The Effects of Tolbutamide on the Myocardium in Experimental Diabetes

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HENRY A. OLDEWURTEL, MICHAEL M. LYONS, M.D., AND TIMOTHY J. REGAN, M.D.

SUMMARY The effects of chronic tolbutamide treatment were examined in a diabetic animal model in which abnormal myocardial function and composition have previously been demonstrated. Eight diabetic dogs were given tolbutamide 250 mg/day orally and compared with seven untreated diabetics, five healthy dogs receiving tolbutamide, and eight normal controls. After one year, resting hemodynamic studies in the intact anesthetized state showed that treated diabetic dogs had a significantly higher left ventricular end-diastolic pressure of 12.1 ± 1.3 mm Hg associated with normal end-diastolic volume, compared to 6.1 ± 0.8 mm Hg in untreated diabetics (P < 0.01) and 6.3 ± 0.5 in normals. Stroke work and ejection fraction were similar to normals. Acute volume expansion revealed a larger rise of left ventricular end-diastolic pressure in treated and untreated diabetics than normals, without a significant stroke volume response in treated diabetics. Enhanced stiffness of myocardium appeared to be related to interstitial accumulation of periodic acid-Schiff staining material, further intensified in treated diabetics by triglyceride accumulation observed on electron microscopy and by chemical analysis. Thus treatment of diabetes with tolbutamide, despite improved glucose tolerance, effected further reduction of left ventricular function and altered morphology of myocardium.

THE USEFULNESS OF ORAL HYPOGLYCEMIC AGENTS in the treatment of diabetes mellitus has been controversial since the initial reports of enhanced cardiovascular mortality in patients so treated. Although the acute effects of tolbutamide on the myocardium have been described in animals and man, there has been no study of the myocardial response to chronic administration of tolbutamide in diabetes.

In a chronic animal model of mild diabetes, we have previously observed abnormal left ventricular function without significant coronary artery disease, associated with accumulation of periodic acid-Schiff positive glycoprotein in the myocardial interstitium and cell triglyceride increments. This study was performed to examine the effects of chronic administration of tolbutamide, at doses which virtually normalized glucose tolerance, on the altered myocardial function and composition associated with experimental diabetes.

Methods

Healthy male mongrel dogs, 2–3 years of age, were selected for study. The dogs were dewormed and treated with distemper vaccine; they had no clinical evidence of disease in eight weeks of observation before the study began. Blood samples were negative for heart worms and both hematocrit and serum albumin were initially normal. All groups received the same diet consisting of 8% fat, 22% protein, 58% carbohydrate, 9% ash and 3% crude fiber.

Group I consisted of eight normal controls. Group II was five healthy dogs given tolbutamide 250 mg daily at mealtime. Fifteen dogs were made diabetic without ketosis using alloxan monohydrate, 20 mg/kg dose i.v., given sequentially for three doses spaced at monthly intervals. We have previously provided evidence that alloxan per se in this dosage regimen produces no alteration of the myocardium. Seven of the 15 diabetic dogs were untreated (group III); the remaining eight (group IV) were treated with tolbutamide given 250 mg orally shortly before mealtime, starting one month after the third dose of alloxan when abnormal glucose tolerance was established.

Fasting plasma glucose and glucose tolerance tests were obtained in each animal before and after induction of diabetes every three months until the terminal study. Plasma glucose was analyzed on an Auto Analyzer by the glucose-oxidase method. The glucose tolerance test was performed by intravenous challenge of 25 g of glucose in the unanesthetized state. Plasma disappearance rates of glucose (Gk) were obtained from a semilogarithmic plot of plasma levels versus time. Fasting venous blood samples were also taken in the control state and intermittently through the observation period for determination of plasma triglyceride, cholesterol and phospholipids.

After approximately six months of treatment tolbutamide blood levels were obtained in groups II and IV at hourly intervals after oral administration of the drug. The serum was frozen for subsequent analysis using a gas chromatographic technique.

Hemodynamic Studies

Each dog was last fed approximately 24 hours prior to study. By the time of study, serum tolbutamide levels were negligible. All dogs were anesthetized with morphine 3 mg/kg subcutaneously and sodium pentobarbital 12 mg/kg intravenously. They were ventilated on a Harvard respirator pump with endotracheal tube. Hemodynamic data were obtained with chest intact. Goodale-Lubin catheters, 8F, 50 cm long, were placed in the left ventricle and the root of the ascending aorta and connected directly to Statham P23Db strain gauge transducers. Photographic recordings were made from a multi-channel oscilloscope-recorder (Electronics for Medicine) as previously described.

Cardiac output and left ventricular volumes were determined by the thermal indicator dilution method. Cardiac output by this technique has been found to correlate well

*Courtesy of Dr. D.G. Kaiser, Upjohn Co., Kalamazoo, Michigan.
with the dye dilution technique\textsuperscript{14, 15} and end-diastolic volume has been correlated with the angiographic method.\textsuperscript{16, 17} A Swan-Ganz thermodilution catheter tip was placed in the main pulmonary artery and 10 ml of room temperature normal saline was injected as a bolus into the right atrium through the proximal lumen. Change of temperature was detected by the thermistor in the pulmonary artery and displayed on a recorder through a Wheatstone bridge; the area of the curve was computed manually for cardiac output calculation. Left ventricular ejection fraction was obtained in duplicate from the left ventricular washout curves obtained just above the aortic valve. Five ml cold saline was injected as a bolus into the inflow site of the ventricle; adequate mixing at this site has been described in our previous report.\textsuperscript{8} End-diastolic volume was calculated from the ratio of stroke volume and ejection fraction and expressed per kg body weight. Stroke work index in g·m/kg was calculated from the product of stroke volume per kg and the mean left ventricular systolic pressure minus end-diastolic pressure × 0.0136. The ratio of left ventricular end-diastolic pressure and volume was used as a simple index of wall stiffness at end diastole.

To evaluate the contractile state of the ventricle \textit{in vivo} we employed an index which normalizes left ventricular dP/dt max for 1) the maximal isovolumic pressure (MIP) and 2) the circumferential fiber length (2πr), assuming a spherical shape at the end of the systolic isovolumic period and deriving the radius from the end-diastolic volume. The formula\textsuperscript{18} is

\[
\frac{dP/dt \text{ max}/\text{MIP}}{2\pi r}
\]

Left ventricular function was assessed during volume expansion.\textsuperscript{8} A second catheter was introduced into the ventricle and normal saline was infused at a rate of 50 ml/min. Left ventricular end-diastolic pressure was continuously recorded at high sensitivity while cardiac output and left ventricular volumes were determined rapidly in duplicate after three to four minutes of infusion when the animals were in a steady state as judged by heart rate, aortic pressure and ventricular end-diastolic pressure.

**Metabolic and Histologic Studies**

Approximately one hour following these studies, a thoracotomy was performed and the heart was arrested with iced Ringer’s solution. Samples of myocardium were rapidly taken from the periaxial region and divided into outer and inner layers. The outer layer was carefully trimmed of epicardial adipose tissue. All samples were placed in liquid nitrogen and homogenized in phosphate buffer. Lipids were extracted by the Folch procedure for determination of triglyceride,\textsuperscript{10} cholesterol\textsuperscript{11} and phospholipid.\textsuperscript{12} Separate portions of these samples were homogenized and electrolytes extracted for 48 hours in distilled water. Potassium and sodium were determined on an Auto Analyzer system with a flame attachment. Water content was calculated from the wet weight–dry weight difference after the samples were dried in an oven at 100°C to constant weight. Statistical analyses included analysis of variance followed by Dunnett’s multiple comparison test.\textsuperscript{19}

Sections were taken from the left ventricular myocardium and the left coronary artery for histochemical ex-

amination by the pathologist without knowledge of the diagnosis. These examinations included periodic acid-Schiff after treating twice with diastase to prevent staining of glycogen.\textsuperscript{20} Also, 12–15 specimens for electron microscopy were rapidly fixed in cold glutaraldehyde buffered with phosphate. The tissue was then washed, postfixed in osmium, exposed to lead and uranyl acetate, and embedded in epon.

**Results**

The diabetic animals (groups III and IV) were observed for 10–12 months after the initial alloxan dose and compared with the normal dogs (group I) observed for a similar duration. The tolbutamide control group (group II) was treated for 7–9 months, which was comparable to the duration of treatment in group IV. Body weight, hematocrit and serum albumin at the terminal study did not differ significantly from the control period in any group. There were no animals with heart worms at postmortem examination.

Fasting plasma glucose remained in the normal range in both diabetic groups before and after the induction of diabetes (table 1). The normal and tolbutamide control groups also had normal fasting plasma glucose. In the latter, no hypoglycemia was observed in fasting samples, presumably because the peak action of the drug was during the postprandial period. The glucose clearance was significantly reduced one month after the last dose of alloxan in both diabetic groups and remained reduced in the untreated dogs of group III throughout the observation period. In those treated with tolbutamide there was significant improvement of glucose intolerance toward normal. The clearance constant remained normal in the tolbutamide control group.

Tolbutamide blood levels were assayed after six months of treatment in groups II and IV. Peak serum levels after drug ingestion at meal time averaged 38 ± 9 μg/ml at four hours and were similar in both groups (fig. 1). This level approximated the values reported in humans after an oral dose of 500 mg tolbutamide.\textsuperscript{21}

The resting hemodynamic data are summarized in table 2. Heart rate and aortic pressures were not significantly different in the four groups but there was a significant elevation of left ventricular end-diastolic pressure in the tolbutamide treated diabetics at rest compared to normals ($P < 0.01$). In the untreated diabetics, left ventricular end-diastolic pressure was normal. There was no significant difference in left ventricular end-diastolic volume, stroke work, ejection fraction and contractility index at rest in the four groups. The ratio of left ventricular end-diastolic pressure and volume was increased in the tolbutamide-diabetic group, but not significantly. In the tolbutamide control group the modest elevation of end-diastolic pressure was accompanied by a nearly proportional increase of end-diastolic volume. The enhanced end-diastolic pressure in the tolbutamide-diabetic group despite a normal end-diastolic volume was consistent with an interpretation of increased diastolic stiffness of the ventricle. This could not be accounted for by hypertrophy since ventricular weights were within the normal range in all dogs.

To examine the response to volume expansion, 200 ml of saline was infused acutely into the left ventricular chamber over a four minute period. There was no significant change
of heart rate or aortic pressure from control levels in any group during the infusion. The normal dogs exhibited a significant rise of left ventricular end-diastolic pressure and stroke volume (fig. 2). In the untreated diabetic group there was a significantly greater rise of left ventricular end-diastolic pressure as compared to normals, with a similar stroke volume increment. The response in the tolbutamide treated diabetic animals showed no significant stroke volume change, despite an increased left ventricular end-diastolic pressure, reflecting a more depressed functional state. In the tolbutamide control group, three animals were tested. Left ventricular end-diastolic pressure rose from 6.6 ± 0.8 to 9.3 ± 1.1 mm Hg and stroke volume rose from 0.95 ± 0.08 to 1.41 ± 0.22 ml/kg. This response was qualitatively similar to the normal group.

Gross and histologic examination of the coronary vessels revealed no evidence of large or small vessel occlusive lesions. There was no indication of hypertrophy, fibrosis, necrosis or inflammatory reaction in any animals of the four groups. Histochemical staining with periodic acid-Schiff reagent after two diastase treatments showed distinct accumulation of a glycoprotein-like material in the myocardial interstitium of all untreated and treated diabetic dogs, without an apparent difference in extent between the two groups.

On electron microscopy, lipid accumulation was observed in group IV with fat particles interspersed between myofibrils in a focal distribution (fig. 3), which was not apparent in the other three groups. The lipid particles, associated with pockets of glycogen particles, were in most

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**Table 1. Diabetic Status**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (kg)</th>
<th>Fasting plasma glucose (mmoles/L)</th>
<th>Glucose clearance constant (Gk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Normal (N = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control state</td>
<td>22.5 ± 0.4</td>
<td>5.38 ± 0.28</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Terminal study</td>
<td>23.9 ± 1.0</td>
<td>5.46 ± 0.33</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>II. Tolbutamide (N = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control state</td>
<td>21.9 ± 0.8</td>
<td>5.12 ± 0.11</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Terminal study</td>
<td>21.5 ± 1.2</td>
<td>5.00 ± 0.23</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>III. Diabetes (N = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control state</td>
<td>22.8 ± 0.6</td>
<td>5.65 ± 0.23</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Postalloxan</td>
<td>22.3 ± 1.1</td>
<td>5.58 ± 0.31</td>
<td>1.6 ± 0.1*</td>
</tr>
<tr>
<td>Terminal study</td>
<td>23.7 ± 1.7</td>
<td>5.79 ± 0.28</td>
<td>1.8 ± 0.1*</td>
</tr>
<tr>
<td>IV. Diabetes and tolbutamide (N = 8)</td>
<td>23.5 ± 0.8</td>
<td>5.17 ± 0.17</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>Control state</td>
<td>23.0 ± 1.0</td>
<td>5.36 ± 0.21</td>
<td>2.4 ± 0.2†</td>
</tr>
<tr>
<td>Terminal study</td>
<td>24.6 ± 1.4</td>
<td>4.67 ± 0.31</td>
<td>3.9 ± 0.6</td>
</tr>
</tbody>
</table>

Values are mean and standard error of mean. Significance was tested by Dunnett's multiple comparison test. *P < 0.05, paired t-test.

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**Figure 1.** Average serum concentrations of tolbutamide in the diabetic animals (group IV) with oral administration after control sample (c).

**Figure 2.** Relationship of left ventricular end-diastolic pressure and stroke volume response before and during the infusion of saline into the left ventricular chamber. The lower panel indicates that resting end-diastolic pressure was higher in tolbutamide treated diabetics (DM + T) than the normals (P < 0.01) and the untreated diabetics (DM) (P < 0.02). All groups showed a significant rise of end-diastolic pressure during saline infusion, while the stroke volume rose significantly in the normal and diabetic group (both P < 0.05), but not in the tolbutamide treated diabetics.
instances contained within single membrane bound structures consistent with sarcoplasmic reticulum. This corresponded to a significant increase of myocardial tissue triglyceride in these animals (table 3), despite the fact that terminal plasma lipid levels were normal.

Myocardial cation and water composition is shown in table 4. Group II had a significant increase of tissue water while the changes in group IV were not significant.

Discussion

This diabetic animal model exhibits reduced glucose tolerance and plasma insulin levels after intravenous glucose and diminished pancreatic levels of insulin during fasting. The mild degree of diabetes enables long-term observations without exogenous hormone treatment. Since abnormalities of myocardial function and composition were not observed in animals in which the pancreatic effects of alloxan and the production of diabetes were prevented, a cardiotoxic effect of this compound appears unlikely. Moreover, similar alterations of the left ventricle were observed in animals with spontaneous diabetes.

Early treatment of the diabetic animal with tolbutamide in a dosage producing serum levels approximating those of treated human diabetics improved glucose intolerance toward normal. Hemodynamic study revealed no corresponding myocardial effect. End-diastolic pressure at rest was significantly greater than in untreated diabetic dogs and normal controls, despite similar levels of end-diastolic volume. Acute volume expansion with saline revealed a higher level of filling pressure without a significant stroke volume response in the group of tolbutamide treated diabetics. This is consistent with the interpretation that there was increased wall stiffness in diastole with an associated,

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart mass (g)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Stroke volume (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>142 ± 7</td>
<td>135 ± 11</td>
<td>133 ± 12</td>
</tr>
<tr>
<td>II</td>
<td>100 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>III</td>
<td>6.3 ± 0.3</td>
<td>0.39 ± 0.4</td>
<td>0.37 ± 0.3</td>
</tr>
<tr>
<td>IV</td>
<td>119 ± 13</td>
<td>108 ± 7</td>
<td>121 ± 1.3*</td>
</tr>
</tbody>
</table>

*Denotes a multiple comparison test by two-tailed method; P < 0.001.
perhaps related, effect on systolic function.

The basis for these hemodynamic abnormalities could be related to altered composition of the myocardium. Interstitial accumulation of periodic acid-Schiff staining material may account for the diminished diastolic compliance in a manner analogous to that of amyloid heart disease. This process, if intensified, might be responsible for the greater hemodynamic abnormality in the treated diabetic animals, but this could not be concluded on morphologic grounds. A distinctive compositional difference was the twofold increment of triglyceride concentration in myocardium, compared to untreated diabetics. On electron microscopy lipid particles were found interspersed between myofibrils only in the diabetics receiving tolbutamide. It is possible that this compositional change enhanced the viscous properties of the cardiac cell and contributed to the alteration of diastolic compliance.

The mechanism of lipid accumulation is not known. In vitro studies of tolbutamide have shown increased cyclic AMP activity in the myocardium. In subacute experimental diabetes, reduced levels of AMP-phosphodiesterase in myocardium have been reported. Additive, if not synergistic, effects of the drug in diabetes could produce increments of cyclic AMP in the myocardial cell, which may effect substantial triglyceride accumulation.

In the nondiabetic animals treated with tolbutamide left ventricular end-diastolic pressure and volume were both elevated. However, the resting pressure/volume ratio and the response to volume expansion with saline were normal. Presumably tolbutamide induced a retention of salt and water to enhance plasma volume in the nondiabetics, a renal action which appeared to be aborted in the diabetics. Moreover, modest changes in tissue composition were observed in these controls. In addition to the elevation of myocardial triglyceride that was intermediate between normals and that of the diabetic group receiving hypoglycemic agent, there was an increment of tissue water that may well have been related to expansion of extracellular volume. Morphologic abnormalities in the form of interstitial glycoprotein or triglyceride accumulation between myofibrils were not observed in the tolbutamide control group.

Extrapolation of these data to man is done with caution for several reasons. The degradation products in the dog are p-toluenesulfamide and p-toluene sulfonamide, in contrast to man where the carboxy metabolite predominates. Since the former are present in relatively low concentrations, they are perhaps unlikely to be responsible for tissue toxicity. After a daily tolbutamide dose of approximately 10 mg/kg, serum determinations in four dogs showed no significant difference in serial samples over 24 hours by an assay method measuring the intact sulfonamide compound and an assay measuring the degradation products as well. It is of interest that while lipid also accumulates in liver after tolbutamide in dogs, feeding of large doses of the tolune metabolites produced no such effect.

Although these experiments were done in diabetic animals with fasting normoglycemia, the drug was given before mealtime so that the time of major activity would correspond to the period of elevated glucose levels. If fasting hyperglycemia was associated with a qualitatively different
cardiac response to chronic tolbutamide use, then these studies would not be applicable to humans with elevated fasting blood glucose. However, such diabetics appear to have myocardial abnormalities marked by triglyceride\textsuperscript{30, 31} and interstitial glycoprotein accumulation.\textsuperscript{31, 32} Since the former at least, is intensified by tolbutamide treatment in this animal model with chemical diabetes, the abnormal metabolism of myocardium in human hyperglycemic diabetics may be similarly affected.

**Acknowledgment**

The authors are grateful to Mrs. B. Jenkins, Mrs. E. Melzer, Miss R. Torres and Mr. E. Wolf for expert technical assistance and Mrs. A. Binetti and Mrs. A. Brown for secretarial services rendered.

**References**


**Table 4. Myocardial Cations and Water Content**

<table>
<thead>
<tr>
<th></th>
<th>Potassium (μEq/g)</th>
<th>Sodium (μEq/g)</th>
<th>Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer</td>
<td>Inner</td>
<td>Outer</td>
</tr>
<tr>
<td>I. Normal</td>
<td>62.1 ± 1.3</td>
<td>61.9 ± 1.2</td>
<td>32.0 ± 1.1</td>
</tr>
<tr>
<td>(N = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Tolbut.</td>
<td>61.6 ± 1.5</td>
<td>61.4 ± 3.7</td>
<td>39.6 ± 3.7</td>
</tr>
<tr>
<td>(N = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Diabetes</td>
<td>63.8 ± 1.1</td>
<td>62.4 ± 1.7</td>
<td>30.7 ± 2.8</td>
</tr>
<tr>
<td>(N = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV. Diabetes</td>
<td>59.8 ± 3.9</td>
<td>58.3 ± 2.0</td>
<td>36.8 ± 2.9</td>
</tr>
<tr>
<td>Tolbut.</td>
<td>(N = 8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dunnett's test; *P < 0.01.

**TOLBUTAMIDE AND HEART/Wu et al.**
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