Increased number of myocardial voltage-gated Ca\textsuperscript{2+} channels and unchanged total \(\beta\)-receptor number in long-term streptozotocin-diabetic rats

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In order to elucidate further the abnormal myocardial Ca\textsuperscript{2+} metabolism in diabetes mellitus, voltage-gated Ca\textsuperscript{2+} channels and \(\beta\)-receptors were quantified in myocardial membranes of short- and long-term diabetic rats. Diabetes was induced by an injection of streptozotocin (STZ). Animals were killed 2, 4, 7, 90 and 200 days after STZ. A group of diabetic animals were treated with insulin for 20 days following 180 days of untreated diabetes. Diabetic animals developed low triiodothyronine syndrome. During short-term diabetes, the maximum binding capacity (MBC) for Ca\textsuperscript{2+} channels was reduced by 25\% at day 4 (p < 0.05) and the \(\beta\)-receptor MBC was reduced by 48\% (p < 0.05). A normalizing tendency was observed at day 7 for both receptor types: insulin-treated rats did not differ from controls at that time. After 90 and 200 days of untreated diabetes the Ca\textsuperscript{2+} channel MBC had increased by 36\% and 27\%, respectively (p < 0.05). Twenty days of strictly regulated blood glucose following 180 days of untreated diabetes totally normalized the Ca\textsuperscript{2+} channel MBC. This is in contrast to a previous report where insulin treatment did not normalize the Ca\textsuperscript{2+} channel MBC. Total \(\beta\)-receptor MBCs did not differ from control values 90 and 200 days after STZ. In conclusion, an increase in rat myocardial Ca\textsuperscript{2+} channel MBC during long-term diabetes was fully normalized by short-term insulin treatment. The increase in sarcolemmal Ca\textsuperscript{2+} channels could serve to compensate for a defect coupling of the \(\beta\)-receptor to adenylate cyclase. An elevated Ca\textsuperscript{2+} channel number may, at least theoretically, lead to increased Ca\textsuperscript{2+} flow across the cardiac sarcolemma and in this way contribute to the diabetic cardiomyopathy by increasing the intracellular Ca\textsuperscript{2+} concentration.

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Calcium ion homeostasis of the myocardial cell has been shown to be affected deeply during experimental diabetes and may play a major role in the development of diabetic cardiomyopathy (1−4). The \(\beta\)-receptor–adenylate cyclase system has been suggested to be involved by means of down-regulation in the \(\beta\)-receptor number (5) or by a defect coupling of the \(\beta\)-receptor to the adenylate cyclase (6). The Ca\textsuperscript{2+}−ATPase activity of the sarcoplasmic reticulum has been shown to be compromised (7, 8), leading to a delay in return of Ca\textsuperscript{2+} from cytoplasm to intracellular deposits. This in turn may lead to derangement of diastolic function and thus to a delay in muscle relaxation (1, 3). The two major mechanisms responsible for extruding excess Ca\textsuperscript{2+} from the cell during initiation of relaxation, i.e. the Na\textsuperscript{+}−Ca\textsuperscript{2+} exchange and sarcolemmal Ca\textsuperscript{2+}−ATPase, have also been shown to be compromised (2, 9), although controversies exist in this field (4). The net result of these changes in Ca\textsuperscript{2+} homeostasis could be a cellular Ca\textsuperscript{2+} overload (1, 10), contributing to cellular damage, fibrosis and, ultimately, clinical cardiomyopathy. Because the amount of released Ca\textsuperscript{2+} from intracellular stores mainly depends on the amount of Ca\textsuperscript{2+} entering the cell through voltage-gated Ca\textsuperscript{2+} channels (by Ca\textsuperscript{2+}−induced Ca\textsuperscript{2+} release (11)), it is surprising that changes in Ca\textsuperscript{2+} channels during diabetes have not attracted more attention. We are only familiar with two other studies where myocardial voltage-gated Ca\textsuperscript{2+} channels in diabetes were evaluated directly by membrane studies. The results are conflicting, probably due to variations in diabetes duration and severity (12, 13). In the present study we re-evaluated changes in myocardial voltage-gated Ca\textsuperscript{2+} channels and \(\beta\)-receptors during short- and long-term diabetes. The influence of short-term insulin treatment following long-term diabetes on Ca\textsuperscript{2+} channels was also investigated.

Materials and methods
Female Wistar rats (Mollegaard, Eiby, Denmark) with
an initial mean body weight of 234 g (age 9 weeks) were used. All animals had free access to a standard diet (Altromin, Lage, Germany) and tapwater. The rats were weight-matched and randomized into different groups. For the short-term study the control animals (N = 6) were killed on day 2 and the placebo-treated streptozotocin (STZ)-diabetic rats and insulin-treated STZ-diabetic rats (N = 4–6 rats/day) were killed on days 2, 4 and 7. For the long-term study the control rats (N = 6/ day) and STZ-diabetic rats (N = 8/day) were killed on days 90 and 200. Some long-term diabetic rats were treated randomly with insulin for 20 days after the initial 180 days of untreated diabetes (N = 8) and were sacrificed on day 200.

Induction of diabetes and metabolic control

Diabetes was induced on day 0 by an iv injection of STZ (55 mg/kg body weight) in acidic 0.154 mol/l NaCl (pH 4.5) following 12 h of food deprivation. Twenty-four hours after administration of STZ the animals were weighed, a urine analysis was performed for glucose and ketones using Neostix 4 (Ames Limited, Stoke Poges, Slough, UK) and tail-vein blood glucose was determined by Hemoglucotest 1–4 and Reflux (Boehringer-Mannheim, Mannheim, Germany). Insulin treatment with a long-acting, heat-treated Ultralente insulin (Novo-Nordisk, Bagsværd, Denmark) was initiated 24 h after administration of STZ in the short-term study when all animals had blood glucose levels above 20 mmol/l. Insulin was given in an initial dose of 8 U, followed by 2–6 U/day, depending on morning blood glucose values. On the day of sacrifice, animals were anaesthetized with sodium barbitral (50 mg/kg body weight) and hearts were rapidly excised, freed from atria and great vessels and frozen immediately in liquid nitrogen. Hearts were stored at −80°C until further processing.

Total adrenergic β-receptors

Total adrenergic β-receptors were quantified under equilibrium conditions as described previously in detail (14). In brief, each ventricle was homogenized in 10 vols of buffer (50 mmol/l TRIS-HCl, 0.1 mmol/l phenylmethylsulfonyl fluoride (PMSF); pH 7.4). The homogenate was centrifuged at 700 g for 5 min at 4°C and the supernatant was centrifuged at 100,000 g for 15 min at 4°C. Crude membrane protein (20 μg) was incubated with increasing amounts (approximately 1–120 pmol/l) of [125I]iodocyanopindolol (ICYP)(NEN, specific activity 2200 Ci/mmol for 60 min at 37°C in a final volume of 0.3 ml. Non-specific binding was determined by incubating parallel tubes with 1 μmol/l unlabeled propranolol ( Sigma, St Louis, MO). Incubates were vacuum-filtered over Whatman’s GF/C filters. Specific binding was calculated by subtracting non-specifically bound ligand from totally bound ligand. Maximum binding capacities (MBCs) and receptor affinities (Kd) were evaluated by means of saturation curves and Scatchard plots (Fig. 1A). A single, saturable set of receptors was identified. Experiments were performed twice on separate occasions.

Voltage-operated Ca2+ channels

Voltage-operated Ca2+ channels were determined by
incubation of \(50-100 \mu g\) of membrane protein (37°C for 60 min), prepared as described above (14), together with increasing concentrations (40-5000 pmol/l) of (+)[3H]PN200-110 (NEN, specific activity 70-86 Ci/mmol). Buffer was added to a final volume of 0.3 ml. Non-specific binding was determined by incubating parallel tubes with 1 \(\mu\)mol/l nifedipin added (Sigma, St. Louis, USA). Separation of free and bound [3H]PN200 was as described for \(\beta\)-receptors. Scintillation cocktail (Ecoscint A, National Diagnostics, NJ) was added and filters counted in a \(\beta\)-counter (Betazint, Berchtold, GmbH; counting efficiency 25%). Total and non-specific binding were determined in triplicate for seven concentrations and revealed one single, saturable set of binding sites for the \(Ca^{2+}\) antagonist. MBCs were determined by means of saturation curves (Fig. 1B). Binding experiments were repeated on different occasions.

Serum thyroid hormones

Serum thyroid hormones were determined by RIA as described elsewhere (15), with the modification that polyethylene glycol was used for separation. Protein was determined by the commercial BioRad kit using calf gamma globulin as standard, and DNA was determined by the method of Burton (16).

Statistical evaluation

Experimental data are given as group mean and sem unless noted otherwise. Comparisons were performed by one-way analysis of variance (ANOVA), followed by multiple comparison by Dunnett’s method. When only two groups were compared, Student’s two-tailed \(t\)-test was used; \(p < 0.05\) was considered significant.

Results

Metabolic parameters

All STZ-treated animals developed hyperglycemia within 24 h. Blood glucose concentrations ranged from 25 to 28 mmol/l and remained at that level throughout the study, while blood glucose levels in insulin-treated animals were around 5 mmol/l after the first injection and remained stable thereafter, with values not different from non-diabetic controls (Table 1). Untreated diabetic animals had glucosuria > 111 mmol/l, but no animal exhibited ketonuria at any time during the study period. The diabetic animals exhibited a small body weight loss on day 2; thereafter the body weight remained stable (Table 1). Insulin-treated diabetic animals did not lose weight, and gained in body weight parallel to non-diabetic control animals. Heart weight was reduced significantly within 2 days after STZ, while insulin treatment prevented this loss of weight. Following 20 days of insulin treatment heart weights did not differ from control values in long-term diabetic rats (Table 1).

Table 1. Experimental data of short- and long-term streptozotocin-diabetic rats.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mmol/l)</th>
<th>Body weight (g)</th>
<th>Heart weight (mg)</th>
<th>Serum T3 (nmol/l)</th>
<th>Serum T4 (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (6)</td>
<td>7.1 ± 0.2</td>
<td>249 ± 4</td>
<td>728 ± 13</td>
<td>1.17 ± 0.09</td>
<td>94.1 ± 7.5</td>
</tr>
<tr>
<td>D (6)</td>
<td>28.3 ± 0.4</td>
<td>223 ± 4*</td>
<td>675 ± 17*</td>
<td>0.59 ± 0.08*</td>
<td>69.5 ± 6.0*</td>
</tr>
<tr>
<td>ID (6)</td>
<td>4.1 ± 0.3</td>
<td>252 ± 4</td>
<td>743 ± 17</td>
<td>1.21 ± 0.08</td>
<td>97.0 ± 5.4</td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.8 ± 0.3</td>
<td>267 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (5)</td>
<td>28.6 ± 0.3</td>
<td>226 ± 3*</td>
<td>659 ± 11*</td>
<td>0.48 ± 0.10*</td>
<td>44.2 ± 5.0*</td>
</tr>
<tr>
<td>ID (5)</td>
<td>7.2 ± 1.0</td>
<td>260 ± 4</td>
<td>680 ± 6</td>
<td>1.10 ± 0.07</td>
<td>102.0 ± 4.5</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>6.9 ± 0.2</td>
<td>281 ± 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (4)</td>
<td>27.2 ± 1.9</td>
<td>224 ± 1*</td>
<td>621 ± 19*</td>
<td>0.67 ± 0.07*</td>
<td>55.5 ± 6.5*</td>
</tr>
<tr>
<td>ID (4)</td>
<td>8.3 ± 1.8</td>
<td>279 ± 5</td>
<td>801 ± 23</td>
<td>1.28 ± 0.09</td>
<td>94.0 ± 6.5</td>
</tr>
<tr>
<td>Day 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (6)</td>
<td>4.7 ± 0.0</td>
<td>284 ± 5</td>
<td>772 ± 23</td>
<td>1.23 ± 0.07</td>
<td>70.0 ± 4.8</td>
</tr>
<tr>
<td>D (8)</td>
<td>25.65 ± 3.0</td>
<td>240 ± 3*</td>
<td>680 ± 17*</td>
<td>0.90 ± 0.08*</td>
<td>57.8 ± 6.5</td>
</tr>
<tr>
<td>ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (6)</td>
<td>5.4 ± 0.2</td>
<td>288 ± 13</td>
<td>773 ± 33</td>
<td>1.39 ± 0.04</td>
<td>81.8 ± 5.7</td>
</tr>
<tr>
<td>D (8)</td>
<td>29.0 ± 0.5</td>
<td>230 ± 7*</td>
<td>641 ± 21*</td>
<td>0.70 ± 0.08*</td>
<td>64.6 ± 7.1</td>
</tr>
<tr>
<td>ID (8)</td>
<td>7.9 ± 2.0</td>
<td>289 ± 10</td>
<td>873 ± 33</td>
<td>1.27 ± 0.11</td>
<td>90.0 ± 8.0</td>
</tr>
</tbody>
</table>

* C = control; D = diabetes; ID = insulin-treated diabetes; DI = diabetic rats treated with insulin for 20 days following 180 days of untreated diabetes. Number of animals in parentheses. Day 2 controls were used for comparisons with short-term diabetic rats (days 2, 4 and 7); data given as means ± sem; *p < 0.05.
significantly in diabetic animals, while insulin treatment totally normalized this parameter. Serum T₄ was lowered initially in the non-insulin-treated, short-term diabetic group. Long-term diabetic rats did not differ significantly from controls with respect to serum T₄.

**Total β-receptor MBC**

The total β-receptor MBC declined during the initial phase of diabetes, reached a minimum at day 4 (p < 0.05) and tended to normalize at day 7 in both insulin-treated and placebo-treated rats (Fig. 2A). After 90 and 200 days of untreated diabetes, β-receptor MBCs did not differ from the respective control groups. Twenty days of strict blood glucose control did not influence the total β-receptor MBC. Receptor affinity did not differ from control values at any time during the experiment (Table 2).

**Calcium ion channel MBC**

The Ca²⁺ channel MBC initially declined during short-term diabetes, with normalization at day 7 only in the insulin-treated group, a similar pattern as for β-receptors (Fig. 2B). After 90 and 200 days the Ca²⁺ channel MBCs were increased by means of 36% (p < 0.05) and 27% (p < 0.05) compared with the respective control groups, as depicted in Fig. 2. Twenty days of insulin treatment following 180 days of untreated diabetes led to a reduction in the Ca²⁺ channel MBC compared to the control level. Receptor affinities did not differ from control values at any time during the experimental period (Table 2).

**Discussion**

In the present study STZ-induced short-term diabetes in the rat gave rise to an early (days 2 and 4) decrease in the MBC for both β-receptors and voltage-gated Ca²⁺ channels. Because the reduction could not be prevented by insulin, this effect may have been due to STZ per se, rather than diabetes alone. In long-term diabetic rats, a 36% and 27% increase in myocardial binding sites for the Ca²⁺ channel antagonist [³H]PN200-110 90 and
Table 2. The maximum binding capacity (MBC) and equilibrium dissociation constants for β-receptors and Ca\textsuperscript{2+} channels in long-term streptozotocin (STZ)-diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>β-receptor MBC ([I\textsuperscript{125}I]ICYP) (fmol/mg P)</th>
<th>K\textsubscript{d} for β-receptor (pmol/l)</th>
<th>Ca\textsuperscript{2+} channel MBC ([\textsuperscript{3}H]PN200-110) (fmol/mg P)</th>
<th>K\textsubscript{d, Ca}\textsuperscript{2+} channel (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2 C (6)</td>
<td>42.2 ± 2.5</td>
<td>9.83 ± 1.45</td>
<td>131.3 ± 8.9</td>
<td>66.6 ± 5.2</td>
</tr>
<tr>
<td>Day 200 C (6)</td>
<td>37.8 ± 3.9</td>
<td>10.25 ± 0.79</td>
<td>171.0 ± 17.1</td>
<td>52.2 ± 3.5</td>
</tr>
<tr>
<td>Day 200 D (8)</td>
<td>40.0 ± 2.8</td>
<td>10.00 ± 1.29</td>
<td>216.8 ± 7.89</td>
<td>45.3 ± 4.5</td>
</tr>
<tr>
<td>Day 200 DF (8)</td>
<td>39.9 ± 3.4</td>
<td>7.25 ± 0.65</td>
<td>176.0 ± 16.7</td>
<td>45.9 ± 5.1</td>
</tr>
</tbody>
</table>

\(^a\) C = control group; D = STZ-diabetes; DI = 20 days of insulin treatment following 180 days of untreated diabetes; P = membrane protein; [\textsuperscript{125}I]ICYP = \textsuperscript{125}I-iodocyanopindolol. Number of animals given in parentheses. The Ca\textsuperscript{2+} channel MBC increased during long-term untreated STZ-diabetes, while 20 days of insulin treatment normalized the MBC. The K\textsubscript{d} values for both receptor types in the different groups did not differ at any time; \(p < 0.05\).

200 days after STZ was observed, without change in receptor affinity. The number of total binding sites for the β-receptor antagonist [\textsuperscript{125}I]ICYP did not differ from control values during long-term diabetes, despite reductions in serum T\textsubscript{3} concentrations of 27% and 50% after 90 and 200 days. A period of insulin treatment following 180 days of untreated diabetes totally normalized the Ca\textsuperscript{2+} channel MBC and serum T\textsubscript{3}, while no changes appeared for the total β-receptor MBC.

**Calcium ion channels**

The observation that the Ca\textsuperscript{2+} channel MBC increased during longstanding diabetes in accordance with the report by Nishio et al. (13), who found a 27% and 42% increase in the number of binding sites for [\textsuperscript{3}H]PN200-110 in cardiac membranes from STZ-diabetic rats after 42 and 70 days, respectively, without change in receptor affinity. In opposition, Lee and colleagues (12) reported a 29% and 50% decrease in the Ca\textsuperscript{2+} channel MBC and also a decrease in receptor affinity for [\textsuperscript{3}H]nitrendipine in STZ-diabetic rats after 21 and 56 days, respectively. The different radioligands employed in these studies can hardly explain the conflicting results, because both drugs belong to the dihydropyridine family, have similar chemical properties and bind to the same site in the Ca\textsuperscript{2+} channel. The different methods of preparing myocardial membranes may have played a role. The most obvious difference between the methods employed seems to be that Lee et al. (12) employed 40°C in their initial homogenate preparation (12) instead of a cold environment as did Nishio (13) and ourselves. The different results on Ca\textsuperscript{2+} channel MBC cannot be explained by the different ages of the animals because the rats of Lee et al. and our rats were of similar age. Lee et al. (12) and Nishio et al. (13) both used Sprague-Dawley rats, whereas we used Wistar rats. Genetic variation between strains thus does not seem crucial, because results were similar in the study of Nishio and ourselves. In the present study we used female rats in order to avoid excessive weight gain due to fat deposits during the prolonged observation period. However, because our results are in accordance with those of Nishio et al., who used male rats, we assume that sex does not influence the results. A biphasic regulation of Ca\textsuperscript{2+} channels, due to different diabetes duration, was suggested by Lee et al. in order to explain the opposing results between their and Nishio’s group (12). In accordance, we found an early reduction in the Ca\textsuperscript{2+} channel MBC, which reached a minimum 4 days after STZ, followed by a tendency towards normalization. We do, however, ascribe the initial reduction in the Ca\textsuperscript{2+} channel MBC to the induction of systemic illness by STZ. The sudden drop in serum T\textsubscript{3} and T\textsubscript{4}, which characterizes severe, non-thyroidal illness (low T\textsubscript{3} syndrome) in rats (22), supports this point of view. Different degrees of systemic illness between the animals of Lee et al. and our animals may have played a role, but this cannot be ruled out, because no data were given on body or heart weight or serum thyroid hormones by Lee (12). The late increase in the Ca\textsuperscript{2+} channel MBC observed during diabetes may be a myocardial response to insulin deficiency per se. However, the upregulation could also be a consequence of several disturbances in the Ca\textsuperscript{2+}-regulating mechanisms in cell or sarclemma. An increase in Ca\textsuperscript{2+} channel number could serve to counter-regulate the defect β-receptor-adenylate cyclase system (6) or defects in the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange mechanism (2, 9). It must also be considered that an increased density in binding sites measured by radioligand binding does not necessarily indicate an increased channel activity, although it is most likely. Streptozotocin-induced myocardial toxicity alone cannot explain the observed changes, because insulin treatment was able to completely return the Ca\textsuperscript{2+} channel MBC as well as other parameters to normal.

**Effect of insulin treatment on Ca\textsuperscript{2+} channels**

Twenty days of close blood glucose control following 180 days of untreated diabetes resulted in total normalization of the Ca\textsuperscript{2+} channel MBC. Nishio et al. (13) prevented an increase in the Ca\textsuperscript{2+} channel MBC by intensively treating STZ-diabetic animals with insulin for 56 days, beginning 14 days after STZ (i.e. before any change in the Ca\textsuperscript{2+} channel MBC had become evident).
However, insulin treatment for 14 days following 56 days of untreated STZ diabetes (when the Ca\(^{2+}\) channel MBC had reached a higher level) did not restore the Ca\(^{2+}\) channels to normal (13). It is possible that the animals used by Nishio et al. were more heavily affected by the diabetic state than those in our study. Nishio et al. report decreases in body and heart weight of 44% and 37% 70 days after STZ, compared with body and heart weight losses of 15% and 12% 90 days after STZ in our set-up. The present study shows for the first time that insulin treatment is able to not only prevent development of changes in the Ca\(^{2+}\) channel MBC, but also to restore binding capacity to normal after 6 months of untreated diabetes.

**Beta receptors**

No significant change in the total \(\beta\)-receptor MBC or receptor-affinity was observed 90 and 200 days after induction of diabetes in the present study. When interpreting the results on quantitative changes in \(\beta\)-receptors it must be borne in mind that in the present study we used crude membrane preparations rather than fractionated membranes. Because sarcolemmal and intracellular membranes were not separated strictly, we cannot exclude that redistribution of \(\beta\)-receptors may have occurred between the different membrane fractions without any measurable net quantitative changes (17). This would be in accordance with the observations by Kashiwagi et al. (18), who reported a specific decrease of 41% in cell surface membrane \(\beta\)-receptors in 10-week-old STZ-diabetic rat hearts, whereas the total \(\beta\)-receptor number was unchanged. Furthermore, Nishio and colleagues (5) reported a 59% decrease in the \(\beta\)-receptor MBC in STZ-diabetic rats 70 days after STZ, without change in receptor affinity. Apart from the method of membrane preparation, various degrees of diabetes may have played a role in the outcome. Accordingly, the animals in Nishio et al.'s experiment may have been more severely affected than ours, as judged by body and heart weight reduction (by 48% and 35% 70 days after STZ vs 15% and 12% 90 days after STZ in the present study).

Others have reported an unchanged (6) or decreased \(\beta\)-receptor MBC (19), apparently depending on the duration as well as the severity of the diabetic state. The presently observed early reduction in the \(\beta\)-receptor MBC (within 7 days), reaching a minimum at day 4, may be attributed to the presence of low T\(_3\) syndrome, as reflected by the drop in serum T\(_3\) and T\(_4\) within 2 days after STZ, as well as STZ itself. Because serum T\(_3\) remained low throughout the study period, some degree of low T\(_3\) syndrome obviously persisted. It has previously been shown that T\(_3\) specifically induces an increase in \(\beta\)-receptor mRNA through interaction with the T\(_3\) nuclear receptor in responsive tissues (2). Moreover, the \(\beta\)-receptor MBC decreases markedly during overt hypothyroidism (21). It was therefore surprising that the diabetes-induced low T\(_3\) syndrome (22) did not result in a reduced \(\beta\)-receptor MBC in the present study. On the other hand, low T\(_3\) syndrome differs from regular hypothyroidism in a number of ways, and the \(\beta\)-receptor state in diabetes may be dependent on conditions other than peripheral thyroid metabolism. From the present findings it seems that, despite reduced serum T\(_3\), the total \(\beta\)-receptor production rate can be maintained at normal levels even in long-term untreated diabetes.

An early down-regulation in voltage-gated Ca\(^{2+}\) channels and adrenergic \(\beta\)-receptors in the myocardium of STZ-diabetic rats was followed by a more protracted increase in the Ca\(^{2+}\) channel MBC and a normalization of the total \(\beta\)-receptor MBC. Insulin treatment at a late stage of disease fully normalized the Ca\(^{2+}\) channel MBC as well as serum thyroid hormones. The increased Ca\(^{2+}\) channel MBC together with other reported diabetes-associated disturbances in myocardial Ca\(^{2+}\) metabolism may contribute to a cellular Ca\(^{2+}\) overload, which again may be part of the pathogenesis in diabetic cardiomyopathy.

**Acknowledgments**

The study was supported by The Danish Heart Foundation, The Danish Diabetes Association, the Nordic Insulin Foundation, the Novo Foundation and the Danish Medical Research Council.

**References**


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Received May 8th, 1995
Accepted August 22nd, 1995