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Diabetes-induced bradycardia is an intrinsic metabolic defect reversed by carnitine

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Abstract

Rats with streptozotocin-induced diabetes (STZ-D) have reduced serum carnitine levels and bradycardia. Heart rates (HRs) of 24 nondiabetic rats (NRs) and 24 STZ-D rats were compared. L-Carnitine (C) was added to the drinking water of rats (12 STZ-D + C) to raise their serum carnitine level. The intrinsic HR for each animal was determined after parasympathetic and sympathetic blockade. The HRs of STZ-D rats (278 ± 15 beats per minute) were less than those of NRs (348 ± 8 beats per minute) ($P < .01$). STZ-D rats had low serum carnitine compared with control and STZ-D + C rats. The difference in HR of STZ-D rats and NRs continued after blockade, indicating that the bradycardia of diabetes is intrinsic to the heart. The metabolic milieu reflected in the rats' urinary organic acid profiles differed between the control and STZ-D rats. The HR of STZ-D + C rats (326 ± 5 beats per minute) did not differ from those of NRs. Increasing either the insulin dose or the serum free carnitine reduced urinary organic acids, but normal HRs were associated only with elevated serum carnitine levels. When glucose is compromised as a myocardial energy source (diabetes mellitus), we propose that elevated levels of serum carnitine may increase myocardial fatty acid metabolism sufficiently to correct the bradycardia of STZ-D rats.

1. Introduction

Cardiovascular disease is the most common serious complication of diabetes mellitus [1]. Coronary atherosclerosis and cardiomyopathy occur as a result of the metabolic abnormalities associated with diabetes [2]. These physical changes require years to develop in humans after the onset of chronic hyperglycemia [3], indicating a slowly progressive process. We have previously shown a declining heart rate (HR) in rats made diabetic with streptozotocin (STZ) at 6 months of age [4]. We and others [5,6] have noted bradycardia in rats 2 weeks after diabetes induction with STZ. Heart rate variability in diabetes is commonly attributed to associated neuropathy [7]. It has been noted, however, that the induction of hyperglycemia in Wistar rats after 6 months of life does not alter peripheral nerve function for an additional 6 months [8] but does reduce the HR [5]. Carnitine deficiency is often encountered in diabetes mellitus [8,9] and is noted within 2 weeks of STZ-induced diabetes (STZ-D) in rats. A recent publication [10] suggests that abnormal myocardial fatty acid metabolism in diabetes leads to energy deprivation and cardiovascular morbidity and mortality. To evaluate the role of primary energy metabolism and autonomic function in cardiac rate in STZ-D rats, we monitored electrocardiograms (ECGs) and metabolic variables to examine their relationship with the intrinsic HRs of nondiabetic (NR) and STZ-D rats.

2. Methods

Forty-eight 6-week-old male Wistar rats (100-120 g) were used in this study. The rats were divided into 2 groups of 24 animals as follows: the first group was composed of the nondiabetic control animals, the second group the diabetic animals (Fig. 1). Diabetes was induced by injecting 50 mg/kg of STZ intraperitoneally after a 12-hour fast. The goal was to make 24 of these animals diabetic and to maintain normal weight gain by giving them 10 U/kg of Ultralente insulin 3 days each week (Monday, Wednesday, and Friday). This procedure sustains normal weight gain while maintaining blood glucose levels above 250 mg/dL.

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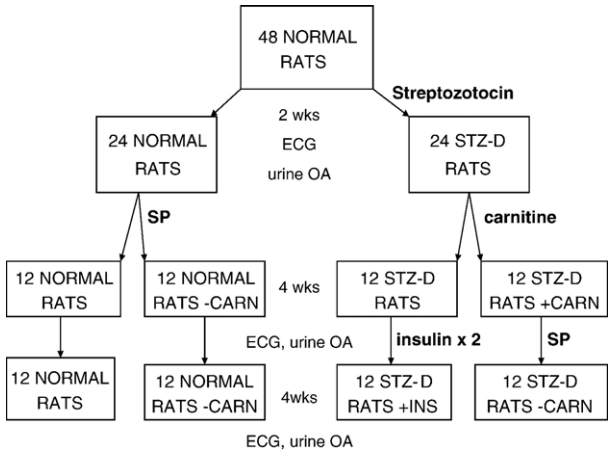

Fig. 1. Study protocol. ECG indicates electrocardiogram; urine OA, urine organic acids; CARN, carnitine; INS, insulin, SP, sodium pivilate.

Blood glucose was measured using tail vein blood and a glucose oxidase reagent strip read by a Free Style blood glucose meter (TheraSense, Alameda, CA) 3 days each week (Monday, Wednesday, and Friday). Six-hour timed urine collections were obtained from all animals 2 weeks after the second group became diabetic. ECG tracings were also performed at 2, 6, and 10 weeks after diabetes onset (Fig. 1) on these animals in the conscious state with the RatPaak Biotelemetry System (CleveMed, Cleveland, OH).

The RatPaak is a wireless data acquisition system used to remotely monitor physiologic parameters in small laboratory animals. The animal-associated hardware consists of a nylon-lycra thoracic jacket, which contains the skin contact electrodes, a battery pack, a microprocessor, and a transmitter. Each rat was placed in an isoflurane induction chamber (2%-4% isoflurane was delivered by a calibrated vaporizer equipped with scavenger) to induce anesthesia. After induction of anesthesia, the rat was removed from the chamber and its thoracic wall fur was clipped while anesthetized. The 3 contact electrodes (nonpenetrating, noninvasive) were fixed to the rat's thorax with electrode paste/gel. The rat jacket was then secured by Velcro fasteners. The entire process (clipping and electrode/jacket placement) took less than 1 minute, and the rat was allowed to recover fully from the isoflurane anesthesia in its home cage before measurement recording was begun.

The first group of animals was then divided into 2 groups of NRs: one group received regular food and water, the other received regular food, but 10% sodium pivilate (SP) (10 mmol/L) was added to their drinking water to induce renal carnitine wasting, which caused severe carnitine deficiency (Fig. 1). The group with STZ-D was divided into 2 groups of 12 animals: one group received food, water, and insulin, whereas the other received the same but was also

Table 2
Metabolites and carnitine in the urine of nondiabetic and 2-week STZ-D rats

Variable	n	Normal rats (mean ± SEM)	n	Diabetic rats (mean ± SEM)	*P*
Lactic acid	23	6.16 ± 0.56	23	471.11 ± 46.5	.002
Pyruvic acid	23	5.79 ± 0.67	23	42.76 ± 9.9	.0003
Acetoacetic acid	23	1.31 ± 0.20	23	8.08 ± 0.81	.001
β-OH butyric acid	23	5.13 ± 0.64	23	8.09 ± .42	.11
Succinic acid	23	24.5 ± 2.2	23	12.56 ± 2.2	.03
Methylsuccinic acid	23	2.46 ± 0.29	23	6.74 ± 1.46	.008
Fumaric acid	21	2.47 ± 0.30	23	9.43 ± 2.27	.03
Adipic acid	23	3.13 ± 0.36	23	12.26 ± 4.54	.058
p-OH phenylacetic acid	23	1.18 ± 0.46	23	29.87 ± 3.21	.004
α-Ketoglutaric acid	22	35.1 ± 3.97	23	10.99 ± 4.53	.002
p-OH phenyllactic acid	13	1.18 ± 0.20	20	7.50 ± 1.65	.006
Oxalic acid	23	14.38 ± 1.82	23	28.28 ± 9.48	.18
Malonic acid	23	0.87 ± 0.67	23	33.79 ± 2.34	.26
Glutaric acid	23	2.34 ± 0.3	21	5.59 ± 2.23	.15
Suberic acid	22	2.48 ± 0.27	22	6.67 ± 0.73	.35
Cisaconinic acid	21	3.85 ± 0.49	21	8.18 ± 2.22	.27
Hippuric acid	22	13.31 ± 1.83	22	13.07 ± 2.94	.96
Citric acid	22	9.08 ± 1.33	23	16.57 ± 5.13	.19
Sebacic acid	14	1.82 ± 0.31	21	11.54 ± 5.10	.073
Total carnitine (mmol/dL)	21	5.42 ± 0.58	23	26.06 ± 2.7	.001
Carnitine esters (mmol/dL)	21	4.2 ± 0.14	23	18.27 ± 0.92	.001

Table 1
Carnitine and cardiac function in STZ-D rats

Variables	Control	Control − carnitine	Diabetic	Diabetic + insulin	Diabetic + carnitine	Diabetic − carnitine
HbA_{1c} (%)	3.62 ± 0.14	3.44 ± 0.06	8.24 ± 0.26*	8.6 ± 0.3*	6.63 ± 0.19*	7.8 ± 0.23*
Weight increase (g/wk)	30.35 ± 3.56	29.65 ± 4.71	22.32 ± 3.5**	29.65 ± 2.25†	18.68 ± 2.7**	17.56 ± 3.5**
Glucose (mg/dL)	103 ± 2.5	104 ± 2.87	381 ± 15.5*	319 ± 11*	380 ± 23*	298 ± 14*
Serum carnitine (mmol/dL)	24.2 ± 0.7	9.8 ± 0.4**	8.0 ± 0.4**,†	10.8 ± 1.8**,†	30.3 ± 2.2	8.0 ± 0.3**,†
HR preblockade (beats/min)	348 ± 8	341 ± 6	278 ± 15**,†	270 ± 8**,†	326 ± 5	225 ± 7*,†
HR postblockade (beats/min)	339 ± 9	338 ± 7	269 ± 16**,†	241 ± 10**,†	300 ± 14	193 ± 7*,†

All values were collected after 4 weeks in the indicated experimental group.

* $P < .01$, results different from control.

** $P < .05$, results different from control.

† $P < .05$, diabetic animal results different from carnitine-supplemented diabetic animals.

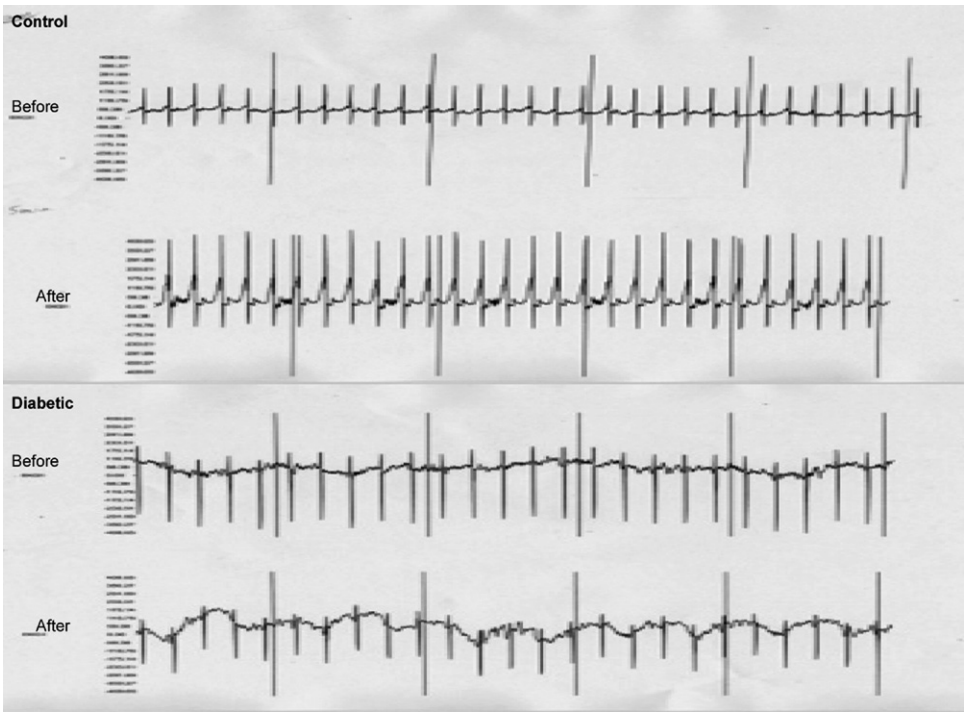

Fig. 2. An example of ECG tracings collected using the RatPaak Biotelemetry System on control and STZ-D rats before and 5 minutes after total sympathetic and parasympathetic blockade after 4 weeks in each of the experimental treatment groups.

given L-carnitine (1 g/L) in their drinking water to prevent carnitine deficiency (Fig. 1).

After 4 weeks, these animals had their ECG tracings performed in the conscious state by remote telemetry and their HRs were determined. The HRs and QTc intervals of these animals were calculated from the telemetry records. To determine intrinsic HR, each animal was maximally blocked with both propranolol hydrochloride (4 mg/kg) and atropine sulfate (3 mg/kg) [11] after each baseline ECG measurement. The cardiac rate was measured for 1 minute at baseline and 5 minutes after administration of the blocking agents. The jacket was then removed, and the rat returned to its home cage. Each rat was monitored and observed during the entire process. If an animal exhibited behavior such as aggressive attempts to remove the jacket, attempts to chew at the jacket, or vocalization, the jacket was immediately removed, and the animal was excluded from this portion of the study.

The daily management of the 2 groups of NRs did not change for the next 4 weeks. However, the daily management of the 2 groups of STZ-D rats did change during the next 4 weeks (Fig. 1). Twelve STZ-D rats had their exogenous insulin dose doubled for the next 4 weeks, whereas those STZ-D rats that received supplemental carnitine in their drinking water had the carnitine removed and SP added to their drinking water for 4 weeks to induce a dramatic reduction of serum carnitine. Urine was again collected to measure organic acids after each 4-week treatment period, and ECGs were performed before and after blockade (Fig. 1). At the end of the experiment, all of the animals were anesthetized with sodium pentobarbital and euthanized. Whole blood was collected for the measurement of hemoglobin A_{1c} (HbA_{1c}) [8] and serum used to measure carnitine [12]. Organic acids in the 6-hour urine collections were measured by gas chromatography using a DB-1 column (oven temperature, 275°C; detector temperature, 280°C) [13]. All data are presented as means ± SEM and were compared by analysis of variance and Student t test for unpaired data, with a $P < .05$ considered significant.

3. Results

The mean blood glucose and weekly weight gain (Table 1) indicate that STZ-D rats have diabetes. The HbA_{1c} was elevated and the serum free carnitine was reduced in the STZ-D rats that did not receive oral carnitine supplementation (Table 1). The urinary organic acid excretion in the STZ-D animals changed significantly from the NRs (Table 2) after 2 weeks of diabetes suggesting a definite difference in Krebs cycle activity. The STZ-D animals also lost more carnitine in their urine (Table 2).

Electrocardiogram tracings (Fig. 2) were used to measure the HRs and QTc intervals of the experimental animals (Table 1). The second tracing for each animal was made 5 minutes after the induction of parasympathetic and sympathetic blockade (Fig. 2). Autonomic blockade of the heart did not result in a significant change in the resting HRs of either the control or diabetic animals. The STZ-D animals continued to have reduced HRs. Then 12 of the STZ-D rats were supplemented with carnitine in their drinking water for

Table 3
Urine organic acid response to insulin and carnitine in STZ-D rats

Variables	Control	Control − carnitine	Diabetic	Diabetic + insulin	Diabetic + carnitine	Diabetic − carnitine
Number	12	11	11	11	12	12
Lactic acid	3.1 ± 0.23	2.6 ± 0.26	211.3 ± 34*	32.3 ± 12.3**,†	30.6 ± 5.8**,†	150 ± 27.1*,‡
Pyruvic acid	3.0 ± 0.25	2.43 ± 0.21	36.7 ± 7.7**	10.3 ± 1.8	8.6 ± 1.2	7.03 ± 1.2
β-OH butyrate	2.8 ± 0.32	2.41 ± 0.19	6.0 ± 0.92	12.5 ± 2.7	19.5 ± 8.2*,†	31.7 ± 9.1*,‡
Acetoacetic acid	2.2 ± 0.2	1.9 ± 0.25	5.0 ± 0.8	1.6 ± 0.2	3.0 ± 0.3	2.23 ± 0.1

All samples were collected after 4 weeks in the designated group.
* $P < .01$, different from controls.
** $P < .05$, different from controls.
† $P < .05$, different from diabetic rats.
‡ $P < .01$, different from diabetic rats.

4 weeks. Both serum free carnitine and HRs returned to normal levels (Table 1). These same animals had no change in their daily insulin dose, but instead of carnitine, SP was added to their drinking water for the next 4 weeks. Although these animals continued to gain weight appropriately for their insulin dose (Table 1), their serum free carnitine dropped 22.2 mmol/dL to a final concentration of 8.0 ± 0.3 mmol/dL, and their HRs (326 ± 5 beats per minute) dropped to 226 ± 7 beats per minute. That group of STZ-D animals also demonstrated prolonged QTc intervals and T-wave inversion. Twelve of the STZ-D animals without carnitine supplementation during the first 4 weeks had their daily insulin dose doubled for the next 4 weeks. They gained more weight and had some mild changes in metabolic activity as indicated by their urinary organic acids (Table 3), but no change in cardiac parameters (Table 1). The presence of diabetes and differing levels of insulin and carnitine had some influence on Krebs cycle metabolism as noted in the 6-hour urinary organic acid excretion (Table 3), but normal HRs were found only in the STZ-D rats with normal serum carnitine levels.

4. Discussion

Resting HRs were reduced in diabetic rats (278 ± 15 beats per minute) when compared with control animals (348 ± 8 beats per minute) after 4 weeks of diabetes. The HRs of the diabetic and NRs did not change significantly after 5 minutes of parasympathetic and sympathetic blockade (Table 1). This indicates that a sympathetic/parasympathetic imbalance is not the cause of the reduced HRs in the diabetic animals. Thus, the intrinsic HR of STZ-D rats was less than the intrinsic HRs of NRs. The free carnitine levels for the diabetic rats (8.0 ± 0.4 μm/dL) were much less than those found in control animals (24.2 ± 0.7μm/dL). Carnitine supplementation of STZ-D rats resulted in correction of their free serum carnitine levels (30.3 ± 2.2 μm/dL) as well as their HRs (326 ± 5 beats per minute). The carnitine-supplemented STZ-D rats received the same STZ to make them diabetic, and the same amount of insulin to treat hyperglycemia, as the STZ-D rats that had bradycardia. Thus, neither STZ nor insulin caused that bradycardia. Control animals receiving SP had a reduction of serum free carnitine levels (9.8 ± 0.4 μm/dL) but no change in their HRs (341 ± 6 beats per minute). Thus, carnitine deficiency alone was not the cause of the reduced HR, unless the animals also had diabetes and impaired glucose metabolism. When urinary organic acid excretion was used as an indicator of general metabolism in the study rats, we see a generalized increase of organic acids in the urine of the diabetic animals (Table 2). This indicates an increase in fatty acid metabolism in our study animals when their insulin levels are fixed by a daily administered dose. The normal plasticity in myocardial substrate preference is reported to be lost in diabetic mice [14] and humans [10]. It has been reported that myocardial glucose extraction and utilization is reduced in diabetic subjects when compared with normal controls in spite of increased myocardial blood flow and higher blood glucose levels [10]. This observation is likely due to inappropriate glucose transport and metabolism caused by nonphysiologic insulin levels, the hallmark of diabetes mellitus. It is recognized that the diabetic heart has a greater dependence on fatty acid metabolism than does the normal heart [14]. The apparent relationship of the HR in STZ-D rats to carnitine levels is consistent with this altered substrate preference in the diabetic heart, because there was no change in the HRs of nondiabetic animals with normal glucose metabolism when made carnitine deficient. The STZ-D rats receiving supplementation of either carnitine or insulin had a significant reduction in urinary pyruvic and lactic acid in their urine, indicating associated changes in their metabolism. The STZ-D animals with greater dependence on fatty acid substrate, however, had a better functional (HR) response to supplemental carnitine than to insulin. The STZ-D rats receiving supplemental carnitine in their drinking water had normal free carnitine levels (30.3 ± 2.2 μm/dL); and normal HRs (326 ± 5 beats per minute) When the same animals received SP in their drinking water to quickly lower their free carnitine (8.0 ± 0.3 mmol/dL), their HRs dropped dramatically to 225 ± 7 beats per minute. These reduced HRs were lower than those found in animals that became carnitine deficient gradually in response to increased organic acid loss in the urine of diabetic animals.

The control animals in this study had a mean HbA_{1c} of 3.62% ± 0.14% during the experiment. The mean HbA_{1c} of the diabetic animals was 8.24% ± 0.26%, indicating more circulating glucose availability for energy metabolism in the hearts of the STZ-D rats. Doubling the administered insulin dose for 4 weeks resulted in greater weight gain for those animals, a reduction of urine organic acids, but no change in their relative bradycardia (Table 1). This finding suggests that increased cellular glucose availability provided by doubling the insulin level in STZ-D rats was not as functionally effective as increasing fatty acid availability with carnitine for normalizing the HR. The STZ-D rats (receiving supplemental carnitine) had normal HRs and elevated HbA_{1c} levels of 8.6% ± 0.3%, but when those animals had SP added to their drinking water to lower their serum free carnitine (8.0 ± 0.3 mmol/dL) with no change in daily administered insulin, there was little effect on their HbA_{1c} levels, but a major reduction in their HRs.

The quick and significant loss of carnitine in STZ-D animals, more dependent upon myocardial fatty acid metabolism, may have caused a dramatic reduction in mitochondrial fatty acid transport essential for energy generation from this substrate. It has been previously noted that increased fatty acid metabolism and decreased myocardial glucose oxidation as measured by positron emission tomography scan occurs in subjects with type 1 diabetes mellitus [10]. It has also been observed that fatty acid oxidation declines as the duration of diabetes increases. This change in substrate utilization noted in humans with type 1 diabetes mellitus could be in response to decreasing carnitine levels associated with increasing duration of excessive urinary carnitine loss, documented in the current study.

The metabolic activity in the diabetic animals in this study differed significantly from the NRs as noted by urinary organic acid levels (Table 2). The greater excretion of organic acids in the urine of STZ-D animals suggests that the mechanism for the chronic loss of carnitine is as an organic acid ester that is not reabsorbed by the renal tubule and therefore is lost in the urine. This was also noted by the increased esterified and total carnitine found in the urine of the diabetic animals (Table 2). The level of urinary lactic acid excretion was increased 10-fold in the STZ-D rats, suggesting increased anaerobic metabolism in these animals. When those rats were given supplemental carnitine, there was a significant reduction in urinary lactic acid levels, which indicates a shift to more aerobic metabolism in those rats. This may indicate that increased mitochondrial fatty acid oxidation in response to increased free carnitine levels causes increased mitochondrial electron transport chain activity, which generates more adenosine triphosphate and nicotinamide adenine dinucleotide to support aerobic metabolism. When the STZ-D rats that were given supplemental carnitine had their serum free carnitine reduced from 30.2 ± 2.2 to 8.0 ± 0.3 mmol/dL with SP there was an increase in urinary lactic acid and a dramatic reduction in their HRs. This finding suggests that the HRs of STZ-D rats are regulated in conjunction with changes in fatty acid metabolism. Because this occurred during autonomic blockade in animals whose myocardium has increased dependence on fatty acid energy generation, one can speculate that this reflects an intrinsic metabolic influence on HR not previously described. This may reflect a cellular metabolic influence on myocardial function.

These same animals showed a significant increase in their QTc (83.48 ± 2.2 vs normal 73.5 ± 1.6 msec) and inversion of their T waves, suggesting other repolarization abnormalities. The observations in humans with diabetes [10] and our data in the STZ-D rat suggest that further studies are indicated to determine the role of carnitine and insulin levels in myocardial energy metabolism. Such studies may help elucidate a cellular mechanism for the cardiomyopathy of diabetes mellitus.

5. Conclusion

This study demonstrates that diabetes and carnitine deficiency directly influence the intrinsic HR independent of sympathetic and parasympathetic regulation. Carnitine had a greater impact than insulin supplementation on ameliorating the bradycardia found in STZ-D rats. This finding suggests that when glucose availability is compromised by diabetes, fatty acid metabolism becomes essential for normal myocardial function. The increased organic acid production associated with diabetes and insulin insufficiency may deplete serum carnitine levels, which then will compromise fatty acids as a myocardial energy source. This suggests that monitoring serum carnitine levels and supplementation of those subjects who have low levels may help manage the many cardiac problems associated with diabetes mellitus.

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