Increase in muscle nerve sympathetic activity in humans after food intake

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INTRODUCTION

Ingestion of food induces a number of cardiovascular changes. In young, healthy subjects heart rate and cardiac output increases [1-4], total peripheral blood flow is increased [5], whereas both increased and decreased calf blood flow have been reported [6, 7]. Minor or no changes in blood pressure (BP) have been observed in healthy young subjects after a meal [2, 3, 7], whereas postprandial hypotension is a well-known feature of autonomic failure [8, 9] and can occur also in the healthy elderly subject [10].

A rise in plasma noradrenaline (NA), indicating an increased sympathetic outflow, has been reported by several authors after carbohydrate intake [7, 11-13], but not after other nutrient intake [7, 11, 13]. We have recently reported a strong and sustained increase in muscle nerve sympathetic activity (MSA), recorded by microneurography, after an oral glucose load [14], which might be due to the recently demonstrated stimulating effect of insulin on MSA [15, 16]. However, the physiological cardiovascular responses to a meal, as well as the postprandial hypotension in autonomic failure and elderly people, seem to take place after ingestion of different types of food, but different meal composition may cause differences in the intensity and time course of the evoked responses [2].

The observations mentioned above imply that sympathetic activation takes place after food ingestion, irrespective of its nutrient composition. The present study aimed at characterizing the MSA response, using the microneurographic technique, after different types of nutrient ingestion. The results unequivocally show that every kind of meal is followed by an increase in MSA.

Part of this work was presented at the 21st International Congress of Neurovegetative Research, Bologna, Italy, 5-7 April 1993, and at the 28th Annual Meeting of the Scandinavian Society for the Study of Diabetes, Stockholm, Sweden, 14-16 May, 1993.

Subjects

Recording of MSA was performed in 39 subjects, who were randomly allocated to groups given glucose (n=8, mean age 25.8 years; four females, four males), fat (n=8, mean age 25.5 years; three females, five males), protein (n=8, mean age 25.6 years; three females, five males), a mixed meal (n=8, mean age 26.2 years; two females, six males) or water (n=7, mean age 24.9 years; three females, four males). All subjects were non-smoking, healthy, lean individuals (body mass index \(21.5 \pm 0.3\) kg/m\(^2\), range 17.1-
25.4 kg/m²), who had given their informed consent before the procedure. The recordings were approved by the Ethical Committee of the Medical Faculty of Uppsala University.

Eleven other subjects were excluded from the study. In one a strong desire to void forced the experiment to be interrupted at 40 min after water intake. In another subject, also given water, the recording position was lost at 50 min and not readily refound. The remaining nine exclusions were due to failure to find a sympathetic recording site of acceptable quality.

Food intake

The different meals were composed as follows.
(1) Glucose meal. Anhydrous D-glucose (100 g) was dissolved in 300 ml of water. The energy content was calculated to be 1750 kJ.
(2) Fat meal. Vegetable cooking oil (50 ml) was mixed with 250 ml of water. The energy content was calculated to be 1700 kJ.
(3) Protein meal. Highest quality fillet of beef was cut into minute pieces and was lightly fried in a teflon frying pan (no fat or spices were added); after frying 100 g was taken with 250 ml of water. The dry weight of this portion of meat was 40 g, and the energy content was calculated to be about 700 kJ. This meant that the glucose, fat and protein meals were similar with respect to volume, but the energy content of the protein meal was lower (an isocaloric protein meal would amount to 250 g of fried meat, which was not considered possible to ingest).
(4) Water (control experiment). Water (300 ml) i.e. isovolaemic with (1-3), was given.
(5) Mixed meal. Creamed potatoes (including milk) with a sauce containing minced beef and chopped tomatoes, onion, salt and pepper were eaten. The wet weight of the portion was 400 g and its energy content was 1750 kJ (44% carbohydrate, 36% fat and 20% protein). In addition, 200 ml of water was taken during this meal. The composition of the meals followed the advice of a dietician.

Recordings

MSA was recorded from the right peroneal nerve at the fibular head by means of microneurography. Details of the recording procedure are described elsewhere [14]. In brief, subjects were lying comfortably on a bed. A tungsten microelectrode (tip diameter 5 μm) was inserted through the unanaesthetised skin. Electrical stimuli were applied through the needle, which was advanced manually towards the nerve, until muscle twitches were evoked. Then minor adjustments of the electrode position were made until the characteristic pattern of multi-unit MSA was encountered. The evidence that this activity is of sympathetic origin is as follows: the impulses are efferent, as shown by application of a local anaesthetic agent proximal and distal to the recording site; the activity is temporarily abolished after intravenous injection of a ganglion-blocking agent; the signals are conveyed at a conduction velocity of about 1 m/s; and the activity displays a close dynamic relationship with BP variations [17].

The nerve signal was amplified in two steps, with a total gain of × 50,000, and was fed through a 700-2000 Hz band pass filter and an amplitude discriminator for optimal signal-to-noise ratio. A resistance-capacitance integrating network (time constant 0.1 s) delivered a mean voltage neurogram, which was used for display and analysis of the recordings (c.f. Fig. 1). During the experiments the nerve signal was monitored on a storage oscilloscope and fed through a loudspeaker.

An ECG was recorded by chest surface electrodes. BP was measured continuously and non-invasively by a photoplethysmographic finger-cuff method, with the cuff applied to the right middle finger (Finapres; Ohmeda, Englewood, CO, U.S.A.) [18, 19].

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

During the experiment the nerve the subject may experience minor discomfort, but once a recording position is found and the electrode is left in this position nothing is felt. Minor and shortlasting paraesthesiae within the innervation area of the nerve are regularly felt by about 10% of subjects [20], and this was reported by seven subjects in a routine follow-up letter.

General procedure

The subjects arrived at the laboratory at 08.00 hours after an overnight fast. Since bladder distension causes an increase in MSA [21], all subjects voided immediately before the experimental procedure. An indwelling teflon catheter for blood specimens was inserted in the left brachial vein. When the BP cuff was applied, the search for a nerve recording site, which took 5-50 min, started (the search for a recording site was abandoned at 60 min in nine subjects, see above).

When a sympathetic recording site with an acceptable signal-to-noise ratio was found, the activity was monitored during 20 min of initial rest, and thereafter the food or water was given to the subject. This meant that the subjects, in the supine position, were given the solid food (protein and the mixed meal) by feeding with a spoon and the fluid through a straw. Some subjects exhibited electromyographic activity that disturbed the nerve recording during eating, but in most cases the recording site was unchanged through the feeding procedure. The fluid meals were finished within 1-2 min (in one subject receiving glucose, 4 min), and
the meals with solid food within 7–11 min (except for one subject who needed 16 min). Then the recorded signals were followed until 90 min after the start of feeding.

Blood samples for determination of glucose, insulin and packed cell volume were taken twice (with a 15 min interval) before feeding, and then at 15, 30, 45, 60, 75 and 90 min after the start of eating.

Analysis procedure

The sympathetic activity was identified by inspection of a 2 mm/s paper display of recorded signals. Bursts of MSA were counted manually over 6 min periods corresponding to the blood sampling occasions, and the outflow for the period in question was expressed as bursts/min for each subject.

The general course of BP was obtained from the Finapres device, which delivered a continuous BP signal and numerical values for systolic and diastolic BP, these being the average of measurements every 2 s. From ten consecutive BP values noted during each minute throughout the experiment a mean value for that minute was calculated. The average of 3–4 consecutive such BP values constituted the individual BP level for each analysis period of 7.5 min (see Fig. 6).

Plasma glucose was measured with a glucose oxidase technique (Beckman model 2; Beckman Instruments, Inc., Palo Alto, CA, U.S.A.). Plasma insulin was measured by radioimmunoassay, and blood packed cell volume was measured by the routine method of the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.

Statistical methods

The general courses of MSA, BP, heart rate, plasma glucose, plasma insulin and packed cell volume for each group of subjects were analysed by analysis of variance with Dunnett's t-test for multiple comparisons. Linear regression and t-test for unpaired variables were applied when appropriate.

No significant changes occurred for any of the variables during the initial control period. Time 0 (c.f. Figs. 2 and 5–8) was chosen as the point of reference when changes after food intake were assessed. Results are expressed as means ± SEM.

RESULTS

MSA

The group receiving oral glucose displayed the highest basal outflow of MSA at rest (20.6 ± 2.1 bursts/min at time 0, c.f. Fig. 2), which was significantly higher than that of the group receiving protein (11.9 ± 2.3 bursts/min; P < 0.05, t-test for unpaired variables), but not significantly different from the groups receiving fat (15.6 ± 4.0 bursts/min), water (15.4 ± 2.3 bursts/min) or the mixed meal (14.6 ± 2.7 bursts/min). The wide range of MSA at supine rest, well known from basic studies [22, 23], inevitably means that significantly different levels of activity will sometimes randomly be found between appropriately selected control groups.

During the intake of protein and the mixed meal, i.e. the solid foods requiring an extended time for eating, MSA tended to increase, but this increase did not reach statistical significance. During the intake of fluids no effect was seen on MSA.

There was an increase in MSA after intake of each kind of nutrient, as shown in Fig. 1 and summarized in Figs. 2 and 3. This increase was seen in every subject receiving any kind of nutrient, except for one subject receiving the fat meal, whose outflow of MSA was unchanged throughout the 90 min monitored. Glucose intake evoked the fastest and most pronounced response (in one case being already obvious at 7 min after intake) with the mean response being significant at 15 min. The response to glucose peaked at 30 min, with a subsequent slight decrease, but the increase was still strongly significant (P < 0.001) at 90 min (Fig. 2). The responses to
fat and protein were more flat, with a sustained maximum at 60–75 min (Fig. 2). The mixed meal elicited a flat response like those to fat and protein, its increase being between that after glucose and those after fat or protein intake (Fig. 2). The increase was still significant at 90 min after each kind of nutrient (Fig. 2). After water intake MSA remained stable throughout the 90 min studied.

The enhancement of MSA after all nutrients given also included an increase in burst amplitude (c.f. Fig. 1), which from inspection by eye could be estimated to at least 25%. No exact measurement of this increase in mean amplitude was performed, since the general response of MSA is already clearly expressed by the increase in number of bursts/min.

For comparison of the strength of the response during the observation time of 90 min after ingestion, the area under the curve was calculated (from a baseline drawn at the value of time 0) for all subjects. This procedure was considered appropriate for this purpose despite the fact that the values had not returned to baseline at 90 min; the area was limited by a vertical line at time 90 min. This calculation confirmed the above-mentioned impression that the response to glucose intake was more pronounced than those to protein and fat intake (Fig. 3), whereas the response to the mixed meal did not differ significantly from any of the others.

Since the group given glucose had a higher basal outflow of MSA than the other groups, the basis for the different degree of response might be an artefact, individuals with a higher output at rest simply responding stronger to food intake. This possibility was ruled out by the lack of any relationship between the individual level of activity at rest and the individual response after nutrient intake (Fig. 4).

**Heart rate**

There was a significant increase in heart rate after intake of all types of food. The course of this increase was sustained, with a tendency to reach a maximum at 90 min (Fig. 5), i.e. at the end of the experimental session.

The act of eating the solid foods was also accompanied by a highly significant transient increase in heart rate (mean heart rate for the two groups receiving solid food was $59.4 \pm 2.0$ beats/min at time 0 and $70.5 \pm 2.3$ beats/min during eating. $p<0.001$; this increase subsided before 15 min and is consequently not shown in Fig. 5).
occurred during the sessions, the significant changes in BP were evident when the subject ingested solid food. There was also a significant transient increase in BP during the eating period, as exemplified in Fig. 6. No relationship was seen between the general course of BP and the increase in MSA after nutrient intake was seen.

**Blood chemistry**

As anticipated, plasma glucose rose considerably after glucose and the mixed meal, whereas protein, fat and water intake were followed by minor or no changes in plasma glucose (Fig. 7). Similarly, intake of glucose and the mixed meal caused a strong increase in plasma insulin. Protein ingestion was followed by a late slight, but significant, increase in plasma insulin, whereas fat and water intake caused no significant changes (Fig. 8).

By inspecting the courses of plasma glucose and plasma insulin (Figs. 7 and 8) and comparing them with the courses of MSA (Fig. 2) and the areas under the curve for MSA (Fig. 3), it can be deduced that neither a rise in plasma glucose nor an increase in plasma insulin was a prerequisite for an increase in MSA. However, the increase in MSA was stronger when high plasma insulin levels were present, as displayed by the responses to glucose and the mixed meal.

Packed cell volume increased from 39.1 ± 1.7% at time 0 to 39.7 ± 1.6% (P < 0.05) at 15 min and to 40.6 ± 1.7% (P < 0.01) at 30 min for the group receiving glucose. Otherwise, there were no significant changes on any occasion.

**DISCUSSION**

The main findings in the present study are, first, that MSA, consisting of baroreflex-governed vasoconstrictor signals involved in cardiovascular homeostasis [17], displays a pronounced and sustained increase after a carbohydrate, protein, fat or mixed meal, and secondly, that insulin secretion is not a prerequisite for this increase, although the response tends to be stronger when insulin secretion is stimulated.

During microneurography the subject has to lie almost immobile (small hand and head movements are allowed), and a long experimental procedure thus entails the risk of an increasingly uncomfortable position. This may act as an artefactual stimulant of MSA [24] and might play a role in the late part of a sustained increase in MSA. The late increase in heart rate in many subjects (Fig. 5) implies that discomfort was present at the end of the experimental session. However, since MSA was stable throughout the 90 min after water ingestion (Fig. 2), it is unlikely that this potential error was of importance in the present study.

MSA is under the control of volume receptors as well as high-pressure baroreceptors [25], and changes in plasma volume after the nutrient intake and the blood samples taken must be considered. The same amount of blood was withdrawn in all experiments, and the lack of change in MSA in the control experiments with water ingestion, rules out the possibility of a volume effect due to blood loss. Packed cell volume is considered to be an approximate measure of plasma volume variation in situations with constant erythrocyte mass [26], and the lack of changes in packed cell volume after all the types of meals makes a fall in plasma volume highly unlikely as a contributing factor to the observed response in MSA (the significant changes on two occasions for the glucose group only is not regarded as indicating a real change in plasma volume). The same conclusion was reached in a previous study on MSA after an oral glucose load [14]. Thus it can be concluded that the increase in MSA is directly related to the intake of food.

The non-significant increase in MSA during the act of eating solid food (protein and the mixed meal), which was considerably less than the late increases seen after the ingestion of all nutrients, does not justify any conclusion about a causal relationship. Most subjects exhibited a tendency to electromyographic disturbance of the neurogram during eating, indicating that the artificial and probably stressful way of eating induced muscle tension. Since static muscle work is known to cause a rise in MSA [27], this might be the explanation for
the non-significant increase in MSA during the eating of solid food. It is worth noting, however, that a significant increase in BP and heart rate during eating was not accompanied by a reduction in MSA, but instead, if anything, by a tendency to increase. These relationships indicate a rapid and transient resetting of the baroreflex working range.

Skin nerve sympathetic activity (SSA), the other subdivision of the sympathetic nervous system that can be studied with microneurography, was not included in the present study. No response of SSA to food intake is expected, since no change in SSA occurs either after a glucose meal (giving rise to the strongest MSA response) or after insulin stimulation without concomitant hypoglycaemia [14, 16]. This difference between the MSA and SSA responses to nutrient intake emphasizes that the present observations must not be generalized to other parts of the sympathetic nervous system [17].

The individual level of MSA correlates with plasma noradrenaline [28], and this correlation is
still seen during some manoeuvres [29, 30]. Therefore the strong increase in MSA after a glucose and mixed meal was expected, since NA elevation has been reported by several investigators after such meals [11–13, 31–33]. On the other hand, studies on protein and fat meals have, with few exceptions [33], shown no increase in NA [7, 11, 13, 32], a finding apparently not consistent with the increase in MSA seen in the present study. Two possible explanations can be put forward for this discrepancy. The present study shows that the MSA response to fat and protein is weaker and more sluggish than that to glucose, and this may explain why an increase in NA has rarely been detected after protein and fat meals. Secondly, the insulin effect involved in the response to glucose and mixed meals (c.f. below) might stimulate sympathetic outflow to other organs and vascular beds, thereby contributing to a more easily detectable rise in NA. Since recording of MSA is a direct monitoring of sympathetic nerve traffic, whereas measurement of NA is an indirect assessment, the present results are considered to represent the true response of this particular part of the sympathetic nervous system to intake of different nutrients.

The mechanism behind this sympathetic stimulation is unclear. BP was monitored continuously throughout the experiments, and both systolic and diastolic BP remained stable or were occasionally slightly but significantly increased (see Fig. 6). Thus the possibility that the increase in MSA could be an expression of baroreflex compensation for a threatened fall in BP seems to be ruled out; the same conclusion was drawn in a previous study of the MSA response to an oral glucose load [14], as well as in studies of the MSA response to euglycaemic hyperinsulinaemia [15, 16].

Insulin has been suggested to play a key role in the cardiovascular responses to feeding. There is evidence for an effect of insulin on the postprandial hypotension seen in autonomic failure [9, 34, 35], and insulin injection in patients with diabetic autonomic neuropathy has been reported to cause hypotension [36]. Insulin infusion without a change in blood glucose has been shown to increase plasma NA [37], and further support for a role of insulin came recently from three independent observations of an increase in MSA during euglycaemic hyperinsulinaemia [15, 16, 38]. A positive correlation between endogenous insulin secretion and increase in MSA was observed after a glucose meal [14]. However, the MSA response to oral glucose intake was found to be considerably stronger than that to physiological and supraphysiological euglycaemic hyperinsulinaemia [16], an observation that is in agreement with the present study, which clearly shows that insulin secretion is not required for a postprandial increase in MSA. The difference in strength between the response to glucose and the responses to fat and protein meals (c.f. Fig. 2 and 3) might represent the effect of insulin per se. The intermediate response of the mixed meal is also in accordance with such an interpretation.

This stimulation of MSA when a nutrient enters the gastrointestinal tract is likely to play a role in the redistribution of blood that occurs after a meal. A rapid increase in mesenteric blood flow has been reported to follow different test meals [39], some investigators reporting that the response to a carbohydrate meal is faster but not certainly stronger than that to protein and fat meals, both in healthy young [5, 7] and elderly subjects [40]. On the other
hand, drinking an isovolaemic amount of water did not change mesenteric blood flow [5, 39]. An increase in MSA, i.e. a vasoconstrictor command to muscle vessels, is functionally logical when mesenteric blood flow is rising after a meal. The lack of change in both MSA and mesenteric blood flow after water ingestion reinforces the functional relationship and suggests that the response is dependent on the chemical composition of what has been ingested, and not on its volume [5]. The precise mechanism behind the activation of MSA remains unexplained. A fall in BP would be a possible consequence of splanchnic vasodilatation, but as discussed above no reduction in BP was observed. Qamar and Read [5] reported time courses for the rise in mesenteric circulation after different types of food that were similar to the time courses for the increase in MSA observed in the present study. Such a detailed time relationship might indicate the presence of a directafferent signal flow from dilating mesenteric vessels [41] or from nutrient-sensitive gastrointestinal receptors [42] to central sympathetic motor neurons. Alternatively, neuropeptides like vasoactive intestinal peptide and somatostatin may play a role [9, 33]. However, few data are available on such influences, and the suggested mechanisms remain conjectural so far.

Even if the postprandial increase in MSA does not seem to be due to a fall in BP, its occurrence is presumably of practical importance for the prevention of postprandial hypotension. It seems reasonable to assume that postprandial hypotension in autonomic failure [8, 9] occurs due to the lack of this response of MSA (and possibly parallel sympathetic outflow to other essential vessel beds) after food intake.

Postprandial hypotension can also be a problem in seemingly healthy elderly subjects [10]. A fall in BP after nutrient intake has been demonstrated in old people, the reduction being more marked after glucose than after fat ingestion [33, 40]. Potter et al. [32] observed a fall in BP after glucose and protein meals, but not after fat intake. Basal MSA increases with age [23, 43, 44], which might mean that the capacity for a further, temporary increase is blunted, thereby allowing a fall in BP. Studies of MSA responses in the healthy, elderly population are needed to shed further light on these phenomena.

Since MSA is a vasoconstrictor command to the muscle vessels, a reduction in calf blood flow would be an expected consequence. A decrease in limb blood flow has been reported after carbohydrate and fat meals [7, 40], but an increase after nutrient ingestion has been observed as well [6, 45]. This inconsistency still awaits explanation; varying vasodilating influence from insulin and vasoactive peptides might play a role. An increase in MSA with a simultaneous, seemingly contradictory, increase in limb blood flow has been observed in hypoglycaemia [30] and euglycaemic hyperinsulinaemia [15].

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