Oral glucose intake inhibits hypothalamic neuronal activity more effectively than glucose infusion

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We previously showed that hypothalamic neuronal activity, as measured by the blood oxygen level-dependent (BOLD) functional MRI signal, declines in response to oral glucose intake. To further explore the mechanism driving changes in hypothalamic neuronal activity in response to an oral glucose load, we here compare hypothalamic BOLD signal changes subsequent to an oral vs. an intravenous (iv) glucose challenge in healthy humans. Seven healthy, normal-weight men received four interventions in random order after an overnight fast: 1) ingestion of glucose solution (75 g in 300 ml) or 2) water (300 ml), and 3) iv infusion of 40% glucose solution (0.5 g/kg body wt, maximum 35 g) or 4) infusion of saline (0.9% NaCl, equal volume). The BOLD signal was recorded as of 8 min prior to intervention (baseline) until 30 min after. Glucose infusion was associated with a modest and transient signal decline in the hypothalamus. In contrast, glucose ingestion was followed by a profound and persistent signal decrease despite the fact that plasma glucose levels were almost threefold lower than in response to iv administration. Accordingly, glucose ingestion tended to suppress hunger more than iv infusion (P < 0.1). We infer that neural and endocrine signals emanating from the gastrointestinal tract are critical for the hypothalamic response to nutrient ingestion.

Oral glucose intake inhibits hypothalamic neuronal activity more effectively than glucose infusion driven by nutrient ingestion may serve to guard energy equilibrium in the face of an environmental challenge.

The mechanism linking glucose intake and changes in hypothalamic neuronal activity in humans is currently unknown. Various metabolic and endocrine cues are worthwhile to consider. Circulating levels of glucose can be sensed by specialized neurons in the arcuate and ventromedial nuclei of the hypothalamus, and these neurons project to other key nuclei (21). Alternatively, insulin inhibits NPY neuronal activity in the arcuate to control other hypothalamic areas (29). Also, various gut peptides, released in response to nutrient ingestion, including peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), inhibit neuronal activity in the hypothalamus to induce satiety and facilitate insulin action (2, 40). To further explore the mechanism driving changes in hypothalamic neuronal activity in response to an oral glucose load, we here compare hypothalamic BOLD signal changes subsequent to an oral vs. an intravenous (iv) glucose challenge in healthy humans. We reasoned that, if glucose sensing is involved, the BOLD response to either stimulus is expected to be similar (or even more pronounced in response to iv administration, depending on ensuing plasma glucose levels). If, on the other hand, insulin and/or gut peptides are involved, iv glucose administration should evoke a considerably less pronounced BOLD response, since infusion of glucose stimulates gut peptide and insulin release to a lesser extent than oral intake (14, 18, 20, 24, 41).

MATERIALS AND METHODS

Subjects. Seven healthy, normal-weight men participated (mean ± SD age 23.1 ± 2.2 yr; mean ± SD body mass index 21.7 ± 0.8 kg/m²). The subjects were recruited with advertisements put up at various locations in the University Medical Center Utrecht. A standard MRI screening form and a health and lifestyle questionnaire were used to assess eligibility. Exclusion criteria were the following: body mass index <19 or >25 kg/m²; age <18 or >28 yr on the study day; current smoker; a history of or current alcohol consumption of >28 units/wk; a history of medical or surgical events that may have significantly affected the study outcome, such as metabolic or endocrine diseases or any gastrointestinal disorder; irregular eating habits; being on a self-imposed or medically prescribed diet; use of medication (except aspirin or paracetamol); claustrophobia; diabetes; metal implants or metal objects on the body which cannot be removed (e.g.,

THE BLOOD OXYGEN LEVEL-DEPENDENT (BOLD) signal, produced by functional magnetic resonance imaging (fMRI), is a noninvasive measure of neuronal activity (19, 30, 35). We have recently shown that the BOLD signal in the upper part of the hypothalamus of healthy, normal-weight humans consistently declines in response to an oral glucose load, which strongly suggests that glucose ingestion blunts hypothalamic neuronal activity in these subjects. The hypothalamus plays a critical role in the control of energy balance. Neural circuits in hypothalamic nuclei perceive and integrate endocrine and metabolic cues reflecting bodily energy content to coordinate behavior and fuel flux so as to maintain energy homeostasis (28, 34). Thus, adaptations of hypothalamic neuronal activity

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a piercing, hearing aid, or brace). Written informed consent was obtained from all subjects according to the Declaration of Helsinki, and the study protocol was approved by the Medical Ethics Committee of the University Medical Center Utrecht, Utrecht, The Netherlands.

Experimental procedures. To compare the hypothalamic BOLD response between ingestion and infusion of glucose, we used a randomized crossover design with four treatments: oral ingestion of glucose (Gpo), intravenous infusion of glucose (Giv), oral ingestion of water (Wpo), and intravenous infusion of saline (Siv). For the oral treatments, subjects ingested a 300-ml glucose solution (standard oral glucose tolerance test solution, 75 g of glucose in 300 ml of water with sorbic acid added as a preservative) or tap water through a tube. For the iv treatments, equal volumes of 40% glucose solution or saline (0.9% NaCl) were infused into an antecubital vein through a canula over ~3 min at a flow rate of 0.50 ml/s, using an infusion pump (Medrad, Indianola, PA). Subjects received 0.5 g glucose/kg body wt with a maximum of 35 g, as recommended by Bingley et al. (5), i.e., a maximum of 87.5 ml 40% glucose or saline. Six of seven subjects had a body weight over 69 kg and thus received 35 g of glucose; one subject received 34 g of glucose. Subjects were randomly assigned to each treatment the day before each visit. As far as possible, subjects were unaware of treatment order: they were unable to discern between glucose and saline infusion but could tell apart glucose from water by taste.

The subjects fasted overnight from 2200 (no food or beverages except water) and were scanned the next morning, starting between 0800 and 1045. The scans were performed on four separate days, at least 1 wk apart, with a 3.0-T Philips Achieva system (Philips Medical Systems, Best, The Netherlands) equipped with a SENSE-head coil. The subjects were placed in a supine position, and their heads were immobilized with cushions. The functional scan consisted of a T2*-weighted gradient-echo, segmented echo-planar imaging sequence (repetition time 20 ms, echo time 30 ms, flip angle 30°, image matrix 256 × 231, field of view 208 × 208 mm, 12 signal averages/scan, 33 k-lines acquired/excitation pulse) with which a 12-mm thick midsagittal slice was scanned. The images were reconstructed to 256 × 256 pixels. The subjects were scanned for 38 min (225 scans). After a reference period (baseline) of 8.4 min (50 scans), the subjects received one of the four treatments. After the functional scan, a T1-weighted anatomic scan was made of the same slice (repetition time 550 ms, echo time 10 ms, field of view 208 × 208 mm).

To assess the effect of treatment on their general motivation to eat, the subjects filled out a set of four visual analog scales (VASs, range 0 to 100 mm) before and after every scan (i.e., 30 min after treatment), on which they reported their feelings of hunger, fullness, desire to eat, and prospective food consumption (16). For every subject, the scores of these four scales were averaged to obtain two single hunger scores, one for the fasted and one for the treated state.

Blood sampling and analysis. On every test day, six blood samples were drawn from a canula that was placed in an antecubital vein (this canula was also used for infusion). Before blood samples were taken, the canula and attached tubing were cleared of saline/old blood. The time points were before scanning (fasted) and during scanning at ~3 min (before treatment) and at 15, 30, 45, and 60 min after the onset of treatment. Samples for determination of serum glucose and insulin concentrations were collected in 4-ml serum separation tubes. The tubes were centrifuged at 1,730 g for 10 min. Serum was stored in aliquots at ~80°C until analysis. Sample handling and storage was done by U-Diagnostics (Utrecht, The Netherlands). After completion of the study, blood samples were transferred to the Laboratory for Clinical Chemistry at Leiden University Medical Center, Leiden, The Netherlands. There, serum glucose concentrations were measured using a fully automated Hitachi Modular P800 system. Serum insulin concentrations were measured by immunoradiometric assay (INS-IRMA; BioSource Europe, Nivelles, Belgium).

Data analysis. First, all 225 functional images of every time series were motion corrected with the Multimodality Image Registration using Information Theory software for image registration by maximization of mutual information (22). The images were aligned to the middle image, and the anatomic T1-weighted image was also coregistered with this image.

Next, for every subject, the hypothalamus was manually segmented with the use of the anatomic image according to predefined criteria (23). Anatomic landmarks were the anterior commissure, optic chiasm, and mammillary body. In addition, a square reference area of about the same size as the hypothalamus (10 × 10 pixels) was delineated in the thalamus. At every time point, the mean gray value in the hypothalamus was calculated. These mean gray values were then normalized to the mean of their 8.4-min baseline value, which yielded the percentage signal change from the mean baseline. The global signal drift (scanner drift) was corrected by subtracting the mean signal in the reference area from that in the hypothalamus. For statistical analysis, the data were pooled per minute (38 time slots). To test for effects of glucose treatment, the mean signal changes in every time slot were compared between glucose and vehicle by use of Student’s t-tests, i.e., Gpo vs. Wpo and Giv vs. Siv. This approach is comparable to differential regression analysis (8). A Bonferroni-corrected threshold of $P = 0.0013$ was employed since 38 t-tests were done.

Mean hunger scores were calculated for every subject by averaging the VAS scores of general hunger, 100 minus fullness, general desire to eat, and prospective food consumption. The effect of treatment on the mean hunger score was calculated by subtracting the score in the fasted state from that in the treated state. With a randomized block design (analysis of variance), the change in hunger score was tested for effects of treatment (glucose), mode of administration, and the interaction between these two.

Statistical analyses were done with SPSS statistical software (version 13.0; SPSS, Chicago, IL). A $P$ value of 0.05 (two sided and Bonferroni corrected for multiple comparisons) was considered significant.

RESULTS

Hypothalamic fMRI signal. The fMRI signal changes in the hypothalamus are shown in Fig. 1. Ingestion of the test solution took 1.5 ± 0.5 min (mean ± SD) and caused well-known signal artifacts that quickly subsided (strong signal decreases, Fig. 1, top). Intravenous stimulus administration did not cause such artifacts (as expected, Fig. 1, bottom). Glucose ingestion evoked a prolonged and significant signal decrease (1.5–2.0%) in the hypothalamus, which began at 9 min and lasted for the remainder of the scan (20 min), whereas iv glucose was associated with a significant, but transient, signal decrease (~1.0%), which began at 4 min, just after glucose infusion, and subsided at 17 min.

Glucose and insulin responses. Serum concentrations of glucose and insulin are shown in Fig. 2. Glucose ingestion was followed by a slow rise in serum glucose concentration which peaked at ~7 mmol/l and was accompanied by a considerable increase in serum insulin concentrations (to ~50 μU/ml). In contrast, serum glucose and insulin concentrations in response to glucose infusion peaked much earlier at ~15 mmol/l and ~40 μU/ml, respectively, and declined rapidly. At 60 min after infusion, the glucose concentration was back at baseline. Administration of water or saline did not affect glucose and insulin concentrations.

Hunger scores. Mean hunger scores are shown in Table 1. Analysis of variance of the change in hunger score revealed a
significant effect of treatment (glucose, $P < 0.05$) and no effect of the mode of administration, although the interaction between the two factors reached borderline significance ($P = 0.09$). Thus, glucose administration was associated with decreased hunger at 30 min after treatment.

**DISCUSSION**

We previously showed (36, 37) that hypothalamic neuronal activity declines in response to oral glucose intake. In view of the critical role of the hypothalamus in the control of (postprandial) energy balance and fuel flux (28, 34, 42), it appears of utmost importance to clarify the mechanism driving the apparent impact of nutrient intake on hypothalamic activity and its biological meaning. To establish whether the oral route of intake is a prerequisite for a consistent hypothalamic response to nutrients, we measured BOLD signal changes in the hypothalamus in response to oral vs. iv glucose administration. Our data clearly show that an oral glucose load exerts more powerful effects. Intravenous administration evoked a statistically significant but minor and transient decline of the BOLD signal in healthy men. In contrast, oral glucose intake was accompanied by a profound and persistent reduction of the hypothalamic BOLD signal in the face of plasma glucose levels that were almost threefold lower than in response to iv administration. Accordingly, glucose ingestion tended to suppress hunger more effectively than iv glucose administration ($P < 0.1$), which is consistent with previous observations (33). It should be noted here that, apart from the physiological effects of glucose, the decrease in hunger score after oral glucose could have been affected by the fact that subjects could recognize oral glucose by its taste. We infer that the magnitude of the increase in serum glucose does not increase the magnitude or duration of the decrease in hypothalamic activity. The exact effect of the circulating glucose concentration per se on the hypothalamic response to oral glucose intake remains to be elucidated. Other factors, like gastrointestinal signals and/or insulin, are most likely involved.

**Table 1. Hunger scores for the four treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fasted*</th>
<th>Treated*</th>
<th>Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral glucose†</td>
<td>73±15</td>
<td>60±17</td>
<td>-13±19‡</td>
</tr>
<tr>
<td>iv glucose</td>
<td>70±11</td>
<td>68±14</td>
<td>-1±14‡</td>
</tr>
<tr>
<td>Oral water†</td>
<td>68±17</td>
<td>68±17</td>
<td>0±5</td>
</tr>
<tr>
<td>iv saline</td>
<td>66±11</td>
<td>68±10</td>
<td>1±8</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n = 7$. *Hunger scores were recorded after an overnight fast (fasted) and 30 min after treatment (treated). Change in hunger score was calculated by subtracting fasted from treated scores; †$n = 6$ due to one missing value; ‡Analysis of variance showed a significant effect of glucose ($P < 0.05$) and no significant effect of the mode of administration ($P = 0.09$).
The plasma concentrations of various metabolites and hormones, including glucose, fatty acids, insulin, and gut peptides, change in response to food intake and serve as barometers of fuel availability. In that capacity, these cues can modulate neuronal activity in the hypothalamus (28). Glucose ingestion, but not glucose infusion, triggers the release of several gut peptides, including GLP-1, glucose-dependent insulintropic peptide (GIP), oxyntomodulin (OXM), and PYY, by intestinal cells into the circulation (17, 27). These peptides are critically involved in the control of postprandial glucose metabolism. GLP-1 and GIP promote glucose-induced insulin release (11). Of particular relevance to the present findings, GLP-1, OXM, and PYY act in the hypothalamus to induce satiety in rodents and humans (1, 2, 9, 10, 15, 39). In vivo functional neuroimaging studies revealed that OXM and GLP-1 inhibit neuronal activity in hypothalamic nuclei of rats (7). Thus, either one of these peptides may also affect hypothalamic neuronal activity in response to glucose ingestion in the human to explain our findings.

Alternatively, or additionally, insulin may be involved in the response of hypothalamic neurons to nutrient ingestion. Insulin profoundly affects neural circuits in the hypothalamus to inhibit food intake and regulate hepatic glucose output (32). Our data show a decline and subsequent rise of the BOLD signal that coincides with the rise and fall of circulating insulin levels in response to iv glucose administration. Moreover, the more profound and persistent reduction of neuronal activity following glucose ingestion was conspicuously accompanied by a more marked and delayed increase of circulating insulin (Fig. 2). Thus, the extent and time frame of changes in circulating insulin levels appear to correspond to changes in BOLD signal intensity in the present experiments, which suggests that insulin may be involved in the control of hypothalamic neuronal activity in response to nutrient intake. In apparent contrast, ingestion of maltodextrin (a nonsweet carbohydrate) does not blunt hypothalamic BOLD signals despite ensuing plasma glucose and insulin levels similar to those in response to glucose ingestion (36). These data indicate that, although circulating insulin may have an impact, it certainly is not the sole mediator of the hypothalamic response to glucose ingestion.

In addition to hormones and metabolites, neural signals are likely involved. Vagal afferents from the gastrointestinal tract project to the hypothalamus (4, 31) and are, among others, important for blood glucose regulation (3, 38). The gastrointestinal tract features glucoreceptors and osmoreceptors (13, 25), as well as sweet-taste receptors (12). Intestinal glucoreceptors take part in glucose regulation by mediating nervous control of insulin release via the vagus nerve (26) and glucose-induced increases in pancreatic islet blood flow (6). These mechanisms, which rely on vagal pathways, are bypassed by direct infusion of glucose into the blood stream. Neural signals from sweet-taste receptors in the mouth, which are also bypassed by infusion, may also contribute to the hypothalamic response. However, the finding that ingestion of an aspartame solution does not elicit a hypothalamic response, as measured by fMRI (36), makes it unlikely that such signals per se play an important role.

Taken together, the data from previous studies (36, 37) and the current study suggest that no factor by itself (e.g., sweet taste or serum insulin) is sufficient for a hypothalamic fMRI response to glucose ingestion. Rather, the picture of an integrated model arises, from which the following factors acting in concert cannot be excluded: neural input from sweet-taste receptors and the gut, endocrine signals in the form of insulin and other peptide hormones, and blood glucose.

In conclusion, we have shown here that oral glucose intake more effectively inhibits hypothalamic neuronal activity than iv glucose administration does. Accordingly, glucose ingestion tended to suppress hunger more effectively than iv infusion (P < 0.1). Therefore, neural and endocrine signals emanating from the gastrointestinal tract appear to be critical for the hypothalamic response to nutrient ingestion.

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GRANTS

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REFERENCES


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