</td>
<td>19.78±2.52</td>
<td>19.50±3.27</td>
<td>24.43±3.38</td>
<td>27.20±3.73</td>
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</tbody>
</table>

**Note:** Values are mean ± s.e. N, number of isolations; C, Wistar control; D, Wistar diabetic; H, spontaneously hypertensive control; HD, spontaneously hypertensive diabetic. K⁺NPPase, the specific activity of the SL fraction. Recovery, the total K⁺NPPase activity in the SL fraction (the product of specific activity in μmol·mgprot⁻¹·h⁻¹ and total protein in SL fraction) expressed as a percentage of total K⁺NPPase activity in the crude homogenate. PI, the purification index defined as the ratio of the specific activity of K⁺NPPase in the SL fraction over that in the crude homogenate.
**Fig. 1.** (a) Initial rates of Na⁺-dependent Ca²⁺ uptake in sarcolemmal vesicles in control (open circles) and diabetic (closed circles) rats as a function of [Ca²⁺]₀. Each data point represents the mean of eight experiments using 4 different preparations for each group, pH 7.0, with reaction time 2 s and temperature 37°C. Error bars represent + (C) and – (D) SEM; * indicates $P < 0.05$. (b) Eadie-Hofstee plot of the data shown in (a). The drawn lines represent the least squares linear regression fit of data from control (open circles) and diabetic (closed circles) hearts. The derived $V_{max}$ values of Na⁺-Ca²⁺ exchange in the control and diabetic hearts were 4.00 ± 0.46 and 2.96 ± 0.26 nmol·mgprot⁻¹·s⁻¹, respectively. The derived $K_m$ (Ca²⁺) values of Na⁺–Ca²⁺ exchange in the control and diabetic hearts were 56 ± 4 and 42 ± 4 μM, respectively.

**Fig. 2.** (a) Initial rates of Na⁺-dependent Ca²⁺ uptake in sarcolemmal vesicles in H (open squares) and HD (closed squares) rats as a function of [Ca²⁺]₀. Each data point represents the mean of eight experiments using 4 different preparations for each group, pH 7.0, with reaction time 2 s and temperature 37°C. Error bars represent + (HD) and – (H) SEM; * indicates $P < 0.05$. (b) Eadie-Hofstee plot of the data shown in (a). The drawn lines represent the least squares linear regression fit of data from H (open squares) and HD (closed squares) hearts. The derived $V_{max}$ values of Na⁺–Ca²⁺ exchange in the H and HD hearts were 3.59 ± 0.24 and 3.84 ± 0.88 nmol·mg·prot⁻¹·s⁻¹, respectively. The derived $K_m$ (Ca²⁺) values of Na⁺–Ca²⁺ exchange in the HC and HD hearts were 51 ± 8 and 33 ± 7 μM, respectively.
other laboratories (Philipson et al. 1988). Two differences were apparent from the blots. First, the 120-kDa band intensity was decidedly weaker in group D in comparison to group C. Second, both the H and HD groups exhibited a more complex pattern of banding in the 120–130 kDa region.

Discussion

The significantly lower heart and body weights in both diabetic groups relative to their respective controls is typical of the diabetic state (Rodrigues and McNeill 1986; Rodrigues and McNeill 1992). The models of diabetes and hypertension used in this study have been well characterized by one of our laboratories (J.H. McNeill) in previous studies. In this model, the D and HD plasma glucose levels are typically increased by ~300% (19–26 mM) over controls from the same strain (Yu and McNeill 1991a; Jian et al. 1996) after 6 weeks. In addition, plasma insulin levels are decreased by ~50% (19–25 µU/mL) in both D and HD in this experimental paradigm. Systolic pressures in both H and HD are elevated by ~30% (150–165 mmHg) under these conditions in comparison to the Wistar controls (Jian et al. 1996).

The initial rates of Na⁺-dependent Ca²⁺ uptake in SL vesicles from C and D are shown in Fig. 1a. The rates of uptake were significantly greater in the control group compared to D at 160 µM Ca²⁺. The Eadie-Hofstee plot of these data is shown in Fig. 1b, and the data in Fig. 3b indicate that there was a significant reduction in the V_max of the exchanger in the D group. This finding confirms the observation by Makino et al. (1987). In their study, SL isolated from 6-week-old STZ-diabetic rats exhibited marked reduction (~50%) in Na⁺-dependent Ca²⁺ uptake compared to control rats. Similarly, Allo et al. (1991) found that Na⁺-dependent Ca²⁺ uptake initial rate was depressed by more than 50% at 30 µM [Ca²⁺]₀ in SL vesicles isolated from the hearts of male Wistar rats made diabetic 10 months previously by STX injection. Although the reduction observed in our study was substantially lower (~25%) than that observed in these other studies, this may be due to differences in animal strain, model of diabetes (compared to Allo et al. 1991), vesicular orientation and (or) quenching times.

The physiological (and pathophysiological) significance of the decreased NCX in the diabetic heart remains elusive. In both isolated heart tissue (Yu and McNeill 1991b) and cardiomyocytes (Yu et al. 1994a, 1994b), defects in mechanical relaxation are exhibited after 6 weeks of diabetes. Although this is associated with a profound decrease in SR Ca²⁺ pump activity, alterations in NCX may also play a significant role. Using isolated myocytes, we have found (Yu et al. 1994b) significant reductions in both rest potentiation (32%) and rapid cooling contractures (38%) in hearts from 6-week diabetic rats compared to controls. Both of these parameters reflect SR Ca²⁺ loading. As the Na⁺–Ca²⁺ exchanger represents the major trans-sarcolemmal Ca²⁺ efflux mechanism.
Composite Default screen

Color profile: Disabled

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Fig. 4. Representative immunoblot of 4.0 µg of sarcolemmal protein from control (C), diabetic (D), SHR-control (H) and SHR–diabetic (HD) on 7.5% SDS-PAGE. Aliquots from all four groups were run on the same gel and blotted to the same membrane simultaneously. Primary antibodies were raised in rabbits against purified canine cardiac Na⁺–Ca²⁺ exchanger. Second antibody was goat anti-rabbit conjugated to horseradish peroxidase. Numbers on right refer to relative molecular weight in kDa. A total of eight immunoblots, each of which included all four samples, were performed.

under physiological conditions (Bers and Bridge 1989; Bridge et al. 1988), the loading of the SR with Ca²⁺ in turn depends on the relative abilities of the Na⁺–Ca²⁺ exchanger to remove Ca²⁺ to the extracellular fluid (ECF) to that of the SR Ca²⁺ pump to sequester Ca²⁺ into the SR. The observation that NCX is only modestly depressed in the diabetic heart as shown in this study clearly suggests that alterations in the SR Ca²⁺ pump may be of greater pathogenic importance towards impaired relaxation in diabetes. This interpretation is supported by our previous study (Yu et al. 1994) using intact cardiomyocytes in which the magnitude of rapid cooling contractures were depressed by 38% in the diabetic group.

In contrast to the observations made above (in normotensive diabetic rats), the V_{\text{max}} of cardiac NCX did not differ between the H and HD groups. A growing body of evidence indicates that hemodynamic overload can induce the expression of the Na⁺–Ca²⁺ exchanger (Ashida et al. 1989; Kent et al. 1993; Nakanishi et al. 1989). It has been found that stenosis of the pulmonary artery resulted in a marked increase in the amount of the exchanger transcript within a few hours of stenosis (Kent et al. 1993). Furthermore, the exchanger protein, as determined by immunoblotting, was markedly increased within 48 h of ligation. Increased activity of the Na⁺–Ca²⁺ exchanger in arterial smooth muscle has been observed (Ashida et al. 1989) in the spontaneously hypertensive rat compared to the age-matched WKY control rat. On the other hand, Na⁺–Ca²⁺ exchanger activity was found to be depressed in the hearts of male Sprague-Dawley rats made 4–12 weeks after they were made hypertensive by the Goldblatt renal artery occlusion technique (Andrawis et al. 1988). The NCX initial activity in this study was depressed by about 50% at 50 µM [Ca²⁺]_o. Thus, the effects of hypertension per se on NCX activity in the heart remain controversial.

As depicted in Fig. 3A, both diabetic groups (D and HD) exhibited reduced K_{\text{m}} (Ca²⁺) values relative to their respective controls (C and H). Similar changes were noted in hearts from rats subjected to treadmill training (Tibbits et al. 1989). While the measurement of K_{\text{m}} (Ca²⁺) is known to be subject to experimental variations, some of which are difficult to control, the differences are statistically significant and may be important physiologically. The mechanism through which diabetes may cause this specific adaptation in both normotensive and hypertensive rats remains unclear. It is possible that this may be secondary to diabetes-induced hyperlipidemia. In the model used in this study, we have shown that 6 weeks after STZ injection, profound changes are observed in plasma lipids (Yu and McNeill 1991a). For example, plasma levels of triglycerides and cholesterol were shown to be increased by 750% and 183%, respectively. It is well known that modifications in lipid bilayer composition can profoundly influence the K_{\text{m}} (Ca²⁺) (Vemuri and Philipson 1988; Philipson and Ward 1987; Philipson and Nicoll 1993). For example, increasing the cardiac SL content of negatively charged phospholipids (with phospholipase D, phosphatidyl serine) or cholesterol profoundly stimulates NCX activity by lowering the K_{\text{m}} (Ca²⁺). The cholesterol content (by weight of total SL lipids) is known to increase by ≈10–15% in the diabetic heart. Thus, it is possible that changes in the lipid composition of the SL attendant to the diabetic condition may cause reductions in the K_{\text{m}} (Ca²⁺) in D and HD groups. However, this hypothesis is speculative and requires direct testing, perhaps by reconstitution experiments. Other mechanisms of adaptation are possible. For example, Schaffer et al. (1997) have shown that in Wistar rats made diabetic by STZ injection and studied 12–14 months later, the V_{\text{max}} of NCX activity was reduced by more than 40% while the K_{\text{m}} was not reduced. Furthermore, they present data indicating that the diabetic condition increased the total protein kinase C (PKC) and PKCβ activity while depressing the PKCδ activity, which may influence NCX activity.

The immunoblots shown in Fig. 4 are similar to those of other species (Tibbits et al. 1992; Philipson et al. 1988). The canine Na⁺–Ca²⁺ exchanger cDNA has been cloned and sequenced (Nicoll et al. 1990) and the deduced molecular weight is 110 kDa and following cleavage of the leader peptide is 105 kDa (Nicoll and Philipson 1991). The 120 kDa band appears to be a result of post-translational modification including glycosylation (Hryshko et al. 1993). Although we cannot explain the more diffuse 120–130 kDa band in the SL of H and HD groups, this may be linked to (1) different isoform expression of the Na⁺–Ca²⁺ exchanger or (2) differences in post-translational modifications. The intensity of staining of the 120-kDa band suggests some important differences. First, the intensity is lower in the D group compared to C group. The reduced expression of the exchanger as reflected in the Western blot is consistent with the reduced V_{\text{max}} observed in the SL uptake studies in this group. The 70 kDa represents a proteolytic fragment typical of chymotrypsin treatment that demonstrates NCX activity.
without the normal Na\(^+\) and Ca\(^{2+}\) regulatory influences (Iwata et al. 1995; Philipson et al. 1996). The more pronounced 70 kDa band in both the H and HD groups likely indicates a greater proteolytic activity in these hearts that may occur in response to the hemodynamic overload or differences in strains.

In summary, chronic diabetes resulted in a modest, yet significant reduction in the \(V_{\text{max}}\) of the myocardial Na\(^+\)-Ca\(^{2+}\) exchanger in D rats. This reduction was consistent with the fainter exchanger protein bands observed in immunoblots of SL from the D group. However, concomitant with the depressed \(V_{\text{max}}\) was a reduction in the \(K_m\) (Ca\(^{2+}\)) in the D heart that may represent a compensatory homeostatic mechanism. By marked contrast, the HD group did not show a reduction in the \(V_{\text{max}}\) of myocardial Na\(^+\)-Ca\(^{2+}\) exchanger relative to the H group. Although we are unable to address this issue, we suggest that this may be a compensatory adaptation to the hemodynamic overload in the hypertensive rats as no differences in the immunoblots were noted.

Acknowledgements

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