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ADRENAL CATECHOLAMINE METABOLISM AND MYOCARDIAL ADRENERGIC RECEPTORS IN STREPTOZOTOCIN DIABETIC RATS

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Abstract—Adrenal medullary function and myocardial adrenergic receptors were investigated in streptozotocin-treated diabetic rats. The animals were rendered diabetic by a single i.v. injection of streptozotocin (STZ, 65 mg/kg) and killed 60 days after treatment. Adrenal tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), and phenylethanolamine *N*-methyltransferase (PNMT) activities were increased by 52, 28 and 39%, respectively, in the STZ diabetic rats. In addition, adrenal concentrations of dopamine (+52%) norepinephrine (+46%), and epinephrine (+33%) were elevated significantly ($P < 0.05$). Increased adrenal TH activity reflected an increased V_{max}, but no change in K_m. Receptor densities (B_{max}), determined by [^{3}H]prazosin and [^{3}H]dihydroalprenolol binding, were decreased by 24 and 25%, respectively, in the myocardium of 60-day diabetic rats. Insulin-induced chronic hypoglycemia in the STZ diabetic rats produced a marked increase in the adrenal TH concentration (V_{max}, +65% or +225%, respectively), as compared to control or diabetic rats, without changes in the affinity (K_m) for the substrate. These results suggest that the STZ diabetic rat has abnormalities of catecholaminergic function of the adrenal medulla and myocardial adrenergic receptors, which may contribute to the development and maintenance of many of the hemodynamic and metabolic defects described in this animal model of diabetes mellitus.

The sympathoadrenal system plays a key role in physiological responses to stressors such as physical exercise, immobilization and hypoglycemia [1–3]. The adrenal catecholamines (CA) have metabolic and hemodynamic effects that range from increasing blood pressure to stimulating glycogenolysis. They enhance hepatic glucose production [4], reduce insulin responses to glucose [5], and elevate plasma concentrations of free fatty acids and ketone bodies [6, 7]. Many of these metabolic alterations are similar to those observed in untreated diabetics [8].

Diabetes, as such, is a stressor [9] and produces increased plasma CA levels [10–15] which, in turn, may have different effects in diabetics compared to normal subjects. Adrenal concentrations of CAs have also been reported to be increased or unchanged in diabetes mellitus [10–15] and exaggerated responses of plasma CAs have been observed during exercise in patients and animals with poorly controlled diabetes [10, 15, 16]. Examination of sympathodrenal function in diabetes is needed due to a possible role of CAs in the pathogenesis of complications of diabetes mellitus, such as hypertension, cardiomyopathy, congestive heart failure and the Somogyi phenomenon [17–24].

Little information is available, however, concerning adrenal CA metabolism in experimental or clinical diabetes. Therefore, we measured changes in CA content and activities of CA-synthesizing enzymes in the adrenal medulla of experimentally diabetic rats. Furthermore, myocardial adrenergic receptors and effects of insulin-induced hypoglycemia on the activity of adrenal tyrosine hydroxylase were quantified in these animals.

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METHODS

Animals

Sprague–Dawley derived rats (175–200 g) were obtained from Charles River Laboratories (Wilmington, MA) and maintained under a standardized light–dark schedule (6:00 a.m.–6:00 p.m.) and temperature (22°) on a diet of Purina Laboratory Chow and water available *ad lib*. Animals were housed two to a cage on hardwood bedding in clear plastic cages.

Treatment

Diabetes was induced by an i.v. injection of streptozotocin (STZ, 65 mg/kg) prepared in 0.05 M citrate buffer, pH 4.5. To prevent the development of a diabetic state, nicotinamide was administered (1000 mg/kg, i.p.) to the control animals 15 min before the STZ injection [25–27].

For the induction of hypoglycemia in STZ diabetic rats, a slow release insulin pellet or placebo pellet was implanted subcutaneously beneath the ventral panniculus carnosus for 8–10 days (slow release from cholesterol/methylcellulose/lactose pellets, 4.5 mg insulin/pellet, 3 weeks duration, Innovative Research, Rockville, MD).

Tissue preparation

Rats were killed between 9:00 and 10:00 a.m. by decapitation, with the order of sacrifice alternating between controls and STZ diabetic rats. The adrenal glands and hearts were removed immediately, cooled on a petri dish, placed on ice, and dissected free of fat. The organs were frozen on solid CO_2 and stored at −70° in tightly sealed vials until the time of the assay. Enzyme activities, receptor densities and affinities, and CA concentrations were determined simultaneously in non-treated diabetic, nicotinamide-treated diabetic, and non-diabetic control animals which received only the buffer.

Adrenal samples were sonicated (sonicator cell disruptor, model W-22 of Heat System Ultrasonics, Plainview, NY) in 100 vol. (w/v) of 50 mM Tris–HCl, pH 7.4, containing 0.25 M sucrose and 0.2% (v/v) Triton X-100. Aliquots (25% of the homogenization volume) for catecholamine assays were withdrawn immediately and precipitated with 0.25 ml of 0.8 M perchloric acid containing 0.1% EDTA and 0.5% sodium metabisulfite. The remaining homogenates were centrifuged at 4° for 15 min at 5000 *g*, and the supernatant fractions were used for the enzyme assays. Homogenates of myocardial membranes were prepared according to the method of Baker and Potter [28].

Enzyme assays

Tyrosine hydroxylase (TH). The activity of TH was measured by the method of Coyle [29] as modified by Bitar *et al.* [30]. [^{3}H]DOPA (produced from L-[2,3-^{3}H]tyrosine) was separated from L-[2,3-^{3}H]tyrosine on acid-activated alumina.

Dopamine-β-hydroxylase (DBH). The activity of DBH was measured according to the radioenzymatic method of Molinoff *et al.* [31] with slight modifications [32].

Phenylethanolamine-N-methyltransferase (PNMT). The activity of PNMT was measured according to a modification of the technique of Saavedra *et al.* [33] as described by Denoroy *et al.* [32].

Catecholamine concentrations

High performance liquid chromatography was used to measure the content of catecholamines in the adrenal medullas. The chromatographic separation of the CAs norepinephrine (NE), dopamine (DA), and epinephrine was achieved using a C18 reverse phase column as described previously [30].

Binding assays

Specific binding sites for [^{3}H]dihydroalprenolol and [^{3}H]prazosin were measured as described by Williams and Lefkowitz [34] and Greengrass and Bremner [35] respectively.

Protein

Protein was quantified by the method of Lowry *et al.* [36] using bovine serum albumin as standard.

Glucose

Serum glucose concentrations were measured by the *o*-toluidine method of Feteris [37].

Statistics

Data are expressed as means ± SEM. Two-tailed Student's *t*-test was used to analyze differences between the treated and control groups.

RESULTS

The effects of diabetes on body, adrenal, and heart weights and on serum glucose concentrations are presented in Table 1. Body, adrenal, and heart weights were decreased and serum glucose concentrations were increased in the diabetic state. The ratios of adrenal and heart weights to body weight were increased in the STZ diabetic rats. The protein concentration remained unaltered in the adrenals and hearts.

Changes in the adrenal medullary CA concentrations and the activities of the key enzymes involved in CA synthesis are detailed in Table 2. In the STZ diabetic rats, TH, DBH and PNMT activities were increased by 52, 28 and 39% respectively. In addition, the concentrations of DA (+52%), NE (+46%) and epinephrine(+33%) were increased.

Further experimentation was undertaken to ascertain whether the observed increase in the adrenal TH activity in the diabetic rats was due to an increased

Table 1. Physical and biochemical variables in the STZ diabetic rats*

Variable	Control	60-Day diabetic
Body weight (g)	250 ± 5.5	130 ± 8.7†
Adrenal weight (mg)	62 ± 2.8	42 ± 3.0†
Heart weight (mg)	550 ± 20	350 ± 22†
Relative adrenal weight × 100 (mg adrenal/g body wt)	2.48 ± 0.15	3.20 ± 0.11†
Relative heart weight (mg heart/g body wt)	2.20 ± 0.13	2.70 ± 0.15†
Adrenal protein × 100 (mg protein/mg wet wt)	1.50 ± 0.05	1.60 ± 0.05
Membrane protein yield (mg/g heart wet wt)	23 ± 1.7	25 ± 2.0
Serum glucose (mg/100 ml)	125 ± 8.1	592 ± 28.5†

* STZ (65 mg/kg) was injected i.v., in 0.05 M citrate buffer, pH 4.5. Values are expressed as means ± SEM for at least four animals.

† Significantly different from corresponding control values at $P < 0.05$.

Table 2. Adrenal activities of catecholamine biosynthetic enzymes in control and STZ diabetic rats*

Variable	Control	60-Day diabetic
Tyrosine hydroxylase (nmoles DOPA/mg protein/hr)	10.88 ± 1.2	16.5 ± 2.6†
Dopamine β-hydroxylase (nmoles octopamine/mg protein/hr)	28.6 ± 1.1	36.8 ± 2.3†
Phenylethanolamine *N*-methyl-transferase (nmoles *N*- methyl-phenylethanolamine/mg protein/hr)	9.6 ± 0.85	13.4 ± 1.95†
Dopamine (μg/mg protein)	0.025 ± 0.004	0.038 ± 0.006†
Norepinephrine (μg/mg protein)	0.588 ± 0.025	0.862 ± 0.052†
Epinephrine (μg/mg protein)	2.750 ± 0.122	3.660 ± 0.152†

* STZ (65 mg/kg) was injected i.v., in 0.05 M citrate buffer, pH 4.5. Values are expressed as means ± SEM for at least four animals.
† Significantly different from corresponding control values at $P < 0.05$.

Table 3. Kinetic parameters of adrenal tyrosine hydroxylase in control and STZ diabetic rats*

Treatment	K_m (mM)	V_{max} (nmoles DOPA/mg protein/hr)
Control	0.084 ± 0.013	12.5 ± 2.1
60-Day diabetic	0.067 ± 0.012	20.3 ± 3.3†

* STZ (65 mg/kg) was injected i.v., in 0.05 M citrate buffer, pH 4.5. Values are expressed as means ± SEM for at least four animals.
† Significantly different from corresponding control values at $P < 0.05$.

affinity of the substrate for the enzyme or an increase in the concentration of the enzyme. An Eadie–Hofstee plot revealed that the affinity (K_m) of the substrate for the enzyme was not increased in the diabetic animals. The maximal enzyme activity (V_{max}), however, showed a 62% increase (Table 3).

Results of the experiments on the effects of chronic diabetes on myocardial adrenergic receptors are given in Table 4. Specific binding of 0.05 to 50 nM [^{3}H]prazosin and 0.5 to 15 nM [^{3}H]dihydroalprenolol to the myocardial membrane preparations was a saturable process. An Eadie–Hofstee plot revealed that the receptor densities (B_{max}) for [^{3}H]prazosin and [^{3}H]dihydroalprenolol binding were reduced by 24 and 25%, respectively, without changes in affinities (K_d) in the 60-day diabetic rats.

Animals receiving the full diabetogenic dose of STZ 15 min after nicotinamide injection did not display any significant changes in body weight, serum glucose concentration, adrenal metabolism of CAs or myocardial receptor binding.

The data concerning adrenal TH activity, serum glucose concentration, and body weight of the insulin-treated diabetic rats are presented in Table 5. Insulin treatment of diabetic rats promoted weight gain and reduced the serum glucose concentration to a hypoglycemic level. An Eadie–Hofstee plot of the activity of the adrenal TH revealed that the affinity (K_m) of the substrate for the enzyme was not changed in chronically hypoglycemic animals. The enzyme concentration (V_{max}) in these animals was increased by 225 and 65% as compared to control and STZ diabetic rats respectively.

DISCUSSION

Whereas the relevance of the sympathoadrenal activity to the metabolic and hemodynamic abnormalities of human diabetes mellitus has been investigated [10, 38, 39], there have been few studies of this system in experimental diabetes. The present report describes changes in adrenal medullary CA function and myocardial adrenergic receptors in an animal model of diabetes mellitus. The results clearly indicate that the adrenal contents of DA, NE, epinephrine, and their rate-limiting biosynthetic enzyme, TH, were elevated markedly in STZ diabetic rats. Furthermore, the content of TH was increased even

Table 4. Myocardial adrenergic receptors in STZ diabetic rats*

	[^{3}H]Dihydroalprenolol		[^{3}H] Prazosin	
Treatment	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)
Control	3.52 ± 0.83	44.6 ± 4.7	0.21 ± 0.03	47.7 ± 3.4
60-Day diabetic	3.20 ± 0.75	33.3 ± 4.3†	0.19 ± 0.03	36.3 ± 4.5†

* STZ (65 mg/kg) was injected i.v., in 0.05 M citrate buffer, pH 4.5. Values are expressed as means ± SEM for at least four animals.
† Significantly different from corresponding control values at $P < 0.05$.

Table 5. Adrenal tyrosine hydroxylase, body weight and serum glucose concentration in chronically hypoglycemic animals*

	Tyrosine hydroxylase			
	K_m (nM)	V_{max} (nmoles/mg protein/hr)	Body wt (g)	Serum glucose (mg/100 ml)
Control	0.083 ± 0.009	14.4 ± 1.2	247 ± 16	110 ± 3.8
Diabetic	0.065 ± 0.011	19.6 ± 2.4†	178 ± 8.6†	449 ± 35†
Diabetic and insulin	0.071 ± 0.012	32.4 ± 5.6‡	259 ± 15.0	25 ± 3.2‡

* STZ (65 mg/kg) was injected i.v., 60 days before the implantation of the insulin pellet. Animals were killed 68–70 days later. Values are expressed as means ± SEM for at least four animals.
† Significantly different from corresponding control values at $P < 0.05$.
‡ Significantly different from control or diabetic values at $P < 0.05$.

further in chronically hypoglycemic STZ diabetic rats. Whether the increased activities of DBH and PNMT found in this experiment were due to changes in V_{max} or K_m requires further study. In addition to the adrenal changes, marked decreases were found in the binding of [^{3}H]dihydroalprenolol to β-adrenergic receptors and [^{3}H]prazosin to α_1-adrenergic receptors in myocardial membranes of the STZ diabetic animals. The metabolic derangements of diabetes mellitus and not the inherent toxicity of the STZ were probably responsible for these findings because animals receiving a full diabetogenic dose of STZ 15 min after nicotinamide injection failed to display any of these abnormalities. Nicotinamide produces this protective effect by antagonizing STZ-induced depletion of nicotinamide adenine dinucleotide which is necessary for proinsulin biosynthesis in the islands of Langerhans [25–27].

The observed increase in TH activity in diabetes suggests that adrenomedullary CA biosynthesis was enhanced, because this enzyme is considered to be the rate-limiting step of adrenal CA synthesis [40]. Indeed, our finding of increased activities of adrenal DBH and PNMT in these animals supports this notion. Whether diabetes-related changes in the V_{max} of TH were due to an increased protein synthesis or a loss of an inhibitor is not known. Previous studies have shown, however, that the enhanced activity of DBH in STZ diabetic rats represents increased enzyme protein rather than a reduction in the concentration of an inhibitor [41]. Our conclusion that the K_m values of the enzyme TH were similar in control and the STZ diabetic rats is tentative. This is because decapitation (method used in this study) results in a marked activation of TH [42].

Although increases in the activities of enzymes mediating CA biosynthesis can account for the elevated level of adrenal CA in diabetic rats, the biochemical events that lead to such alterations are unknown. It is noteworthy that glucocorticoids have been reported to regulate CA metabolism in the adrenal gland. Hypophysectomy causes marked reductions in the activities of the adrenal CA-synthesizing enzymes including TH, DBH, and PNMT in rats, and the administration of ACTH and/or glucocorticoids restores the activities of these enzymes to a normal level [43–45]. Furthermore, the induction of adrenocortical hyperplasia by unilateral adrenalectomy markedly elevates the activity of DBH in the remaining adrenal medulla [46]. A case in point is the recent finding that the administration of ACTH to human subjects markedly enhances the release of adrenal epinephrine and NE [47]. Thus, diabetes-related alterations in adrenal CA enzymes may be mediated via abnormalities in adrenal glucocorticoid function. This hypothesis is in accordance with previous findings on hyperfunction of the pituitary-adrenal axis in the STZ diabetic rat [9]. Alternatively, the adrenal CA-increasing effect of diabetes could be neurogenic as it is well known that synthesis of adrenal medullary hormones is stimulated by increased impulse flow in the cholinergic nerves of the adrenal medulla [48]. The latter hypothesis can be tested by administering anticholinergic drugs to STZ-diabetic rats.

The enhanced activity of TH in STZ diabetic rats may lead to increased release of adrenal medullary CA into the circulation [49, 50]. This concept is consistent with previous studies demonstrating an elevation in plasma CA and DBH concentrations in humans and animals with diabetes mellitus [10–13, 41]. The effects of elevated circulating CAs on cardiovascular function in diabetes are not known. Previous studies have shown, however, that long-term exposure of various cell types to β-adrenergic agonists leads to a decreased number of β-adrenergic receptors and a reduced responsivity to a further challenge with agonists [51–54]. Thus, increased adrenal medullary function and, presumably, increased plasma CA concentration in STZ diabetic rats could be expected to down-regulate cardiac β-adrenergic receptors and to alter their responsivity to a drug that stimulates adenylate cyclase. The previously reported data showing that the sensitivity of adenylate cyclase to isoproterenol stimulation is depressed and abnormalities in cardiac contractility are evident in the STZ-diabetic rat [55–57], together with our data demonstrating a decrease in the binding of [^{3}H]dihydroalprenolol to myocardial β-adrenergic receptors, suggest that the reduced binding documented by us may have functional significance.

Hormonal changes also have been shown to alter myocardial adrenergic receptors. For example, hypothyroidism decreases β-adrenergic receptor number and increases α-adrenergic receptor binding [58]. Because diabetes mellitus in animals is associated with hypothyroidism [59, 60], the decrease in β-adrenergic receptor binding observed in STZ diabetic rats may be due to hypofunction of the thyroid

gland in these animals. Our data of a concomitant decrement of myocardial α_1-adrenergic receptors in these animals render this argument unlikely. The present data on myocardial adrenergic receptor antagonist binding in female diabetic rats are in accordance with those in male diabetic rats [61, 62]. Contrary to these findings, others have reported no change in the binding sites for [^{3}H]dihydroalprenolol in 8-day diabetic animals [63]. The reasons for the seemingly discrepant findings may be related, at least in part, to the duration of diabetes.

Neuropathy is a well-documented phenomenon among diabetic patients [38]. No evidence for neuropathy of the sympathetic nerves has been observed in STZ diabetic rats [41, 64]. For example, the pattern of noradrenergic innervation was not changed in the hearts of diabetic rats even after 4 months of STZ diabetes [64]. Thus, it is unlikely that diabetes-related changes in myocardial adrenergic receptors are due to neuropathy.

Hypoglycemia is frequent in patients with insulin-dependent diabetes mellitus. Glucagon is the most important factor in normal glucose counter-regulation, but epinephrine can partially compensate for a deficient glucagon response [65, 66]. Insulin-dependent diabetics have commonly blunted or absent glucagon secretory responses to hypoglycemia [67–69]. Thus, they exhibit increased dependency upon epinephrine-mediated adrenergic mechanisms for recovery from hypoglycemia. Abnormality in the sympathoadrenal activation or interference with the action of epinephrine could, therefore, leave insulin-dependent diabetics vulnerable to hypoglycemia. Our present data suggest that the sympathoadrenal activation which promotes recovery from hypoglycemia was probably functioning in the STZ diabetic rats, because a similar elevation of adrenal TH activity has been reported in normal animals made hypoglycemic with insulin [70].

In conclusion, our data clearly demonstrate that the sympathodrenal system is strongly activated in STZ diabetic rats.

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