The Cephalic Insulin Response to Meal Ingestion in Humans Is Dependent on Both Cholinergic and Noncholinergic Mechanisms and Is Important for Postprandial Glycemia

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We studied the mechanisms and physiological relevance of the cephalic insulin response to meal ingestion in 12 healthy women (age 63 ± 0.4 years; BMI 27.7 ± 1.7 kg/m²). The ganglionic antagonist, trimethaphan, which impairs neurotransmission across parasympathetic and sympathetic autonomic ganglia, or atropine or saline was given intravenously during the first 15 min after ingestion of a standard meal (350 kcal). During saline infusion, insulin levels increased during the first 10 min after meal ingestion, whereas the first increase in glucose was evident at 15 min. The preabsorptive 10-min insulin response was reduced by 73 ± 11% by trimethaphan (P = 0.009), accompanied by impaired reduction of glucose levels from 25 to 60 min after meal ingestion (dGlucose = −1.27 ± 0.5 [with saline] vs. 0.1 ± 0.4 mmol/l [with trimethaphan]; P = 0.008). This reduction at 25–60 min in glucose levels correlated significantly to the 10-min insulin response (r = 0.65, P = 0.024). The 10-min insulin response to meal ingestion was also reduced by atropine, but only by 20 ± 9% (P = 0.045), which was lower than the reduction with trimethaphan (P = 0.004). The preabsorptive insulin response was not accompanied by any increase in circulating levels of gastric inhibitory polypeptide (GIP) or glucagon-like peptide 1 (GLP-1). In conclusion, 1) the early preabsorptive insulin response to meal ingestion in humans can be largely attributed to autonomic activation mediated by noncholinergic and cholinergic mechanisms, 2) this cephalic insulin response is required for a normal postprandial glucose tolerance, and 3) GIP and GLP-1 do not contribute to the preabsorptive cephalic phase insulin response to meal ingestion. Diabetes 50:1030–1038, 2001

The preabsorptive or cephalic phase insulin response, which lasts for ~10 min, is initiated by meal ingestion, as has been demonstrated in humans and rats (1–9). It is abolished by vagotomy in rats (1) and by atropine in rats (1) and humans (10), suggesting mediation by cholinergic mechanisms. However, the mechanism is probably more complex than that executed solely by cholinergic actions on islet β-cells; noncholinergic mechanisms might also contribute to the response. For example, islet parasympathetic nerves harbor several neuropeptides in addition to acetylcholine, such as vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase–activating polypeptide (PACAP), and gastrin-releasing polypeptide (GRP) (2). These neuropeptides are released after vagal nerve activation of the pancreas (11–13) and stimulate insulin secretion (2,14). Prevention of their effects inhibits the insulin response to oral administration of glucose in mice (15–17). It is also possible that the gut hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), contribute to the cephalic phase insulin response to meal intake, as both these hormones are potent insulin secretory hormones released during meal ingestion (18) and their secretion is under neural control (19–21). Finally, there is a problem in the interpretation of atropine data in previous studies, as atropine reduces postprandial glycemia, thereby also indirectly influencing insulin secretion (10). Therefore, the mechanism of the cephalic phase insulin response to meal ingestion has not been established in humans.

It has recently been established that the early insulin response to meal ingestion is of great importance for subsequent glucose tolerance. This was first suggested by results demonstrating a negative correlation between the 30-min insulin response to oral glucose, as a marker for early insulin secretion, and the 120-min glucose value, as a marker of glucose tolerance (22). Furthermore, prevention of the early insulin response by somatostatin results in glucose intolerance (23), and sham feeding, which increases circulating insulin, improves glucose tolerance after intragastric glucose (9). The importance of the early insulin response for postprandial glucose tolerance is also illustrated by studies reporting that brief administration of a minute amount of insulin during the first 15 min after
food intake markedly improves glucose tolerance in obese (10) and type 2 diabetic subjects (24). Whether specifically the neurally mediated cephalic insulin response to meal ingestion is of importance for postprandial glucose homeostasis has, however, not been established.

The current study was designed to explore the mechanism and significance of the preabsorptive insulin response to meal ingestion in humans. Healthy overnight fasted subjects were given a standard meal in the presence of the ganglionic blocker, trimethaphan, or the muscarinic antagonist, atropine. Trimethaphan inhibits neurotransmission across autonomic ganglia (25) and therefore blocks the neural influences of islet function. Its usefulness for studies of neural regulation of islet function in humans was shown in a previous study, in which we explored the neural contribution to the islet hormone responses to insulin-induced hypoglycemia (26). By comparing the results obtained with trimethaphan with those obtained with saline, the contribution of neural influences to the insulin response to meal ingestion could be established; furthermore, by comparing results with trimethaphan with those of atropine, the relative contribution of cholinergic versus noncholinergic neural mechanisms could be established. We also determined the GIP and GLP-1 responses to meal ingestion with or without administration of trimethaphan or atropine to examine whether any cephalic phase response of these incretins might contribute to the cephalic phase insulin response. Finally, examining glycaemia in the presence or absence of trimethaphan, the importance of the neurally mediated insulin response to meal ingestion for postprandial glycaemia could be established.

RESEARCH DESIGN AND METHODS

Subjects. We recruited 18 healthy postmenopausal women from a large population screened for the prevalence of diabetes in Malmö, Sweden (27). All subjects had normal glucose tolerance, as judged by results of a 75-g oral glucose tolerance test performed before entry in the study and measured according to World Health Organization criteria. All subjects were in good health and not taking any medication. Of the 18 subjects, 12 underwent two separate meal experiments in random order, with 4–6 weeks between studies. In 6 of those 12 women, the two studies were a meal ingestion with infusion of saline or trimethaphan (protocol 1), whereas in 6 other women, the two studies were a meal ingestion with infusion of saline or atropine (protocol 2). In six other healthy women with normal glucose tolerance, the effect of trimethaphan or atropine on the insulin secretory response to arginine was assessed (protocol 3). The study protocol was approved by the ethics committee of the Faculty of Medicine, Lund University. Written informed consent was obtained from all participants.

Experimental protocols 1 and 2. In protocol 1, the effect of trimethaphan on the meal-induced insulin secretion was examined; subjects' mean age was 63 ± 0.4 years and mean BMI was 27.6 ± 2.1 kg/m² (means ± SD). In protocol 2, the effect of atropine was examined; those subjects' mean age was 64 ± 0.3 years and mean BMI was 27.8 ± 1.8 kg/m². Studies began at 0800, after subjects fasted overnight. Indwelling catheters were inserted into the antecubital veins of both arms. One arm was used for the infusion of saline, trimethaphan, or atropine, whereas the contralateral arm was used for intermittent blood sampling. The catheters were kept patent with the slow infusion of 0.9% saline. Blood pressure was recorded regularly during the experiments. Following two baseline samples, an intravenous infusion of saline, trimethaphan (Hoffman LaRoche, Nutley, NJ), or atropine (NM Pharma, Stockholm, Sweden) was given for 30 min. The initial trimethaphan infusion rate was 0.3 mg · kg⁻¹ · min⁻¹ at a volume infusion rate of 55 ml/h. This infusion rate was kept constant in three women, who displayed a reduction in systolic blood pressure by ~10 mmHg. In another three women, the infusion rate was increased after 10 min to 0.45 mg · kg⁻¹ · min⁻¹ when those women showed a reduction in systolic blood pressure by ~10 mmHg. Atropine was given as a bolus injection (0.5 mg/kg) followed by a constant infusion of 0.01 mg · kg⁻¹ · min⁻¹. After 15 (trimethaphan) or 10 min (atropine) of infusion, a standard breakfast was served, consisting of two slices of bread, 10 g margarine, 10 g marmalade, a slice of cheese containing 15% fat, and a cup of black coffee (350 kcal with 28, 22, and 50% of energy coming from protein, fat, and carbohydrate, respectively). The breakfast was ingested during 5 min.

Blood was sampled throughout the following 120-min study period. Infusions of saline, trimethaphan, or atropine were stopped at 15 min.

Experimental protocol 3. In six women (mean age 58 ± 1.0 years; mean BMI 25.9 ± 1.5 kg/m²), the effect of trimethaphan or atropine on the acute insulin response to arginine was studied to determine whether trimethaphan and atropine block the beta-cell secretion. The women fasted and catheters were inserted as described above. Saline, trimethaphan (0.3 mg · kg⁻¹ · min⁻¹ in four subjects, 0.45 mg · kg⁻¹ · min⁻¹ in two subjects), or atropine (bolus injection of 0.5 mg/kg followed by a constant infusion of 0.01 mg · kg⁻¹ · min⁻¹) was infused intravenously. After 15 (trimethaphan) or 10 min (atropine), and after taking two baseline blood samples at −5 and −2 min, 5 g of arginine hydrochloride was administered intravenously, and new blood samples were collected at 2, 3, 4, and 5 min.

Assays. Blood samples for plasma analysis of glucose, pancreatic polypeptide (PP), epinephrine, GIP, and GLP-1, and for serum analysis of insulin were immediately centrifuged at 5°C and serum frozen at −20°C (for epinephrine, at −70°C) until analyzed in duplicate. Serum insulin concentrations were analyzed by a double-antibody radioimmunoassay technique. Guinea pig anti-human insulin antibodies, human insulin standard (Linco Research, St. Louis, MO) and aprotinin (250 KIU/ml blood; Sigma Chemical, St. Louis, MO) were used. With the insulin antibody, the intra-assay CV was 4% and the inter-assay CV was 5%. Samples for analysis of GIP and GLP-1 were obtained in prefilled test tubes containing EDTA (2.8 mmol/l blood; Sigma Chemical, St. Louis, MO) and aprotinin (250 KIU/ml blood; Bayer AG, Leverkusen, Germany). Analyses of GIP concentrations were performed with a double-antibody radioimmunoassay technique using rabbit anti-human GIP antibodies (Bios), 125I-labeled human GIP, and human GIP standard (28). The antibody used crossreacts fully with human GIP, but not with 8-kDa GIP, the nature and relationship of which to the synthesis or secretion of GIP is still unclear (29). The intra-assay CV was 6% and the inter-assay CV was 8–12%. Plasma concentrations of GLP-1 were measured with a radioimmunoassay after extraction with ethanol, as previously described (30). The antisera (code no. 89990) is directed against the amidated COOH-terminus of GLP-1, and therefore mainly measures GLP-1 of intestinal origin. The intra-assay CV was 6% and the inter-assay CV was 8–12%. PP levels were determined with a double-antibody radioimmunoassay using rabbit anti-human PP antibodies (Linco), 125I-labeled human PP, and human PP standard (Peninsula Labs, Merseyside, U.K.). The intra-assay CV was 8% and the inter-assay CV was 10%. Plasma epinephrine levels were determined by high-performance liquid chromatography, as previously described (31,32). The intra-assay CV was 4% and the inter-assay CV was 5%. Plasma glucose concentrations were determined using the glucose-oxidase method. All samples were analyzed in duplicate, and the mean values for each time point are given in the results.

Statistical analysis. Data are presented as means ± SE unless otherwise noted. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago) for Windows system. The areas under the curve (AUCs) for the suprabasal glucose, insulin, GIP, and GLP-1 levels were calculated by the trapezoid rule. Differences in AUCs among the experimental series were evaluated with Student's t test for paired samples or the Mann-Whitney U test for unrelated samples. Differences among individual time points were evaluated by analysis of variance with the Bonferroni post hoc test.

RESULTS

Experimental protocol 1 (trimethaphan) Blood pressure. Systolic blood pressure decreased from 129 ± 6 to 115 ± 5 mmHg and diastolic blood pressure from 82 ± 3 to 74 ± 4 mmHg (P = 0.036) during the 15-min trimethaphan infusion before meal intake (P = 0.018), but did not change significantly with saline infusion. In contrast, heart rate was not altered during trimethaphan infusion, changing from 76 ± 4 beats/min before start of trimethaphan vs. 78 ± 5 beats/min after 10 min of infusion. Similarly, heart rate was not altered during saline infusion. Following meal ingestion, no significant change in blood pressure or heart rate was observed in any of the series. Insulin and glucose. Figure 1 shows that baseline serum insulin and plasma glucose levels did not change signifi-
significantly during the 15-min infusion of trimethaphan or saline before ingestion of the meal. After meal ingestion, in the control experiments, serum insulin increased significantly over premeal values at 5 (by 12.9 ± 3.8 pmol/l; \( P = 0.008 \)), 7 (by 14.6 ± 2.8 pmol/l; \( P = 0.012 \)), and 10 min (by 14.8 ± 4.0 pmol/l; \( P = 0.026 \)), whereas plasma glucose did not start to increase significantly until 15 min (by 0.6 ± 0.2 mmol/l; \( P = 0.034 \)). In contrast, after trimethaphan infusion, no significant increase in serum insulin was observed during the initial 15 min after meal ingestion, and at 5, 7, and 15 min after meal ingestion, serum insulin levels were lower in the presence of trimethaphan than in the control series (\( P < 0.05 \)). At 25 min, serum insulin levels did not differ significantly between the series. The suprabasal AUC\(_{\text{insulin}}\) for the preabsorptive 10 min after meal ingestion was 15.4 ± 3.2 nmol/l \( \times \) 120 min in the control series vs. 15.4 ± 1.8 nmol/l \( \times \) 120 min in the trimethaphan series (NS). The fractional insulin response during the first 10 min in relation to the entire 120-min insulin response was 0.80 ± 0.22% in the control series, which was reduced to 0.15 ± 0.05% by trimethaphan (\( P = 0.040 \)). Plasma glucose did not differ significantly during the first 25 min after meal ingestion between the two series. However, the postprandial change of glucose levels from 25 to 60 min was inhibited by trimethaphan, reaching 2.1.3 ± 0.5 mmol/l in the control series versus 0.1 ± 0.4 mmol/l in the trimethaphan series (\( P = 0.008 \)). The AUC\(_{\text{insulin}}\) for the initial 10 min after meal ingestion correlated inversely with this change in glucose levels (\( r = -0.65, P = 0.024 \) (Fig. 2).

**GIP and GLP-1.** Figure 3 shows that baseline levels of GIP or GLP-1 were not affected by trimethaphan infusion. After meal ingestion, GIP levels did not increase significantly during the first 10 min in either of the two series. In the control series, the first significant increase in GIP levels was observed after 15 min (by 15.3 ± 5.2 pmol/l; \( P = 0.041 \)) whereas in the trimethaphan series the increase after 15 min was not significant (7.3 ± 5.0 pmol/l; \( P = 0.36 \)). The increase in GIP levels at min 25 was significantly reduced by trimethaphan (55.0 ± 9.0 [control series] vs. 38.6 ± 6.5 pmol/l [trimethaphan series], with a reduction of 26.4 ± 11.6%; \( P = 0.046 \)). The 15-min GIP and insulin levels displayed significant correlation (\( r = 0.70, P = 0.011 \)). GLP-1 levels did not show any significant change during the first 10 min after meal ingestion, but increased thereafter in both experiments. However, the GLP-1 response to meal intake was not affected by trimethaphan.

**PP and epinephrine.** Figure 4 shows that trimethaphan reduced baseline PP levels from 128 ± 12 to 96 ± 9 pmol/l (\( P = 0.031 \)). The initial 10-min PP response to meal ingestion was 15.4 ± 3.2 nmol/l \( \times \) 120 min in the control series vs. 15.4 ± 1.8 nmol/l \( \times \) 120 min in the trimethaphan series (NS). The fractional insulin response during the first 10 min in relation to the entire 120-min insulin response was 0.80 ± 0.22% in the control series, which was reduced to 0.15 ± 0.05% by trimethaphan (\( P = 0.040 \)). Plasma glucose did not differ significantly during the first 25 min after meal ingestion between the two series. However, the postprandial change of glucose levels from 25 to 60 min was inhibited by trimethaphan, reaching 2.1.3 ± 0.5 mmol/l in the control series versus 0.1 ± 0.4 mmol/l in the trimethaphan series (\( P = 0.008 \)). The AUC\(_{\text{insulin}}\) for the initial 10 min after meal ingestion correlated inversely with this change in glucose levels (\( r = -0.65, P = 0.024 \) (Fig. 2).

**POSTPRANDIAL CEPHALIC INSULIN RESPONSE**

![FIG. 1. Serum insulin and plasma glucose levels in six healthy women subjected to a 30-min intravenous infusion of the ganglionic antagonist, trimethaphan, or saline (from -15 to +15 min). At time 0, a standardized meal was served. Small insert in upper panel shows the insulin levels between 0 and 10 min. Data shown are means ± SE. *\( P < 0.05 \), **\( P < 0.01 \) for random differences between groups for the various time points as revealed by analysis of variance (ANOVA).](image1)

![FIG. 2. Correlation between the AUC\(_{\text{insulin}}\) during the first 10 min after meal ingestion and the change in plasma glucose at 25–60 min after meal ingestion in six healthy women subjected to a 30-min intravenous infusion of the ganglionic antagonist, trimethaphan, or saline (from -15 to +15 min). At time 0, a standardized meal was served. The correlation coefficient was \( r = -0.65, P = 0.024 \).](image2)
ingestion, which was 28 ± 6 pmol/l in the control series (P < 0.018), was completely abolished by trimethaphan. In contrast, the subsequent slight increase in circulating PP was not different between the two series, although the absolute levels were constantly higher in the control series (P, 0.05 or less). Plasma epinephrine levels, which were 0.09 ± 0.03 nmol/l at baseline in the control series, were not altered by meal ingestion. Trimethaphan did not alter epinephrine levels in the baseline condition or after meal ingestion.

**Experimental protocol 2 (atropine)**

**Blood pressure.** Systolic or diastolic blood pressures did not change during the 10-min premeal treatment with atropine or saline during or after meal ingestion. However, atropine slightly increased the heart rate from 71 ± 5 to 85 ± 7 beats/min (P = 0.011) during the 10 min preceding meal intake. Thereafter, heart rate was stable and unaltered throughout the experiment.

**Insulin and glucose.** Figure 5 shows that atropine did not alter basal levels of insulin and glucose. Also, in this experimental protocol, there was a preabsorptive increase in insulin levels during the first 10 min after meal ingestion, as insulin levels increased over basal in the control experiments at 3, 5, 7, and 10 min (P < 0.03), whereas the first significant glucose change was observed at 15 min. Atropine reduced the preabsorptive 10-min AUCinsulin from 130.0 ± 13.6 to 104.3 ± 13.6 pmol/l × 10 min, which was equivalent to a reduction of 20 ± 9% (P = 0.045). This reduction was significantly lower than the 73 ± 11% reduction in the 10-min preabsorptive insulin response induced by trimethaphan in protocol 1 (P = 0.004). After the initial 15 min, the glucose increase was significantly lower with atropine than with saline, which resulted in a corresponding reduction in the increase in insulin levels, resulting in significantly lower circulating levels of glucose at 25 and 45 min and of insulin at 15 and 25 min (P < 0.05) in the presence of atropine versus saline. The AUCglucose or AUCinsulin over the entire 120-min period did not differ significantly between the series (Table 1).

**GIP and GLP-1.** Baseline GIP and GLP-1 were not affected by atropine (Fig. 6). After meal ingestion, GIP levels did not increase significantly during the first 10 min in either series. In the control series, the first significant increase in GIP levels was observed after 15 min, whereas with atropine, the first increase in GIP was observed at 25 min. GIP levels were significantly reduced by atropine at 15 and 25 min after meal ingestion (P < 0.06). As in the first experimental protocol, GLP-1 levels did not show any significant change during the first 10 min after meal ingestion, but increased thereafter. The postabsorptive GLP-1 response during 15–25 min was significantly reduced by atropine (P < 0.05). The AUCGIP or AUCGLP-1 over the initial 10-min or the entire 120-min period did not differ significantly between the series (Table 1).
A cephalic phase that is induced by sensory stimulation in the oral cavity and is involved in the regulation of insulin secretion after meal ingestion in humans has been demonstrated in several previous reports (3,5,7–10,33,34). Because atropine reduces the cephalic phase insulin release, it has been thought that cholinergic mechanisms may contribute (10). In the present study, we used the ganglionic blocker trimethaphan to examine the degree of total neural contribution to the preabsorptive insulin response, as trimethaphan interrupts all autonomic influences that are transduced through autonomic ganglia (25). We have previously documented the usefulness of trimethaphan in exploring neural contribution to the glucagon response to insulin-induced hypoglycemia in humans (26). In the current study we verified that a sufficient ganglionic blockade was achieved by trimethaphan by showing a marked reduction in both basal PP levels and the PP response to meal ingestion (PP secretion is largely dependent on vagal activity and may be used as a marker of cholinergic activation of islet function) (35). Although infusions of trimethaphan or atropine were not performed in the absence of meal intake in the present study, we do not consider the observed differences in the present study among results obtained during trimethaphan or atropine versus saline as being attributable to changes in baseline levels of the analyzed variables. This consideration is also supported by the absence of any change in basal insulin and glucose levels during the 15 or 10 min preceding meal ingestion, but after administration of trimethaphan or atropine in the present study.

Trimethaphan reduced the preabsorptive insulin response to meal ingestion by 73%, indicating that this phase of insulin secretion is largely mediated by the autonomic nerves. The remaining trimethaphan-resistant insulin response might be explained by a slight contribution by a nondetectable increase in circulating glucose. Also, atropine reduced the preabsorptive insulin response to meal ingestion, which confirms the results of a previous human study (10) and several rat studies (1). The failure of atropine to prevent the cephalic phase insulin response >20% is at variance with previous findings of a more potent action of atropine in this respect (1,10). This discrepancy may be partially explained by an incomplete inhibition of muscarinic receptors because of a short run in time for atropine before the meal was given, although a significant increase in heart rate was observed. A previous study has showed a more marked increase in heart rate after infusion of atropine (10). That study, however, was performed in men, whereas the present study was undertaken in women, making direct comparison on sensitivity to atropine difficult because of observed gender differences in such sensitivity (38). The discrepancy may also be explained by the use of different types of stimuli, such as the different composition of the meals or different experimental situations. In any case, our study suggests that the neural cephalic phase insulin response to meal ingestion is

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**DISCUSSION**

This study showed that the autonomic nerves are essential for the majority of the preabsorptive insulin response to meal ingestion in humans, explaining >70% of the initial 10-min response, and that this effect involves both cholinergic and noncholinergic mechanisms. The study also showed that there is no preabsorptive increase in GIP or GLP-1 levels in humans, and that the neurally mediated 10-min insulin response to meal ingestion is of importance for maintaining postprandial glucose tolerance.
attributable to both cholinergic and noncholinergic mechanisms. The nature of these noncholinergic autonomic mechanisms has not been established, but most likely includes noncholinergic neurotransmitters in the parasympathetic nerves, such as VIP, PACAP, and GRP (2,11–14). Results of recent animal studies also support the suggestion that these neuropeptides might be involved in the postprandial neurally mediated insulin secretion (15–17). However, the extent to which these neuropeptides are involved in the noncholinergic neural contribution to cephalic phase insulin secretion in humans remains to be studied.

Trimethaphan also interrupts the neurotransmission across the sympathetic ganglia and in the adrenal medulla. Contribution to cephalic phase insulin release by interruption of sympathetic activity is less likely, however, given that sympathetic nerve activation is known to inhibit insulin secretion (2). In contrast, contribution by epinephrine would be a possibility, as this catecholamine can stimulate insulin secretion through a $\beta_2$-adrenoceptor mechanism (2). In fact, an important recent study in dogs showed that epinephrine, in addition to parasympathetic nerves, contributes to the insulin response to meal ingestion in this species (39). Another study in dogs (40) and a study in rats (41) have documented increases in circulating epinephrine during food intake. In the present study,

### TABLE 1

Area under the 10- and 120-min glucose, insulin, GIP, and GLP-1 curves after meal ingestion in the absence or presence of trimethaphan or atropine during the first 15 min after initiation of meal ingestion

<table>
<thead>
<tr>
<th></th>
<th>Experimental series 1</th>
<th>Experimental series 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Trimethaphan</td>
</tr>
<tr>
<td>$AUC_{glucose}$ 10 min (mmol/l × 10 min)</td>
<td>1.2 ± 0.9 0.604</td>
<td>2.0 ± 1.5 0.604</td>
</tr>
<tr>
<td>$AUC_{insulin}$ 10 min (pmol/l × 10 min)</td>
<td>98.2 ± 14.8 0.009</td>
<td>24.5 ± 9.2 0.009</td>
</tr>
<tr>
<td>$AUC_{GIP}$ 10 min (pmol/l × 10 min)</td>
<td>1.1 ± 1.9 0.598</td>
<td>-1.9 ± 1.3 0.598</td>
</tr>
<tr>
<td>$AUC_{GLP-1}$ 10 min (pmol/l × 10 min)</td>
<td>-1.6 ± 4.6 0.689</td>
<td>1.9 ± 3.9 0.689</td>
</tr>
<tr>
<td>$AUC_{glucose}$ 120 min (mmol/l × 120 min)</td>
<td>90.1 ± 35.8 0.061</td>
<td>150.1 ± 36.3 0.061</td>
</tr>
<tr>
<td>$AUC_{insulin}$ 120 min (nmol/l × 120 min)</td>
<td>15.4 ± 3.2 0.861</td>
<td>15.4 ± 1.8 0.861</td>
</tr>
<tr>
<td>$AUC_{GIP}$ 120 min (nmol/l × 120 min)</td>
<td>6.6 ± 1.5 0.789</td>
<td>6.9 ± 1.5 0.789</td>
</tr>
<tr>
<td>$AUC_{GLP-1}$ 120 min (nmol/l × 120 min)</td>
<td>0.86 ± 0.25 0.771</td>
<td>0.92 ± 0.21 0.771</td>
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Data are ± SE. There were six subjects in each experimental series. $P$ values indicate probability level of random difference by paired t test.

FIG. 6. Plasma levels of GIP and GLP-1 in six healthy women subjected to a 25-min intravenous infusion of the muscarinic antagonist, atropine, or saline (from –10 to +15 min). At time 0, a standardized meal was served. Data shown are means ± SE. *$P < 0.05$ for random difference between the groups for the various time points as revealed by ANOVA.

FIG. 7. Serum insulin levels before and after an intravenous administration of arginine hydrochloride in six women subjected to intravenous infusion of trimethaphan or saline from min –15 throughout the study (left panel) and in six other women subjected to intravenous infusion of atropine or saline from min –10 throughout the study (right panel).
however, we found that circulating epinephrine did not change significantly during or after meal ingestion. This suggests that 1) it is unlikely that epinephrine contributes to the early insulin response after meal ingestion in humans, and 2) there is a difference between our present human study versus the previous studies in dogs (39,40) and rats (41) with regard to epinephrine response to food intake.

Both GIP and GLP-1 are of importance for the insulin response to meal ingestion (18,20,21); their physiological relevance is illustrated by findings that mice in which the GIP or GLP-1 receptor has been deleted are glucose intolerant, with impaired insulin response to oral glucose (42,43). However, whether the two hormones contribute to the neurally mediated early insulin response has not been established. Also, although it is known that the secretion of GIP and GLP-1 is affected by autonomic nerves and neurotransmitters (19–21), the extent of neural mediation in relation to the secretion of the two incretins after meal ingestion is not known. We found no increases in the circulating levels of GIP or GLP-1 during the initial preabsorptive 10-min period after meal ingestion, suggesting that no cephalic phase regulation of GIP and GLP-1 secretion is evident in humans. This implies that the incretin hormones do not contribute to the preabsorptive insulin response to meal ingestion in humans. However, the increase in GIP levels at 15 min after meal ingestion was lowered by trimethaphan and atropine. Furthermore, the reduction in the GIP response at 15 min correlated to the lowered insulin response at 15 min. It is therefore likely that the GIP response at 15 min was in part neurally mediated and may have contributed to the insulin response at this time point. In contrast, the GLP-1 response to meal ingestion was not affected by trimethaphan. In addition, atropine reduced GIP and GLP-1 levels at 15–40 min after meal ingestion. These reductions, however, were accompanied by a reduction in postprandial glycemia, and therefore most likely could be explained by reduced gastric emptying and/or inhibited glucose absorption, as is known to be induced by atropine (44,45), causing both delayed glucose absorption and inhibited secretion of GIP and GLP-1.

This study also demonstrated that inhibition of the cephalic phase insulin release to meal ingestion by trimethaphan was accompanied by impaired reduction of glucose levels at 25–60 min, which is a sign of glucose intolerance. Furthermore, the increase in insulin during the first 10 min after meal intake did inversely correlate to the change in glucose levels between 25 and 60 min, suggesting that neurally mediated preabsorptive insulin secretion is causally related to postprandial hyperglycemia. It should be emphasized, however, that other potential actions of trimethaphan cannot be excluded as having contributed (e.g., effects on muscle and liver). Nevertheless, contribution by the cephalic phase insulin response to postprandial glycemia is supported by the correlation between the insulin response at 10 min and glucose disposal. The importance of the early insulin response to food intake for normal glucose tolerance has been demonstrated before in humans (9,24), as has the rapid first phase of insulin secretion after intravenous glucose administration (46). The mechanism of the preservation of normal glucose tolerance by the early insulin response probably involves insulin’s regulation of liver glucose metabolism, as recently proposed by Teff (3). Maximal postprandial hepatic glucose uptake has been shown to be achieved at 15 min after meal ingestion in dogs (47); if portal insulin delivery is retarded during the initial minutes after meal intake, impairment of hepatic glucose uptake with exaggerated glycemia at 25–45 min after meal intake would be expected, as observed in the present study. However, the time course of the changes in hepatic glucose flux after meal ingestion in humans has not been established.

In the present study, the impaired glucose elimination after meal ingestion during trimethaphan infusion was not compensated for by an increased total AUCinsulin. This might suggest that additional effects are induced by trimethaphan on insulin metabolism or sensitivity, although the failure to detect any increased AUCinsulin might also be because of the minimal degree of increased glycemia. During recent years, the importance of prandial glucose levels for the long-term control of type 2 diabetes has come into focus (48). It has, for example, been shown that HbA1c levels correlate mainly to postlunch levels rather than to fasting glucose levels (49). Hence reduction of prandial glycemia might be a major target for antidiabetic treatment; in the context of the present study, it is of interest that the prandial glycemia is dependent on the immediate insulin response, which is largely neurally mediated. In fact, animal studies have presented evidence of the importance of neural-islet interactions in diabetes. Thus, defective neural islet mechanisms seem to underlie the development of diabetes in an animal model of type 2 diabetes, as has been seen in the Chinese hamster (50); furthermore, cholinergic agonism has been shown to improve the glucose intolerance seen in high fat–fed mice (51).

Because gastric emptying and intestinal transit are processes under neural control (45), it may be argued that the results of the series with autonomic antagonists are influenced by altered glucose delivery to the gut and glucose absorption rate. However, this is unlikely after trimethaphan, as 1) the time from start of meal intake until the first detectable increase in circulating glucose was the same whether saline or trimethaphan was given, and 2) the rate of increase in glucose levels during the first 30 min after meal intake was not affected by trimethaphan. In contrast, after atropine, the increase in glucose levels after meal ingestion was impaired, probably because of the inhibiting effect of atropine on gastric emptying and glucose absorption (38,44,45). Consequently, reliable conclusions on the islet effects of atropine can be drawn only for the preabsorptive period.

Our current results therefore show that autonomic nerves are of major importance for the early islet hormone secretion after food ingestion and for the preservation of glucose tolerance in humans through both cholinergic and noncholinergic mechanisms. Based on this finding, we suggest that a failure of islet innervation might contribute to the development of glucose intolerance, and we propose that augmentation of neural-induced insulin secretion might be a target for treatment of islet dysfunction in diabetes.
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REFERENCES


