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Effects of Streptozotocin-Induced Diabetes on the Response of Male Rats to Immobilization Stress

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The effects of streptozotocin-induced (STZ) diabetes on the response to immobilization stress were evaluated in adult male rats. Rats were injected with STZ or vehicle and handled daily to minimize stress. Four weeks later, half of the animals were lightly anesthetized with ether and immobilized for 20 min. At that time the stressed and nonstressed controls were sacrificed, and blood and tissue collected for hormone and amine determinations. Immobilization caused an increase in plasma glucose levels in the controls, but caused no further increase in the already high levels seen in the diabetic rats. Basal corticosterone levels did not differ between the STZ and control rats, and the increase after immobilization was of similar magnitude. The stress-induced increase in prolactin was attenuated in the diabetic rats. Immobilization caused a significant rise in plasma norepinephrine (NE) levels in control, but not in diabetic rats. Adrenal NE content and tyrosine hydroxylase activity were not significantly affected by stress or STZ treatment. Dopamine (DA) and NE content was increased in the hypothalamus of immobilized diabetic rats as compared to nondiabetic immobilized controls. These results demonstrate that diabetic rats respond to immobilization stress, but the endocrine and sympathetic nervous system response is impaired. Changes in the stress response may be related to changes in hypothalamic amine metabolism.

Key Words: Diabetes; norepinephrine; stress; corticosterone; prolactin; hypothalamus.

Introduction

It has been well-documented that human patients with insulin dependent diabetes mellitus (IDDM) exhibit altered sympathoadrenal responses to stress and exercise (Giorgino, 1988; Fagius, 1991; Daly and Landsberg, 1991). Elevated levels of plasma catecholamines are seen in uncontrolled diabetes, but there is much conflicting data about catecholamine levels in patients with good glycemic control (reviewed by Christensen, 1979). Many of these conflicts can probably be explained by differences in diabetic severity and duration, as these factors correlate with the incidence of autonomic neuropathies and adrenal pathology. In noninsulin dependent diabetes mellitus (NIDDM), insulin levels are initially high, and insulin is known to stimulate the sympathetic nervous system (Daly and Landsberg, 1991). Obesity is also a confounding factor in such studies as it is associated with reduced sympathetic activity either in the presence or absence of diabetes (Astrup et al., 1991). It has also been reported that patients with poorly controlled diabetes exhibit exaggerated norepinephrine (NE) responses to exercise that could explain abnormal increases in systolic pressure, heart rate, and platelet aggregation (Vignati and Cunningham, 1985; Hendra et al., 1988).

There have been relatively few studies of adrenal medullary function in diabetic animals, but Bitar and colleagues (1987) have reported that adrenal contents of dopamine (DA), NE, and epinephrine (EPI) are elevated in rats with streptozotocin-induced diabetes. Increases in the activity of the rate limiting enzymes for the synthesis of these catecholamines were also elevated suggesting that plasma levels were also elevated. Unfortunately, plasma catecholamine levels were not measured. Adrenal catecholamine secretion after splenic nerve stimulation in vitro is reduced in spontaneously diabetic BB-Wistar rats (Wilke et al., 1993). There have been few studies on the adrenal medullary responses to stress in animal models of diabetes, and the results are conflicting. Lee and coworkers (1989), demonstrated two distinct subgroups of alloxan diabetic rats; one with normal EPI levels and response to stress, and the other with elevated basal and enhanced responses to footshock. Similar results were all seen when rats were made diabetic with streptozotocin (STZ). Rats with STZ-induced diabetes show an attenuated catecholamine response to insulin-induced hypoglycemia (Patel, 1983) whereas cold exposure and exsanguination may lead to enhanced NE responses in diabetic rats (Fushimi et al., 1984; Bellush and Henley, 1990).

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The hypothalamic-pituitary-adrenal-cortical axis is also altered in diabetic patients and animals (DeNiccola et al., 1977; Kozak and Cooppan, 1985; Scribner et al., 1991). Plasma corticosterone concentrations have been reported to be either unchanged or elevated in diabetic as compared to control rats, but an analysis of diurnal plasma corticosterone levels leads to the conclusion that rats were made diabetic with STZ hypersecrete corticosterone (L'Age et al., 1974; Scribner et al., 1991). Additional evidence that STZ-treated rats hypersecrete corticosterone include increased urinary corticosterone excretion, increased adrenal weights and decreased thymus weights that are prevented by adrenalectomy (Scribner et al., 1991).

In the present study, we report the effects of immobilization stress on both the adrenal medulla and cortical response in rats made diabetic with STZ. We also report the effects of stress on hypothalamic monoamine levels in these same animals.

Methods and Materials

Animals and Experiments

Adult male Sprague Dawley rats (225–250 g) were purchased from Harlan Industries (Madison, WI). The rats were housed in a temperature controlled (22°C) room on a 12:12 light:dark cycle (lights on at 0700 h). Food (TekLab Rat Diet; Madison, WI) and tap water were provided *ad libitum*. Two weeks after arrival, the rats were injected with streptozotocin (50 mg/kg ip in 0.01*M* citrate buffer, pH 4.5) or the injection vehicle (day 1). All rats were weighed at least weekly and handled daily to minimize stress.

Four weeks after vehicle or STZ treatment, rats were randomly grouped into stressed or nonstressed groups. The rats in the stress group were lightly anesthetized with ether, then immobilized by taping them to a plastic block. Since the immobilized rats were subjected to ether, the stress response in actuality is the combined effect of ether and immobilization. The nonstressed controls remained in their cages in a separate room. Twenty minutes later, the rats were killed by decapitation. At the time of sacrifice, trunk blood was collected and the adrenal and brain were rapidly removed and the median eminence (ME) was separated with irridectomy scissors. The adrenals, brain, and ME were then immediately frozen on dry ice and stored at –70°C. Blood was kept on ice for not longer than 45 min at which time serum was separated by centrifugation and then frozen and stored at –70°C.

Catecholamine Assays

Serum was thawed and 1-mL aliquots were pipetted into 2-mL conical centrifuge tubes to which 200 μL of internal standard (100 ng dihydroxybenzylamine/mL 0.32*M* $HClO_4$) and 10 mg of alumina oxide were added. After addition of 750 μL Tris buffer (0.4*M*; pH 8.6), the tubes were mixed for 15 min by rotation through an ice bath. The tubes were then centrifuged (11,000*g*), the supernatant aspirated, and the alumina and adsorbed catecholamines were washed three times. The wash step consisted of the addition of 1 mL Tris buffer (0.004*M*; pH 8.6) containing $10^{-3}M$ $NaHSO_3$, vortexing for 45 s and 1 min of centrifugation. After aspiration of the third wash solution, the catecholamines were resuspended in 50 μL of 0.16*M* $HClO_4$. The samples were stored in the dark at 4°C until high-performance liquid chromatography (HPLC) analysis (Steger et al., 1983). All samples were run within 4 wk of collection.

Adrenal glands were sonicated in 0.1*M* $HClO_4$ containing the internal standard for the catecholamine assay (dihydroxybenzylamine) and 1 m*M* sodium bisulfite prior to alumina extraction as previously described (Fernandez-Ruiz et al., 1989). Catecholamines were separated by HPLC and quantitated by electrochemical detection.

Prior to assay, the brains were partially thawed, and medial basal hypothalamus (MBH) and anterior hypothalamus (AH) were dissected free as previously described (Steger et al., 1983). The MBH and AH were weighed and then sonicated in 0.1*M* $HClO_4$ containing the internal standards for the catecholamine assay (dihydroxybenzylamine) and the serotonin (5-HT) assay (methyl 5-HT), and 1 m*M* sodium bisulfite. The ME was sonicated in the same solution, but without the methyl 5-HT. Median eminence supernatants were separated by HPLC and quantitated by electrochemical detection (Steger et al., 1983). The MBH and AH supernatants were subjected to alumina extraction prior to HPLC separation (Steger et al., 1983).

Tyrosine Hydroxylase Assay

Adrenals were homogenized in 30 vol of 0.25*M* sucrose. Homogenates were assayed according to the method described by Nagatsu et al. (1979) with the following modifications: The use of dihydroxybenzylamine as an internal standard rather than α-methyl-DOPA; the measurement of L-DOPA was performed by HPLC with electrochemical detection. The alumina extraction and HPLC procedure were similar to those described in the previous paragraphs. The results are expressed in terms of μg L-DOPA formed/gland/h.

Radioimmunoassay (RIA) and Glucose Determinations

Plasma levels of LH and PRL were measured by RIA using reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) as described previously (Smith and Bartke, 1987). Corticosterone levels were measured using a commercial RIA kit (ICN Biomedicals, Inc., Costa Mesa, CA). Plasma glucose was assayed by a glucose oxidase procedure using a kit purchased from Sigma (St. Louis, MO).

Data Analysis

The effects of treatment on hormone levels and neurotransmitter content were evaluated using analysis of variance or Student's *t*-test. Mean values between groups were considered significantly different when $p < 0.05$.

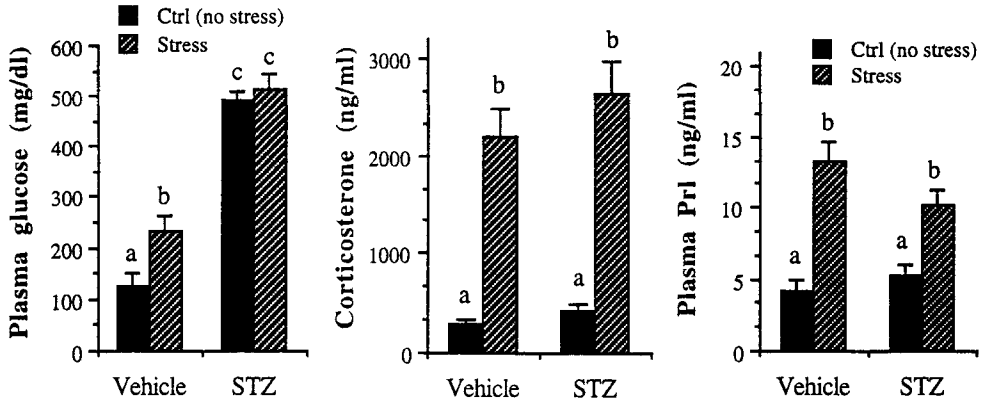

Fig. 1. Effects of STZ-induced diabetes on plasma glucose **(A)**, corticosterone **(B)**, and prolactin **(C)** levels in rats subjected to 20 min of immobilization stress immediately prior to blood collection by decapitation. Immobilized rats were lightly anesthetized with ether prior to immobilization. The values represent the mean ± SEM ($n = 6$ rats/group). Bars with different subscripts are statistically different ($p < 0.05$).

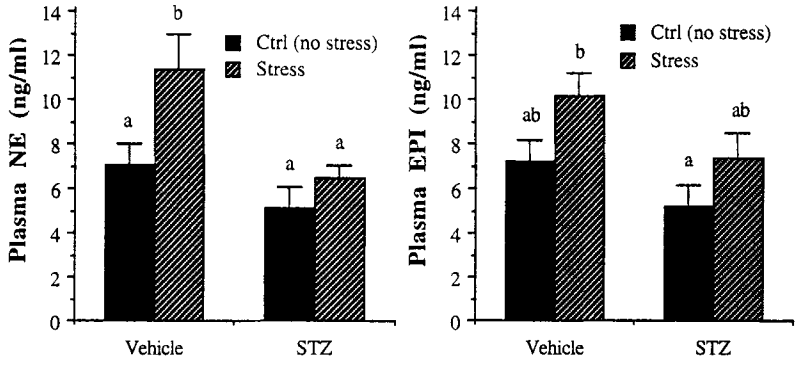

Fig. 2. Effects of STZ-induced diabetes on plasma norepinephrine **(A)** and epinephrine **(B)** levels in rats subjected to 20 min of immobilization stress immediately prior to blood collection by decapitation. Immobilized rats were lightly anesthetized with ether prior to immobilization. Bars with different subscripts are statistically different ($p < 0.05$).

Results

Body weights at the time of autopsy were significantly less in the diabetic rats than in the nondiabetic controls (327 ± 7 vs 404 ± 7 g). Absolute adrenal weights were higher in the STZ than in the control rats (28.5 ± 1.3 vs 25.2 ± 1.5) but the difference was not significant. However, relative adrenal weight (i.e., per gram body weight) was significantly elevated in the diabetic rats.

Plasma glucose levels were significantly elevated in the nonstressed diabetic rats as compared to the nonstressed nondiabetic controls (Fig. 1A). Immobilization stress resulted in a significant increase of plasma glucose levels in the controls, but not in the diabetic animals. Basal plasma corticosterone levels did not differ between the STZ and control rats, and the increase after immobilization stress was of similar magnitude (Fig. 1B). Prolactin levels increased in both groups of stressed rats, but the magnitude of increase was greater in the nondiabetic controls (Fig. 1C). Plasma LH levels did not differ between any of the treatment groups (data not shown).

Nonstress plasma NE and EPI levels appeared to be lower in the diabetic than the control rats, but the difference was not significant (Fig. 2A, B). Immobilization stress caused a significant rise in NE levels in the control rats that was not seen in the diabetic animals. Plasma EPI levels appeared to increase after immobilization, but neither the rise in the control or diabetic animals was significant (Fig. 2B).

Adrenal NE and EPI content were similar in diabetic and control rats (Table 1). Immobilization stress did not effect adrenal catecholamine content. Tyrosine hydroxylase activity appeared to be elevated in the nonstressed diabetic rats, but the apparent increase was not statistically different (Table 1). Levels of activity tended to increase after immobilization stress in both groups, but again, the change was not significant.

Norepinephrine, DA, 5-HT, and 5-hydroxy-indole-acetic acid (5-HIAA) content in the ME, MBH, and AH was not different between nonstressed control and diabetic rats (Table 2). Norepinephrine content was unaffected by stress except in the MBH of diabetic rats where an increase in NE content was observed. The content of DA in the AH and

Table 1
Effects of STZ-Induced Diabetes on Adrenal Catecholamine Content and Tyrosine Hydroxylase Activity in Rats Subjected to Immobilization Stress

	Control		Diabetic	
	Unstressed	Stressed	Unstressed	Stressed
NE content (ng/adrenal)	3465 ± 497	3392 ± 284	2928 ± 424	3162 ± 279
EPI content (ng/adrenal)	20,236 ± 2021	20,288 ± 697	21,579 ± 1245	22,220 ± 996
TH activity (ng DOPA/h)	6448 ± 891	8417 ± 862	8114 ± 947	9810 ± 638

The values represent the mean ± SEM (n = 6 rats/group).

Table 2
Effects of STZ-Induced Diabetes on Hypothalamic Amine Content in Rats Subjected to Immobilization Stress

	Control		Diabetic	
	Unstressed	Stressed	Unstressed	Stressed
Median eminence				
Dopamine	2.63 ± 0.25	2.65 ± 0.19	2.91 ± 0.23	2.95 ± 0.08
Norepinephrine	4.92 ± 0.57	4.56 ± 0.30	5.61 ± 0.41	6.28 ± 0.86
5-HT	1.15 ± 0.08	1.83 ± 0.20*	1.63 ± 0.52	1.48 ± 0.18
5-HIAA	1.75 ± 0.21	1.08 ± 0.21*	1.82 ± 0.36	2.35 ± 0.12
Medial Basal H.				
Dopamine	0.268 ± 0.025	0.343 ± 0.048	0.257 ± 0.033	0.411 ± 0.046*
Norepinephrine	1.272 ± 0.058	1.385 ± 0.157	1.428 ± 0.113	1.937 ± 0.150*
5-HT	0.728 ± 0.054	0.900 ± 0.131	0.632 ± 0.040	0.953 ± 0.033*
5-HIAA	1.220 ± 0.090	0.783 ± 0.073*	1.033 ± 0.105	0.739 ± 0.038*
Anterior H.				
Dopamine	0.232 ± 0.049	0.418 ± 0.046*	0.260 ± 0.038	0.446 ± 0.065*
Norepinephrine	2.217 ± 0.168	2.367 ± 0.153	2.248 ± 0.335	2.544 ± 0.372
5-HT	0.762 ± 0.017	0.768 ± 0.050	0.763 ± 0.102	0.821 ± 0.118
5-HIAA	3.490 ± 0.191	4.210 ± 0.339	3.523 ± 0.279	3.800 ± 0.419

*$p < 0.05$ unstressed vs stressed.
The values represent the mean ± SEM (n = 6 rats/group).

MBH increased after stress in both treatment groups, although the rise in MBH DA in the nondiabetic controls was not significant. Stress induced an increase in ME and MBH 5-HT content, but only the increase in ME 5-HT in the controls and MBH 5-HT content in the diabetic rats reached statistical significance. Immobilization stress resulted in a decrease in 5-HIAA levels in both the ME and MBH of nondiabetic controls, but a similar decrease was seen only in the MBH of diabetic rats.

Discussion

The results of the present study demonstrate that diabetic rats are affected by immobilization stress, but the physiologic response differs qualitatively and quantitatively from nondiabetic control rats. The results also demonstrated that under basal (nonstress) conditions, many parameters of sympatho-adrenal function appeared to be unaffected by diabetes. Thus, plasma catecholamine levels in the unstressed rats were not different between control and diabetic rats in agreement with previously published results (Lee et al., 1989). However, plasma NE levels increased in the control, but not in the diabetic rats during immobilization.

In the rat, plasma EPI levels exclusively reflect adrenal secretion, but NE in the nonstressed animal primarily comes from sympathetic nerves (McCarty and Kopin, 1979; Goldstein et al., 1983). Under conditions of acute stress, up to 45% of plasma NE may come from the adrenal medulla. The present study could not differentiate whether the diabetes-associated change in NE secretion was a result of

adrenal medullary and/or sympathetic nerve changes, adrenal NE content, and adrenal TH activity was not affected by diabetes.

The effects of diabetes and stress on plasma EPI levels was qualitatively similar to NE levels, but the increase in EPI in the control rats was not significant. The lack of a significant increase in plasma EPI could be the result of sampling time since plasma EPI and NE rise to a peak within 1 min of restraint and decline thereafter (DeTurck and Vogel, 1980). Even though immobilization stress was maintained until the time of sacrifice, the animals response to the stress probably diminished during the 20 min of immobilization. Previous studies have demonstrated that the rise in EPI in diabetic rats undergoing exsanguination was normal 2 wk after STZ-treatment, but attenuated after 6 wk (Fushimi et al., 1984).

Adrenal NE and EPI content and the activity of the rate limiting enzyme for catecholamine synthesis, tyrosine hydroxylase, did not differ between control and diabetic rats suggesting that the observed changes in plasma NE responses were secondary to altered sympathetic activity, rather than altered adrenal medullary function. Increased adrenal catecholamine content and biosynthetic activity in diabetic rats was reported by Bitar et al. (1987), but the rats in this study were more severely diabetic (higher STZ dose) and were diabetic for a longer duration (60 vs 28 d). Fushimi et al. (1984) demonstrated that adrenal EPI content was unaffected at 6 wk after induction of diabetes, but was elevated at 13 wk.

In the present study, rats with STZ-induced diabetes had basal corticosterone levels similar to those seen in controls. Furthermore, there was no difference attributable to diabetes in the corticosterone response to immobilization suggesting that the hypothalamic-pituitary-adrenal-cortical axis was intact. These results are in conflict with several studies demonstrating corticosterone hypersecretion in diabetic rats (DeNiccola et al., 1977; Scribner et al., 1991) but in agreement with other studies showing normal adrenal-cortical function (Tornello et al., 1981). These differences could be the result of the severity or duration of the diabetic state or because of diabetes-related changes in the circadian periodicity of corticosterone release (Gibson et al., 1985).

Despite normal corticosterone and nearly normal catecholamine responses to immobilization stress in the diabetic rats, there was no apparent effect of these hormones on plasma glucose levels. It is possible that plasma glucose levels did not increase in the diabetic rats because they were already markedly elevated, but in previous studies short-term diabetic rats (7 d post-STZ) showed a glucose response to footshock stress comparable to nondiabetic controls. Thus, duration of diabetes may be an important component determining physiologic response.

The PRL response to immobilization was blunted in the diabetic rats similarly to previously published results (Ratner et al., 1991). Several labs have reported abnormal PRL responses in diabetic patients (Coiro et al., 1987), diabetic rats (Tesone, 1985; Kampa et al., 1986), and studies from our labs indicate this may be secondary to changes in hypothalamic function since in vitro studies demonstrate normal or enhanced PRL secretion from diabetic pituitaries (Steger et al., 1989; Steger et al., 1990). Diabetes-related changes in the hypothalamic catecholamine and indoleamine responses to stress were demonstrated in the present study, but additional pharmacologic studies or measurements of amine turnover are needed to determine if these factors might be associated with altered PRL release in response to stress.

In summary, we have demonstrated that the diabetic rat is capable of responding to immobilization stress, but that certain parameters of this response appear abnormal. Previous reports in the literature indicate that these changes may become progressively greater as the duration or the severity of the diabetic state increases.

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