Decreased Adrenal Medullary Catecholamine Release in Spontaneously Diabetic BB-Wistar Rats

Role of Hypoglycemia

Russell A. Wilke and Cecilia J. Hillard

We have demonstrated previously that spontaneously diabetic BB-Wistar rats exhibit decreased adrenal medullary catecholamine secretion in response to splanchnic nerve terminal stimulation. We hypothesized that this abnormality is caused by changes in the sensitivity of the adrenomedullary chromaffin cells to acetylcholine (ACh). To study this hypothesis, we isolated adrenal glands from control and spontaneously diabetic BB-Wistar rats, perfused them with ACh, and measured catecholamine secretion. Adrenal catecholamine release in response to ACh was significantly decreased at 2, 8, and 16 weeks after the onset of diabetes compared with age-matched, nondiabetic control rats. Catecholamine release in response to perfusion with 20 mM K^+ was the same in adrenals from diabetic and control rats. The decreased responsiveness of diabetic rat adrenals to perfusion with ACh was significantly correlated with a decrease in the release of catecholamines in response to splanchnic nerve stimulation. A similar defect in catecholamine secretion was also seen in adrenals harvested from nondiabetic BB-Wistar rats following a 3-h period of acute hypoglycemia; however, the adrenal response to potassium was also decreased as was the catecholamine content of the adrenal. Conversely, nondiabetic BB-Wistar rats made diabetic with streptozocin (STZ) and maintained in a hyperglycemic state did not exhibit catecholamine hyposecretion 2 weeks after STZ administration. Collectively, the data describe decreased adrenomedullary response to cholinergic stimulation in spontaneously diabetic rats as early as 2 weeks after the onset of diabetes and that a similar, although more severe, hyposecretion occurs after acute, severe hypoglycemia. ***Diabetes*** **43:724–729, 1994**

Epinephrine (EPI) is the primary catecholamine released from the chromaffin cells of the adrenal medulla (1) and functions as a counterregulatory hormone in the maintenance of glucose homeostasis (2). Adrenomedullary EPI secretion is decreased in a subpopulation of human patients with insulin-dependent diabetes mellitus, placing them at risk for rapid metabolic decompensation (3,4). Because these patients normally depend on EPI-mediated symptoms to detect periodic episodes of iatrogenic hypoglycemia, loss of adrenomedullary EPI secretion may contribute to the development of a phenomenon known as hypoglycemia unawareness (3). Very little is known about the etiology or pathogenesis of the loss in adrenomedullary function in diabetes.

Since its introduction more than a decade ago, the spontaneously diabetic BB-Wistar rat has served as an excellent model system for studying the neural and metabolic complications associated with diabetes (5–7). Recent studies conducted in our laboratory have demonstrated that spontaneously diabetic BB-Wistar rats exhibit a defect in adrenomedullary catecholamine secretion in response to splanchnic nerve stimulation (8). Several possible mechanisms exist for this effect, including decreased acetylcholine (ACh) release from presynaptic terminals, decreased responsiveness of the chromaffin cell cholinergic receptors, or a generalized hypofunctioning of the catecholamine release process. We have begun to explore the mechanism for the decrease in adrenomedullary secretion by investigating the effect of diabetes on the release of catecholamines in response to ACh and to depolarization with high concentrations of potassium.

We have conducted studies to explore the etiologic basis for the change in adrenomedullary catecholamine release. We have investigated the relationship between the time of diabetes onset to the decrease in catecholamine secretion, and we have found that catecholamine secretion in response to perfusion with ACh is impaired in the adrenals of the spontaneously diabetic rats as early as 2 weeks after the onset of diabetes. Because of the very early onset of this effect, we have begun to explore the hypothesis that the decrease in catecholamine release occurs in response to recent changes in the glycemic status of the animal. In support of this hypothesis, we present data showing that catecholamine release is significantly decreased in adrenals isolated from nondiabetic BB-Wistar rats following 3 h of acute hypoglycemia but is not altered in rats maintained in a hyperglycemic state.

From the Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin.

Address correspondence and reprint requests to Dr. Cecilia J. Hillard, Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 West Watertown Plank Road, Milwaukee, Wisconsin 53226.

Received for publication 2 September 1993 and accepted in revised form 13 January 1994.

EPI, epinephrine; ACh, acetylcholine; STZ, streptozocin; ANOVA, analysis of variance.

RESEARCH DESIGN AND METHODS

All experiments in this study were conducted in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Spontaneously diabetic male BB-Wistar rats were obtained from the Department of Pathology at the University of Massachusetts Medical Center (Worcester, MA), along with age-matched nondiabetic BB-Wistar control rats. The rats were received at ~80 days

TABLE 1
Adrenal catecholamine secretion in response to electrical stimulation and perfusion with 20 mM KCl in glands from BB-Wistar spontaneously diabetic rats at disease duration of 2 weeks, 2 months, and 4 months

	2 weeks		2 months		4 months	
	Control	Diabetic	Control	Diabetic	Control	Diabetic
n	3	3	4	3	5	5
Electrical stimulation (ng/600 cycles)	218 ± 15	124 ± 34*	291 ± 13	121 ± 51*	296 ± 22	128 ± 49*
Direct depolarization (ng/4 min)	230 ± 101	274 ± 41	232 ± 51	359 ± 220	211 ± 90	258 ± 138

Data are means ± SE. Electrical stimulation: after a 60-min equilibration period, each gland was stimulated transmurally for 600 cycles at 3 Hz at 80 V. The perfusate was collected, and the catecholamine content was determined. Direct depolarization: after a 90-min recovery period, the perfusion medium was switched to a modified Krebs buffer containing 20 mM KCl, and the catecholamine release was determined in 4-min fractions. *$P < 0.05$ significantly different from age-matched nondiabetic control rats.

of age and were kept on a 12-h light-dark schedule (lights from 0600 to 1800) with food (Purina Rat Chow) and water available continuously. Maintenance of the diabetic rats was conducted as described previously (8).

In some studies, diabetes was induced in diabetes-resistant BB-Wistar rats by intravenous injection of the pancreatic β-cytotoxin streptozocin (STZ) (Sigma, St. Louis), at doses of 50 (n = 5) or 65 mg/kg (n = 5). Those rats receiving 65 mg/kg STZ were given daily maintenance injections of insulin (according to the same protocol used for the spontaneously diabetic BB-Wistar rats). Rats made diabetic with 50 mg/kg STZ were not injected with insulin. Mild hypoglycemia was induced in diabetes-resistant BB-Wistar rats by overnight fasting (food removed at 1600; n = 3). In some cases, the fasted rats were made severely hypoglycemic by subcutaneous injection of regular insulin (U100, Lilly, Indianapolis, IN), at 3 U/kg at 1100 the following morning (n = 3). Catecholamine release from adrenal glands of rats in these treatment groups was compared with age-matched, diabetes-resistant BB-Wistar rats (n = 6).

Ex vivo perfusion of adrenal glands. We have used the retrograde perfusion technique of Wakade (9) with a few minor modifications as described previously (8). One adrenal gland from each rat was isolated and perfused by a peristaltic pump at a rate of 0.4 ml/min. Unless otherwise specified, the perfusion medium was a prewarmed, Krebs bicarbonate buffer of the following composition: 119 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 5.5 mM glucose, and 100 μg/ml ascorbate. The solution was equilibrated with 95% O_2 and 5% CO_2, and the final pH was 7.4. For the potassium depolarization experiments, the KCl concentration was increased to 20 mM, and NaCl was decreased to 104 mM. ACh was added to the perfusate at concentrations between 0.01–1 mM. Transmural stimulation of the splanchnic nerve terminals in this preparation was conducted by placing a stimulating electrode in contact with the gland. The following stimulation parameters were used: pulse duration of 1 ms and frequency of 1, 3, or 10 Hz to a total of 600 pulses at 80 V. The stimulation interval was 15 min.

Catecholamine content of perfusate fractions. Total catecholamine content of the perfusate fractions was determined by the fluorometric method of Anton and Sayre (10), as modified by Wakade (9) without intermediate purification on alumina. A 200-μl aliquot was taken from each perfusate fraction and oxidized by the addition of 20 μl of 0.25% $K_3Fe(CN)_6$. After 1 min, the reaction was stopped by the addition of 400 μl of 5 N NaOH containing 1 mg/ml ascorbate. The reaction mixture was then diluted to 1 ml, and the fluorescence (excitation wavelength = 409 nm; detection wavelength >470 nm) was determined using a model 8000C spectrofluorometer (SLM, Urbana, IL). Calculations were based on a standard curve generated from a 70:30 mixture of EPI:NE (norepinephrine).

Catecholamine content of adrenal glands. Right adrenal glands were harvested, gently blotted dry, and weighed. Medullas were separated from cortex and capsule using a binocular dissecting microscope and were homogenized in 1 ml of 0.1 M $HClO_4$. After centrifugation at 15,000 g for 5 min, the catecholamine content of the supernatant was determined using the assay described above.

Data analysis. All experiments were repeated at least three times on different days. STZ-induced (or diabetic) and control rats were matched with regard to age and were killed within 2 days of each other. Results are presented as means ± SE. Comparisons between diabetic and control rats were made using a two-way analysis of variance (ANOVA) with treatment as one variable and either stimulation frequency or ACh concentration as the other. Individual comparisons were made using unpaired Student's t tests.

RESULTS

Adrenal glands from control BB-Wistar rats released catecholamines at a rate of ~30 ng/5 min when perfused with normoglycemic Krebs buffer. Transmural electrical stimulation, which induces catecholamine release indirectly by stimulating residual splanchnic nerve terminals (8,11), produced an 10-fold increase in catecholamine secretion (Table 1). Previous studies of Wakade (9,12) have demonstrated that variation of the stimulation frequency between 1 and 10 Hz results in selective stimulation of neuronal populations. In agreement with the results of Wakade (9), the frequency response relationship in adrenals from the control animals is biphasic (Fig. 1). Transmural electrical stimulation of adrenal glands isolated from male, spontaneously diabetic BB-Wistar rats 4 months after the onset of diabetes resulted in a catecholamine secretory response that was significantly lower than the response in control rats at all frequencies tested, indicating a nonselective depression of all neuronal input to the gland (Fig. 1).

Catecholamine secretion in response to 20 mM KCl, which releases catecholamines via a direct effect on chromaffin cells (13), was the same in glands from control and diabetic (diabetes duration 4 months) rats (Table 1). Similar patterns of effect were seen in adrenals taken from rats that had been diabetic for 2 weeks or 2 months, i.e., decreased secretion in response to electrical stimulation but no difference in the response to perfusion with 20 mM KCl. In agreement with our earlier study (8), no differences were found between catecholamine content of nonperfused glands from control and diabetic rats at any of the time points examined (data not shown).

Catecholamine release from control rat adrenals increased in a concentration-dependent fashion when ACh (0.01 to 1.0 mM) was included in the perfusion medium (Fig. 2). When the adrenal glands harvested from spontaneously diabetic rats were perfused with ACh, catecholamine secretion was significantly lower than in age-matched nondiabetic control rats. Decreased responsiveness to ACh was detected in all spontaneously diabetic rats tested, regardless of disease duration (2 weeks through 4 months). Among the glands taken from diabetic rats, the decreased catecholamine secretion in response to ACh was significantly correlated with the decreased secretion in response to electrical stimulation in the same gland (r^2 = 0.8) (Fig. 3).

We conducted studies to explore factors involved in the decrease in catecholamine release in response to electrical

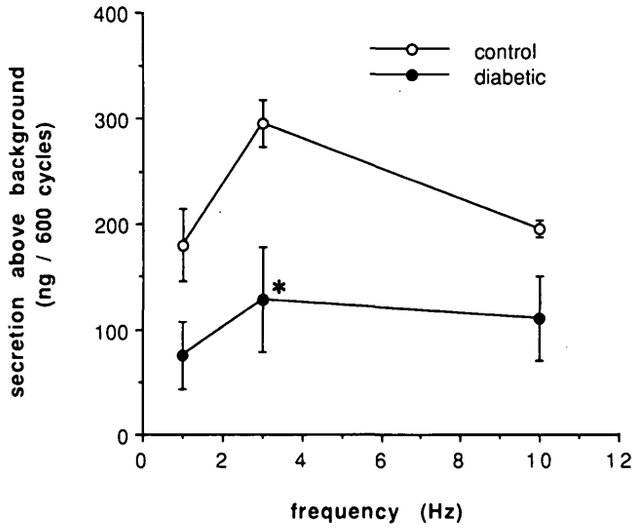

FIG. 1. Electrically induced catecholamine release from rat adrenal glands taken from control (n = 5) or spontaneously diabetic (n = 5) rats. Adrenal glands were transmurally stimulated at 80 V (for a total of 600 cycles) at each of the frequencies indicated. Basal catecholamine release was 37.6 ± 7 ng/5 min for the control glands and 32.6 ± 9 ng/5 min for the glands from diabetic rats. A 15-min recovery period was included before each change in frequency. Results are expressed as means ± SE. *Significantly different from control rats ($P < 0.05$).

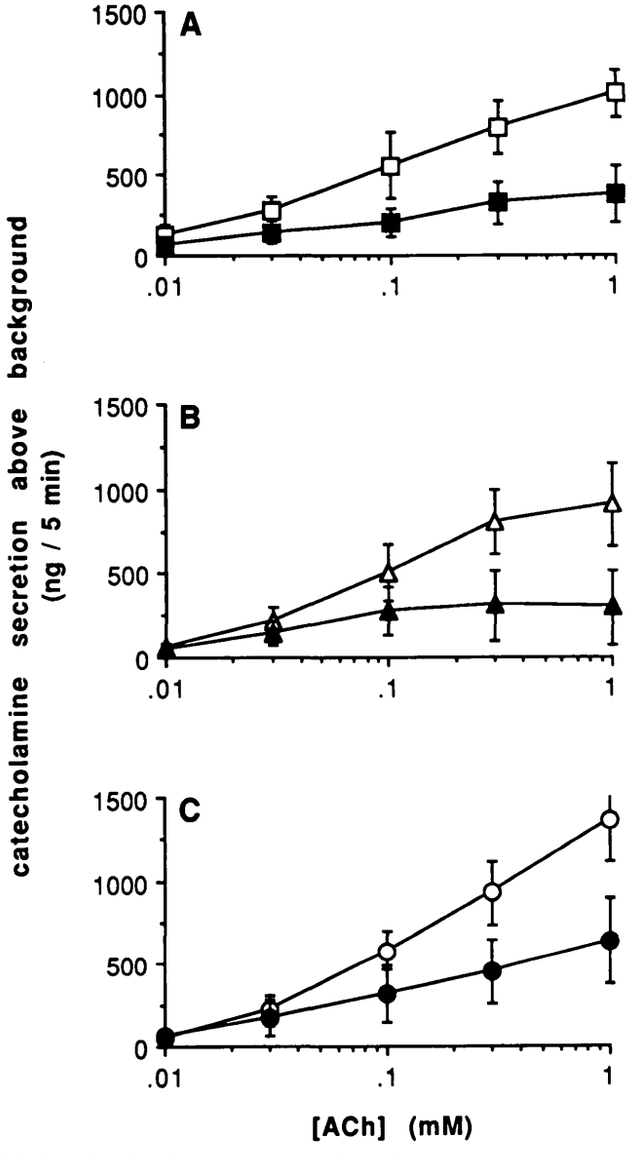

FIG. 2. ACh-induced catecholamine release from the perfused left adrenal glands of spontaneously diabetic BB-Wistar rats at 2 (*A*), 8 (*B*), and 12 (*C*) weeks after the onset of diabetes. Each adrenal preparation was perfused for 5 min with ACh at the concentrations indicated. Perfusate fractions were collected and analyzed fluorometrically for total catecholamine content. Each gland was perfused with buffer alone until baseline catecholamine release was maintained for at least 20 min before exposure to the next concentration of ACh. Basal release rates for the control glands were (in ng/5 min): 15.0 ± 9 (*A*), 11.9 ± 4 (*B*), and 9.1 ± 3 (*C*); and the glands from diabetic rats were 12.2 ± 3 (*A*), 12.5 ± 3 (*B*), and 19.8 ± 6 (*C*). □, △, ○: Data from control adrenals; ■, ▲, ●: data from diabetic adrenals. Results are expressed as means ± SE, n = 3–5. At all three times, the response of the diabetic rat adrenals was significantly different from the control response ($P < 0.05$, according to two-way ANOVA).

stimulation and ACh perfusion as early as 2 weeks after the onset of diabetes. In these studies, diabetes-resistant (control) BB-Wistar rats were used to investigate the role of recent glycemic status and diabetes itself in the secretory defect. In the first group of rats, a mild diabetic state was induced by injection with STZ. These rats received no exogenous insulin. On the day of killing, blood glucose levels were significantly higher and body weight was significantly lower in this group than in age-matched euglycemic control rats (Table 2). Adrenal catecholamine secretion in response to either ACh perfusion, electrical stimulation, or perfusion with 20 mM KCl were not different from the control values (Fig. 4 and Table 3).

The second group of rats was given an insulin-dependent form of diabetes by injection of a higher dose of STZ (65 mg/kg). Although this group of rats received daily subcutaneous injections of insulin, they remained continuously hyperglycemic throughout the course of their maintenance on insulin and had signifcantly higher blood glucose levels than the control group when killed (Table 2). Similar to the STZ-induced diabetic group, catecholamine secretion from glands isolated from the STZ-induced/insulin-treated rats was not different from control secretion (Fig. 4 and Table 3).

In our experience of caring for the spontaneously diabetic BB-Wistar rats, we have observed that they tend to have many hypoglycemic episodes under the insulin treatment protocol used in these studies. Because STZ-induced diabetes did not mimic the change in catecholamine secretion that occurred in the spontaneously diabetic rats, we hypothesized that the change in secretion was attributable to hypoglycemia. To test this hypothesis, a group of rats were fasted overnight to produce mild hypoglycemia and were killed the next morning. Blood glucose concentrations in these rats tended to be lower than control concentrations but were not statistically different (Table 2). Adrenal catecholamine secretion was unaffected by the overnight fast (Fig. 4 and Table 3).

To further test this hypothesis, fasted rats were made severely hypoglycemic by subcutaneous injection of a bolus of regular insulin (3 U/kg). Rats were killed after 3 h of maintaining blood glucose levels <2 mM. Catecholamine secretion in response to perfusion with ACh was significantly decreased in glands removed from the insulin-treated rats compared with the control and fasted groups (Fig. 4). Furthermore, catecholamine secretion in response to electrical stimulation was decreased at all stimulus frequencies compared with the control response (Fig. 5) and followed a pattern similar to the change seen in spontaneously diabetic rats. In contrast to the results seen in the spontaneously

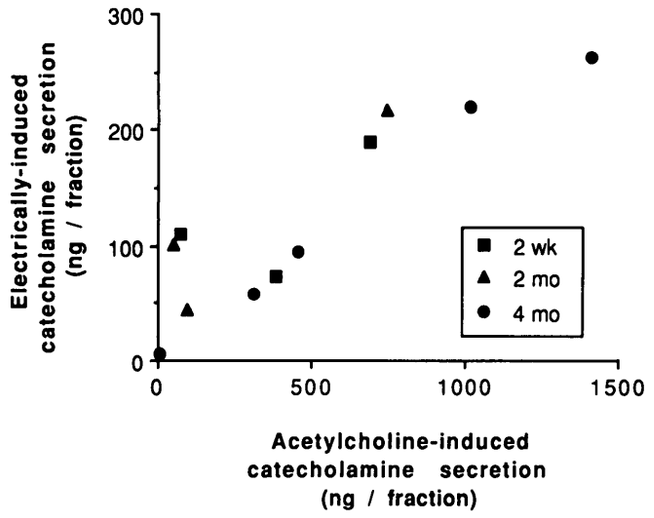

FIG. 3. Correlation between catecholamine secretion in response to transmural stimulation and in response to perfusion with ACh. Adrenal glands were isolated from diabetic rats with disease durations of 2 weeks, 2 months, and 4 months. The response to electrical stimulation at 3 Hz was plotted against the response to perfusion with 1 mM ACh (data taken from Fig. 1) for each gland. The correlation coefficient of the data is 0.895 (correlation is statistically significant with $P < 0.01$).

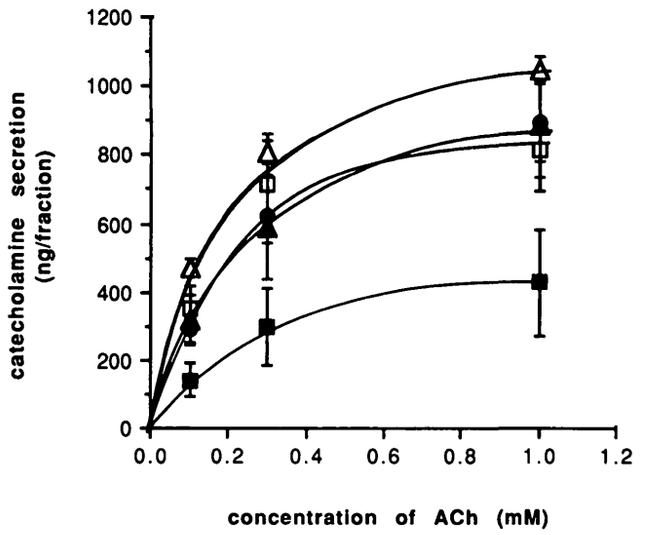

FIG. 4. ACh-induced catecholamine release from adrenal glands from hyperglycemic and hypoglycemic BB-Wistar rats. Each adrenal preparation was perfused for 5 min with ACh at the concentrations indicated. Perfusate fractions were collected and analyzed fluorometrically for total catecholamine content. Each gland was perfused with buffer alone until baseline catecholamine release was maintained for at least 20 min before exposure to the next concentration of ACh. □, Control group; ▲, STZ-induced diabetic group; ●, STZ-induced diabetic plus insulin group; △, fasted group; ■, fasted plus insulin group. Results are expressed as means ± SEM. The fasted plus insulin group (■) is significantly different from all other groups ($P < 0.05$, according to two-way ANOVA).

diabetic rats, adrenal glands from hypoglycemic rats exhibited a decrease in catecholamine secretion in response to perfusion with 20 mM KCl (Fig. 6). We have measured the catecholamine content of nonperfused glands taken from rats in each of the treatment groups (Table 3). The catecholamine content of the glands from the hypoglycemic group were significantly decreased compared with the control rats. The catecholamine content values of all other groups were not different from control group.

DISCUSSION

In an earlier study, we reported that female BB-Wistar rats with a diabetes duration of 4 months had decreased adrenal medullary catecholamine release in response to electrical stimulation of splanchnic terminals (8). The results reported in this study extend those findings and demonstrate that adrenal glands from spontaneously diabetic BB-Wistar rats also exhibit decreased catecholamine secretion in response to perfusion with ACh, the major transmitter released by the splanchnic nerves. Among the individual diabetic rats, the catecholamine secretory response to electrical stimulation and the response to ACh are highly correlated. Furthermore, we have demonstrated that the decrement in catecholamine release in response to both electrical stimulation and ACh occur 2 weeks after diabetes onset. These results suggest that the hyposecretion of adrenomedullary catecholamines seen in response to electrical stimulation is caused by decreased responsiveness of the chromaffin cells to stimulation by ACh rather than decreased presynaptic ACh release.

The time course studies have demonstrated that the decrease

TABLE 2
Blood glucose concentrations and body weights of hyperglycemic and hypoglycemic rats at time of death

	Blood glucose (mM)	Body weight (g)
Control	4.4 ± 0.4	389 ± 9
STZ alone	18.9 ± 1.5*	316 ± 14*
STZ and insulin	16.2 ± 2.4*	365 ± 6
Fasted	2.7 ± 0.4	380 ± 17
Fasted and insulin	<2*	366 ± 10

Data are means ± SE. Blood glucose concentrations in tail blood were determined at death using an enzymatic glucose monitor (Tracer II, Boeringer Mannheim, Mannheim, Germany). STZ alone: diabetes was induced by tail vein injection of 50 mg/kg STZ, no insulin was administered. Duration of diabetes was 16 ± 1 days. STZ and insulin: diabetes was induced by tail vein injection of 65 mg/kg STZ. Ultralente insulin was administered daily. Duration of diabetes was 22 ± 2 days. Fasted: rats were fasted overnight (at least 20 h) before being killed. Fasted and insulin: rats were killed after 3 h of blood glucose levels <2 mM. Hypoglycemia was induced by subcutaneous injection of regular insulin (3 U/kg) following an overnight fast. *$P < 0.05$ significantly different from control value.

TABLE 3
Catecholamine secretion in response to electrical stimulation and depolarization with 20 mM KCl, and catecholamine content of adrenal glands from hyperglycemic and hypoglycemic rats

	Electrical stimulation (ng/600 cycles)	20 mM KCl (ng/5 min)	Content (μg/gland)
Control	179 ± 11	490 ± 111	23.6 ± 1.8
STZ alone	209 ± 30	502 ± 58	24.9 ± 2.5
STZ and insulin	223 ± 12	440 ± 71	29.1 ± 1.9
Fasted	207 ± 8	335 ± 107	21.6 ± 2.2
Fasted and insulin	92 ± 36*	123 ± 46*	12.0 ± 3.4*

Data are means ± SE. Adrenal medullary catecholamine content was determined after isolation and homogenization of a nonperfused gland. STZ alone: diabetes was induced by tail vein injection of 50 mg/kg STZ, no insulin was administered. Duration of diabetes was 16 ± 1 days. STZ and insulin: diabetes was induced by tail vein injection of 65 mg/kg STZ. Ultralente insulin was administered daily. Duration of diabetes was 22 ± 2 days. Fasted: rats were fasted overnight (at least 20 h) before being killed. Fasted and insulin: rats were killed after 3 h of blood glucose levels <2 mM. Hypoglycemia was induced by subcutaneous injection of regular insulin (3 U/kg) following an overnight fast. *$P < 0.05$ significantly different from control value.

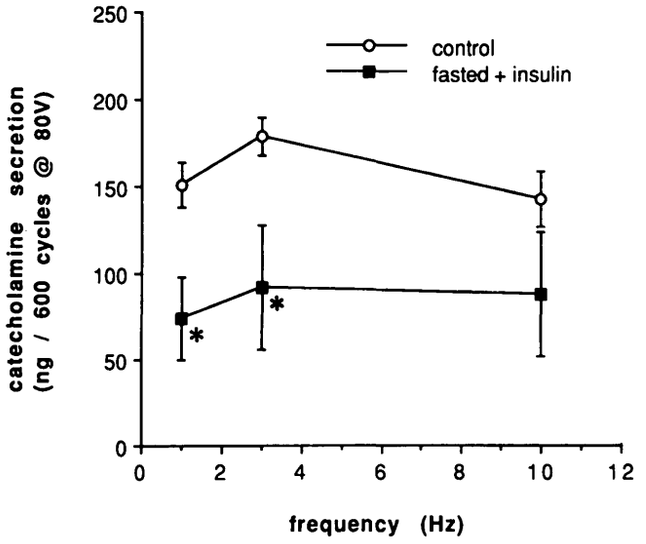

FIG. 5. Electrically induced catecholamine release from control and hypoglycemic rat adrenal glands. Adrenal glands from control BB-Wistar rats or overnight fasted rats given 3 U/kg insulin 3 h before being killed were transmurally stimulated at 80 V (for a total of 600 cycles) at each of the frequencies indicated. A 15-min recovery period was included before each change in frequency. Basal catecholamine release was 10.9 ± 4 ng/5 min for the control glands and 1.7 ± 2 ng/5 min for the glands from diabetic rats. Results are expressed as means ± SE. *Significantly different from control glands ($P < 0.05$).

in catecholamine secretion in response to both electrical stimulation and ACh occurs and is fully developed at 2 weeks after the onset of diabetic symptoms. Based on previous studies of the progression of neuropathy in the BB-Wistar rat, it is unlikely that the adrenal medullary hyposecretion is a result of the development of a classical neuropathy. Although decreased conduction velocity of some large nerves has been demonstrated as early as 3 weeks after the onset of diabetes in the BB-Wistar rat (14), abnormalities in neuronal function have not been seen before 2 months of the disease (15). The morphological damage, which we have observed previously in the adrenomedullary splanchnic nerves of diabetic animals (8), appears to be a separate phenomenon that is not causally related to the decrease in catecholamine secretion. This interpretation is

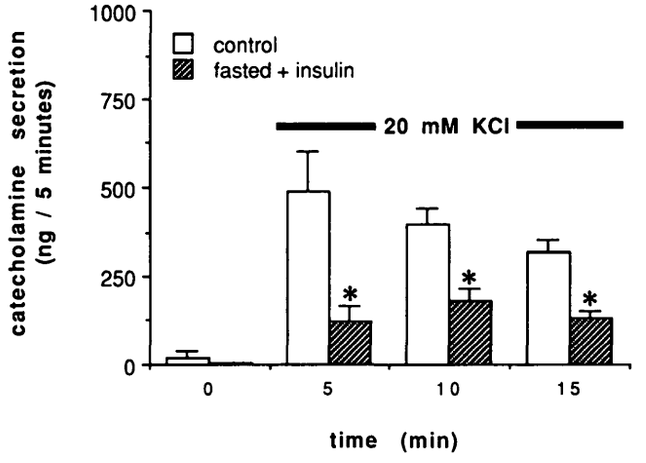

FIG. 6. Catecholamine secretion from control and hypoglycemic rat adrenal glands in response to perfusion with 20 mM KCl. Adrenal glands from control BB-Wistar rats or overnight fasted rats given 3 U/kg insulin 3 h before being killed were perfused with 20 mM KCl as indicated, and the fractions were analyzed for catecholamine content. Results are expressed as means ± SE. *Significantly different from control adrenal glands ($P < 0.05$).

consistent with that of Sima et al. (16), who suggested that classic autonomic neuropathy and other forms of nerve dysfunction (e.g., hypoglycemia-mediated) exist as separate pathological entities that may cosegregate in this diabetic rat model.

We have conducted studies to explore possible factors involved in the development of catecholamine hyposecretion. Several reports in the literature have indicated that both human diabetic patients (17) and nondiabetic subjects (18) experiencing intermittent episodes of hypoglycemia exhibit impairment of glucose counterregulation and signs of autonomic failure. In humans, the counterregulatory hormones increase as expected during short-term hypoglycemic episodes; however, plasma EPI responses are significantly decreased after subsequent hypoglycemic episodes (18). Based on these and other studies, the hypothesis has been put forth that hypoglycemic episodes impair the physiological defense against subsequent episodes by elevating the threshold for autonomic responses to hypoglycemia (17). The mechanism for the impairment is not known, although it has been suggested that it may be because of adaptation of the CNS glucoregulatory centers (19). Available data does not rule out the possibility that alterations in the sensitivity of the adrenal medulla to neural input is part of the adaptive mechanism. A few reports show diminished adrenal catecholamine responsiveness following hypoglycemia in rats. Rats subjected to chronic hypoglycemia by infusion with insulin demonstrate hyposecretion of EPI from adrenal medullary slices in response to ACh (20). Furthermore, rats that were swim-trained for several weeks have a lower EPI response to insulin than control rats, which the authors attribute to repeated bouts of hypoglycemia leading to adaptation of the adrenal medulla (21).

In light of these studies, which suggest that hypoglycemia induces a form of physiological adaptation that may include adrenal medullary hyposecretion, we have begun to explore the hypothesis that the decrease in adrenomedullary catecholamine secretion in spontaneously diabetic rats is secondary to hypoglycemia. Although we did not continuously monitor blood glucose levels in the diabetic rats, we noted several hypoglycemic episodes during the course of diabetes in the spontaneously diabetic rats. In agreement with the earlier reports, we have found that rats kept hypoglycemic 3 h before being killed exhibited decreased adrenomedullary catecholamine secretion in response to both ACh and electrical stimulation. Overnight fasting alone was not sufficient to affect catecholamine release. However, in contrast to the results of the studies with spontaneously diabetic rats, severely hypoglycemic rats also exhibited decreased catecholamine secretion in response to KCl as well as decreased catecholamine content. These results suggest that the decreased release in response to electrical stimulation and ACh were a result of a reduction in the catecholamines available for release. Further studies using hypoglycemic conditions that more closely match those of the diabetic animal are needed to clarify these differences.

Control BB-Wistar rats maintained in a hyperglycemic state for 2 weeks (i.e., STZ-induced diabetes) exhibited normal catecholamine release. Furthermore, STZ-induced diabetic rats that were injected with insulin but were maintained in a hyperglycemic range also had normal catecholamine release, which indicates that insulin is not the causative factor. This finding is important in light of reports that insulin itself may suppress catecholamine release in human diabetic patients (22,23). Although these findings are

not definitive, they are consistent with the hypothesis that the defect in adrenal catecholamine secretion seen in the spontaneous diabetic rats is caused by something other than diabetes. At this point, we cannot rule out the possibility that the defect in adrenal catecholamine secretion in the spontaneously diabetic rats is caused by an unknown genetic alteration that cosegregates with diabetes or another pathological change that accompanies the development of diabetes, such as adrenal medullitis (24).

Catecholamine secretion in response to direct chromaffin cell depolarization by perfusion with 20 mM KCl was normal in the adrenals taken from male spontaneously diabetic BB-Wistar rats. This differs from the results we obtained earlier in female diabetic animals in which potassium-induced secretion was significantly decreased (8). Female humans reportedly are more prone to hypoglycemia and have lower catecholamine responses to hypoglycemia than males (25). The female diabetic patients possibly experienced a greater number of hypoglycemic episodes than the males and, therefore, had more global adrenomedullary dysfunction.

Defective glucose counterregulation and hypoglycemia unawareness appear to affect ~20% of human diabetic patients treated with conventional insulin therapy (26). Because these phenomena are even more common in diabetic patients attempting to maintain strict control (27) and the frequency of iatrogenic hypoglycemia is inherently much higher in such patients, it has been proposed that the abnormal adrenomedullary catecholamine secretion responsible for these phenomena is related to a patient's previous history of hypoglycemic insults (3). The results reported in this study suggest that spontaneously diabetic rats develop similar adaptive changes in response to insulin-induced hypoglycemia and provide a model for further studies of the mechanisms involved in these important clinical problems.

ACKNOWLEDGMENTS

This study was funded in part by U.S. Public Health Service Grant R29-DA-04800. R.A.W. is funded by the Medical Scientist Training Program at the Medical College of Wisconsin.

The authors wish to thank Dr. Arun Wakade, Dr. Alan Bloom, and Jody Pounds.

REFERENCES

1. Wakade AR, Wakade TD: Contribution of nicotinic and muscarinic receptors in the secretion of catecholamines evoked by endogenous and exogenous acetylcholine. *Neuroscience* 10:973–978, 1983
2. Cryer PE, Gerich JE: Glucose counterregulation, hypoglycemia, and intensive insulin therapy in diabetes mellitus. *N Engl J Med* 313:232–241, 1985
3. Cryer PE: Decreased sympathochromaffin activity in IDDM. *Diabetes* 38:405–409, 1989
4. Hoeldtke RD, Boden G, Shuman CR, Owen OE: Reduced epinephrine secretion and hypoglycemia unawareness in diabetic autonomic neuropathy. *Ann Intern Med* 96:459–462, 1982
5. Nakhooda AF, Like AA, Chappel CI, Murray FT, Marliss EB: The spontaneously diabetic Wistar rat: metabolic and morphologic studies. *Diabetes* 26:100–112, 1977
6. Nakhooda AF, Like AA, Chappel CI, Wei CN, Marliss EB: The spontaneously diabetic Wistar rat (the "BB rat"): studies prior to and during the overt syndrome. *Diabetologia* 14:199–207, 1978
7. Brown MR, Dyck PJ, McClearn GE, Sima AAF, Powell HC, Porte D: Central and peripheral nervous system complications. *Diabetes* 31 (Suppl. 1):65–70, 1982
8. Wilke RA, Riley DA, Lelkes PI, Hillard CJ: Decreased catecholamine secretion from the adrenal medullae of chronically diabetic BB-Wistar rats. *Diabetes* 42:862–868, 1993
9. Wakade AR: Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J Physiol* 313:463–480, 1981
10. Anton AH, Sayre DF: A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J Pharmacol Exp Ther* 138:360–375, 1962
11. Wakade AR: Facilitation of secretion of catecholamines from rat and guinea-pig adrenal glands in potassium-free medium and after ouabain. *J Physiol* 313:481–498, 1981
12. Wakade AR: Noncholinergic transmitter(s) maintains secretion of catecholamines from rat adrenal medulla for several hours of continuous stimulation of splanchnic neurons. *J Neurochem* 50:1302–1308, 1988
13. Malhotra RK, Wakade TD, Wakade AR: Comparison of secretion of catecholamines from the rat adrenal medulla during continuous exposure to nicotine, muscarine, or excess K. *Neuroscience* 26:313–320, 1988
14. Sima AAF, Hay K: Functional aspects and pathogenetic considerations of the neuropathy in the spontaneously diabetic BB-Wistar rat. *Neuropathol Appl Neurobiol* 7:341–350, 1981
15. McEwen TAJ and Sima AAF: Autonomic neuropathy in BB rat: assessment by improved method for measuring heart-rate variability. *Diabetes* 36:251–255, 1987
16. Sima AA, Zhang WX, Greene DA: Diabetic and hypoglycemic neuropathy: a comparison in the BB rat. *Diabetes Res Clin Pract* 6:279–296, 1989
17. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus: recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. *J Clin Invest* 91:819–828, 1993
18. Widom B, Simonson DC: Intermittent hypoglycemia impairs glucose counterregulation. *Diabetes* 41:1597–1602, 1992
19. Lingenfelser T, Renn W, Sommerwerck U, Jung MF, Buettner UW, Zaiser-Kaschel H, Kaschel R, Eggstein M, Jakober B: Compromised hormonal counterregulation, symptom awareness, and neurophysiological function after recurrent short-term episodes of insulin-induced hypoglycemia in IDDM patients. *Diabetes* 42:610–618, 1993
20. Carbonaro DA, Mitchell JP, Hall FL, Vulliet PR: Altered reactivity of rat adrenal medulla. *Brain Res Bull* 21:451–458, 1988
21. Stallknecht B, Kjaer M, Mikines KJ, Maroun L, Ploug T, Ohkuwa T, Vinten J, Galbo H: Diminished epinephrine response to hypoglycemia despite enlarged adrenal medulla in trained rats. *Am J Physiol* 259:R998–R1003, 1990
22. Diamond MP, Hallarman L, Starick-Zych K, Jones TW, Connolly-Howard M, Tamborlane WW, Sherwin RS: Suppression of counterregulatory hormone response to hypoglycemia by insulin per se. *J Clin Endocrinol Metab* 72:1388–1390, 1991
23. Davis MR, Shamoon H: Deficient counterregulatory hormone responses during hypoglycemia in a patient with insulinoma. *J Clin Endocrinol Metab* 72:788–792, 1991
24. Brown FM, Smith AM, Longway S, Rabinowe SL: Adrenal medullitis in type I diabetes. *J Clin Endocrinol Metab* 71:1491–1495, 1990
25. Amiel SA, Maran A, Powrie JK, Umpleby AM, Macdonald IA: Gender differences in counterregulation to hypoglycemia. *Diabetologia* 36:460–464, 1993
26. Goldgewicht C, Slama G, Papoz L, Tchobroutsky G: Hypoglycemic reactions in 172 type I (insulin-dependent) diabetic patients. *Diabetologia* 24:95–99, 1983
27. Arias P, Kerner W, Zier H, Navascues I, Pfeiffer EF: Incidence of hypoglycemic episodes in diabetic patients under continuous subcutaneous insulin infusion and intensified conventional insulin treatment: assessment by means of semiambulatory 24-hour continuous blood glucose monitoring. *Diabetes Care* 8:134–140, 1985