Altered Acetylcholine and Norepinephrine Concentrations in Diabetic Rat Hearts

Role of Parasympathetic Nervous System in Diabetic Cardiomyopathy

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The concentrations of acetylcholine (ACh) as a parasympathetic marker and norepinephrine (NE) as a sympathetic marker were investigated in the hearts of rats 2, 4, and 8 wk after the induction of diabetes by an injection of streptozocin (STZ; 65 mg/kg i.v.). ACh and NE were measured by high-performance liquid chromatography with electrochemical detection. Diabetic rats showed low body weight and heart weight at 2, 4, and 8 wk and higher heart-to-body weight ratio and bradycardia at 8 wk, almost all of which were normalized after insulin treatment. Myocardial ACh and NE concentrations in the diabetic rats at 2 and 4 wk were not significantly different from those in age-matched control rats. However, ACh and NE concentrations in the diabetic rats at 8 wk significantly increased compared with the control rats. Diabetic rats at 8 wk also had increased myocardial choline concentration and choline acetyltransferase activity and decreased acetylcholinesterase activity. Insulin treatment normalized all of these changes in the diabetic rats. Thus, in STZ-induced diabetes (STZ-D), the concentrations of both cholinergic and noradrenergic neurotransmitters in the myocardium increased. The results of this study confirm a previously reported increase in sympathetic activity to the heart and also indicate that there is an increase in the synthesis and a decrease in the metabolism of ACh in STZ-D and that adequate insulin treatment normalizes these changes. *Diabetes* 38:231–36, 1989

Myocardial dysfunction, independent of atherosclerotic coronary artery disease, and hypertension have been suggested to occur in experimental animals (1) and humans (2) with diabetes mellitus. This has been postulated to be due to diabetic cardiomyopathy or to result from diabetic microangiopathy. Diabetic cardiomyopathy involves various biochemical, functional, and ultrastructural alterations in cardiac cells (3–5). In addition, diabetic cardiomyopathy is known to be associated with an abnormally enhanced sympathetic activity (6,7) followed by chronic sympathetic denervation of the heart (8,9). Based on the previously reported down-regulation of cholinergic receptors (10), enhanced concentrations of coenzyme A (CoA) in cardiac tissue (11), and bradycardia in streptozocin-induced diabetes (STZ-D) (7, 12), a similar enhancement of parasympathetic activity to the heart might be expected in this model of diabetes. Although Kuntscherová and Vlk (13) indicated that the concentration of acetylcholine (ACh) in the atrial tissue from alloxan-induced diabetic (ALX-D) rats was decreased, their results were obtained with a relatively nonselective bioassay of ACh. This investigation was designed to investigate the effect of STZ-D on parasympathetic activity to cardiac tissue by means of a more sensitive and specific assay of ACh. To properly interpret metabolic changes in parasympathetic nerves, additional parameters, including choline (Ch) concentration and the activities of choline acetyltransferase (ChAT, EC 2.3.1.6) and acetylcholinesterase (AChe, EC 3.1.1.7) in the myocardium, were also determined. The effect of insulin treatment on these parameters was also investigated.

MATERIALS AND METHODS

Materials. Male Wistar rats of the same age (8 wk at the time of STZ injection) were used in this study. Diabetes was induced by an injection of STZ (65 mg/kg body wt i.v.) in 0.1 M citrate buffer (pH 4.5) into the tail vein while rats were under light ether anesthesia. Control rats were injected with citrate buffer only. All rats were allowed food and water ad libitum throughout the experiment. The diabetic animals were subdivided randomly into two groups. The first group of diabetic animals were killed 2, 4, and 8 wk after the STZ injection. The control animals were age matched accordingly.

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Received for publication 7 April 1988 and accepted in revised form 7 September 1988.

The animals in the second group received injections of 3 U protamine zinc insulin s.c. daily for the last 4 wk before being killed at 8 wk. Insulin was given at ~1700 daily. Heart rate and systolic blood pressure were measured by the tail-cuff method in all rats before death. After the administration of anesthesia, the heart was excised and divided rapidly into five portions in chilled saline: right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), and interventricular septum (IVS). Each portion was frozen immediately in liquid N_2 and stored at −70°C until assay. Plasma samples were collected at the time of death and analyzed for glucose by the glucose oxidase method and for insulin and circulating thyroid hormone triiodothyronine (T3) by radioimmunoassay.

Tissue preparation. The procedure for sample preparation from heart tissue was essentially the same as that described for brain tissue (14,15) and for heart tissue (16). Each portion was weighed and homogenized in 3 ml of 1 N formic acid–acetone (vol/vol 15:85) containing 2 nmol ethylhomocholine (EHC) as an internal standard for ACh and Ch and 2 nmol 3,4-dihydroxybenzylamine as an internal standard for norepinephrine (NE). The homogenate was centrifuged at 20,000 × *g* for 15 min at 0°C. After removing the supernatant lipid with the diethyl ether, the aqueous phase was divided into two portions, one for the NE assay and the other for the ACh and Ch assay. Each portion was evaporated at 40°C under N_2. The extract for the NE assay was dissolved in 100 μl of 0.1 N HCl, and 20 μl of an aliquot was applied to a high-performance liquid chromatography with electrochemical detection (HPLC-ECD).

The extracts for ACh and Ch assay were dissolved in 200 μl of water and added to 50 μl of 1 mM tetraethylammonium and 20 μl KI-I_2 solution (2 g KI and 1.8 g I_2 dissolved in 10 ml water). The quaternary compounds were precipitated by centrifugation at 2000 × *g* for 10 min. The precipitate was then dissolved in 2 ml acetonitrile, and AGI-X8 anion-exchange resin (Bio-Rad, Richmond, CA) was added to convert the periodides of the quaternary compounds to chlorides. After centrifugation at 2000 × *g* for 5 min, the supernatant was evaporated under N_2. The extracts were dissolved in 100 μl water, and 20 μl of samples was applied to the HPLC system.

ACh and Ch quantification. A recently developed HPLC-ECD was used for the determination of ACh and Ch (15,16). A modification of Potter et al.'s method (14), with an immobilized enzyme column as a postcolumn reactor, was used for the quantification of ACh and Ch in rat heart. Because Brown and Salata (17) reported that cardiac ACh and Ch were stable after death, microwave irradiation, which stabilizes such components, was not used. The HPLC system comprised L-5000 and RP-203 pumps, a Rheodyne 7125 injector with a 100-μl sample loop (Berkeley, CA), a Yanapak ODS-AP precolumn, a Yanapak ODS-A reverse-phase column (4.6 × 250 mm), and an Enzympak ACh (immobilized-enzyme column). Except for the injector, all HPLC units were from Yanaco (Kyoto, Japan).

The hydrogen peptide converted from ACh, Ch, and EHC was detected by an ECD cell equipped with a platinum electrode set to the potential +0.45 V against an Ag/AgCl reference electrode (VMD 501, Yanaco). The recorder was a Chromatocorder 11 (SIC, Tokyo).

NE quantification. The NE assay was conducted by means of the HPLC-ECD (pump unit: model L-2000, analytical column: Yanapak ODS-T, detector cell: VMD 101; Yanaco) as described previously (16).

ChAT quantification. The assay for ChAT activity was almost the same as that reported by Kaneda and Nagatsu (18). The enzyme solution for the measurement of ChAT activity was obtained from frozen RA by homogenization in 12.5 ml of 25 mM sodium phosphate buffer (pH 7.4) per gram wet weight, by means of a Teflon homogenizer, followed by centrifugation at 20,000 × *g* for 60 min at 4°C. The supernatant was used as an enzyme solution. To distinguish between the activities of ChAT and carnitine acetyltransferase (EC 2.3.1.7), 2 μM bromoacetylcholine was used to inhibit ChAT (19). The standard incubation mixture was the same as that reported by Kaneda and Nagatsu (18). Incubation was carried out at 37°C for 20 min, and the reaction was stopped with 50 μl of 1 M perchloric acid in an ice bath. After 10 min, 10 μl of 0.5 mM EHC in 0.01 M hydrochloric acid was added as an internal standard. Then, the reaction mixture was centrifuged at 1600 × *g* for 10 min at 4°C, and 10 μl of the supernatant was applied to the HPLC system. The ChAT activity was expressed as the rate of the formation of ACh during the incubation. The protein in tissue suspension was determined by the method of Lowry et al. (19a).

AChe quantification. The assay for AChe activity was similar to that reported by Kaneda et al. (20). The enzyme solution for the measurement of AChe activity was prepared from frozen RA by homogenization in 12 ml of 25 mM potassium

TABLE 1
General characteristics of experimental animals

	2 wk		4 wk		8 wk		
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Diabetic + insulin
Body weight (g)	297.5 ± 8.7	250.8 ± 8.1*	349.3 ± 8.3	237.8 ± 18.4*	493.8 ± 19.0	295.6 ± 9.7*	425.8 ± 23.6†‡
Heart weight (g)	0.85 ± 0.02	0.72 ± 0.03*	0.96 ± 0.03	0.66 ± 0.05*	1.22 ± 0.06	0.84 ± 0.03*	1.12 ± 0.05‡
Heart weight:body weight (mg:g)	2.87 ± 0.04	2.88 ± 0.10	2.74 ± 0.03	2.79 ± 0.06	2.47 ± 0.03	2.84 ± 0.03*	2.64 ± 0.07†‡
Plasma glucose (mg/dl)	184.5 ± 3.9	440.3 ± 27.7*	169.4 ± 5.3	605.6 ± 18.1*	166.1 ± 3.2	608.9 ± 23.5*	203.1 ± 17.1‡
Plasma insulin (μU/ml)	16.7 ± 1.8	11.4 ± 2.2	16.1 ± 0.7	9.9 ± 2.2†	19.1 ± 1.6	10.4 ± 0.8*	54.1 ± 2.5*‡
Plasma triiodothyronine (ng/dl)	60.0 ± 4.5	41.7 ± 3.1*	68.8 ± 2.3	48.3 ± 3.1*	65.0 ± 3.7	41.3 ± 4.4*	73.3 ± 3.3‡
Systolic blood pressure (mmHg)	114.4 ± 1.8	113.8 ± 2.0	115.3 ± 2.9	113.9 ± 2.3	115.6 ± 1.9	114.3 ± 2.5	118.1 ± 1.3
Heart rate (beats/min)	411.0 ± 11.4	395.1 ± 8.3	393.8 ± 10.5	378.0 ± 8.5	405.6 ± 15.5	341.9 ± 6.1*	380.5 ± 11.8§

Values are means ± SE of 10–12 experiments.
*$P < .01$ and †$P < .05$ vs. corresponding control values.
‡$P < .01$ and §$P < .05$ vs. age-matched diabetic animals.

TABLE 2
Acetylcholine concentration in rat myocardium

	2 wk		4 wk		8 wk		
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Diabetic + insulin
Right atrium	20.9 ± 0.7	21.0 ± 0.7	21.0 ± 1.4	24.5 ± 1.6	20.2 ± 1.1	27.3 ± 1.9*	22.3 ± 0.8†
Left atrium	6.1 ± 0.4	6.2 ± 0.4	6.4 ± 0.3	7.1 ± 0.8	6.3 ± 0.9	8.7 ± 0.5	7.3 ± 0.9
Right ventricle	2.9 ± 0.3	3.1 ± 0.1	3.2 ± 0.2	3.5 ± 0.4	3.0 ± 0.1	3.8 ± 0.2*	2.8 ± 0.2‡
Left ventricle	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.8 ± 0.2§	1.2 ± 0.1‡
Interventricular septum	1.7 ± 0.2	1.9 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1

Values are in nanomoles per gram wet weight and are means ± SE of 10–12 experiments.
*$P < .01$ and §$P < .05$ vs. corresponding control values.
†$P < .05$ and ‡$P < .01$ vs. age-matched diabetic animals.

phosphate buffer (pH 7.0) per gram wet weight. The homogenate was diluted 5 times with the same buffer and used as an enzyme solution. To distinguish between the activities of AChe and butyrylcholine esterase (EC 3.1.1.8), 3 μM 1.5-bis (allyldimethylammoniumphenyl)-pentane-3-1-dibromide (BW 284-C-51) was used to inhibit AChe (21). The standard incubation mixture was the same as that reported by Kaneda et al. (20). Incubation was carried out at 37°C for 15 min, and the reaction was stopped with 40 μl of 5% metaphosphoric acid in an ice bath. After 10 min, 10 μl of 2 mM EHC in water was added as an internal standard. Then, the reaction mixture was centrifuged at 1600 × *g* for 10 min at 4°C, and 10 μl of the supernatant was applied to the HPLC system. The AChe activity was expressed as the rate of the formation of Ch during the incubation resulting from the enzymatic hydrolysis of ACh. The concentration of protein was determined by the method of Lowry et al. (19a).

Statistical analyses. All data are presented as means ± SE. For data comparison between the diabetic and the control groups, two-way analysis of variance (ANOVA) was carried out. When the *F* value was significant, differences among the groups, including the insulin-treated diabetic group, were assessed by one-way ANOVA followed by Duncan's multiple-range test or Student's *t* test for unpaired data.

RESULTS

General characteristics of experimental animals. Two, 4, and 8 wk after STZ injection, diabetic rats displayed significantly lower body and heart weights (Table 1). Elevated heart-to-body weight ratios and a decreased heart rate were observed in the 8-wk-diabetic rats but not in the 2- and 4-wk-diabetic rats. None of the diabetic rats showed any alteration in blood pressure compared with the control rats. Diabetes in the STZ-injected animals was evident from elevated plasma glucose and depressed insulin levels in comparison with the control rats. Although these changes were almost normalized by insulin treatment, diabetic rats treated with insulin had a lower body weight and higher heart-to-body weight ratio than the control rats.

Insulin treatment maintained the plasma insulin at a high level. Experimental diabetes was accompanied by the depressed level of T3. This result is in agreement with the data from other studies in which a similar experimental protocol was used (6,7). Insulin treatment returned the plasma value of T3 to the control level.

ACh concentration in heart. The myocardial ACh concentration in both the diabetic and control rats was in the order of RA > LA > RV > IVS > LV at 2, 4, and 8 wk after the induction of diabetes (Table 2). The atrium had a higher concentration than the ventricle, and the right side showed a higher concentration than the left side. Insulin treatment did not alter the pattern of ACh distribution in the heart.

Two and 4 wk after STZ injection, the ACh concentration in diabetic rats did not significantly differ from that in the control rats. Eight weeks after STZ injection, ACh concentration in RA ($P < .01$), RV ($P < .01$), and LV ($P < .05$) significantly increased in diabetic rats compared with concentrations in the control rats. Insulin treatment in the diabetic rats inhibited the increase in ACh concentration in these portions of the heart. There was no significant difference in ACh concentration in LA and IVS among the three groups at 8 wk.

NE concentration in heart. The myocardial NE concentration in both diabetic and control rats was in the order of

TABLE 3
Norepinephrine concentration in rat myocardium

	2 wk		4 wk		8 wk		
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Diabetic + insulin
Right atrium	18.7 ± 0.8	20.8 ± 1.6	19.9 ± 1.2	20.9 ± 1.3	18.0 ± 0.8	25.7 ± 1.5*	19.8 ± 0.6†
Left atrium	8.3 ± 0.6	8.8 ± 0.8	8.6 ± 0.4	9.0 ± 0.8	8.6 ± 0.4	9.1 ± 0.8	8.3 ± 0.6
Right ventricle	6.2 ± 0.5	6.4 ± 0.6	6.5 ± 0.3	6.9 ± 0.4	6.7 ± 0.4	8.9 ± 0.6*	7.0 ± 0.4†
Left ventricle	4.8 ± 0.4	5.2 ± 0.3	4.8 ± 0.4	5.6 ± 0.4	4.4 ± 0.3	6.0 ± 0.4*	4.8 ± 0.3‡
Interventricular septum	3.8 ± 0.2	3.9 ± 0.5	4.1 ± 0.1	4.3 ± 0.1	3.7 ± 0.3	5.5 ± 0.2*	3.9 ± 0.3†

Values are in nanomoles per gram wet weight and are means ± SE of 10–12 experiments.
*$P < .01$ vs. corresponding control values.
†$P < .01$ and ‡$P < .05$ vs. age-matched diabetic animals.

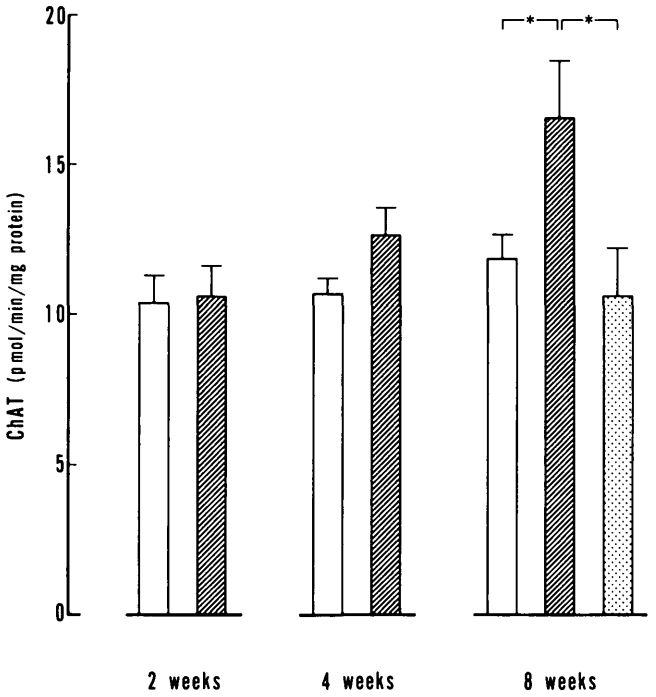

FIG. 1. Choline acetyltransferase (ChAT) activity in right atrium from control (*open bars*), diabetic (*hatched bars*), and diabetic rats treated with insulin (*stippled bar*) 2, 4, and 8 wk after streptozocin injection. Each *bar* represents mean ± SE of 10–12 experiments. *$P < .05$.

RA > LA > RV > LV > IVS at 2, 4, and 8 wk after the induction of diabetes (Table 3). This result is nearly the same as the ACh distribution in the heart. Insulin treatment also did not change the pattern of NE distribution in the heart.

Two and 4 wk after STZ injection, the NE concentration in diabetic rats was not significantly different from that in the control rats. Eight weeks after STZ injection, the NE concentration in RA ($P < .01$), RV ($P < .01$), LV ($P < .01$), and IVS ($P < .01$) was significantly higher in diabetic rats than in the control rats. Insulin treatment in the diabetic rats inhibited the increase in NE concentration in these portions. There was no significant difference in NE concentration in LA among the three groups at 8 wk.

ChAT activity in RA. Two and 4 wk after STZ injection, ChAT activity in the diabetic rats was not significantly different from that in the corresponding control rats (Fig. 1). However, 8 wk after STZ injection, ChAT activity in the diabetic rats (16.7 ± 1.9 pmol · min^{-1} · mg^{-1} protein) was significantly higher than that in the control rats (11.9 ± 0.8 pmol · min^{-1} · mg^{-1} protein). There was no significant difference in ChAT activity between insulin-treated diabetic (10.7 ± 1.6 pmol · min^{-1} · mg^{-1} protein) and age-matched control rats.

AChe activity in RA. There was no significant difference in the AChe activity between diabetic and control rats 2 wk after the induction of diabetes (Fig. 2). The AChe activity in diabetic rats was 4.3 ± 0.4 nmol · min^{-1} · mg^{-1} protein at 4 wk and 4.6 ± 0.3 nmol · min^{-1} · mg^{-1} protein at 8 wk, which was significantly lower than the values from the corresponding control rats (7.4 ± 0.5 and 6.9 ± 0.2 nmol · min^{-1} · mg^{-1} protein at 4 and 8 wk, respectively). Insulin treatment in the diabetic rats completely returned the AChe activity to the control level (6.5 ± 0.3 nmol · min^{-1} · mg^{-1} protein).

Ch concentration in heart. Two and 4 wk after STZ injection, there was no significant difference in Ch concentration between diabetic and control rats (Table 4). Eight weeks after STZ injection, the Ch concentration in the diabetic rats significantly increased in the RV ($P < .05$), LV ($P < .01$), and IVS ($P < .01$) compared with that in the control rats. Insulin treatment in the diabetic rats decreased the Ch concentration to the control level.

DISCUSSION

Biological assay (22), pyrolysis gas chromatography (23), and radioisotope assay (24) have been used for the measurement of ACh concentration. However, these methods are complicated and do not have a high sensitivity or specificity. In this study, we measured the ACh and Ch concentrations in rat myocardium by a modified method of Potter et al. (14), which is simple, specific, and sensitive for quantification. NE, ACh, and Ch were extracted from the same sample and detected by HPLC-ECD. We also applied HPLC-ECD for the measurement of ChAT and AChe activities in the diabetic rat heart.

Recently, Ganguly et al. (6,7) reported an increased cardiac concentration, turnover, release, uptake, synthesis, and metabolism of NE in chronic diabetic rats and indicated an increased sympathetic activity in diabetic myocardium. They also reported that these changes were normalized by insulin treatment. Our findings on NE concentration in the myocardium were similar to theirs.

Evaluation of the parasympathetic innervation of the heart contributes to our understanding of complex autonomic regulation of this organ in the normal status and how the control is altered in the pathological status. In this study, both ACh concentration and ChAT activity significantly increased in the diabetic rat heart 8 wk after STZ injection, which suggests the enhanced myocardial ACh synthesis in this model of diabetes. Insulin treatment prevented these changes as the increased sympathetic activity in diabetic myocardium was normalized by insulin treatment (6,7).

In contrast with our observations, Kuntscherová and Vlk (13) reported a decrease in atrial ACh concentration in ALX-D rats. Such an apparent discrepancy may be due to differences in the method applied for studying ACh con-

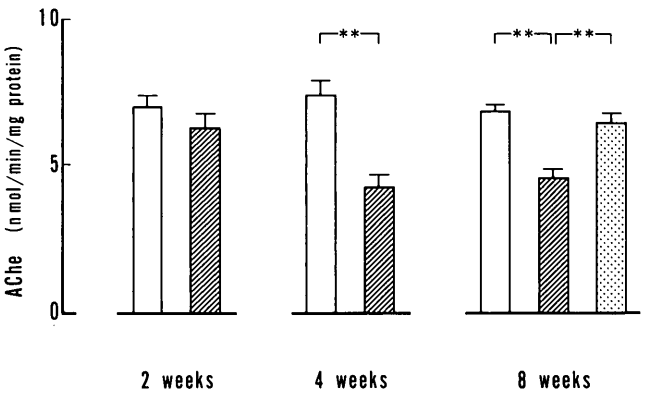

FIG. 2. Acetylcholinesterase (AChe) activity in right atrium from control (*open bars*), diabetic (*hatched bars*), and diabetic rats treated with insulin (*stippled bar*) 2, 4, and 8 wk after streptozocin injection. Each *bar* represents mean ± SE of 10–12 experiments. **$P < .01$.

TABLE 4
Choline concentration in rat myocardium

	2 wk		4 wk		8 wk		
	Control	Diabetic	Control	Diabetic	Control	Diabetic	Diabetic + insulin
Right atrium	150.4 ± 7.3	158.4 ± 7.6	150.5 ± 3.0	162.3 ± 8.2	137.4 ± 6.4	152.4 ± 10.3	140.8 ± 8.7
Left atrium	72.5 ± 3.9	79.2 ± 4.1	103.0 ± 4.0	109.8 ± 6.7	70.2 ± 6.3	85.3 ± 7.3	75.3 ± 3.6
Right ventricle	66.5 ± 4.6	78.5 ± 4.1	94.5 ± 5.2	105.8 ± 6.8	71.3 ± 4.6	92.1 ± 6.7*	80.5 ± 5.7
Left ventricle	51.0 ± 3.0	53.8 ± 4.9	81.3 ± 3.3	83.8 ± 7.3	53.8 ± 2.5	96.5 ± 7.0†	62.4 ± 4.8‡
Interventricular septum	58.3 ± 5.7	67.9 ± 5.9	84.0 ± 3.0	92.5 ± 6.4	62.0 ± 2.8	82.8 ± 3.9†	67.7 ± 5.0§

Values are in nanomoles per gram wet weight and are means ± SE of 10–12 experiments.
*$P < .05$ and †$P < .01$ vs. corresponding control values.
‡$P < .01$ and §$P < .05$ vs. age-matched diabetic animals.

centration and the experimental model used. Their method was a biological assay that is less sensitive and less specific compared with the HPLC-ECD. They used female albino rats. In experimental diabetes, cardiac function in male rats has been reported to differ from that in female rats (25). The time course of changes associated with their model may differ from that in this study.

There have been no reports concerning myocardial ChAT activity in diabetes. ChAT activity in spinal medulla has been reported to increase in experimental diabetes (26). Preganglionic fibers of cardiac vagal nerve are known to originate from medulla, and ChAT is thought to be produced in the cell body in medulla and transported in the axon to the site of ACh synthesis in the heart (27). Therefore, the increased ChAT activity in RA might result from the activity in spinal medulla.

There have been several lines of indirect evidence suggesting the increased ACh synthesis in diabetic hearts. The number of muscarinic receptors is decreased by chronic exposure to agonists or by inhibition of AChe, whereas it is increased by exposure to antagonists (28,29). The number of myocardial muscarinic receptors has been shown to decrease in diabetes (10). This decrease may be due to down-regulation as a result of elevated levels of myocardial ACh. ACh synthesis from Ch and acetyl-CoA catalyzed by ChAT is accompanied with CoA synthesis. The myocardial level of CoA is reported to increase in diabetes (11). This could be related to the enhanced ACh synthesis in diabetes. ACh synthesis in the heart has been shown to depend on the availability of Ch (30). Therefore, the increased myocardial Ch concentration in diabetes may acclerate ACh synthesis.

A significant decrease in heart rate was observed in diabetic rats at 8 wk. Bradycardia has been a consistent finding in this model of diabetes (7,12). Possible explanations for it include alterations in sinoatrial nodal electrical activity resulting from hyperglycemia (31), glycoprotein-induced ventricular stiffness (1), decreased cardiac adrenergic receptors (12), and a decrease in circulating thyroid hormone (12). However, the precise mechanism responsible for this bradycardia is still unknown. In this study, decreased plasma T3 was observed in diabetic rats 2, 4, and 8 wk after STZ injection, whereas bradycardia developed at 8 wk coincided with the increase in ACh concentration. Because the administration of T3 to diabetic rats has been shown to fail to improve bradycardia (7), it is unlikely that decreased plasma T3 was responsible for bradycardia. The parasympathetic nervous system has been reported to predominate over the sympathetic nervous system in the control of heart rate (32). Therefore, bradycardia may have resulted from the augmented parasympathetic activity in this model of diabetes.

Autonomic neuropathy is one of the major complications in chronic diabetes, and the cardiac vagal nerve is more prone to dysfunction than are the cardiac sympathetic nerves (33). Tomlinson and Yusof (8) have reported the predominant parasympathetic denervation of the heart with some degree of sympathetic denervation in rats induced with ALX for 8 mo. This contrast to our findings is presumably due to the difference in the duration of diabetic status. In short-term diabetes, like that produced in this study, decreased responsiveness of the heart to exogenous cholinergic and noradrenergic agonists (34,35), increased sensitivity in baroreflex (36), increased myocardial NE concentration (6,7,9), and increased myocardial noradrenergic fiber density (9) have been reported. By contrast, enhanced responsiveness of the heart to exogenous cholinergic and noradrenergic agonists (8,34), decreased sensitivity in baroreflex (36), decreased myocardial NE concentration (9), degenerated noradrenergic nerves, and the absence of cholinergic terminals in the atria (8) have been reported in long-term diabetes. Although whether similar phenomena exist in humans is not known, the functional status of the autonomic nervous system changes with time in experimental diabetes. Physiological compensatory mechanisms may exist and may also change with time. Although further investigations are required, our results seem to indicate that the cardiac parasympathetic activity was augmented to compensate for the augmented sympathetic drive in the early stage of diabetes. A similar cardiac sympathetic-parasympathetic interaction has been suggested for hypertension (16).

AChe activity, which terminates the action of ACh by hydrolysis, decreased in diabetic rats 4 and 8 wk after STZ injection. A similar observation has been demonstrated in atria (10) and in erythrocyte membrane (37) in experimental diabetes. A decrease in AChe activity may increase the concentration of ACh that affects myocardial receptors. Although AChe is less specific for cholinergic innervation compared with ACh and ChAT, the relation between the increase in ChAT activity and the decrease in AChe activity is unclear. Because a high concentration of Ch could inhibit AChe (38), the decrease in AChe activity might partly be due to the increased myocardial Ch concentration.

In conclusion, this study suggests that early stages of STZ-D are associated not only with the augmented sympathetic activity but also with the augmented parasympathetic activity and that adequate insulin treatment prevents these alterations. These findings lead to a better understanding of development of autonomic neuropathy and cardiomyopathy in diabetes mellitus.

ACKNOWLEDGMENTS

We thank Tomoyuki Moriya of the Research Institute for Experimental Animals for care of the rats and Keiko Sakai for secretarial assistance.

This research was supported in part by grants-in-aid for developmental scientific research (62624507 and 62570388) from the Ministry of Education, Science and Culture of Japan.

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