Altered Norepinephrine Turnover and Metabolism in Diabetic Cardiomyopathy

Pallab K. Ganguly, Ken S. Dhall, Ian R. Innes, Robert E. Beamish, and Naranjan S. Dhall

Cardiac norepinephrine turnover and metabolism were examined in rats 8 weeks after the induction of chronic diabetes by an intravenous injection of streptozotocin (65 mg/kg). Cardiac norepinephrine concentration, norepinephrine turnover, and norepinephrine uptake were markedly increased in chronic diabetes in comparison with control values; these changes were reversible by 28-day insulin therapy. When the animals were exposed to cold for 6 hours, norepinephrine turnover rate constant increased in control and decreased in diabetic animals; cold exposure also increased norepinephrine concentration in diabetic hearts. Both cardiac norepinephrine concentration and turnover rate in diabetic rats were restored toward control values by ganglionic blockade with pentolinium. The conversion of \(^{3}H\)tyrosine to \(^{3}H\)catecholamine was enhanced and tyrosine hydroxylase as well as dopa decarboxylase activities were increased in diabetic hearts. The higher concentrations of \(^{3}H\)normetanephrine and deaminated catechols indicated a faster metabolic rate of norepinephrine metabolism in hearts from diabetic rats; both monoamine oxidase and catechol-O-methyltransferase activities were also increased. The increased activities of the enzymes for the synthesis and metabolism of norepinephrine were not evident on treating the diabetic animals with insulin. These data not only support the view that chronic diabetes in rats is associated with increased sympathetic activity but also indicate that the cardiac norepinephrine concentration in diabetic rats may be maintained at a higher than normal level by an increased synthesis and uptake of norepinephrine in the adrenergic nerve terminals. (Circulation Research 1986;59:684-693)

It is now well accepted that norepinephrine (NE), which acts as a neurotransmitter in the sympathetic system, is stored in the intracellular membrane-bound granules in the adrenergic nerve terminals. Although sympathetic innervation contributes very little to the intrinsic contractile state of the heart muscle, it plays a major role in the augmentation of myocardial dynamics in response to stress. Recent data from studies with both diabetic patients and diabetic animals have indicated the possibility of an abnormal activity of the sympathetic nervous system. In diabetes the levels of plasma catecholamines are reported to be decreased, \(^{10-12}\) increased, \(^{4,13,14}\) both increased and decreased, \(^{15}\) or unchanged. \(^{16}\) The apparent discrepancy in these results may be due to the fact that many of the studies are comparing diabetes of short duration in animals caused by chemicals with the human disease of long duration. Furthermore, despite the existing information about plasma catecholamines in diabetes, the validity of inferences about the activity of the sympathetic nervous system based only on circulating plasma catecholamines is questionable. \(^{17}\) This view is derived from the fact that the relation between circulating catecholamine levels and activity of the sympathetic system is obscured by the lack of information regarding NE concentration, uptake, turnover, and metabolism in the diabetic myocardium. Paulson and Light\(^{14}\) and Fushimi et al\(^{19}\) have shown an increase in cardiac NE and have suggested some disturbance of NE release in diabetic hearts. However, NE concentration in the myocardium was found to be unchanged \(^{9}\) or decreased \(^{18}\) in diabetes. In addition to these controversial findings very little information concerning NE turnover, uptake, and metabolism is available in the literature. The present study was undertaken to examine NE levels during the development of diabetic cardiomyopathy and to test the status of NE turnover, uptake, and metabolism in diabetic myocardium. Experiments were also done to investigate whether the observed changes in diabetes are reversible on treatment with insulin. Previous studies from this laboratory have demonstrated that chronic diabetes, produced by injecting streptozotocin in rats, results in cardiomyopathy, which involves various biochemical, functional, and ultrastructural alterations in cardiac cell. \(^{19-22}\) Accordingly, streptozotocin-induced diabetes in rats was used as an experimental model in this investigation. It may also be pointed out that this model of diabetes is known to be accompanied by a low level of thyroid hormone in plasma, \(^{4,19}\) and since hypothyroidism has been shown to increase the sympathetic activity \(^{23-24}\) it is possible that any defect in cardiac NE concentration during diabetes may be due to a chronic deficiency of thyroid hormone. Experiments were undertaken to examine if changes in cardiac NE concentration are reversible on treatment of diabetic animals with thyroid hormone.
Materials and Methods

Experimental Model

Male Sprague-Dawley rats (175–200 g) of the same age were rendered diabetic by a single intrafemoral vein injection of streptozotocin (65 mg/kg body wt) dissolved in a citrate-buffered vehicle (pH 4.5). Age-matched normal rats injected with citrate buffer alone were used as controls. All rats were provided with food and water ad libitum during the entire experiment. The diabetic animals were subdivided randomly into three groups. The first group of diabetic animals was sacrificed 3, 7, 14, 28, or 56 days after the streptozotocin injection; the control animals were matched accordingly. The animals in the second group were injected with 3 U protamine zinc insulin/day subcutaneously for the last 4 weeks before sacrifice at 8 weeks. The third group received triiodothyronine (T3) (10 µg/kg body wt, subcutaneous) daily beginning the third day after streptozotocin injection until sacrifice at 8 weeks. The rats were quickly decapitated with minimal handling, and an initial 2–2.5 ml of blood from the trunk was collected through a funnel into a heparin-containing tube. Plasma samples were analyzed for insulin and T3 by the standard radioimmunoassay techniques (Amer sham Canada Ltd., Oakville, Ont.) and for glucose levels by the Worthington Statzyme glucose reagent kit (Worthington Diagnostics, New Jersey). The above experimental protocol is similar to that employed previously by us and others for establishing diabetic cardiomyopathy.19–22,25

Catecholamine Determination

Ventricular tissue was homogenized by a Polytron homogenizer with 1% Na2S2O3 in 0.4 N HClO4 (1:10 wt/vol) and the homogenate was centrifuged at 30,000g for 20 minutes. The supernatant was carefully aspirated, volume measured, and pH adjusted to 8.6 with 1 M Tris. Catecholamines in this supernatant were extracted by using activated aluminium oxide and measured by employing high-performance liquid chromatography (HPLC) with electrochemical detection as described by Sole et al.26 The chromatographic apparatus included a Spectra-Physics SP 8700 solvent delivery system equipped with an LC-4A amperometric detector (Bioanalytical Systems) in an oxidation mode. The column was a NOVA PAC™ C18 (part number 086344) reverse phase (Water Associates). The results were recorded on a Hewlett-Packard integrator 3390A. HPLC coupled with electrochemical detection offers a high degree (picogram) of sensitivity and specificity in the determination of catecholamine levels.

Norepinephrine Turnover

Norepinephrine turnover was examined in the control and diabetic hearts 8 weeks after the onset of the experiment. Each rat was given 6 µCi/100 g dl-[7-3H-N-NE] (New England Nuclear Corp., specific activity 9.4 Ci/mmole) in 100 µl of 10−4 N acetic acid by i.p. injection. Preliminary studies showed less than 0.5% of the radioactivity was detectable in the peritoneal cavity 30 minutes after the injection. Specific activity was determined as nanoCuries of NE per gram divided by nanogram NE per gram to yield nCi/ng. The rate of change in the specific activity of NE was measured between 1 and 8 hours after [3H]NE injection. At appropriate times the rats were sacrificed, hearts removed quickly, the atria and connective tissue dissected, and the ventricular tissue was processed to separate catecholamines as described by Sole et al.27 A 500-µl sample of the alumina eluate was added to 10 ml of scintillation fluid (Ready-Solv HP, Beckman) for counting radioactivity. [3H]NE recovery accounted for almost 90% of the total radioactivity present in the supernatant. Rate of disappearance of [3H]NE from the heart was used to estimate the turnover of NE in this tissue.27 The overall rate of NE turnover was expressed as K[NE],27 where K is the fractional turnover rate constant and [NE] is the steady-state level of NE. The fractional turnover rate constant was calculated from the rate of decline of the logarithm of the specific activity of the labelled NE stores (regression coefficient). Since the radioactivity in these experiments was determined in an aliquot of the alumina extract, it is possible that contaminating radioactivity, not related directly to labelled NE, may affect the data on tissue specific activities. However, it is unlikely that such an artifact could affect conclusions based on turnover determinations in control and experimental preparations that were handled simultaneously under identical conditions.

Ganglionic Blockade and Cold Stress

Pentolinium, a ganglionic blocker that does not enter the central nervous system, was used to inhibit the peripheral sympathetic activity in some of the experiments. The drug was injected i.p. (10 mg/kg body wt) every 2 hours for 6 hours, and then NE concentration and turnover rate were measured as described earlier. For cold stress, rats were kept in the cold room (4°C) for 6 hours. The [3H]NE disappearance curve for hearts from rats kept in the cold was then compared with that for hearts from rats kept at room temperature.

Uptake of [3H]NE

In a separate study, some of the experimental animals were sacrificed, hearts removed and slices of ventricle prepared in Krebs-bicarbonate solution at 4°C. To determine the uptake of [3H]NE, slices of comparable weights (both control and diabetic) were placed in beakers containing 15 ml of Krebs-bicarbonate solution for 45 minutes. The solution was prewarmed to 37°C in the incubator and pre-equilibrated with 95% O2–5% CO2; 2 ng/ml of dl-(-7-[3H]NE) and 0.75 mg/ml of ascorbic acid were added in the solution. The heart slices were removed at various time intervals, processed for catecholamine separation and the radioactivity was counted. Drugs were added at the beginning of the preincubation time. The data were plotted as the concentration ratio (i.e., the ratio of counts per gram of tissue over counts per milliliter of medium).
Synthesis of NE and Related Enzyme Activities

Each control and diabetic rat received l-[3H]tyrosine (New England Nuclear Corp., specific activity 72.5 Ci/mmol) according to the method of Levitt et al.28 Rats were sacrificed at various times and cardiac tissue was processed as described earlier for the determination of [3H]catecholamine compounds. Assays for tyrosine hydroxylase and dopa decarboxylase activities were carried out according to the method described by Nagatsu et al29 and Kuntzman et al, respectively. Briefly, tyrosine hydroxylase activity was measured by the conversion of l-[14C]tyrosine to l-[14C]dopa. The reaction mixture was incubated for 1 hour in an atmosphere of nitrogen at 37° C in 1.3 ml of a solution containing 100 mg homogenized tissue, 200 μmol sodium acetate buffer, pH 6.1, 0.5 μmol ferrous sulfate, 1 μmol 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine (DMPH), 20 μmol 2-mercaptoethanol, 1 μmol sodium phosphate, 2 nmol l-[14C]tyrosine (6 × 105 cpm) and 1 μmol 3-hydroxy-4-bromobenzyloxyamine dihydrogen phosphate (NSD 1055). The incubation was carried out at 30° C in air for 15 minutes on a metabolic shaker. The 3H-dopa formed was adsorbed on alumina, eluted, and assayed for radioactivity. Dopa decarboxylase activity was determined fluorometrically by measuring the dopamine formed from l-dopa. The reaction mixture contained 100 mg homogenized tissue, 750 μmol sodium acetate buffer, pH 6.9, and 150 nmol pyridoxal-5-phosphate. After a preincubation period of 15 minutes the reaction mixture was incubated for 1 hour in an atmosphere of nitrogen at 37° C in 1.3 ml of a solution containing 1.52 μmol of l-dopa.

Metabolites of NE and Related Enzyme Activities

Rats were given [3H]NE (6 μCi/100 g) and sacrificed 1 hour after [3H]NE. Major nonconjugated metabolites were isolated from the myocardium and estimated as described by Landsberg and Axelrod.23 Monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) activities were measured by the procedures described by Wurtmann et al19 and Axelrod, respectively. MAO activity was assayed by measuring the conversion of [3H]tryptamine to indoleacetic acid. The incubation mixture (0.3 ml) contained 5 mg tissue, 60 nmol [3H]tryptamine (0.1 μCi), and 50 μmol phosphate buffer, pH 7.4. The incubation was carried out at 37° C in air for 20 minutes on a metabolic shaker. Boiled enzyme was used as a blank. COMT activity was assayed with epinephrine as substrate and an aliquot of the 40,000g supernatant used for this purpose. The incubation mixture (50 μl) contained 5 μmol phosphate buffer (pH 7.6), 2.5 μmol MgCl2, 2.1 nmol S-adenosyl-L-methionine (3H-AdoMet, 0.1 μCi), 0.25 μmol epinephrine, and 25 μl of the supernatant.

Statistical Analysis

Results were expressed as the mean ± SE. Analysis of variance was used for calculating the standard error of the regression coefficient (fractional NE turnover rate constant), the significance of the regression coefficients, and the difference between them. For comparing more than two groups, the multiple-range test was adopted to determine differences between the means within the population. The statistical differences between mean values for two groups were evaluated by Student's t test.

Results

General Features of Experimental Animals and Cardiac Concentration of Norepinephrine

Eight weeks after streptozotocin injection, diabetic rats had lower body weight and elevated ventricular-to-body weight ratios (Table 1). Diabetes was evident in this group by the presence of highly elevated plasma glucose and depressed insulin levels in comparison with control rats. Cardiac NE concentration was markedly increased. Although these changes were almost normalized by insulin treatment, insulin-treated diabetic rats still had lower body and ventricular weights. Experimental diabetes was accompanied by a depressed circulating plasma T3 level, and this is in agreement with data from other studies using a similar experimental protocol.6 19 T3 administration restored the plasma T3 level back to control level but did not correct changes in cardiac NE concentration and other parameters in diabetic rats. Figure 1 shows that there was no significant difference in cardiac NE concentration between control and diabetic groups for 14 days after streptozotocin injection. By 28 and 56 days, NE concentration in myocardium was markedly increased (more than twofold) in diabetic animals. Since elevated plasma glucose and depressed plasma insulin levels

Table 1. General Characteristics of Experimental Rats

<table>
<thead>
<tr>
<th>Rats</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + Insulin</th>
<th>Diabetic + T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>430 ± 12</td>
<td>260 ± 11*</td>
<td>335 ± 9</td>
<td>270 ± 12*</td>
</tr>
<tr>
<td>Ventricular wt (g)</td>
<td>1.10 ± 0.04</td>
<td>0.80 ± 0.04*</td>
<td>0.89 ± 0.02*</td>
<td>0.82 ± 0.03*</td>
</tr>
<tr>
<td>Ventricular/body wt ratio (mg/g)</td>
<td>2.56 ± 0.03</td>
<td>3.07 ± 0.02*</td>
<td>2.65 ± 0.06</td>
<td>3.03 ± 0.04*</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>155 ± 12</td>
<td>465 ± 14*</td>
<td>104 ± 14</td>
<td>400 ± 16*</td>
</tr>
<tr>
<td>Plasma insulin (μU/ml)</td>
<td>31 ± 1.8</td>
<td>14 ± 1.5*</td>
<td>40 ± 5.2</td>
<td>15 ± 1.2*</td>
</tr>
<tr>
<td>Plasma T3 (ng/dl)</td>
<td>79 ± 6</td>
<td>33 ± 3*</td>
<td>80 ± 5</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>Cardiac norepinephrine (ng/g)</td>
<td>225 ± 10</td>
<td>510 ± 15*</td>
<td>230 ± 13</td>
<td>495 ± 11*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 experiments. *p < 0.05.
are evident within 3 days of streptozotocin injection, it appears that elevated NE concentration in the heart is associated with chronic diabetes.

Cardiac NE Turnover

Figure 2 shows the decline of specific activity of cardiac NE with time in control and diabetic (8 weeks) animals. The disappearance of the specific activity of cardiac NE followed first-order kinetics over the time period examined (1-8 hours after the injection); the slope of the [H]NE disappearance curve in the diabetic group was steeper than that in the control group. At 1 hour, the [H]NE content was 54.3 ± 4.6 nCi/heart for control and 33.2 ± 2.5 nCi/heart for diabetic animals. Table 2 indicates that cardiac NE concentration and content were elevated in diabetes. Furthermore, turnover rate constant (K) was greatly increased in diabetes and the NE turnover rate as expressed by ng/g t\(^{-1}\) or ng/heart t\(^{-1}\) was also significantly higher than that in control animals. However, treatment of the diabetic animals with insulin for 28 days completely restored the endogenous NE and turnover rate constant toward control values.

Effect of Cold Stress on NE Turnover

To determine the effect of stress on the cardiac NE rate constant animals were exposed to cold for 6 hours. Cold exposure enhanced sympathetic activity as reflected by about a threefold increase of NE turnover rate constant in control rats (Table 3); cardiac NE was not changed. On the other hand, NE turnover rate constant was significantly decreased (about 30%) while cardiac NE was further elevated in diabetic animals exposed to cold. It should be pointed out that cardiac NE was decreased by about 60% in both control and diabetic rats upon exposure to cold for 24 hours (data not shown). These data on NE turnover rates suggest that the sympathetic reserve, i.e., the amount by which resting NE turnover rate can be increased during cold stress, was markedly depressed in the diabetic heart (Table 3).

Effect of Ganglionic Blockade

Cardiac NE turnover following ganglionic blockade with pentolinium was studied in order to determine whether or not the observed high NE turnover rate in diabetic animals was due to enhanced sympathetic activity. The results are presented in Table 4. After pentolinium treatment, both control and diabetic hearts had identical, low NE turnover rate constants and a complete restoration of cardiac NE stores was observed in the diabetic hearts.

Uptake of [H]Norepinephrine

In order to understand the mechanism responsible for the increased NE stores in diabetic heart, uptake of [H]NE by heart slices in both control and diabetic animals was studied under in vitro conditions. It is now

### Table 2. Effect of Insulin on Cardiac Norepinephrine (NE) Levels and Turnover in Diabetic Rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Endogenous NE (ng/g)</th>
<th>Rate constant (K(t(_0)) ng/g t(^{-1}))</th>
<th>Half-life (t(_{1/2})) (hr)</th>
<th>NE turnover rate (ng/heart t(^{1/2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>220.18 ± 14.62</td>
<td>0.088 ± 0.01</td>
<td>7.87</td>
<td>22.00 ± 1.32</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>503.48 ± 17.26*</td>
<td>0.161 ± 0.01*</td>
<td>4.30</td>
<td>64.74 ± 7.11*</td>
</tr>
<tr>
<td>Diabetic + insulin</td>
<td>238.98 ± 16.55</td>
<td>0.102 ± 0.01</td>
<td>6.79</td>
<td>23.01 ± 1.86</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4–5 experiments. *p < 0.05. Insulin (3 U/day) was injected subcutaneously for 4 weeks to diabetic animals as described in "Materials and Methods." Half-life was calculated as 0.69/K to provide further information on the characteristics of NE turnover.
generally accepted that uptake of NE by the adrenergic nerve terminals is one of the major factors for removing both exogenous and locally released catecholamines. Figure 3 shows that $[^3H]NE$ accumulation was significantly increased in diabetic hearts at all time periods of incubation. Treatment of diabetic animals with insulin completely reversed the observed changes in $[^3H]NE$ uptake. It is likely that the techniques employed would underestimate the velocity of the NE uptake process, which may be more rapid in vivo, but the in vivo results for NE uptake are difficult to interpret because of the simultaneous involvement of several factors such as NE release, synthesis, and catabolism. Since diabetic hearts were reduced in weight, the possibility exists of increased density of sympathetic innervation with a greater number of nerve terminals per gram weight. This could account for the observed increase in NE uptake. However, NE uptake values (per heart) for diabetic rats were also higher as compared with control and were maximum at 60 minutes (control 2.07 ± 0.10; diabetic 2.68 ± 0.21). It is pointed out that extrapolation of the data expressed per gram of heart tissue to those per heart values has to be made with caution since our incubation media contained comparable amounts of heart slices and $[^3H]NE$ for both control and diabetic rats. This is particularly important when the data are expressed as counts per gram of tissue divided by counts per milliliter of medium.

To understand whether the higher values of NE uptake in diabetic hearts are related to the neuronal uptake process, $[^3H]NE$ uptake was carried out in the presence of cocaine, amitriptyline, and desmethylimipramine. These drugs inhibit neuronal uptake of NE and are used to distinguish between the extraneuronal and neuronal components of the uptake process. Table 5 shows that all these agents were inhibitory to $[^3H]NE$ uptake and the extent of inhibition in each case was approximately the same in the control and diabetic hearts.

Table 4. Effect of Pentolinium on Cardiac Norepinephrine (NE) Levels and Turnover in Control and Diabetic Rats

<table>
<thead>
<tr>
<th>Endogenous NE</th>
<th>Control rats</th>
<th>Diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>ng/g</td>
<td>215.12 ± 12.18</td>
<td>193.45 ± 20.02</td>
</tr>
<tr>
<td>ng/heart</td>
<td>244.19 ± 14.66</td>
<td>224.33 ± 23.31</td>
</tr>
<tr>
<td>Rate constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K$ ($t_{0.5}^{-1}$)</td>
<td>0.085 ± 0.010</td>
<td>0.048 ± 0.009*</td>
</tr>
<tr>
<td>Half life ($t_{0.5}$)</td>
<td>8.15</td>
<td>14.41</td>
</tr>
<tr>
<td>NE turnover rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ng/g $t_{0.5}^{-1}$</td>
<td>18.28 ± 1.93</td>
<td>9.28 ± 2.02*</td>
</tr>
<tr>
<td>ng/heart $t_{0.5}^{-1}$</td>
<td>20.75 ± 1.56</td>
<td>10.76 ± 1.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 experiments. Pentolinium (10 mg/kg; i.p.) was injected every 2 hours for a total of 3 doses before sacrificing the treated animals. The untreated animals received saline injections. *Treated vs. untreated control and diabetic (8 weeks after streptozotocin) animals ($p < 0.05$). Half-life was calculated as in Table 2.
Biosynthesis of Norepinephrine

An increased turnover of cardiac NE stores in diabetics should be accompanied by increased synthesis if the endogenous catecholamine levels are to be maintained at a higher level. Figure 4 demonstrates that diabetic hearts had, in fact, higher synthesis of catecholamine from [3H]tyrosine at all points studied. This is further supported by our observations in which the activities of both tyrosine hydroxylase and dopa decarboxylase, enzymes involved in NE synthesis, were found to be increased (Figure 5). Such increase in the enzyme activities of the diabetic hearts does not appear to be due to an increased density of sympathetic innervation as a result of the decrease in total heart weight since both the enzyme activities were higher in diabetic rats when the values were expressed as nmol/g heart/hr (tyrosine hydroxylase-control 10.4±2.1, diabetic 16.0±1.2; dopa decarboxylase-control 69.5±5.4, diabetic 86.1±2.5). However, insulin-treated diabetic animals showed activities of these enzymes comparable to those of the control rats. Since the activity of tyrosine hydroxylase is suppressed by an endogenous inhibitor in brain,25 it is possible that the observed increase in the tyrosine hydroxylase activity may be due to reduced concentration of the inhibitor in the diabetic heart.

Metabolic Fate of Norepinephrine

In order to examine whether the increased NE turnover in diabetic heart was associated with an increase in intraneuronal metabolism of NE, both control and diabetic rats were injected 6 µCi/100 g [3H]NE. One hour later, the hearts were removed and analyzed for principal unconjugated metabolites of [3H]NE. As shown in Table 6, the diabetic animals had more [3H]normetanephrine and deaminated catechols per gram of heart tissue. When these metabolites were expressed per heart, we found a persistent increase in the [3H]normetanephrine and deaminated catechols of

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**Table 5. Effects of Drugs on Uptake of [3H]Norepinephrine in Control and Diabetic Hearts**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>[3H]NE uptake</th>
<th>% inhibition by drug</th>
<th>[3H]NE uptake</th>
<th>% inhibition by drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>1.48±0.20</td>
<td>—</td>
<td>2.53±0.32*</td>
<td>—</td>
</tr>
<tr>
<td>Cocaine 10⁻⁶ M</td>
<td>0.61±0.09</td>
<td>58.7</td>
<td>1.06±0.12*</td>
<td>58.1</td>
</tr>
<tr>
<td>Cocaine 10⁻⁵ M</td>
<td>0.52±0.05</td>
<td>64.8</td>
<td>0.93±0.10*</td>
<td>63.2</td>
</tr>
<tr>
<td>Amitriptyline 10⁻⁶ M</td>
<td>0.66±0.08</td>
<td>55.4</td>
<td>1.08±0.10*</td>
<td>57.3</td>
</tr>
<tr>
<td>Amitriptyline 10⁻⁵ M</td>
<td>0.60±0.07</td>
<td>59.4</td>
<td>0.99±0.09*</td>
<td>60.8</td>
</tr>
<tr>
<td>Desmethylimipramine (10⁻⁶ M)</td>
<td>0.98±0.06</td>
<td>33.7</td>
<td>1.77±0.21*</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 experiments and are expressed as tissue/medium ratio.
*Significantly different from respective control values (p < 0.05).
Heart slices were incubated for 30 minutes in the presence and absence of different drugs. In a typical experiment where no drug was added, the [3H]NE uptake was 46.24 and 59.36 nCi/heart for control and diabetes, respectively.
diabetic rats compared with that of controls. Assays of MAO and COMT indicate an almost twofold increase in the activities of these enzymes in diabetic animals (Figure 6). The activities of these enzymes when expressed as nmol/g heart/20 min also indicate that diabetic rats had higher values (MAO-control 20.9 ± 4.2, diabetic 33.6 ± 1.8 and COMT-control 5.2 ± 0.7, diabetic 7.5 ± 0.3). Both activities tended to normalize in diabetic animals on treatment with insulin. All these results suggest increased metabolism of NE in the diabetic hearts.

Discussion

The present study demonstrates that both cardiac NE concentration and NE turnover were significantly increased in rats 8 weeks after the induction of diabetes by streptozotocin. Since these changes were inhibited by ganglionic blockade with pentolinium, the data support the view that there is increased sympathetic activity following chronic diabetes. Because NE turnover, under steady-state conditions, has been reported to provide a comparative assessment of the NE synthesis, it is possible that NE synthesis is increased in the diabetic heart, and this may account for the increased cardiac NE in chronic diabetes. It is pointed out that the NE turnover rate should not be accepted as a true reflection of the rate of NE synthesis because the NE turnover constant is determined not only by the rate of local NE synthesis but also by the amount of specific activity of the NE taken up from the blood stream.

Nonetheless, the activities of enzymes involved in the synthesis of cardiac NE and the rate of conversion of [3H]tyrosine to [3H]NE were higher in the diabetic group. Increased NE synthesis as a result of increased sympathetic nervous stimulation has been observed in different types of failing hearts. Since the ability of the diabetic heart to take up NE under in vitro conditions was found to be higher than the control, this mechanism may also partly contribute to the observed increase in NE concentration. Marked increase in the cardiac normetanephrine and deaminated catechols level indicates increased NE metabolism by MAO and COMT. In fact, increased excretion of epinephrine, norepinephrine, and 3-methoxy-4-hydroxymandelic acid in the urine has been reported earlier in experimental diabetes. Because diabetic animals are considered to lose weight due to caloric deprivation, it is possible that the increased NE turnover rate in diabetic heart may be due to insufficient food intake. This does not appear to be the case because starvation has been demonstrated to decrease cardiac NE turnover and sympathetic activity.

Increased sympathetic tone is also suggested by elevated levels of plasma NE in diabetes. However, it may be noted that the level of circulating catecholamines as a reflection of sympathetic tone at a given stage of disease should be viewed with caution. This is further complicated by the fact that there is no ideal method for completely stressless blood collection, and thus it may obscure any interpretation. Berkowitz et al have shown increased circulating dopamine β-hydroxylase activity, and this can be taken to indicate

![Figure 5. Activities of cardiac tyrosine hydroxylase and dopa decarboxylase in control (C), diabetic (D) and diabetic animals treated with insulin (D + I).](image)

![Figure 6. Activities of cardiac monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) in control (C), diabetic (D), and diabetic animals treated with insulin (D + I).](image)

### Table 6. Metabolic Fate of [3H]Norepinephrine in Control and Diabetic Rats

<table>
<thead>
<tr>
<th></th>
<th>3H-Normetanephrine</th>
<th>Deaminated catechols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(dpm/g) x 10^3 (%)</td>
<td>(dpm/g) x 10^3 (%)</td>
</tr>
<tr>
<td>Control</td>
<td>4.2 ± 0.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Diabetic</td>
<td>14.6 ± 0.8  *</td>
<td>4.6 ± 0.3  *</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 experiments.

Deaminated catechols include 3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylglycol. Total counts (T.C.) refer to radioactivity of perchloric acid extract.

*p < 0.05.
increased sympathetic activity in diabetic animals. Elevated levels of plasma catecholamines as a consequence of increased sympathetic activity may explain the reduced number of β-adrenergic receptors and defective adrenergic receptor-adenylate cyclase coupling in diabetic heart due to down-regulation of the system. It is also possible that elevated levels of plasma catecholamines due to increased sympathetic activity may result in the development of cardiomyopathy in chronic diabetes. In fact, high doses of catecholamines have been shown to produce myocardial cell damage similar to that seen in the diabetic animals.

While this paper was in preparation, Yoshida et al. have reported a decrease in cardiac NE turnover in diabetic rats. This observation is in contrast to the present findings, and such an apparent discrepancy may be due to differences in the methods employed for studying NE turnover and the experimental models used. It should be noted that Yoshida et al. have calculated the NE turnover rates from decline of endogenous NE contents at 3 and 6 hours after injecting 80 mg/kg α-methyl-p-tyrosine, an inhibitor of tyrosine hydroxylase. However, the dose of the inhibitor employed by these investigators was very low in comparison with others who used 200 mg/kg of α-methyl-p-tyrosine, and it was repeated before sacrificing the animals. Furthermore, Yoshida et al. have calculated the NE turnover rate on the basis of two points, and it is difficult to interpret their data because several points must be included to evaluate turnover measurement. Finally, Yoshida et al. have used female rats in their study, and it is possible that the time course of changes associated with streptozotocin-induced diabetes may be different from those in this study in which male rats were used.

The maintenance of a relatively constant amount of cardiac NE during the first 14 days of diabetes may be dependent on the balance between NE synthesis and NE release from the noradrenergic nerve terminals. Enhanced catecholamine synthesis and release as a result of increased nervous stimulation has been demonstrated in the adrenal medulla, and it is possible that a similar mechanism may be operating in the noradrenergic nerve terminals of the diabetic heart. However, unlike control rats, the rate constant for the cardiac NE turnover was decreased in 8-week diabetic animals on activation of sympathetic system by cold exposure. Such a finding could be due to the development of autonomic neuropathy in chronic diabetic animals. It is also possible that the smaller increase in NE rate constant in diabetic rats, compared with control rats, could be a result of differences in arterial pressure and/or baroreflex inhibition of cardiac sympathetic function during cold stress. Although diabetic rats had lower heart rates, no significant difference was found in blood pressure between control and diabetic rats.

Whether the changes in hemodynamic parameters in diabetes are altered differentially during cold stress remains to be investigated. The decrease in rate constant of NE turnover in these animals during cold stress could also be due to hypothyroidism associated with this experimental model. Thus the altered response of the diabetic animals to thermal challenge is an intriguing question, and the possibility of the effect of hypothyroid condition in diabetes cannot be ruled out at present. Nevertheless, a decreased response of plasma catecholamines to stress in diabetic rats has been reported earlier, and, accordingly, further activation of the sympathetic tone due to stressful stimuli may not be possible in chronic diabetes.

It appears unlikely that streptozotocin, rather than diabetes per se, could have produced the observed changes in the present study. Streptozotocin does not have any cardiotoxic effect either at a tissue or subcellular level. However, it is possible that the increased sympathetic tone in this animal model may become complicated by the hypothyroidism associated with diabetes. This view is based on the observations that cardiac NE turnover and level of plasma NE are increased in hypothyroidism. However, it may be pointed out that unlike the diabetic animals, endogenous cardiac NE concentration is reduced and NE uptake is unchanged in thyroidectomized rats. Furthermore, daily injections of T₃ in diabetic animals to maintain the plasma concentration of thyroid hormone at a physiological level failed to prevent the increased endogenous NE concentration of diabetic myocardium. Berkowitz et al. also failed to correct changes in plasma dopamine-β-hydroxylase activity in diabetic animals upon treatment with thyroid hormone. Increased cardiac NE turnover but decreased cardiac NE metabolism in these experimental models of heart disease may differ from those in the streptozotocin diabetic cardiomyopathy. Although more detailed information is required to settle the exact role of hypothyroidism, it is unlikely that the hypothyroid condition in our diabetic model could entirely explain the observed changes in sympathetic activity.

It seems appropriate to discuss some possible reservation in the interpretation of the results obtained in this study. It is possible that diabetic rats are chronically hypervolemic secondary to hyperglycemia and a high obligatory water excretion could account for high sympathetic activity. Since we do not have data on plasma volumes of control and diabetic rats or NE turnover from any other peripheral tissue, the hypothesis that hypervolemia in diabetic rats results in pronounced sympathetic activation needs future experimentation. Our present report suggests that cardiac NE level, turnover, uptake, synthesis, and metabolism were increased in diabetic rats. Such increases in the diabetic hearts could be due to a denser sympathetic innervation as a result of the decrease in total ventricular mass. However, our data do not support this contention because higher activities were still evident in diabetic animals when the values were expressed per heart instead of per gram weight. Furthermore, we have observed an increase in the synthesis of catecholamines from [3H]tyrosine. It can be argued that the observed synthesis of [3H]catecholamines from [3H]ty-
rosine in the diabetic heart may also be due to enhanced uptake of labelled tyrosine. Since we do not have data on tyrosine uptake or its specific activities in plasma and ventricular tissue, the possibility of increased [3H]catecholamine synthesis due to greater availability of the precursor pool cannot be ruled out at present. Nonetheless, NE biosynthesis in diabetic heart can be conceived to increase in order to compensate for the mounting demand resulting from enhanced breakdown and turnover of NE as well as to account for the higher level of tissue NE. Recently, the activity of dopamine β-hydroxylase, which is involved in the synthesis of NE, has been shown to decrease in diabetic heart. This would appear to be at variance with the increases in cardiac tyrosine hydroxylase and dopamine β-hydroxylase activities reported here. It is possible that a high exocytotic release of dopamine β-hydroxylase secondary to high sympathetic activity depletes this enzyme in the heart. This view is substantiated by the fact that the level of dopamine β-hydroxylase activity has been reported to increase in plasma from diabetic rats. Although the data concerning high NE turnover is consistent with the view that sympathetic activity is increased in diabetes, the significance of such an abnormality in the development of diabetic cardiomyopathy remains to be established.

References


Key Words • diabetic myocardium • norepinephrine turnover in diabetes • norepinephrine metabolism in diabetes • cardiac norepinephrine uptake • norepinephrine synthesis in diabetes
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