Sympathetic Outflow in Human Muscle Nerves Increases During Hypoglycemia

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SUMMARY

The normal response to insulin-induced hypoglycemia bears many characteristics of activation of the sympathetic nervous system. In this study, the impulse pattern of muscle nerve sympathetic activity (MSA) involved in cardiovascular homeostasis was identified by microneurography in the peroneal nerve of seven healthy and two adrenalectomized subjects. After recordings at rest and an intravenous injection of 0.15 IU insulin/kg body wt (0.10 IU insulin/kg body wt in adrenalectomized subjects), MSA was followed for 90 min. Nadir of hypoglycemia (2.0 ± 0.1 mM) was reached at 30 min. All subjects, including the two adrenalectomized subjects, exhibited an increase of MSA, which peaked at the glucose nadir. The time course of MSA increase was a mirror image of the blood glucose curve. This directly measured increase of MSA may be part of the hemodynamic adjustment to the fall in plasma volume known to occur in hypoglycemia. Another possible cause is direct stimulation of central sympathetic motoneurons. DIABETES 1986; 35:1124–29.

The normal response to hypoglycemia is characterized by symptoms and signs traditionally ascribed to an increase of sympathetic discharge, such as tachycardia, sweating, and a rise of blood pressure. Considerable hemodynamic adjustment occurs in insulin-induced hypoglycemia, implying changes of sympathetic vasoconstrictor activity.[1] The hormonal response to hypoglycemia includes an increase in the plasma level of the sympathetic transmitter norepinephrine.[2] However, because such an increase is difficult to detect in bilaterally adrenalectomized individuals, it has been proposed that the increase in norepinephrine during hypoglycemia is derived from the adrenal medulla and that hypoglycemia does not stimulate the sympathetic nervous system.[3,4]

By use of microneurography, sympathetic discharges can be recorded directly in extremity nerves in awake cooperating humans.[5] Such nerve recordings have shown that sympathetic nerve activity appears as multiunit volleys of impulses with interposed intervals of neural silence. Sympathetic outflow exhibits different characteristics in muscle and skin nerve fascicles. Muscle nerve sympathetic activity (MSA) is involved in blood pressure regulation. The activity appears as bursts of vasoconstrictor impulses time-locked in the cardiac rhythm[6] and is governed by inhibitory baroreflexes, which are responsible for its pulse synchrony.[7] There is a close feedback control between blood pressure variations and MSA: the MSA bursts occur during transient reductions in blood pressure.[8] The amount of MSA at rest varies widely between individuals, whereas in a given subject the outflow is virtually constant from one recording to another.[9] The individual level of MSA correlates to the level of plasma norepinephrine at rest,[10] with no apparent association to resting blood pressure level.[8] Our study was undertaken to characterize the relationship between MSA and insulin-induced hypoglycemia.

SUBJECTS AND METHODS

Subjects. MSA was recorded in seven healthy volunteers (3 men and 4 women) aged 20–41 (mean, 31) yr and in two adrenalectomized patients. One patient was a 51-yr-old woman, treated for Cushings disease with adrenalectomy and radiation of the pituitary body, and the other was a 48-yr-old man, treated with adrenalectomy for bilateral pheochromocytoma. Both patients received hormonal substitution therapy; the ordinary cortisol supply was changed to dexamethasone on the study day.The woman also received treatment for hypertension with the selective β-blocker atenolol (100 mg daily), which for psychological reasons could not be discontinued before the recording.

All subjects gave their informed consent to the procedure, and the study was approved by the Ethics Committee of the Medical Faculty, University of Uppsala.

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Nerve recordings. An insulated tungsten microelectrode (tip diam, ~5 μm) was inserted manually through the intact skin into the underlying right peroneal nerve at the fibular head. A low-impedance reference electrode was placed subcutaneously at a distance of 1–2 cm. The nerve was localized by electrical stimuli delivered through the recording electrode. When the nerve was encountered, an electrode position within a muscle nerve fascicle was identified by muscle twitches evoked by the electrical stimuli and by the appearance of afferent mechanoreceptive activity elicited by stretching or tapping the appropriate muscle. Thereafter, minor adjustments of the electrode position were made until the characteristic pattern of multiunit MSA was recorded. The evidence that the recorded signals derive from sympathetic fibers have been summarized previously.[7] The procedure may cause minor discomfort during the search for the nerve; when a suitable electrode position is found, nothing is felt during the recording. Two healthy subjects reported temporary paresthesia in the foot for 10 and 4 days, respectively, from the 4th day after the recording; otherwise no undesirable effect of the procedure appeared.

The nerve signal was amplified in two steps (total gain, 50,000×) and fed through a 700- to 2000-Hz band-pass filter and an amplitude discriminator for improving signal-to-noise ratio. A resistance-capacitance integrating network (time constant, 0.1 s) delivered a mean voltage display of the multiunit neural activity (relationship between discriminated original and mean voltage neurogram is shown in Fig. 1).

Electrocardiogram (ECG) was recorded by chest electrodes, and respiratory movements were recorded by a strain gauge strapped around the chest with a rubber band. All recorded signals were stored on tape (FM tape recorder Sangamo Sabre VI, Sangamo Weston-Schlumberger, Sarasota, FL) for subsequent analysis. During the experiments, the signals were displayed on a storage oscilloscope.

General procedure. The experiments were carried out in a laboratory with an ambient temperature of 22–24°C. Subjects were fasting and did not smoke after midnight, and the experiments began at 0800 h. Subjects were supine on a comfortable table. After insertion of separate indwelling intravenous catheters for blood specimens and insulin injection, the nerve recording was initiated—the search for a suitable intraneural electrode position lasted for 10–60 min. The sympathetic signals were then recorded continuously throughout the experimental procedure. After recording MSA at rest for 15–30 min, hypoglycemia was induced by rapid intravenous injection of regular insulin (0.15 IU/kg body wt in healthy subjects and 0.10 IU/kg body wt in adrenalectomized patients). Blood samples for the analyses of glucose and catecholamines were drawn at −15, 0, 15, 30, 45, 60, 75, and 90 min in relation to the insulin injection.

The experimental procedure with the subjects supine thus lasted for up to 3 h. Three subjects reported that lying still was uncomfortable during the last 30 min of the procedure.

In six of the experiments (4 healthy subjects), blood pressure was measured noninvasively every 3rd min by an automatic blood pressure recorder (Dinamap, Criticon, Tampa, FL).

Analysis procedure. A 2.5-mm/s paper display of the recorded signals was obtained from an ink-jet recorder (Siemens-Elema, Stockholm, Sweden). Thus the mean voltage neurogram was amplified to a degree giving a similar amplitude of bursts in all recordings; i.e., amplification differed between subjects. Heart rate and the number of bursts of MSA (counted per 100 heartbeats and per min) were determined for selected 6-min periods (mainly at time for drawing blood sample). Thus a burst incidence was obtained for every analyzed period.

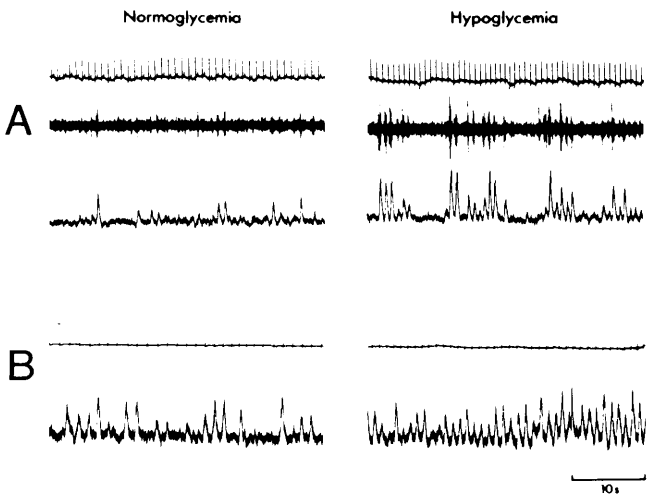

FIG. 1. Increase in muscle nerve sympathetic activity from normoglycemia to hypoglycemia in normal (*A*) and adrenalectomized (*B*) subject. *Top traces*, electrocardiogram; *middle traces* (in *A* only), original (discriminated) neurogram; *bottom traces*, mean-voltage neurogram. Same time scale in all panels.

The total outflow of MSA/unit time can be defined as the product of the number of bursts and the number of impulses/burst, i.e., the strength of the burst (corresponding to burst amplitude in mean voltage neurogram). Because the recorded burst amplitude is critically dependent on the intraneural position of the electrode, the amplitude cannot be used for comparison of the strength of the sympathetic outflow between individuals. Within a given recording, however, relative burst amplitude is representative of the strength of the activity when the MSA response during a certain procedure is compared with a control period (provided electrode position is unchanged). In three recordings (healthy subjects), in which no signs of change of electrode position were seen, the total MSA during each period was calculated as the product of the number of bursts/min and mean burst amplitude and expressed in arbitrary U/min. Burst amplitude was measured in millimeters on a digitizing board (Hipad, Houston Instruments, Austin, TX) connected to a computer (Digital Dec 11/40, Digital Equipment, Maynard, MA).

Blood chemistry. Blood glucose was measured with glucose dehydrogenase (Merck AG, Darmstadt, FRG)[11] in an automated analysis system (Greiner G-300, Langenthal, Switzerland). The catecholamine concentration in venous plasma was determined by high-performance liquid chromatography with a cation-exchange column and electrochemical detection.[12]

Blood samples for catecholamine analysis were collected in 10-ml ice-chilled vacutainer tubes to which 0.2 ml of glutathione and EGTA had been added to yield a final concentration of 4 and 5 mM, respectively (chemicals from Sigma, St. Louis, MO). After centrifugation at +4°C, serum was immediately frozen and kept at −20°C until analysis.

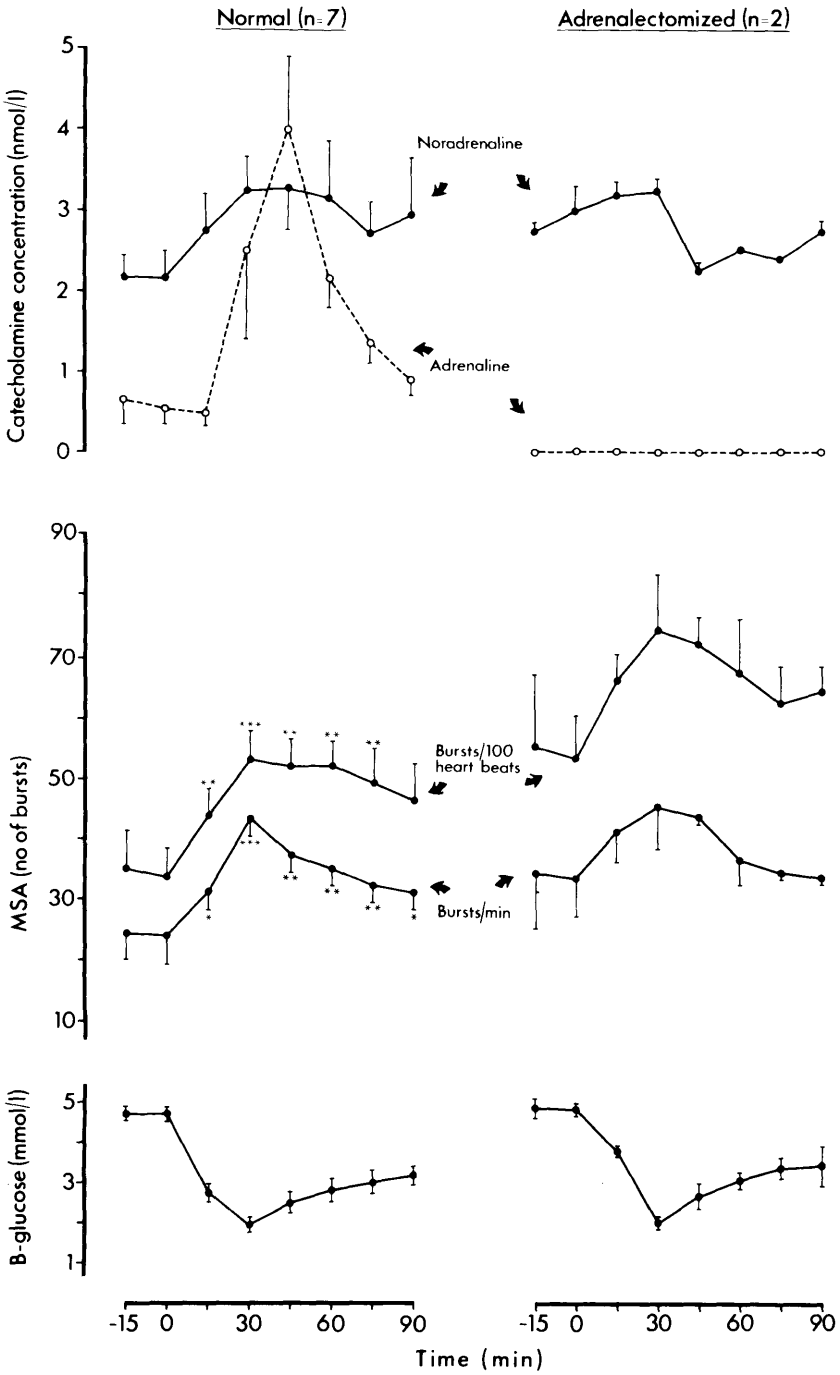

FIG. 2. Time course of insulin-induced changes in venous plasma catecholamine concentration (*top*), muscle nerve sympathetic activity (MSA) (*middle*), and blood glucose (*bottom*) in normal (*left*) and adrenalectomized (*right*) subjects. Intravenous administration of insulin at time 0. Values are means ± SE. Significance levels (change from time 0) indicated only for MSA in normals: *$P < .05$; **$P < .01$; ***$P < .001$.

Statistics. Results are expressed as means ± SE. Statistical methods used were Student's *t* test for paired observations and linear-regression analysis with *t* test of regression coefficient; *P* values represent two-tailed statistical inference.

RESULTS

In the healthy subjects, mean fasting blood glucose before insulin injection was 4.7 mM (range, 3.9–5.3). The nadir of blood glucose [2.0 mM (range, 1.3–2.8)] was reached at 30 min after the insulin injection (1 subject exhibited lowest value at 45 min) and then counterregulation occurred. Fasting blood glucose values were 4.6 and 5.1 mM in the two adrenalectomized patients, both of whom showed a hypoglycemic reaction similar to that of the healthy subjects (cf. Fig. 2).

The healthy subjects reported expected symptoms during hypoglycemia (feeling of hunger and warmth, palpitations, and drowsiness in a few cases); five subjects exhibited profuse sweating and two exhibited moderate sweating. Tachycardia was present between 20 and 40 min after insulin injection, with a peak at 30 min; tachycardia was pronounced in four subjects (33–42% increase in heart rate), moderate in two (20%), and minor in one (12%).

Symptoms during hypoglycemia were less pronounced in the adrenalectomized subjects; in both, only a little sweating occurred. Heart rate increased 35% in one patient (with very

low resting heart rate; cf. Fig. 1), whereas the patient on β-blocking treatment remained at a stable heart rate throughout the experimental procedure.

As expected, the level of MSA at rest differed markedly between subjects, ranging between 14 and 67 bursts/100 heartbeats or 10 and 43 bursts/min. No change occurred during the first 6–8 min after insulin injection. Then a successive increase in the number of bursts and an increase in burst amplitude occurred in all healthy and both adrenalectomized subjects, as shown in Fig. 1. The nadir of blood glucose and the peak of sympathetic outflow coincided in the male adrenalectomized and five healthy subjects, whereas maximal MSA was seen 15 min after the lowest blood glucose level in the female adrenalectomized and one healthy subject and 30 min after glucose nadir in one healthy subject. The time course of the mean outflow of MSA was a mirror image of the blood glucose curve (Fig. 2). The increase of MSA was more protracted than the short-lasting tachycardia.

The individual increase in MSA varied considerably: the ranges of percentage increase were 34–236 and 30–300% for number of bursts/100 heartbeats and bursts/min, respectively. The increase was lowest for the male adrenalectomized patient, who had the highest outflow at rest. On the whole, the individual maximal increase in MSA tended to correlate inversely to the resting level of MSA, expressed as number of bursts/100 heartbeats ($r = -.63$; $P < .10$; $df = 7$).

The inclusion of relative burst amplitude in the analysis of the sympathetic activity (3 subjects) showed that on the average, the net maximal increase in MSA was 25% higher than estimated by counting the number of bursts/minute.

With the exception of one healthy and one adrenalectomized subject, there was an individual negative linear correlation between blood glucose level and sympathetic outflow, whether MSA was expressed as net outflow in arbitrary U/min or counted as bursts/min and bursts/100 heartbeats (correlation coefficients ranged from $-.63$ to $-.99$).

Blood pressure (monitored in 4 healthy subjects) exhibited only small changes that did not coincide with the nadir of glucose and the peak of MSA increase. At 45 min, mean systolic blood pressure was higher than during the control period ($P < .10$), and mean diastolic blood pressure reached a shallow nadir at 60 min ($P < .10$). Blood pressure remained stable in the male adrenalectomized patient. In the female patient (on β-blocking treatment), blood pressure fell from an initial 175/96 to a nadir at 45 min of 144/81, with a subsequent increase to 157/86 at 90 min (each value is mean of 3 observations); in this patient, the lowest blood pressure level coincided with the maximum outflow of MSA.

The epinephrine response (Fig. 2) showed a peak coinciding with the nadir of blood glucose and a rapid return toward control values. Plasma epinephrine was below the detection limit of the assay in the adrenalectomized subjects. In the normal subjects, plasma norepinephrine was significantly increased at the nadir of blood glucose ($P < .05$ at 30 min) and thereafter fell gradually. Excluding one person, who exhibited more signs of initial stress in view of the experimental situation than the others, baseline plasma norepinephrine correlated to baseline MSA ($r = .86$; $P < .01$). Despite the simultaneous increase in MSA and norepinephrine (Fig. 2), the correlation between individual peak plasma norepinephrine and peak MSA was weak ($r = .65$; $P < .10$).

DISCUSSION

Our study provides the first direct evidence that acute insulin-induced hypoglycemia causes a considerable increase in sympathetic discharge in human extremity muscle nerves.

Only the peroneal nerve was explored in this study, but it is likely that the observations are valid for all extremity muscle nerves because MSA has been shown to occur strictly in parallel in different extremity muscle nerves at rest.[9]

A clear increase in sympathetic activity was observed in all subjects, regardless of different levels of activity during control conditions. Because MSA is time-locked in the cardiac rhythm, an enhanced outflow can result either from an increase in pulse rate or from an increase in the number of bursts/100 heartbeats. Finally, recruitment of more fibers in every single burst (i.e., increase of burst strength) may increase MSA. All three changes occurred during hypoglycemia. The increase in number of bursts/100 heartbeats was protracted, whereas the number of bursts/min displayed a more well-defined peak that coincided with the nadir of blood glucose (Fig. 2) due to simultaneous short-lasting tachycardia. The increase in burst amplitude further reinforced the sympathetic outflow at the nadir of blood glucose.

The mean level of MSA at rest was higher in the adrenalectomized patients than in the healthy subjects (cf. Fig. 2), an observation that does not allow any conclusion due to the profound interindividual difference in resting MSA seen normally.[9] The inverse relationship between the resting level of MSA and the increase in MSA is also recognized from several maneuvers affecting the outflow of MSA.[13,14]

No change in MSA was seen after the intravenous administration of insulin until after 6–8 min, suggesting that the increase in MSA is related to hypoglycemia and not to insulin per se. This assumption is supported by the close relationship between blood glucose level and sympathetic outflow in most subjects.

The physiologic responses to hypoglycemia shown to be of critical importance for glucose counterregulation are release of glucagon and epinephrine.[2] There is no reason to believe that an increase in vasoconstrictor nerve activity to muscle vessels should play a primary role in the counterregulation of hypoglycemia. This notion is in accordance with the fact that patients with cervical transection of the spinal cord counterregulate hypoglycemia only slightly less effectively than normal subjects.[15] Instead, the altered sympathetic outflow more likely represents an adaptation to hemodynamic changes during hypoglycemia. Thus the increase in systolic and decrease in diastolic blood pressure[1,16] should influence the outflow of MSA to some extent. The adrenalectomized patient receiving β-blocking treatment in this study exhibited a fall in both systolic and diastolic blood pressure (probably related to inhibition of tachycardia), presumably contributing to the increase in MSA. However, in all other subjects, the small changes in blood pressure and the different time courses for blood pressure fluctuations and changes in MSA make blood pressure unlikely as a major determinant of the effects observed.

Hilsted and co-workers[1] described a decrease in plasma volume with its nadir coinciding with maximum hypoglycemia

at 30 min after intravenous bolus injection of 0.15 IU insulin/kg body wt. This should cause unloading of intrathoracic volume (low-pressure) receptors, resulting in reduced inhibitory afferent activity followed by an increase in MSA. A similar mechanism eliciting increase in MSA is working when subatmospheric pressure is applied to the lower part of the body[17] and when posture is changed from a lying to an upright position.[18] It is not known whether there is any similar decrease in plasma volume during hypoglycemia in adrenalectomized subjects. Because epinephrine infusion reduces plasma volume in intact subjects,[19] the hypoglycemic decrease in plasma volume was suggested to be secondary to epinephrine release.[1] Such a mechanism cannot be operating after adrenalectomy and consequently cannot explain the observed increase in MSA. Local metabolic autoregulatory mechanisms contributing to plasma volume homeostasis may exist, but such mechanisms are difficult to assess. Alternatively, entirely different explanations (e.g., direct stimulation of central sympathetic motoneurons) must be sought for the response of MSA to hypoglycemia.

MSA is presumed to be purely vasoconstrictive,[5] and an increase in extremity vascular resistance in hypoglycemia would be expected in view of the strong increase in MSA found in this study. Several investigators[1,20-22] instead found a significant reduction in upper and lower extremity vascular resistance in hypoglycemia. Our results show that this muscle vasodilation is not due to withdrawal of vasoconstrictor tone.

A possible explanation for these seemingly contradictory observations might be that the epinephrine release during hypoglycemia causes β-adrenergic dilation of muscle vessels,[19,23,24] the effect of which overrides the strong sympathetic vasoconstrictor command. However, increased blood flow to the forearm in adrenalectomized subjects[25] speaks against such a mechanism.

MSA, being only one subdivision of the sympathetic nervous system, correlates to individual venous plasma norepinephrine levels at rest, possibly due to spillover from sympathetic nerve endings.[10] This correlation was corroborated by our study. The observed increase in MSA probably accounts for part of the well-known increase in the plasma level of norepinephrine in hypoglycemia.[2] A sympathetic origin of this increase has been doubted, however, due to difficulties in demonstrating a significant increase of norepinephrine during hypoglycemia after adrenalectomy.[3,16,26] Therefore, the adrenal medulla was put forward as the one source of extra norepinephrine secretion in hypoglycemia, and sympathetic activation was proposed not to occur. This possibility is ruled out by our findings. Due to the complexity of the sympathetic nervous system, one part might be activated while another is inhibited, thus making it difficult to interpret changes in plasma norepinephrine as a measure of sympathetic nerve activation. Our observations of a clear-cut increase in MSA do not exclude the possibility that other components of the sympathetic nervous system have been subjected to inhibition.

In resting conditions, there is evidence for a coupling between MSA and cardioacceleration.[27] The observed short-lasting tachycardia and protracted increase in MSA bursts/100 heartbeats indicate a dissociated influence of hypoglycemia on the release mechanisms for the two types of sympathetic activity. On the other hand, in acute insulin-induced hypoglycemia in humans, there is a coinciding activation of the adrenal medulla and sympathetic fibers subserving muscle vasculature, speaking against a general dissociation between adrenal and sympathetic responses as proposed previously.[4]

Our findings may be important for the understanding of some phenomena in diabetic autonomic neuropathy.[28] Sympathetic outflow in human muscle (and skin) nerves is often not detectable in patients with diabetic polyneuropathy,[29] implying that an increase in MSA cannot occur in hypoglycemia. This particular defect probably plays only a minor role, if any, for the patients' insufficient ability to detect hypoglycemia because even a pronounced increase in MSA does not cause any subjective sensation.[7] Sympathetic activation of the heart and sympathetic outflow in skin nerves, giving rise to tachycardia and sweating, respectively, may be more important as the symptoms making the patient aware of hypoglycemia. However, defective outflow of MSA may at least partly account for the hypoglycemia-associated postural hypotension in diabetic patients with autonomic neuropathy.[30,31]

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