Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5412–5416, September 1983
Medical Sciences

Alterations in Ca^{2+} binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes

(rat heart cell membrane/diabetic cardiomyopathy/membrane sialic acid/membrane phospholipids)

GRANT N. PIERCE, MICHAEL J. B. KUTRYK, AND NARANJAN S. DHALLA*

Experimental Cardiology Section, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E 0W3

Communicated by George B. Koelle, May 19, 1983

ABSTRACT **Chronic streptozotocin-induced diabetes in rats was associated with a significant loss in the ability of isolated cardiac sarcolemmal membranes to bind Ca^{2+}. Administration of insulin to the diabetic rats normalized the sarcolemmal Ca^{2+} binding capacity. The content of sialic acid residues, which are considered to represent a superficial Ca^{2+} pool in sarcolemma, was decreased in preparations from diabetic rats, and this change also was reversible upon insulin treatment of the diabetic rats. Treatment of sarcolemma with neuraminidase decreased Ca^{2+} binding by 37% in control preparations but had no effect on diabetic preparations. Diphosphatidylglycerol content was decreased but other acidic phospholipids such as phosphatidylinositol and phosphatidylserine, which also bind Ca^{2+}, were not altered during diabetes. An increase in lysophosphatidylcholine and a decrease in phosphatidylethanolamine contents were observed in membranes isolated from diabetic rats. These results suggest that some alterations occur in Ca^{2+} binding and composition of heart sarcolemma in chronically diabetic rats and may provide further insight into the pathogenesis of diabetic cardiomyopathy.**

Chronic diabetes mellitus has been associated with primary defects in the contractile function of the heart (1–3). The precise subcellular mechanism responsible for this diabetic cardiomyopathy is unknown; however, it has been suggested (4) that alterations in Ca^{2+} metabolism in the heart occur. Depressed Ca^{2+} uptake by the sarcoplasmic reticulum as well as alterations in Ca^{2+}-activated ATPase have been demonstrated for sarcoplasmic reticular and myofibrillar fractions from diabetic rat hearts (5, 6). In view of the importance of the movement of extracellular Ca^{2+} across the myocardial membrane in modulating the contractile performance of the heart (7, 8), it is possible that alterations in trans-sarcolemmal Ca^{2+} flux may participate in this cardiomyopathy. This hypothesis has been indirectly supported by a preliminary study in which an abnormal response of isolated heart preparations from diabetic animals to extracellular Ca^{2+} was found (9). In addition, the presence of a general cardiac sarcolemmal membrane lesion has been supported by data documenting altered tissue Na^+,K^+ and Ca^{2+} contents (3, 10, 11).

The present study was undertaken to examine sarcolemmal characteristics in an experimental model of chronic diabetes. By virtue of its content of acidic phospholipids and sialic acid residues, heart sarcolemma is known to have a remarkable ability to bind Ca^{2+} and so is considered to serve as a superficial Ca^{2+} pool for the generation of contractile activity (7, 8, 12). Thus, identification of changes in the sarcolemmal composition and Ca^{2+} binding activity could extend our knowledge concerning the molecular mechanisms associated with defective cardiac muscle function in diabetic cardiomyopathy.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Male Sprague–Dawley rats weighing 200–250 g were used in this study. Animals were randomly separated into control and experimental groups. Experimental animals (n = 75) were made diabetic by a single intravenous injection of streptozotocin (65 mg/kg of body weight) delivered in a citrate-buffered vehicle (pH 4.5). Control animals (n = 55) received a similar injection of the vehicle alone. All animals were fed normal rat chow and water ad lib and were sacrificed 8 weeks after injection. To determine if the effects observed in this study were reversible, some animals (n = 40) were injected with streptozotocin and maintained in a diabetic state for 6 weeks instead of 8 weeks. These animals were then given subcutaneous injections of insulin (2–3 units of Connaught protamine zinc insulin per day) for 2 additional weeks and then sacrificed. Blood samples were taken and the plasma was analyzed for glucose (Worthington Statzyme reagent kit) and insulin (Amersham radioimmunoassay techniques).

The heart was removed and the ventricular tissue was washed and homogenized in 10 mM Tris·HCl, pH 7.4/1 mM EDTA. Sarcolemmal membranes were isolated by the hypotonic shock/LiBr method (13) and suspended in 1 mM Tris·HCl at pH 7.4. The vesicles obtained by this procedure are predominantly right-side-out in orientation and have a substantial basement membrane (14, 15). Ca^{2+} binding by the sarcolemmal membranes was studied by the Millipore filtration technique (14, 15). Membranes (150–200 μg) were incubated at 37°C for 5 min in 1 ml of a medium containing 50 mM Tris·HCl (pH 7.4) and 0.05 mM or 1.25 mM $^{45}CaCl_2$.

In some experiments, membranes were treated with neuraminidase (specific activity, 5 units/mg of protein) prior to the determination of Ca^{2+} binding. Specifically, 2 mg of sarcolemmal protein was incubated for 20 min at 37°C in 50 mM Tris·HCl, pH 7.4/20 mM KCl containing 20 or 200 μg of neuraminidase; this represents a ratio of 0.05 or 0.5 unit of neuraminidase per mg of sarcolemmal protein, respectively. These concentrations are similar to those used in previous studies on cardiac sarcolemmal membranes (14, 16). Separate experiments were performed to determine if the endogenous proteolytic activity associated with this neuraminidase preparation (1 microunit/mg of protein) could be responsible for the alteration of Ca^{2+} binding. However, the presence of 0.011 ng of trypsin inhibitor (Sigma T-9003) in the incubation medium, which is sufficient to block the nonspecific proteolytic activity, has no effect on Ca^{2+} binding results with neuraminidase. This suggests that the effects of a neuraminidase reported here were not due to action of contaminating proteases in the enzyme preparation.

Ouabain-sensitive Na^+,K^+-ATPase, adenylate cyclase, and 5′-nucleotidase activities were used as sarcolemmal marker en-

* To whom reprint requests should be addressed.

zymes and were measured according to procedures outlined in detail elsewhere (14–17). Cytochrome *c* oxidase activity (18), K^+-EDTA ATPase activity (19), and Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity (6) were measured to determine the extent of contamination of the sarcolemmal fraction with mitochondria, contractile proteins and sarcoplasmic reticulum, respectively. Inorganic phosphate released in the ATPase reaction and the procedure for protein determinations was estimated as described (6).

Sialyltransferase activity was determined in the sarcolemmal fraction by the disk method of Baxter and Durham (20) by incubating 0.3 mg of membrane protein for 90 min at 37°C in 100 μl of a medium containing 50 mM imidazole·HCl (pH 7.0), 5 mM $MgCl_2$, 0.5 mg of desialylated human α_1-acid glycoprotein and 1.85 pM CMP-sialic acid (170 mCi/mmol; 1 Ci = 3.7×10^{10} Bq). This medium also contained 0.05 or 0.1 mM Na_2ATP to avoid the influence that endogenous phosphatase activity may have on the results. The reaction was terminated by placing the tubes on ice. Aliquots (60 μl) were spotted on 2.5-cm Whatman no. 1 filter disks and the disks were immersed in 10% trichloroacetic acid. The disks were washed three times in 10% trichloroacetic acid, twice in ethanol/ether, 2:1 (vol/vol), and finally in ether. The disks were dried, immersed in 1 ml of 0.05 M H_2SO_4, and incubated at 80°C for 1 hr. The H_2SO_4 was neutralized with 1 M NaOH and the disks were assayed in scintillation medium.

Sialic acid content of the sarcolemma was determined by the thiobarbituric acid method of Warren (21). For measuring the phospholipid content, 2 mg of the sarcolemmal protein was suspended in chloroform/methanol, 2:1 (vol/vol), and the phospholipid extract was streaked on two-directional TLC plates. Phospholipid phosphorus was measured as described (17). $NaDodSO_4$ gel electrophoresis was performed on sarcolemmal samples as described (17) in a 10% acrylamide gel. Molecular weights of the vesicular proteins were estimated through comparison to molecular weights of known standards run alongside the unknown samples. Densitometric scanning was accomplished at 660 nm in a Unicam SP 1800 spectrophotometer fitted with a gel scanner, and individual protein peaks were quantified as outlined elsewhere (22).

Statistical analysis of the data was accomplished through the use of Student's *t* test or analysis of variance with Duncan's multiple range *post hoc* test.

RESULTS

The experimental rats showed all of the diabetic symptoms 8 weeks after streptozotocin injection (Table 1). Body and ventricular growth were significantly retarded in diabetic animals. The ventricular:body weight ratio suggested that the hearts from diabetic animals were in a state of hypertrophy. Plasma glucose concentrations were markedly increased and plasma insulin values were depressed in comparison to control animals. These characteristics are similar to those reported by others using similar protocols (2, 5, 6). Daily injection of the diabetic animals for 2 weeks with insulin normalized plasma glucose and insulin concentrations. However, this treatment was not of a long enough duration to reverse all of the effects of the diabetic state as evidenced by the continued presence of morphometric alterations in these rats in comparison to control.

The results in Table 1 also indicate that yields of sarcolemmal protein from the ventricular tissue of control, diabetic, and insulin-treated diabetic animals were not different from each other ($P > 0.05$). ATP-independent sarcolemmal Ca^{2+} binding in diabetic rat heart in the presence of both 0.05 mM and 1.25 mM Ca^{2+} was significantly depressed (Table 1). *In vivo* treatment

Table 1. Body and ventricular weights, plasma glucose and insulin levels, and sarcolemmal Ca^{2+} binding in control, diabetic, and insulin-treated diabetic rats

	Control	Diabetic	Diabetic; then insulin
Body weight, g	462 ± 8	276 ± 11*	368 ± 12†
Ventricular weight, g	1.16 ± 0.02	0.85 ± 0.03*	1.02 ± 0.03†
Ventricular/body weight ratio, mg/g	2.55 ± 0.02	3.11 ± 0.04*	2.77 ± 0.02†
Plasma glucose, mg/dl	154 ± 8	529 ± 21*	182 ± 10
Plasma insulin, microunits/ml	28.0 ± 1.7	11.4 ± 1.03*	34.6 ± 6.0
Sarcolemmal yield, mg protein/g heart	3.12 ± 0.24	3.27 ± 0.24	2.69 ± 0.13
Ca^{2+} binding, nmol/mg:			
At 0.05 mM Ca^{2+}	15.99 ± 1.55	11.58 ± 0.07†	15.58 ± 1.19
At 1.25 mM Ca^{2+}	193.4 ± 16.7	87.2 ± 4.0*	160.7 ± 6.1

Values represent mean ± SEM of at least five experiments.
* For difference from control, $P < 0.005$.
† $P < 0.05$.

of diabetic animals with insulin resulted in a normalization of Ca^{2+} binding to the cardiac sarcolemmal membrane fraction. Our control values for Ca^{2+} binding by rat heart sarcolemma are in agreement with those reported elsewhere (15, 23, 24). When the hearts were removed from diabetic animals 2 weeks after injection with streptozotocin, no difference in Ca^{2+} binding activities were observed between the control and diabetic preparations ($P > 0.05$).

In order to clarify whether or not differential contamination in control and experimental preparations was confounding the results, activities of some selected marker enzymes were determined in the sarcolemmal membrane fractions from both control and diabetic animals. Ouabain-sensitive Na^+,K^+-ATPase activities were similar ($P > 0.05$) in both preparations (control, 12.21 ± 0.18 μmol P_i/mg per hr; diabetic, 11.41 ± 0.21 μmol) and these represented 8.09- and 7.82-fold increases in purity over homogenate values. Similar values and purification indices for ouabain-sensitive Na^+,K^+-ATPase activity in rat heart sarcolemma isolated by a sucrose-density gradient method have been reported (23). Sarcolemmal adenylate cyclase activity showed 10.21- and 10.07-fold purification with respect to the homogenate values in control (30.19 ± 5 pmol of cAMP per mg of protein per min) and diabetic (15.11 ± 4 pmol), respectively. 5′-Nucleotidase activity was enriched in sarcolemmal preparations from control and diabetic samples 5.18- and 5.26-fold over homogenate values (control, 28.12 ± 1.59 nmol of adenosine per mg per min; diabetic, 22.23 ± 1.01 nmol), respectively. These data indicate that the sarcolemmal membranes from control and experimental animals exhibited similar increases in purity.

Cytochrome *c* oxidase activity of the sarcolemmal preparations was about 50% of the homogenate values in control (260 nmol of cytochrome *c* oxidized per mg per min) and diabetic (230 nmol) hearts and this suggests minimal contamination by mitochondria (approximately 3–5%). Both K^+-EDTA ATPase and Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activities were undetectable in control and diabetic sarcolemmal fractions, suggesting that contamination by contractile protein and sar-

coplasmic reticulum was negligible. In addition, $NaDodSO_4$ gel electrophoretic separation of the sarcolemmal proteins revealed the appearance of no new proteins in the diabetic preparations in comparison to control (Fig. 1) which would offer additional gross evidence of a similar degree of purity in these membrane fractions. However, the results in Fig. 1 indicate a significant increase in a sarcolemmal protein of approximately M_r 70,000 in the diabetic preparations. This peak represented 2.3% of the total protein in control preparations but increased to 6.6% in sarcolemma from diabetic animals.

The mechanism that may explain the observed depression in sarcolemmal Ca^{2+} binding may reside in some alteration in those components of the sarcolemmal membrane that bind Ca^{2+}. Because some phospholipids are known to be able to bind Ca^{2+} (12), the phospholipid composition of both control and diabetic rat heart sarcolemma was investigated. Phospholipid values for control rat sarcolemma reported here are similar to those in the literature (17, 25). The concentration of lysophosphatidylcholine was increased and that of phosphatidylethanolamine and of diphosphatidylglycerol were depressed in sarcolemma from diabetic rat heart (Table 2). Other phospholipids were unaltered.

Another sarcolemmal Ca^{2+} binding pool in the heart is reported to be associated with the sialic acid residues (7, 14, 16, 26). Sialic acid content of myocardial sarcolemmal membranes was significantly depressed in diabetic rat hearts (Table 3); this effect was reversible by insulin. Control values for the sialic acid content are similar to those reported elsewhere (14, 16, 27). The sensitivity of the sarcolemma-bound Ca^{2+} to neuraminidase treatment was examined; neuraminidase specifically cleaves the sialic acid residue from membrane-bound glycoprotein (7, 26). The highest concentration of neuraminidase used in this study released approximately 50–60% of membrane-bound sialic acid (14, 16). Neuraminidase treatment of sarcolemmal membranes from diabetic rats did not decrease their Ca^{2+} binding capacity whereas Ca^{2+} binding by control membranes was significantly reduced by up to 37%.

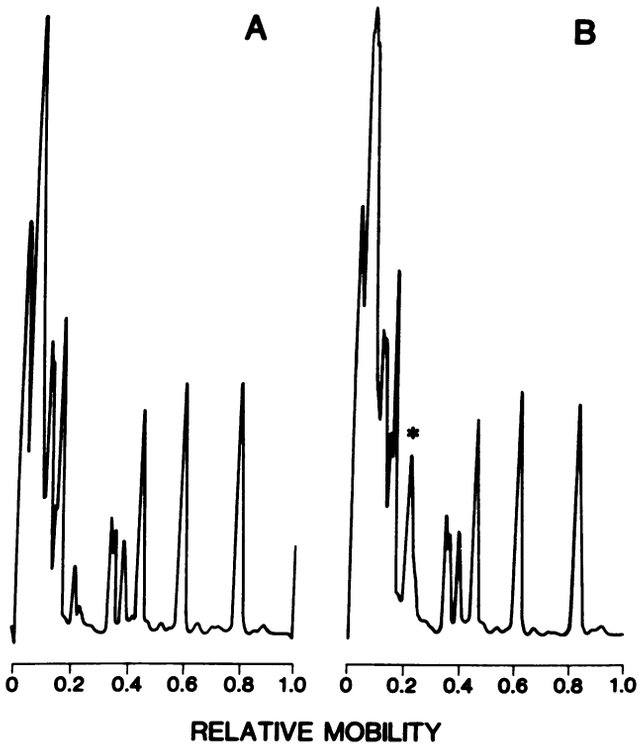

FIG. 1. Densitometric scans of sarcolemmal protein separated by $NaDodSO_4$/10% polyacrylamide gel electrophoresis. Sarcolemmal membrane protein was isolated from control rats (*A*) and diabetic rats (*B*). *, Alteration in percentage composition of underlying peak in comparison to respective control protein peak.

Table 2. Phospholipid composition of cardiac sarcolemmal membranes from control and diabetic rats

Phospholipid	Phospholipid, % of total	
	Control	Diabetic
Phosphatidylcholine	38.5 ± 3.5	42.0 ± 3.2
Lysophosphatidylcholine	0.5 ± 0.3	1.9 ± 0.3*
Phosphatidylethanolamine	38.0 ± 0.9	30.5 ± 2.2*
Sphingomyelin	5.3 ± 1.4	8.7 ± 1.3
Phosphatidylserine	5.9 ± 1.5	7.6 ± 1.8
Phosphatidylinositol	2.7 ± 0.5	3.3 ± 1.3
Diphosphatidylglycerol	9.1 ± 0.5	6.0 ± 1.1*

Results are expressed as mean ± SEM of three experiments.
*$P < 0.05$.

Sialyltransferase activity was unaffected by the diabetic condition of the animal (Table 3). The addition of 0.05 or 0.1 mM ATP to the incubation medium to inhibit any endogenous nonspecific phosphatase activity had no significant effect on either control or diabetic sample sialyltransferase activity. The sialyltransferase activity reported here is similar to that reported for cardiac ghost plasma membranes (28).

DISCUSSION

The major alterations in the ability of the myocardial sarcolemma from diabetic rats to bind Ca^{2+} described here do not seem to be confounded by any differences in the relative purity of these fractions as determined by marker enzyme activities. On the basis of studies in which the diabetogenic action of streptozotocin was blocked with 3-*O*-methyl glucose (1, 2), it is highly improbable that streptozotocin itself has any cardiotoxic effect at either the membrane or tissue level. This contention is reinforced by the insulin-reversible nature of the defects observed in the sarcolemma obtained from diabetic rats in the present study. Furthermore, no changes in sarcolemmal Ca^{2+} binding were seen in hearts removed from rats 2 weeks after induction of diabetes with streptozotocin. Therefore, the observed alterations in sarcolemmal Ca^{2+} binding appear to be associated with the chronic diabetic condition *per se*.

The defect in cardiac sarcolemmal Ca^{2+} binding capacity may be of critical importance to the function of the diabetic rat heart. Ca^{2+} bound to the sarcolemmal pool has been integrally related to force generation in the heart (7, 8, 23). Accordingly, a pathophysiological alteration in the capacity of this superficial Ca^{2+} pool may severely hamper the mechanical performance of the heart. A similar conclusion has been reached in other types of cardiomyopathies (8, 29). Therefore, this sarcolemmal defect may contribute, to some extent, to the diabetic cardiodepression reported (1–3). However, the results in this study in no way rule out the involvement of defects in Ca^{2+} transport by the sarcoplasmic reticulum or myofibrillar Ca^{2+}-stimulated ATPase in the development of cardiac dysfunction in diabetes (5, 6).

The mechanism responsible for the observed decrease in Ca^{2+} binding appears to reside in the low sialic acid content of the diabetic membranes. Such a depression in Ca^{2+} binding may be due partly to a reduction in the neuraminidase-sensitive sialic acid residues because neuraminidase treatment of the diabetic preparation, unlike the control membranes, failed to decrease the Ca^{2+}-binding activity. This decrease in membrane sialic acid

Table 3. Sialic acid content, sensitivity of Ca^{2+} binding to neuraminidase, and sialyltransferase activity in cardiac sarcolemmal membranes isolated from control, diabetic, and insulin-treated diabetic rats

	Sialic acid, nmol/mg	Sensitivity of Ca^{2+} binding to neuraminidase, nmol Ca^{2+}/mg			Sialyltransferase, pmol/mg/hr
		0 unit/mg	0.05 unit/mg	0.5 unit/mg	
Control	35.15 ± 2.77	178.9 ± 10.5	140.3 ± 9.2*	113.6 ± 8.1*	1.49 ± 0.16
Diabetic	25.23 ± 1.36†	98.2 ± 8.6†	109.6 ± 10.1	122.3 ± 31.6	1.84 ± 0.16
Diabetic; then insulin	31.31 ± 1.99	ND	ND	ND	ND

Values represent mean ± SEM of four experiments. Ca^{2+} binding data were observed before and after treatment of the sarcolemmal membranes with 0, 0.05, or 0.5 unit of neuraminidase per mg of sarcolemmal protein. ND, not determined.
*For effect of neuraminidase in comparison to untreated membranes, $P < 0.05$.
†For difference from control, $P < 0.05$.

content during diabetes appears to be a generalized phenomenon in the body—lowered contents of sialic acid have been reported in connective tissue (30), erythrocytes (31), and glomerular basement membrane (32) from human and animal models of diabetes. In addition, the decrease in diphosphatidylglycerol content in the diabetic membranes may also contribute to the decrease in Ca^{2+} binding because this acidic phospholipid has been shown to bind substantial quantities of Ca^{2+} (12). This Ca^{2+} pool is believed to be involved in contractile force regulation (12).

The M_r 70,000 peak that was increased in content in the diabetic preparations corresponds to the sarcolemmal Ca^{2+}-dependent ATPase protein (33). This enzyme has been suggested to be involved in regulating Ca^{2+} entry into the cell (8). Its precise status in this condition, however, is not known.

The cause of the depression in sarcolemmal sialic acid in the diabetic cardiomyopathy is unclear because sialyltransferase activity was not decreased in diabetic preparations. Sialyltransferase is responsible for the attachment of sialic acid to the membrane and has been reported to be depressed in renal tissue from diabetic mice (34) and heart muscle from cardiomyopathic hamsters (28). It is possible that the defect in diabetic cardiomyopathy may lie elsewhere along the pathway of ultimate attachment of the sialic acid to the proper position on the glycoprotein (35) rather than at the level of sialyltransferase. In this regard, a recent study has demonstrated a defect in pyrimidine nucleotide metabolism in diabetic rat heart (36). Because pyrimidine nucleotides function as cofactors in the synthesis of sialic acid (36), it is possible that the reduction in sarcolemmal sialic acid content shown in the present study may be a result of this alteration.

Although alterations in the basic phospholipids are not likely to contribute to the depression in Ca^{2+} binding demonstrated in the diabetic preparations, these changes may be of some importance to cellular integrity. Lysophosphatidylcholine accumulation in the myocardium has been associated with electrophysiological abnormalities (37) and, in fact, alterations in electrical activity in the diabetic rabbit heart have been reported (38). In addition, an increased level of lysophosphatidylcholine has been shown to inhibit Na^+,K^+-ATPase activity (39) and, therefore, could be causally related to the inhibited sodium pump activity observed in hearts from diabetic animals (40). Although patent Na^+,K^+-ATPase activity (that activity expressed in the absence of a membrane-perturbing agent) in diabetic membranes was not different from that in the control preparations, latent Na^+,K^+-ATPase activity (as seen after the treatment of membranes with different membrane-disruptive agents) is depressed in diabetic heart (41). Phosphatidylethanolamine levels were also decreased in cardiac sarcolemmal preparations from diabetic rats in the present investigation, and degradation of this phospholipid has been demonstrated to be associated with defects in sarcolemmal permeability in the myocardium (42).

This observation is particularly interesting because removal of sarcolemmal sialic acid residues in the heart has also been closely associated with an increase in membrane permeability (7, 26). Furthermore, lysophosphatidylcholine accumulation has been shown to result in augmented permeability and enhanced Ca^{2+} entry into the myocardium (43). It is possible, therefore, that the decrease in membrane sialic acid and phosphatidylethanolamine content in concert with the increase in lysophosphatidylcholine level observed in the present investigation may alter permeability characteristics of the cell membrane from diabetic rat hearts. The decreased binding of Ca^{2+} to the sarcolemma from diabetic rats itself may also change permeability characteristics by altering physical properties of the membrane (44). In this regard, increases in vascular tissue permeability have been documented early in the diabetic disease process (45). This hypothesis of a change in the integrity of the cardiac sarcolemmal membrane would agree well with published accounts of altered cation contents in the myocardium of diabetic animals (3, 10, 11); however, it would need more direct analysis to establish its validity conclusively.

The present investigation has demonstrated the existence of a defect in the Ca^{2+}-related function of sarcolemmal membranes from hearts of chronically diabetic rats. This defect appears to be associated with alterations in membrane composition. The lesion is largely insulin-reversible and therefore correlates well with results of studies showing changes in cardiac function during diabetes and its treatment with insulin (1). Thus, alterations in sarcolemmal integrity may be associated with the diabetic cardiomyopathy process, but the etiology of this defect within the framework of the diabetic condition is uncertain. It is known that streptozotocin-induced diabetes is accompanied by a hypothyroid condition as well (1, 2). This is unlikely to have any influence on the results of the present investigation because sarcolemmal preparations from hypothyroid animals exhibit the same sialic acid content and Ca^{2+}-binding characteristics as do samples prepared from euthyroid animals (ref. 27; unpublished data). Future investigations, however, may need to examine a myriad of metabolic disturbances that accompany diabetes (1–4) in order to determine the factors that may influence primarily the sarcolemmal damage in the heart. It also remains to be investigated whether sarcolemmal changes have any cause-and-effect relationship with other defects in the sarcoplasmic reticulum and contractile proteins during the development of diabetic cardiomyopathy (5, 6).

The authors express their appreciation to Dr. Niels Haugaard (University of Pennsylvania) for his helpful criticism of this manuscript. This study was supported by a grant from the Medical Research Council of Canada. G.N.P. and M.J.B.K. are Predoctoral Fellows of the Medical Research Council and of the Canadian Heart Foundation, respectively.

1. Fein, F., Strobeck, J. E., Malhotra, A., Scheuer, J. & Sonneblick, E. H. (1981) *Circ. Res.* **49,** 1251–1256.
2. Penpargkul, S., Schaible, T., Yipintsoi, T. & Scheuer, J. (1980) *Circ. Res.* **47,** 911–921.
3. Regan, T. J., Wu, C. F., Yeh, C. K., Oldewurtel, H. A. & Haider, B. (1981) *Circ. Res.* **49,** 1268–1277.
4. Feuvray, D., Idell-Wenger, J. A. & Neely, J. R. (1979) *Circ. Res.* **44,** 322–329.
5. Penpargkul, S., Fein, F., Sonnenblick, E. H. & Scheuer, J. (1981) *J. Mol. Cell. Cardiol.* **13,** 303–309.
6. Pierce, G. N. & Dhalla, N. S. (1981) *J. Mol. Cell. Cardiol.* **13,** 1063–1069.
7. Langer, G. A. (1978) *Am. J. Physiol.* **235,** H461–H468.
8. Dhalla, N. S., Pierce, G. N., Panagia, V., Singal, P. K. & Beamish, R. E. (1982) *Basic Res. Cardiol.* **77,** 117–139.
9. Bielefeld, D. R. & Boshell, B. R. (1980) *Diabetes* **29,** Suppl. 2, 76 (abstr.).
10. Nagase, N., Tamura, Y., Kobayashi, S., Saito, M., Niki, T., Chikamori, K. & Mori, H. (1981) *J. Mol. Cell. Cardiol.* **13,** Suppl. 2, 70 (abstr.).
11. Pettit, G. W. & Vick, R. L. (1974) *Am. J. Physiol.* **226,** 319–324.
12. Philipson, K. D., Bers, D. M. & Nishimoto, A. Y. (1980) *J. Mol. Cell. Cardiol.* **12,** 1159–1173.
13. Dhalla, N. S., Anand-Srivastava, M. B., Tuana, B. S. & Khandelwal, R. L. (1981) *J. Mol. Cell. Cardiol.* **13,** 413–423.
14. Matsukubo, M. P., Singal, P. K. & Dhalla, N. S. (1981) *Basic Res. Cardiol.* **76,** 16–28.
15. Takeo, S., Duke, P., Taam, G. M. L., Singal, P. K. & Dhalla, N. S. (1979) *Can. J. Physiol. Pharmacol.* **57,** 496–503.
16. Takeo, S., Daly, M. J., Anand-Srivastava, M. B. & Dhalla, N. S. (1980) *J. Mol. Cell. Cardiol.* **12,** 211–217.
17. Panagia, V., Lamers, J. M. J., Singal, P. K. & Dhalla, N. S. (1982) *Int. J. Biochem.* **14,** 387–397.
18. Wharton, D. C. & Tzagoloff, A. (1967) *Methods Enzymol.* **10,** 245–250.
19. Martin, A. F., Pagani, E. D. & Solaro, R. J. (1982) *Circ. Res.* **50,** 117–125.
20. Baxter, A. & Durham, J. P. (1979) *Anal. Biochem.* **98,** 95–101.
21. Warren, L. (1959) *J. Biol. Chem.* **234,** 1971–1975.
22. Potter, J. D. (1974) *Arch. Biochem. Biophys.* **162,** 436–441.
23. Bers, D. M. & Langer, G. A. (1979) *Am. J. Physiol.* **237,** H332–H341.
24. Harrow, J. A. C., Das, P. K. & Dhalla, N. S. (1978) *Biochem. Pharmacol.* **27,** 2605–2609.
25. Owen, K., Pang, D. C. & Weglicki, W. B. (1979) *Biochem. Biophys. Res. Commun.* **89,** 368–373.
26. Frank, J. S., Langer, G. A., Nudd, L. M. & Seraydarian, K. (1977) *Circ. Res.* **41,** 702–714.
27. McConnaughey, M. M., Jones, L. R., Watanabe, A. M., Besch, H. R., Williams, L. T. & Lefkowitz, R. J. (1979) *J. Cardiovasc. Pharmacol.* **1,** 609–623.
28. Bailey, L. E. & Ma, T. S. (1980) *Adv. Myocardiol.* **1,** 91–112.
29. Dhalla, N. S., Das, P. K. & Sharma, G. P. (1978) *J. Mol. Cell. Cardiol.* **10,** 363–385.
30. Berenson, G. S., Radhakrishnamurthy, B., Dalferes, E. R., Ruiz, H., Srinivasan, S. R., Plavidal, F. & Brickman, F. (1972) *Diabetes* **21,** 733–743.
31. Baba, Y., Kai, M., Setoyama, S. & Otsuji, S. (1978) *Clin. Chim. Acta* **84,** 247–249.
32. Westberg, N. G. & Michael, A. F. (1973) *Acta Med. Scand.* **194,** 39–47.
33. Tuana, B. S. & Dhalla, N. S. (1982) *J. Biol. Chem.* **257,** 14440–14445.
34. Bardos, P., Lacord-Bonneau, M., Rakotoarivory, J., Muh, J. P. & Weill, J. (1980) *Int. J. Biochem.* **12,** 505–507.
35. Spiro, R. G. (1969) *N. Engl. J. Med.* **281,** 1043–1056.
36. Gertz, B. J. & Haugaard, E. S. (1979) *Metabolism* **28,** 358–362.
37. Katz, A. M. & Messineo, F. C. (1981) *Circ. Res.* **48,** 1–16.
38. Senges, J., Brachmann, J., Pelzer, D., Hasslacher, C., Weihe, E. & Kubler, W. (1980) *J. Mol. Cell. Cardiol.* **12,** 1341–1351.
39. Karli, J. N., Karikas, G. A., Hatzipaylou, P. K., Levis, G. M. & Moulopoulos, S. N. (1979) *Life Sci.* **24,** 1869–1876.
40. Ku, D. D. & Sellers, B. M. (1982) *J. Pharmacol. Exp. Ther.* **222,** 395–400.
41. Pierce, G. N. & Dhalla, N. S. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,** 1090 (abstr.).
42. Chien, K. R., Reeves, J. P., Buja, L. M., Bonte, F., Parkey, R. W. & Willerson, J. T. (1981) *Circ. Res.* **48,** 711–719.
43. Sedlis, S. P., Corr, P. B., Sobel, B. E. & Ahumada, G. G. (1983) *Am. J. Physiol.* **244,** H32–H38.
44. Gordon, L. M., Sauerheber, R. D. & Esgate, J. A. (1978) *J. Supramol. Struct.* **9,** 299–326.
45. Joyner, W. L., Mayhan, W. G., Johnson, R. L. & Phares, C. K. (1981) *Diabetes* **30,** 93–100.