The increase in sympathetic nerve activity after glucose ingestion is reduced in type I diabetes

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ABSTRACT

Food intake is followed by an increase in baroreflex-governed sympathetic outflow to muscle vessels. It is established that insulin contributes to this stimulation; however, the increase occurs (to a lesser degree) even in the absence of enhanced insulin secretion. To further elucidate the role of insulin, muscle nerve sympathetic activity was recorded by microneurography, and the increase after an oral 100-g glucose load in eight C-peptide-negative patients with type I diabetes without any signs of neuropathy was compared with that in 16 healthy control subjects. The level of sympathetic activity at rest was similar in the two groups (type I diabetes patients, 19.5 ± 2.4 bursts/min; controls, 20.4 ± 4.8 bursts/min; means ± S.D.). Following glucose intake there was a significant increase in activity in both groups, with maximum values at 30 min of 24.3 ± 3.7 bursts/min for type I diabetes patients and 34.4 ± 9.1 bursts/min for controls. The summarized response (during 90 min) of the diabetic patients was less than half that of the control subjects (P = 0.0003). It is concluded that the response of muscle nerve sympathetic activity to glucose ingestion is reduced to about half of its normal strength in the absence of insulin, and that there is no difference in sympathetic outflow at rest between healthy subjects and diabetic patients without polyneuropathy.

INTRODUCTION

Nutrient intake is followed by activation of the sympathetic nervous system in healthy humans [1–5]. In type I diabetes the cardiovascular and noradrenaline response to glucose intake is reduced, but is normalized by insulin infusion [6]. In 1981 insulin was shown to exert a direct effect, unrelated to hypoglycaemia, on human plasma noradrenaline levels, indicating sympathetic nervous system activation [7]. In view of the finding that hypertension is associated with insulin resistance [8–10], increasing attention has been paid to the influence of insulin on sympathetic nerve activity and on blood pressure regulation [11].

Using microneurography, it has been shown that the blood pressure regulating and baroreflex-governed muscle nerve sympathetic activity (MSA) [12] markedly increases after food ingestion [3–5], and also increases significantly during hyperinsulinaemic euglycaemic clamps in both healthy and diabetic subjects [13–20]. Intake of nutrients (fat, protein) or xylose, which elicits marginal or no insulin secretion, is followed by increased MSA [3,4], indicating that insulin only accounts for a part of the MSA response to a meal.

Key words: baroreflex, blood pressure regulation, eating, glucose ingestion, insulin, microneurography, sympathetic nervous system, type I diabetes.

Abbreviation: MSA, muscle nerve sympathetic activity.

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In order to discriminate further between the role of insulin and the effects of ingestion of glucose, we investigated the response of MSA to an oral glucose load in young, C-peptide-negative patients with type I diabetes without signs of polyneuropathy, and compared it with that in young, healthy subjects.

MATERIALS AND METHODS

Subjects

Eight C-peptide-negative subjects (three men and five women) with type I diabetes participated in the study. Their age was 27.8 ± 6.0 years (mean ± S.D.), and the time since onset of diabetes was 9.9 ± 6.4 years. Haemoglobin A1c was 7.3 ± 1.1% (range 5.7–8.8%) and body mass index was 23.3 ± 2.1 kg/m². All patients were treated with three or four doses of soluble insulin before meals, and with protamine or lente insulin at bedtime. Their mean daily insulin dose was 56.3 ± 1.1% (range 5.7–8.8%) and body mass was 70.2 ± 2.3 kg (range 65.0–75.0 kg). All patients were treated with three or four doses of soluble insulin before meals, and with protamine or lente insulin at bedtime. Their mean daily insulin dose was 56.3 ± 1.1% (range 5.7–8.8%) and body mass index was 23.3 ± 2.1 kg/m². All patients were treated with three or four doses of soluble insulin before meals, and with protamine or lente insulin at bedtime. Their mean daily insulin dose was 56.3 ± 1.1% (range 5.7–8.8%).

No patient had any symptoms of polyneuropathy. Achilles tendon reflexes and vibration sense in the toes were normal. The extensor digitorium brevis muscles were intact. Motor nerve conduction velocity of the right peroneal nerve, measured by standard techniques, was normal (46.0 ± 3.6 m/s; —1.0 ± 0.6 S.D. from the age and body height weighted normal value). The sural sensory nerve conduction velocity was 11.7 ± 0.6 m/s (—0.7 ± 0.5 S.D. from the normal value). All individual neurophysiological parameters were within normal limits.

Autonomic nerve function was assessed in three ways: R-R interval variation during deep breathing at 0.1 Hz (expressed as the ratio between the longest and the shortest R-R interval), the Valsalva ratio (longest R-R interval after a 15-s Valsalva manoeuvre divided by shortest R-R interval during the strain), and R-R interval variation with active movement from lying to standing (the ‘30/15-ratio’). The first of these tests evaluates vagal heart rate regulation, whereas the other two are considered to include both vagal and sympathetic influence on the heart [21]. The resulting ratios were 1.44 ± 0.1, 1.79 ± 0.4 and 1.64 ± 0.3 respectively, i.e. normal values for all participating patients [22,23]. The control group comprised 16 young, lean, healthy, non-smoking volunteers (10 men and six women). Their age was 25.9 ± 2.4 years and their body mass index was 21.5 ± 2.3 kg/m² (not significantly different from the diabetic subjects). The sex ratio was not exactly the same in the patient and control groups, but levels of MSA are similar in male and female subjects [24].

All subjects participated after providing informed consent. The study was carried out in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee at the Medical Faculty of Uppsala University.

Glucose intake

Anhydrous d-glucose (100 g) was dissolved in 300 ml of water [energy content 1750 kJ (420 kcal)] and ingested in the lying position through a straw once the MSA recording position was attained (see below).

Recordings

Subjects lay supine and comfortably in a quiet room with an ambient temperature of approx. 23 °C. For MSA recording, a tungsten microelectrode was inserted manually into the peroneal nerve at the right fibular head, as described in detail previously [3,4,12,14]. Blood pressure was measured continuously by a photoplethysmographic finger cuff method, with the cuff applied to the right middle finger (Finapres®; Ohmeda, Enghed, CO, U.S.A.) [4]. ECG was recorded by chest surface electrodes.

All recorded signals were printed out on-line on paper, and in addition were stored on tape for subsequent analysis (FM tape recorder; Sangamo Weston-Shlumberger, Sarasota, FL, U.S.A.).

General procedure

The subject arrived at the laboratory at 08.00 hours after an overnight fast. No insulin was taken on the morning of the study. An indwelling Teflon catheter was inserted into the left brachial vein. The procedure was continued only if plasma glucose in the diabetic patients was > 3.5 mmol/l, since hypoglycaemia acts as a potent activator of MSA [25,26].

When an electrode site yielding MSA with an acceptable signal-to-noise ratio was found, the activity was monitored at rest for 20 min, whereupon the glucose solution was given over 1–4 min.

In 13 healthy subjects and six diabetic subjects the recording was stable throughout the experimental procedure. Therefore assessment of both burst frequency and amplitude (see below) could be made. In three healthy and two diabetic subjects, leg movements changed the MSA recording position, and hence only burst frequency was assessed.

Blood samples for determination of glucose were taken 15 min before, immediately before, and at 15, 30, 45, 60, 75 and 90 min after glucose ingestion.

In two of the diabetic patients the procedure was repeated with insulin infusion at a rate corresponding to the physiological secretion of insulin in healthy subjects (algorithm according to Hegedus et al. [6]). Otherwise the procedure was identical. This procedure
Analysis procedure

Bursts of MSA were counted in 6-min periods corresponding to the blood sampling occasions, and the outflow was expressed as bursts/min (burst frequency). Heart rate was recorded simultaneously.

The strength of an individual burst is reflected by the burst amplitude in the mean voltage neurogram, and is critically dependent on the intraneural electrode position. Thus it cannot be compared between recordings, but within a given recording assessment of burst amplitude can be made, provided that the electrode position is unchanged [3,25]. The recording remained stable in 13 healthy and six diabetic subjects, thus allowing measurement of burst amplitude at baseline and at the time of maximal outflow of MSA. Thereby the amplitude of 50 consecutive bursts constituted the mean amplitude for the period of analysis; the measurement was made, in arbitrary units, on a digitizing board (Hipad; Houston Instruments, Austin, TX, U.S.A.) connected to a computer.

The course of blood pressure was obtained from the Finapres device, which delivers a continuous pressure signal and numerical values for systolic and diastolic pressure; these are the averages of measurements every 2 s. From ten consecutive values noted every 1–2 min throughout the experiment, a mean value for those periods was obtained. The average of three or four such mean values constituted the individual blood pressure levels for each analysis period. Mean blood pressure was calculated as diastolic pressure + 1/3 x pulse pressure.

Plasma glucose was analysed using a glucose oxidase technique (Beckman model 2; Beckman Instruments, Palo Alto, CA, U.S.A.). Fasting C-peptide was measured by radioimmunoassay (RIA-gnost C-peptide; Svenska Hoechst, Stockholm, Sweden), with a lower detection limit of < 0.05 nmol/l. Haemoglobin A1c (reference range 3.8–5.2%) was measured by FPLC. Microalbuminuria was assessed by immunoturbidimetry. The analyses were carried out in the routine service of the Department of Clinical Chemistry, University Hospital, Uppsala.

Statistical methods

Results are expressed as means ± S.D. The courses of MSA, blood pressure, heart rate and plasma glucose for each group of subjects were analysed by analysis of variance (ANOVA, repeated measures) with Dunnett’s t-test for multiple comparisons. Student’s t-test for unpaired observations was applied when appropriate. A P value < 0.05 was considered statistically significant.

RESULTS

Plasma glucose

The course of plasma glucose for the two groups is displayed in Figure 1. In the diabetic subjects the fasting plasma glucose was relatively high (9.7 ± 6.3 mmol/l), since morning insulin was omitted. Their plasma glucose reached 19.5 ± 5.7 mmol/l at 90 min.

Sympathetic nerve activity

MSA at rest was similar in the control and diabetic subjects, with values of 20.4 ± 4.8 and 19.5 ± 2.4 bursts/min respectively at time 0 (P = 0.6).

In both groups of subjects, glucose ingestion was followed by an increase in MSA, as exemplified in Figure 2 and summarized in Figures 3 and 4. For control subjects the increase was highly significant (P < 0.001) between 15 and 90 min after glucose intake, whereas the diabetic subjects displayed a more moderate, albeit significant, increase between 15 and 75 min (Figure 3). MSA peaked at 30 min, reaching 24.3 ± 3.7 bursts/min for the diabetic patients and 34.4 ± 9.1 bursts/min for the controls. For
Figure 3  Time course of MSA (burst frequency) after glucose intake in 16 healthy subjects (●) and eight patients with type I diabetes (▲)

Upper and lower asterisks indicate the level of significance compared with the value at time 0 for the healthy and diabetic subjects respectively: *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 4  Response of MSA to glucose ingestion

Values represent areas under the curves in Figure 3 for 16 healthy subjects (A) and eight patients with type I diabetes (B) (***P < 0.001). Bar C represents results from two of the diabetic subjects, redisplayed separately, for comparison with results after a second procedure (D), whereby insulin was added at a physiological post-prandial level (see the text). No statistics apply to bars C and D, since n ≠ 2.

comparison of the total response of the two groups of subjects, the areas under the curves of Figure 3 were calculated, from baseline to a vertical line drawn at time 90 min. This area was significantly greater for the controls than for the diabetic subjects (61.5 ± 26.5 and 18.8 ± 14.0 arbitrary units respectively; P = 0.0003; Figure 4, bars A and B).

Both groups also displayed an increase in burst amplitude, with a mean increase of 20 ± 9% in the diabetic patients (n = 6; see the Materials and methods section) and 38 ± 25% in the control subjects (n = 13). The range for the increase in amplitude was wide, and the difference did not reach statistical significance (P = 0.10).

Since the possibility cannot be ruled out that some long-acting insulin was still present in some diabetic patients from the pre-study day, attempts were made to correlate the bedtime insulin dose and the MSA response to glucose, but no such correlation was found.

In the two diabetic patients given an insulin infusion, the increase in MSA burst frequency was twice that obtained in the absence of insulin (mean 50.8 compared with 24.5 arbitrary units). As shown in Figure 4, the mean response to glucose ingestion in these two subjects was similar to that of all diabetic subjects, whereas the increase in MSA on insulin infusion reflected that of the controls.

Blood pressure and heart rate

Mean blood pressure remained stable in the diabetic patients, whereas the control subjects displayed a minor but significant increase towards the end of the experiment (from 91 ± 7 mmHg at time 0 to 95 ± 7 mmHg at 90 min; P < 0.01).

Mean heart rate was 62 beats/min at time 0 and 68 beats/min at 30 min in the control subjects (P < 0.001); the corresponding values for the diabetic subjects were 61 and 64 beats/min respectively (P < 0.05).

DISCUSSION

The main results of the present study were that patients with type I diabetes without polyneuropathy displayed an MSA response to glucose ingestion that was less than half that of a group of healthy controls, and that the level of MSA at rest was similar in the two groups.

Food intake is followed by a marked and sustained increase in MSA. This response is greatest following ingestion of glucose, whereas the response to other nutrients is weaker [4]. Euglycaemic hyperinsulinaemia is also accompanied by enhanced MSA [13–20], suggesting that insulin secretion contributes to the increase in MSA after food intake. The magnitude of the response after insulin is, however, weaker than that after glucose intake [14]. Moreover, ingestion of protein and fat meals [4] or intake of the metabolically inert hexose xylose [3], which are followed by a minor or no insulin response, also causes a significant and sustained increase in MSA, which is also weaker than that after glucose intake. Vehicle (water) ingestion causes no change in MSA [3,4].

Thus MSA seems to be dually stimulated after food ingestion, directly from the gastrointestinal tract and by secreted insulin. This assumption is corroborated by the present observations in C-peptide-negative diabetic patients, showing an increase in MSA after glucose intake in the absence of endogenous insulin. Secondly, the importance of insulin for the normal MSA response is reflected by the diminished increase in MSA in diabetic patients compared with control subjects. The difference between MSA responses in control and diabetic subjects is similar to that in healthy subjects following glucose
compared with protein or fat ingestion [4], the latter eliciting negligible insulin secretion.

In the two patients in whom we could successfully carry out a second investigation with insulin infused at a level corresponding to normal post-prandial physiological secretion, the responses in the absence and presence of insulin corresponded well with the mean responses of the diabetic and control subjects respectively (see Figure 4). This observation is in agreement with that of Hegedüs et al. [6], who reported a low noradrenaline response to oral glucose in patients with type I diabetes and a normalization when insulin was added at a physiological dose. Since our observations are restricted to two subjects, however, conclusions must be cautious.

Delayed gastric emptying is a feature of diabetic autonomic neuropathy [27], but may also be a consequence of hyperglycaemia itself, according to recent observations [28]. Our patients had no signs of neuropathy, but the possibility cannot be ruled out that different time courses of gastric emptying in control and diabetic subjects contributed to a minor degree to the observed differences in MSA responses to glucose intake. The high plasma glucose level in the diabetic subjects and the weak and transient increase in MSA corroborates and reinforces previous observations that hyperglycaemia itself does not stimulate MSA [3,16].

The increase in MSA after food intake is believed to be a compensatory phenomenon, preventing a fall in blood pressure during the post-prandial redistribution of blood [4,24]. Our control subjects displayed a slight increase in blood pressure, which was not observed in the diabetic patients, but the blunted increase in MSA in the latter group was not accompanied by any detectable fall in blood pressure. In subjects with autonomic neuropathy, MSA is scarce and difficult to detect [29]. In this situation the MSA response (and presumably sympathetic outflow to other vessel beds) to both injected insulin and food ingestion is likely to be defective, thereby contributing to post-insulin and post-prandial hypotension [30,31].

The diabetic patients were selected to have intact peripheral and autonomic nerve function, and MSA at rest did not differ between the diabetic and control subjects. Hoffman et al. [32] reported a lowered burst frequency in diabetic subjects without overt polyneuropathy, and interpreted this as a subclinical sign of polyneuropathy (an interpretation that might be questioned [33]). The peripheral nerve function of our patients, documented in detail, was within normal limits. With a rigorous interpretation, the fact that the patients’ conduction velocities were 0.7–1 S.D. below the age- and height-weighted normal values might be regarded as a slight tendency towards reduced nerve function. If so, resting MSA would be expected to be decreased in our patients to conform with the quoted results [32], but we were unable to confirm this observation.

In conclusion, the present study shows that there is a dual stimulation of MSA after food intake, and that the total response is reduced by about one-half in the absence of endogenous insulin secretion. It is also concluded that MSA at rest is similar in healthy subjects and in those with type I diabetes without polynuropathy.

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