Effect of Acute and Antecedent Hypoglycemia on Sympathetic Neural Activity and Catecholamine Responsiveness in Normal Rats

William I. Sivitz,[1,2] Judith A. Herlein[1,2] Donald A. Morgan,[1,3] Brian D. Fink,[1,2] Bradley G. Phillips,[4] and William G. Haynes[1,3]

Adrenergic responsiveness to acute hypoglycemia is impaired after prior episodes of hypoglycemia. Although circulating epinephrine responses are blunted, associated alterations in adrenal sympathetic nerve activity (SNA) have not been reported. We examined adrenal nerve traffic in normal conscious rats exposed to acute insulin-induced hypoglycemia compared with insulin with (clamped) euglycemia. We also examined adrenal SNA and catecholamine responses to insulin-induced hypoglycemia in normal conscious rats after two antecedent episodes of hypoglycemia (days −2 and −1) compared with prior episodes of sham treatment. Acute insulin-induced hypoglycemia increased adrenal sympathetic nerve traffic compared with insulin administration with clamped euglycemia (165 ± 12 vs. 118 ± 21 spikes/s [$P < 0.05$]; or to 138 ± 8 vs. 114 ± 10% of baseline [$P < 0.05$]). In additional experiments, 2 days of antecedent hypoglycemia (days –2 and –1) compared with sham treatment significantly enhanced baseline adrenal SNA measured immediately before subsequent acute hypoglycemia on day 0 (180 ± 11 vs. 130 ± 12 spikes/s, respectively; $P < 0.005$) and during subsequent acute hypoglycemia (229 ± 17 vs. 171 ± 16 spikes/s; $P < 0.05$). However, antecedent hypoglycemia resulted in a nonsignificant reduction in hypoglycemic responsiveness of adrenal SNA when expressed as percent increase over baseline (127 ± 5% vs. 140 ± 14% of baseline). Antecedent hypoglycemia, compared with sham treatment, resulted in diminished epinephrine responsiveness to subsequent hypoglycemia. Norepinephrine responses to hypoglycemia were not significantly altered by antecedent hypoglycemia. In summary, prior hypoglycemia in normal rats increased adrenal sympathetic tone, but impaired epinephrine responsiveness to acute hypoglycemia. Hence, these data raise the intriguing possibility that increased sympathetic tone resulting from antecedent hypoglycemia downregulates subsequent epinephrine responsiveness to hypoglycemia. Alternatively, it is possible that the decrease in epinephrine responsiveness after antecedent hypoglycemia could be the result of reduced adrenal sympathetic nerve responsiveness. *Diabetes* 50:1119–1125, 2001

From the Departments of [1]Internal Medicine, Divisions of [2]Endocrinology and [3]Cardiovascular Disease, and [4]Pharmacology, University of Iowa and the Iowa City Veterans Affairs Medical Center, Iowa City, Iowa.

Address correspondence and reprint requests to Dr. William Sivitz, Department of Internal Medicine, The University of Iowa Hospitals and Clinics, 3E-17 VA, Iowa City, IA 52246. E-mail: william-sivitz@uiowa.edu.

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CV, coefficient of variation; MAP, mean arterial pressure; SNA, sympathetic nerve activity.

Impaired hypoglycemic counterregulation and hypoglycemia unawareness are well-recognized complications of long-standing diabetes (1,2). Hypoglycemia unawareness has also been observed as a reversible consequence of transient prior hypoglycemic events (3–5) and has been observed even in individuals without diabetes (6). However, the pathophysiology of hypoglycemic counterregulation and hypoglycemia unawareness is still unclear. Although much has been learned of catecholamine and other hormonal responses to hypoglycemia, the role of sympathetic neural activity per se to adrenal and other tissues has received only limited study.

Peripheral sympathetic nerve activity (SNA) originating in hypothalamic centers may be important in the counterregulatory response to hypoglycemia. Hoffman et al. (7) found that skeletal muscle SNA in type 1 diabetic and nondiabetic human subjects increased during insulin-induced hypoglycemia above that observed during euglycemia at the same insulin infusion rate. Lu et al. (8) reported that renal SNA in anesthetized rats exposed to insulin-mediated hypoglycemia increased above that observed for hyperinsulinemia with maintained euglycemia. Further, Carlsson et al. (9) found that acute insulin-induced hypoglycemia as well as 2-deoxy-D-glucose administration (which induces central neuroglycopenia) increase sympathetic adrenal nerve traffic in anesthetized rats.

However, it is not clear whether hypoglycemia alters adrenal sympathetic traffic in conscious animals or whether this is associated with hypoglycemia-induced catecholamine release. Moreover, to our knowledge, the effect of antecedent hypoglycemia on adrenal sympathetic traffic in response to subsequent hypoglycemia has not been reported. Davis et al. (10–12) demonstrated that antecedent hypoglycemia induced by insulin in healthy human subjects impaired muscle SNA in response to subsequent acute hypoglycemia. In these studies, muscle SNA was reported only as an incremental increase from baseline (nerve activity during subsequent hypoglycemia minus activity immediately before subsequent hypoglycemia), because the baseline nerve recordings demonstrated considerable subject-to-subject variation. These findings are at variance with studies of forearm norepinephrine spillover in response to acute hypoglycemia in which this parameter was not impaired by antecedent hypoglycemia (13).

In the current study, we examined the effects of insulin-induced hypoglycemia compared with insulin infusion with clamped euglycemia on adrenal SNA in conscious rats. In addition, we investigated the effect of antecedent hypoglycemia on adrenal sympathetic traffic and plasma catecholamine concentrations before and during subsequent exposure to insulin-induced hypoglycemia.

RESEARCH DESIGN AND METHODS

Animal experiments. Rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Animals were fed and maintained according to standard National Institutes of Health guidelines. The room temperature was maintained at 25°C.

In pilot studies, adrenal SNA was recorded in anesthetized rats. Rats were anesthetized with methohexital sodium (50 mg/kg i.p.), and a catheter was inserted into the femoral vein for maintenance of anesthesia with intravenous chloralose (50 mg/kg initial dosage, then 25 $mg \cdot kg^{-1} \cdot h^{-1}$). To prevent upper respiratory tract obstruction and hypoxia, the trachea was cannulated for spontaneous respiration of O_2-enriched air. Sodium bicarbonate (0.1 mmol) was administered intravenously every 60 min. Rectal temperature was monitored continuously and maintained at 37.5°C using a heated surgical table and lamps. A catheter was inserted into the left femoral artery for continuous arterial pressure measurement. A left adrenal nerve branch was exposed through a flank incision, a bipolar platinum-iridium electrode (Cooner Wire, Chatsworth, CA) was attached, and SNA was recorded as described below.

Two experimental protocols were then carried out to measure sympathoexcitation in conscious and unrestrained rats, after recovery from brief anesthesia for placement of the electrode. These rats did not require intubation or a heating lamp during nerve recording under anesthesia.

In the first protocol, adrenal SNA was recorded in rats exposed to acute insulin-induced hypoglycemia or equivalent insulin administration, with glucose clamped at the baseline level (glucose concentration immediately before insulin administration). In these experiments, rats were transiently anesthetized with methohexital sodium (50 mg/kg i.p.). Additional methohexital sodium was administered intravenously every 10 min to sustain the level of anesthesia until 30 min after the surgical procedure was completed. Catheters were placed in the carotid artery and jugular vein and tunneled to exit the skin at the nape of the neck. A nerve branch to the left adrenal nerve was exposed through a flank incision and the platinum-iridium electrode was attached. When an adequate recording was obtained, the electrode was fixed in place using silicon gel (Kwikcast; World Precision Instruments, Sarasota, FL). The wound was closed with 4.0 silk and the skin was glued shut with Vetbond tissue adhesive (3 mol/l; St. Paul, MN). The vascular catheters, protected by a spring tether, were then connected to a swivel-mount apparatus (Instech Laboratories, Plymouth Meeting, PA) at the top of the cage, allowing free movement. The procedure itself required ~20 min, and recovery from anesthesia occurred over ~60 min. Then 1 h after recovery, all rats received an intravenous bolus injection of 0.75 U human regular insulin (Lilly, Indianapolis, IN), followed by continuous intravenous infusion at 1.8 $U \cdot kg^{-1} \cdot h^{-1}$ for 60 min. Glucose was measured using a Yellow Springs Instruments glucose analyzer (Yellow Springs, OH) on a drop of whole blood obtained from the carotid artery at 5-min intervals. A variable-rate 25% glucose infusion was used to maintain the glucose at 50% of the baseline concentration in the hypoglycemic group or at baseline in the group exposed to clamped euglycemia.

In the second protocol, rats were exposed to transient insulin-induced hypoglycemia for 2 consecutive days (days –2 and –1) or to sham treatment for 2 consecutive days. Hypoglycemia was induced by subcutaneous injection of 1.5 U of human regular insulin. Sham-treated rats received a subcutaneous injection of an equivalent volume of saline. Blood glucose was determined 150 min after injection by tail vein puncture using the glucose analyzer (Yellow Springs Instruments). On day 0, all rats were transiently anesthetized for placement of an adrenal nerve electrode and vascular catheters, as in the first protocol. Then 1 h after recovery from anesthesia, all rats were exposed to acute insulin-induced hypoglycemia, and glucose was clamped at no lower than 25 mg/100 ml. In a subset of these animals, consisting of the last six in each of the antecedent hypoglycemia and last seven of the sham groups, 1.0 ml of blood was obtained from the carotid arterial line at time 0, 60, and 180 min relative to the start of the insulin infusion. Volume was replaced by infusing 1.0 ml of saline. In the initial rats used in these studies, these samples were not obtained, as there was concern about potential changes in the neural recordings affected by volume withdrawal. However, institution of this procedure did not actually alter the neural recordings.

Sympathetic nerve recordings. Sympathetic activity innervating the adrenal nerve was measured by multifiber recording, as we have previously described (14). SNA was recorded every 5 min as average activity over 1-min intervals. Using a dissecting microscope, a nerve branch to the left adrenal was carefully dissected and the bipolar electrode was inserted. After an optimum recording of SNA was obtained, the electrode was fixed in place using silicon gel. The electrode was connected to a high-impedance probe (HIP-511; Grass Instruments, Warwick, RI), amplified by 10^5, and filtered at low- and high-frequency cutoffs of 100 and 1,000 Hz with a nerve traffic analysis system (model 662-C; University of Iowa Bioengineering, Iowa City, IA). The filtered, amplified nerve signal was routed to *1*) an oscilloscope (model 54501A, Hewlett-Packard) for monitoring, *2*) a MacLab analogue-digital converter (CB Sciences, Milford, MA) for permanent recording of the neurogram on a Macintosh 9500 computer, and *3*) a nerve traffic analyzer (model 706C; University of Iowa Bioengineering) that counts action potentials above a threshold voltage level set just above background (determined postmortem). To document that the nerve recordings represented sympathetic nerve impulses, ganglionic blockade was induced with chlorisondamine (5 mg/kg i.v.) at the end of each experiment. This reduced nerve activity to low-grade background "noise" that could be subtracted from the recorded measurements. Also, a characteristic burst activity pattern was seen as a result of sympathetic outflow, which, although subjective, provided a measure of confirmation. Furthermore, in past studies (14) and in pilot experiments with rats under anesthesia, we transected adrenal nerves distal to the recording site (electrode). In six separate determinations, the neurograms were not altered, documenting the efferent rather than afferent origin of the neural signals.

Blood pressure and heart rate determinations. These parameters were continuously monitored in all rats in both studies using a pressure transducer (Gould Stathan P23ID) attached to the carotid arterial line. The data was acquired by computer through the MacLab analogue-digital converter. Blood pressure and heart rate were recorded every 5 min as average values over 1-min intervals.

Catecholamine determination. Plasma epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography with electrochemical detection (15). The assay has an inter- and intraassay coefficient of variation (CV) of 3.4 and 3.1%, respectively, and a lower limit of detection of 25 pg/ml.

Statistics. Data were analyzed by *t* test or two-way analysis of variance, as indicated.

RESULTS

In pilot experiments, we first examined adrenal SNA in normal anesthetized rats. In 11 animals, the mean SNA ± SD measured 82 ± 56 spikes/s, with a calculated CV of 67%. As compared with the above data for anesthetized rats, mean ± SD adrenal SNA in conscious rats was 108 ± 38 spikes/s, with a CV of 35%. These results were obtained in the baseline state in the 14 conscious rats (see below) used in the studies of acute hypoglycemia (7 each subsequently exposed to insulin-induced hypoglycemia or insulin with euglycemia) (Fig. 1; Table 1). The animal-to-animal variability in baseline adrenal SNA for the rats used in the studies of antecedent hypoglycemia compared with antecedent sham treatment (Fig. 2; Table 2) was further improved (22 and 32% CV, respectively).

Effect of acute hypoglycemia on adrenal SNA in conscious rats. Figure 1 depicts the time course of change in adrenal SNA in conscious rats exposed to insulin-induced hypoglycemia compared with insulin administration at maintained (clamped) baseline glycemia. SNA increased rapidly in the insulin-hypoglycemic rats compared with at clamped euglycemia. Quantitative analysis was carried out for time-averaged SNA over the intervals −15 to 0 min before insulin infusion and 45–60 min after initiation of the 60-min infusions (Table 1). Adrenal SNA increased significantly in the hypoglycemic compared with euglycemic animals, whether measured as absolute activity (spikes/second) or as percent of baseline. Mean arterial pressure (MAP) and heart rate were monitored in these animals, but no significant changes in these parameters were observed (Table 1).

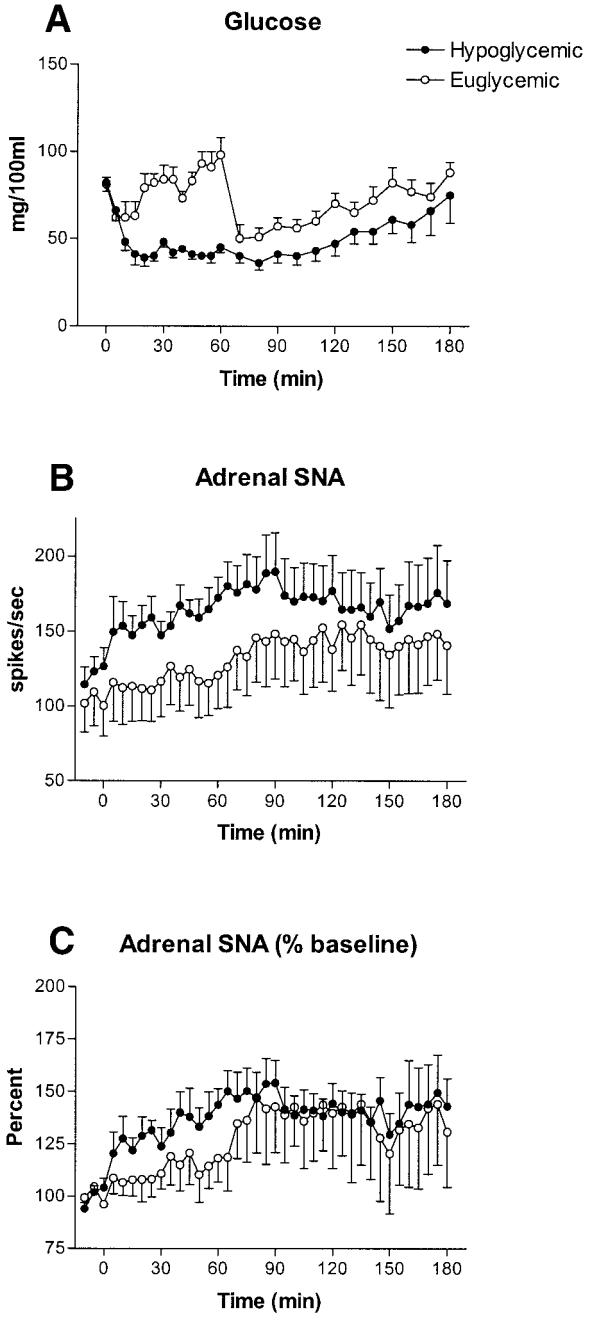

FIG. 1. Effect of acute insulin-induced hypoglycemia (●) compared with insulin administration with clamped baseline glycemia (○) on adrenal SNA. Regular human insulin (0.75 U) was administered as an intravenous bolus at time 0, followed by continuous intravenous infusion of 1.8 U · kg^{-1} · h^{-1} until time 60 min. *A*: glucose concentrations; *B*: absolute SNA (spikes/s); *C*: SNA expressed as percent of baseline (mean SNA from time –15 to 0 min before insulin). Data represent means ± SE; n = 7 rats for each group.

At the 60-min time point, the insulin and glucose infusions were abruptly discontinued. This resulted in a drop in glucose concentrations in the clamped euglycemic rats (presumably a result of slow dissipation of the insulin effect in the absence of continued infusion of glucose) associated with an increase in adrenal sympathetic traffic (Fig. 1).

Effect of antecedent hypoglycemia on adrenal SNA in response to subsequent hypoglycemia. Antecedent hypoglycemia, compared with prior sham treatment, significantly increased absolute SNA (spikes/second) both at baseline (−15 to 0 min relative to acute hypoglycemia) and after acute insulin-induced hypoglycemia (Fig. 2; Table 2). Prior hypoglycemia did not significantly change adrenal SNA responsiveness to acute hypoglycemia when activity was quantified as percentage of baseline or as mean incremental increase over baseline (Table 2). MAP and heart rate did not change significantly as a result of these manipulations.

Glucose concentrations during subsequent insulin-induced acute hypoglycemia and after termination of insulin administration (recovery) were essentially no different in rats exposed to antecedent hypoglycemia compared with sham treatment. At baseline (time 0 relative to insulin), glucose concentrations were slightly higher in the rats exposed to antecedent hypoglycemia (conceivably an effect of increased SNA), although not statistically different from the sham group (Fig. 2). Therefore, the glucose data after subsequent hypoglycemia were also expressed as percentage of initial glucose (Fig. 2). In this case, glucose recovery may have been slightly impaired, but, again, this effect was not statistically significant.

Effect of antecedent hypoglycemia on catecholamine response to subsequent hypoglycemia. We measured catecholamine concentrations in a subset of each group of animals shown in Fig. 2. Epinephrine concentrations increased in both groups (antecedent hypoglycemia and sham) after subsequent acute hypoglycemia (Fig. 3*A*). However, antecedent hypoglycemia significantly reduced the magnitude of epinephrine responsiveness to acute hypoglycemia (Fig. 3*A*). In contrast to the epinephrine results, antecedent hypoglycemia had no significant effect on norepinephrine concentrations after subsequent hypoglycemia (Fig. 3*B*).

DISCUSSION

Previous studies have demonstrated increased sympathetic traffic to lumbar and renal (8) and adrenal nerves (9) in response to acute hypoglycemia. Insulin-induced hypoglycemia has also been shown to increase plasma norepinephrine after adrenalectomy, consistent with sym-

TABLE 1
Quantitative parameters measured in rats exposed to acute insulin-induced hypoglycemia or insulin infusion with clamped baseline glycemia (euglycemia) from time 0 to 60 min

	Euglycemia	Hypoglycemia
Weight (g)	380 ± 9	380 ± 6
SNA		
Baseline (spikes/s)	104 ± 21	121 ± 11
During treatment (spikes/s)	118 ± 21	165 ± 12*
During treatment (% baseline)	114 ± 10	138 ± 8*
MAP (mm Hg)		
Baseline	143 ± 4	137 ± 6
During treatment	149 ± 5	151 ± 4
Heart rate (bpm)		
Baseline	382 ± 19	364 ± 19
During treatment	368 ± 9	371 ± 15

Data are means ± SE; n = 7 for each group. SNA, MAP, and heart rate represent time-averaged values recorded over time −15 to 0 min relative to insulin infusion (baseline) and over time 45–60 min after initiation of insulin infusion (during treatment). *$P < 0.05$ vs. euglycemia by unpaired, two-tailed t test.

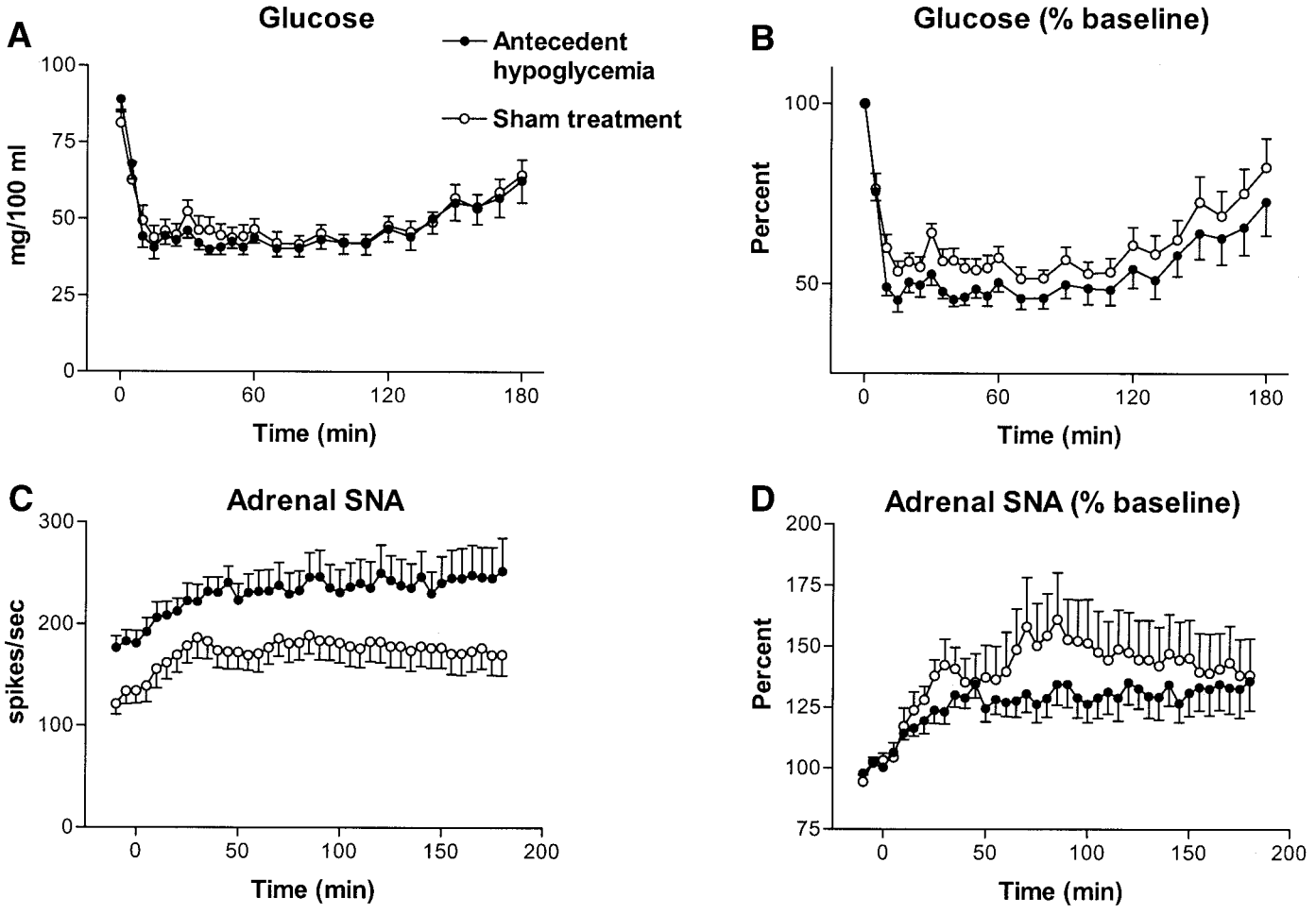

FIG. 2. Effect of 2 days (days –2 and –1) of antecedent hypoglycemia (●) compared with sham treatment (○) on adrenal SNA. On day 0, all rats received 0.75 U regular human insulin as an intravenous bolus at time 0, followed by continuous intravenous infusion at 1.8 U · kg^{-1} · h^{-1} until time 60 min. *A*: glucose concentrations in absolute units (mg/100 ml); *B*: glucose concentrations as percentage of baseline (time = 0 min); *C*: absolute SNA (spikes/s); *D*: SNA expressed as percent of baseline (mean SNA from time –15 to 0 min before insulin). Data represent means ± SE; *n* = 13 rats for each group.

pathoexcitation and catecholamine release from peripheral nerves (16). Hence, we were not surprised to find that in our studies acute insulin-induced hypoglycemia increased adrenal sympathetic nerve traffic. On the other hand, our data are unique in demonstrating that hypoglycemia enhances adrenal nerve traffic independent of hyperinsulinemia. This is of some importance, as insulin in the presence of euglycemia increases muscle SNA in humans (17,18), lumbar SNA in rats (19,20), and lumbar and adrenal SNA in spontaneously hypertensive rats (19).

Our data are also unique in showing that insulin-induced hypoglycemia increases adrenal SNA in conscious unrestrained animals. As noted under RESULTS, the animal-to-animal variability in adrenal nerve activity was reduced considerably when the measurements were made in the conscious as compared with the anesthetized rats. This improvement in variability proved important, as it facilitated our ability to compare absolute SNA after antecedent hypoglycemia with activity after sham treatment, those exposures having occurred on days –2 and –1 relative to initiation of the neural recordings. In past studies of anesthetized rats, SNA has generally been reported as percentage change from baseline (activity after a given manipulation as a function of activity before that manipulation) rather than as absolute activity.

The major findings of the current studies concern the effect of antecedent hypoglycemia on adrenal nerve traffic and the sympathetic response to subsequent hypoglycemia. This is an important issue, as the mechanisms responsible for the well-known clinical phenomena of hypoglycemia unawareness and impaired hypoglycemic counterregulation are only partially understood. Although plasma epinephrine responses to hypoglycemia in human subjects are blunted as a result of antecedent hypoglycemia (6,21–23), the role of adrenal neural activity per se has not been investigated.

Given the blunted epinephrine responses, we had hypothesized that adrenal sympathetic traffic in normal rats would be impaired after antecedent hypoglycemia. Hence, we were somewhat surprised that this was not the case. Rather, adrenal nerve traffic was actually enhanced by antecedent hypoglycemia (Fig. 2*C*; Table 2). This was the case whether adrenal SNA was examined in the baseline state, immediately before subsequent acute hypoglycemia, or after induction of subsequent hypoglycemia. Nonetheless, antecedent hypoglycemia resulted in a nonsignificant reduction in hypoglycemic responsiveness of adrenal SNA when expressed as percentage increase over baseline (Fig. 2*D*; Table 2).

As expected based on the above-mentioned human

TABLE 2
Quantitative parameters measured in rats exposed to sham treatment or insulin-induced hypoglycemia on days −2 and −1 relative to neural recording (day 0)

	Sham treatment	Antecedent hypoglycemia
Weight (g)		
Day −2	399 ± 5	390 ± 4
Day 0	404 ± 6	400 ± 4
Glucose (mg/100 ml)†		
Day −2	78 ± 2	26 ± 2**
Day −1	76 ± 3	32 ± 3**
SNA		
Baseline (spikes/s)	130 ± 12	180 ± 11**
During treatment (spikes/s)	171 ± 16	229 ± 17*
During treatment (% baseline)	140 ± 14	127 ± 5
Incremental increase from baseline (spikes/s)	41 ± 16	49 ± 9
MAP (mm Hg)		
Baseline	136 ± 5	142 ± 3
During treatment	145 ± 5	154 ± 2
Heart rate (bpm)		
Baseline	355 ± 13	364 ± 7
During treatment	363 ± 7	369 ± 9

Data are means ± SE; $n = 13$ for each group. All rats were exposed to acute insulin-induced hypoglycemia from time 0 to 60 min on day 0. SNA, MAP, and heart rate represent time-averaged values recorded over time −15 to 0 min relative to insulin infusion (baseline) and over time 45–60 min after initiation of insulin infusion (during treatment). Percentages of baseline values and incremental increases over baseline compare values in individual animals of each group recorded from 45 to 60 min with values from −15 to 0 min relative to initiation of insulin infusion. *$P < 0.05$, **$P < 0.005$ by unpaired, two-tailed t test compared to sham; †glucose measured 150 min after a single subcutaneous dose of 1.5 U human regular insulin.

studies (6), epinephrine responsiveness to acute hypoglycemia was impaired as a result of antecedent hypoglycemia. However, in the current studies, we showed that this occurred in spite of increased adrenal nerve activity. This surprising observation raises the question of whether enhanced SNA may be important in the mechanism leading to impaired epinephrine responses. Although clearly speculative at this point, it seems plausible that enhanced chronic adrenal sympathetic traffic resulting from prior episodes of hypoglycemia may desensitize adrenal tissue or deplete epinephrine stores, leading to impaired catecholamine release to subsequent stimuli.

An alternative explanation might involve the effect of prior hypoglycemia on the responsiveness of adrenal SNA to subsequent acute hypoglycemia. In fact, we did observe a reduction in adrenal neural responsiveness when SNA was expressed as percent of baseline (Fig. 2*D*; Table 2). On the other hand, that change did not achieve statistical significance, and there was no decrease in adrenal nerve responsiveness when expressed as incremental increase from baseline (Table 2). Nonetheless, it is possible that impaired SNA responsiveness could lead to decreased adrenal epinephrine release.

As previously mentioned, studies of the effect of antecedent hypoglycemia on muscle SNA in normal human subjects (10–12) have revealed a decrease in the incremental response of muscle SNA to subsequent acute hypoglycemia. Thus, these results differ from our findings for adrenal SNA, as we observed that antecedent hypoglycemia did not significantly alter incremental activity over baseline (Table 2). Differences between our results for adrenal SNA and those of muscle SNA are possibly attributable to differences in *1*) activation of the nerve fibers innervating different tissues, *2*) species, or *3*) protocols (e.g., timing and extent of antecedent hypoglycemic exposures). In the above muscle SNA studies, baseline or absolute nerve activities were not reported.

In contrast to epinephrine, antecedent hypoglycemia had no differential effects on circulating norepinephrine concentrations. This finding differs from results in human studies in which prior hypoglycemia impaired epinephrine and norepinephrine responses to subsequent hypoglycemia (6, 12). This discrepancy may be attributable to species differences. Alternatively, we may have had difficulty detecting differences in plasma norepinephrine related to relative complexity (compared with epinephrine) of the factors regulating circulating concentrations. Whereas bioeffective plasma epinephrine derives from adrenomedullary chromaffin cells (24), norepinephrine is also released by peripheral postganglionic sympathetic neurons. Hence, plasma norepinephrine reflects the balance between release and reuptake within multiple tissues and signifies more gener-

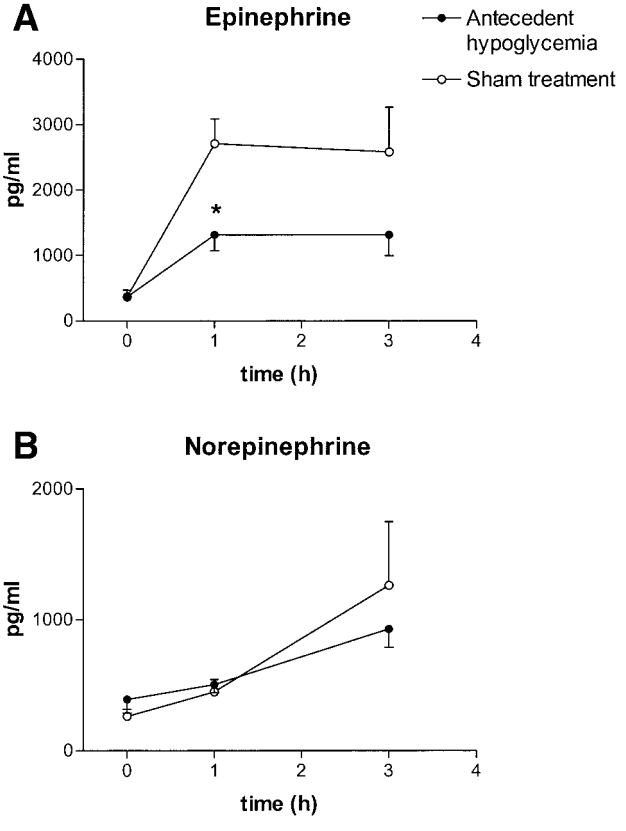

FIG. 3. Effect of 2 days (days –2 and –1) of antecedent hypoglycemia (●) compared with sham treatment (○) on plasma concentrations of epinephrine (*A*) and norepinephrine (*B*). Catecholamine concentrations were determined on the last six rats exposed to antecedent hypoglycemia and the last seven exposed to antecedent sham treatment, as described in the legend to Fig. 2. Data represent means ± SE. *$P < 0.05$ vs. antecedent sham treatment as measured by two-way analysis of variance and Bonferroni's post tests. For plasma epinephrine, the overall treatment and time effects were both significant at $P < 0.01$.

alized sympathetic activation of sympathetic nerve terminals within multiple tissues. Moreover, there is evidence that hypoglycemia, at least in the rat, may preferentially stimulate adrenomedullary cells producing epinephrine compared with those producing norepinephrine (25).

Although epinephrine responsiveness to acute hypoglycemia was reduced in the rats exposed to antecedent hypoglycemia compared with sham treatment, glycemic recovery from insulin-induced hypoglycemia was not impaired by antecedent hypoglycemia (Fig. 2). The reason for this may be multifactorial, as several mechanisms beyond epinephrine have been implicated in the counterregulatory response to hypoglycemia (26). Hypoglycemia-induced glucagon release would be a likely explanation. Glucagon comes into play early in the hierarchy of glucose counterregulatory responses and facilitates glycemic recovery, even in the face of epinephrine deficiency or α- and β-adrenergic blockade (26). Other possibilities, probably less important or acting later in the counterregulatory hierarchy than glucagon, include enhanced endogenous glucose production secondary to neural activation of liver tissues, enhanced glucose output as a direct inverse effect of circulating glucose concentration (autoregulation), or increased circulating substrates, such as free fatty acids, that can decrease glucose utilization as well as increase hepatic output (27–29).

A limitation to our studies was that antecedent hypoglycemia, as studied here, was induced by insulin. Although the sham treatments reproduced the trauma of injections and animal handling, we cannot sort out an effect of prior hypoglycemia per se from an effect of antecedent insulin per se. This may be relevant, given that, as indicated above, insulin administration in the presence of clamped euglycemia can increase sympathetic traffic to peripheral nerves (19). Nonetheless, the current experiments are more relevant to clinical antecedent hypoglycemia in diabetic patients, which, with rare exception, is induced by insulin.

It may be of interest to note here that a different form of altered sympathetic sensitivity induced by hypoglycemia has been previously described in human subjects. Prior exposure to hypoglycemia has been found to decrease heart rate responsiveness to β-adrenergic agonist treatment in subjects with type 1 diabetes, but to increase adrenergic sensitivity when measured this way in nondiabetic healthy subjects (30). In addition, hypoglycemia unawareness has been associated with a decrease in β-adrenergic sensitivity (31). Of course, these changes in sensitivity refer to catecholamine responsiveness, whereas, in the current study, we addressed sympathetic neural effects.

In summary, we have shown that insulin-induced hypoglycemia triggers SNA to adrenal nerves in normal conscious rats. Furthermore, the data suggest that basal SNA is enhanced by two prior episodes of antecedent hypoglycemia. Despite this enhanced adrenal sympathetic tone, catecholamine responsiveness to acute hypoglycemia is impaired by prior hypoglycemia. This raises the possibility that antecedent hypoglycemia, through chronically enhanced sympathetic tone, impairs catecholamine responsiveness. Alternatively, it is possible that the decrease in epinephrine responsiveness could be the result of reduced adrenal neural responsiveness after previous hypoglycemia.

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