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

Paul A.M. Smeets\textsuperscript{1,3}, Solrun Vidarsdottir\textsuperscript{2}, Cees de Graaf\textsuperscript{3,4}, Annette Stafleu\textsuperscript{3}, Matthias J.P. van Osch\textsuperscript{5}, Max A. Viergever\textsuperscript{1}, Hanno Pijl\textsuperscript{2}, Jeroen van der Grond\textsuperscript{5}

\textsuperscript{1} Image Sciences Institute, University Medical Center Utrecht, Utrecht, The Netherlands.
\textsuperscript{2} Department of Endocrinology and Metabolism, Leiden University Medical Center, Leiden, The Netherlands.
\textsuperscript{3} Department of Food and Chemical Risk Analysis, TNO Quality of Life, Zeist, The Netherlands.
\textsuperscript{4} Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands.
\textsuperscript{5} Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands.

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Corresponding author:

Paul A.M. Smeets

University Medical Center Utrecht

Image Sciences Institute

Heidelberglaan 100, room Q0S.459

3584 CX Utrecht, The Netherlands.

Telephone: +31-30-250 6682, Fax: +31-30-251 3399

E-mail: paul@isi.uu.nl

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Abstract

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 (i.v.) 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); 3) intravenous infusion of 40% glucose solution (0.5 g/kg body weight, maximum 35 g) or 4) infusion of saline (0.9 % NaCl, equal volume). The BOLD signal was recorded as of 8 minutes prior to intervention (baseline) until 30 minutes 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 3-fold lower than in response to i.v. administration. Accordingly, glucose ingestion tended to suppress hunger more than i.v. 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.

Keywords

Functional MRI, glucose homeostasis, insulin, glucose, incretins
Introduction

The blood oxygen level-dependent (BOLD) signal, produced by functional magnetic resonance imaging (fMRI), is a non-invasive 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 (36; 37). 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 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 (i.v.) 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 i.v. administration, depending on ensuing plasma glucose levels). If, on the other hand, insulin and/or gut peptides are involved, i.v. 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 y; 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 years 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., 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 to 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 glucose in 300 mL water, with sorbic acid added as a preservative) or tap water through a tube. For the intravenous 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 Inc., Indianola, U.S.A.). Subjects received 0.5 g glucose per kg body weight 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 out 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 22:00 h (no food or beverages except water) and were scanned the next morning, starting between 08:00 and 10:45 h. The scans were performed on four separate days, at least one week 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 $T_2^*$-weighted gradient-echo, segmented echo-planar imaging sequence [repetition time = 120 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 $T_1$-weighted anatomical 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 analogue 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 taking blood samples, 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 $1730 \times g$ for 10 min. Serum was stored in aliquots at -80 °C until analysis. Sample handling and storage was done by U-Diagnostics Corporate (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 an immuno-radiometric assay (INS-IRMA; BioSource Europe S.A., 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 anatomical T$_1$-weighted image was also co-registered with this image.
Next, for every subject, the hypothalamus was manually segmented with the use of the anatomical image according to predefined criteria (23). Anatomical landmarks were the anterior commissure, optic chiasm and the 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 using Student’s t-tests, i.e., Gpo versus Wpo and Giv versus Siv. This approach is comparable to differential regression analysis (8). A Bonferroni-corrected threshold of P = 0.0013 was employed since 38 t-test were done.

The areas under the serum glucose and insulin concentration curves (AUCs) above baseline (i.e., the first measurement) were calculated for every subject with a trapezoidal integration. With the use of randomized block designs (analyses of variance), the AUCs were tested for effects of treatment (glucose administration) and the mode of administration. In addition, the interaction between treatment and mode of administration was assessed.

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 the use of 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 Inc, 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 Figure 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, see left pane). Intravenous stimulus administration did not cause such artifacts (as expected, right pane). 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 intravenous 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 Figure 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 that hypothalamic neuronal activity declines in response to oral glucose intake (36; 37). 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 if 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. intravenous glucose administration. Our data clearly show that an oral glucose load exerts more powerful effects. I.v. 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 3-fold lower than in response to i.v. administration. Accordingly, glucose ingestion tended to suppress hunger more effectively than i.v. glucose administration (P < 0.1), which is consistent with previous observations (33). It should be noted here that, apart from the physiologic 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
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 glucagon-like peptide-1 (GLP-1), glucose dependent insulinotropic peptide (GIP), oxyntomodulin (OXM) and peptide YY (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 man (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 i.v. 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 (Figure 2). Thus, the extent and timeframe of changes in circulating insulin levels appear to correspond with 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 non-sweet 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 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 glucoregulation 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 here show that oral glucose intake more effectively inhibits hypothalamic neuronal activity than intravenous glucose administration. Accordingly, glucose ingestion tended to suppress hunger more effectively than i.v. 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.
Acknowledgements

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References


7. Chaudhri OB, Parkinson JR, Kuo YT, Druce MR, Herlihy AH, Bell JD, Dhillo WS, Stanley SA, Ghatei MA and Bloom SR. Differential hypothalamic neuronal activation following


**Figure legends**

**Figure 1.** Mean ± SD fMRI signal changes per minute in the hypothalamus in response to oral (left) and to intravenous glucose administration (right). Legend: ■ oral glucose (Gpo), ▲ intravenous glucose (Giv), □ oral water (Wpo), Δ intravenous saline (Siv). T = 0 min is the onset of treatment. Asterisks (*) indicate a significant difference between treatment and vehicle (Student’s t-tests with a Bonferroni-corrected threshold of P = 0.0013). The horizontal black bar indicates the approximate duration of treatment: drinking took ~2 min and intravenous treatments took ~3 min to complete.

**Figure 2.** Mean ± SE glucose and insulin responses for the four treatments (n = 7). T = 0 min is the onset of treatment. Legend: ■ oral glucose (Gpo), ▲ intravenous glucose (Giv), □ oral water (Wpo), Δ intravenous saline (Siv).
**Tables**

**Table 1.** Mean ± SD hunger scores for the four treatments ($n = 7$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hunger score</th>
<th>Hunger score</th>
<th>Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fasted</td>
<td>treated</td>
<td></td>
</tr>
<tr>
<td>Oral glucose†</td>
<td>73 ± 15</td>
<td>60 ± 17</td>
<td>-13 ± 19†</td>
</tr>
<tr>
<td>I.v. 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>I.v. saline</td>
<td>66 ± 11</td>
<td>68 ± 10</td>
<td>1 ± 8</td>
</tr>
</tbody>
</table>

* Hunger scores were recorded after an overnight fast (fasted) and 30 min after treatment (treated). The 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 ($P = 0.09$).
Figure 1. Mean ± SD fMRI signal changes per minute in the hypothalamus in response to oral (left) and to intravenous glucose administration (right). Legend: ■ oral glucose (Gpo), ▲ intravenous glucose (Giv), □ oral water (Wpo), Δ intravenous saline (Siv). T = 0 min is the onset of treatment. Asterisks (*) indicate a significant difference between treatment and vehicle (Student’s t-tests with a Bonferroni-corrected threshold of P = 0.0013). The horizontal black bar indicates the approximate duration of treatment: drinking took ~2 min and intravenous treatments took ~3 min to complete.
Mean ± SE glucose and insulin responses for the four treatments (n = 7). T = 0 min is the onset of treatment. Legend: ● oral glucose (Gpo), ▲ intravenous glucose (Giv), □ oral water (Wpo), Δ intravenous saline (Siv).