Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories\textsuperscript{1–3}

Paul AM Smeets, Cees de Graaf, Annette Stafleu, Matthias JP van Osch, and Jeroen van der Grond

ABSTRACT

Background: Evidence exists that beverages do not trigger appropriate anticipatory physiologic responses, such as cephalic phase insulin release. Therefore, it is of interest to elucidate the food properties necessary for triggering adaptive responses. Previously, we found a prolonged dose-dependent decrease in the hypothalamic functional magnetic resonance imaging signal after ingestion of a glucose solution.

Objectives: The aims of the present study were to measure the effects of sweet taste and energy content on the hypothalamic response to glucose ingestion and to measure the concomitant changes in blood glucose and insulin concentrations.

Design: Five healthy, normal-weight men participated in a randomized crossover design trial. The subjects were scanned 4 times for 37 min on separate days with functional magnetic resonance imaging. After 7 min, they ingested 1 of the following 4 stimuli (300 mL of each): water (control), a glucose solution, an aspartame (sweet taste) solution, or a maltodextrin (nonsweet carbohydrate) solution.

Results: Glucose ingestion resulted in a prolonged and significant signal decrease in the upper hypothalamus ($P < 0.05$). Water, aspartame, and maltodextrin had no such effect. Glucose and maltodextrin ingestions resulted in similar increases in blood glucose and insulin concentrations. However, only glucose triggered an early rise in insulin concentrations. Aspartame did not trigger any insulin response.

Conclusions: Our findings suggest that both sweet taste and energy content are required for a hypothalamic response. The combination of sweet taste and energy content could be crucial in triggering adaptive responses to sweetened beverages. Am J Clin Nutr 2005;82:1011–6.

KEY WORDS Functional magnetic resonance imaging, hypothalamus, glucose, insulin, aspartame, maltodextrin, taste, carbohydrates, men

INTRODUCTION

Beverages are an increasingly important source of carbohydrate intake (1, 2). Unfortunately, consumption of caloric beverages does not elicit appropriate dietary compensation and leads to excessive energy intake (1, 3, 4), which promotes obesity. A likely cause for this excessive energy intake is that beverages cause less sensory stimulation because they are swallowed and not chewed and therefore lack the olfactory and visual stimulation of normal foods. Sensory stimulation, such as the sight, smell, and taste of food, can trigger anticipatory physiologic responses, also called cephalic phase responses, which improve the digestion and absorption of nutrients (5–7). In view of the prevalent consumption of beverages, it is of interest to elucidate the properties of foods that are necessary for triggering these adaptive responses. Some evidence exists that liquids do not induce cephalic phase responses; for example, tasting sweet liquids does not elicit cephalic phase insulin release (CPIR) (8, 9), which is important for the prevention of peaks in blood glucose (10–12). In contrast, tasting food can trigger CPIR (5, 13) and so can the combination of sweet taste and the presentation of a meal (8). Recently, we reported a prolonged dose-dependent decrease in the blood oxygen level dependent (BOLD) magnetic resonance imaging (MRI) signal in the hypothalamus a few minutes after the ingestion of a glucose solution (14). BOLD functional MRI (fMRI) measures changes in neuronal activity levels based on the associated changes in the local concentrations of oxygenated and deoxygenated hemoglobin (15, 16). We suggested that the initial part of this response could be triggered by the sweet taste of the glucose solution and could be associated with changes in the blood insulin concentration during the cephalic phase (ie, before glucose has entered the blood stream). The subsequent rise in blood glucose and insulin concentrations could relate to the additional hypothalamic response (14). The aim of the present study was to investigate the separate effects of sweet taste and energy content on this hypothalamic response as well as on blood glucose and insulin concentrations.

SUBJECTS AND METHODS

Subjects

Five healthy, normal-weight men participated in the present study [$\bar{x}$ ± SEM age: 20.4 ± 2.5 y; $\bar{x}$ ± SEM body mass index (in kg/m$^2$): 21.7 ± 1.1]. The subjects were recruited with advertisements that were put up at various locations in the University.

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dextrin; W, water. All treatments were 300-mL clear liquids.

subjects that were relevant to the study. Exclusion criteria were

naire to assess the general health and aspects of lifestyle of the

Medical Center Utrecht. We used a health and lifestyle question-

TABLE 1

Order of treatment for every subject

<table>
<thead>
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<th>Study day</th>
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A, water + aspartame; G, water + 75 g glucose; M, water + malto-
dextrin; W, water. All treatments were 300-mL clear liquids.

Medical Center Utrecht. We used a health and lifestyle question-
naire to assess the general health and aspects of lifestyle of the
subjects that were relevant to the study. Exclusion criteria were
the following: body mass index < 19 or > 25; <18 or >28 y of
age on the study day; current smoker; a history of alcohol con-
sumption or current alcohol consumption >28 units/wk; a his-
tory of medical or surgical events that may have significantly
affected the study outcome, such as metabolic or endocrine dis-

eases or any gastrointestinal disorder; irregular eating habits;
being on a self-imposed or medically prescribed diet; use of
medication (except aspirin or paracetamol); claustrophobia; di-
abetes; and metal implants or metal objects on the body which
cannot be removed (eg, 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 Ethical Committee of the University Medical Center
Utrecht, Utrecht, Netherlands.

Experimental procedures

We used a randomized crossover design with 4 stimuli: water, water + glucose, water + aspartame, and water + maltodextrin
(a nonsweet glucose dimer). Conditions were randomly assigned
by the subjects to drawing lots the day before every visit. The
order of treatment is shown in Table 1. The sweetness of the
aspartame solution and energy-content of the maltodextrin solu-
tion were matched to those of the glucose solution. The details
and preparation of the stimuli were as follows: 300-mL tap water
was used for water, the 75-g glucose solution was obtained by
dissolving 82.5 g glucose monohydrate (D-uctose; Avebe Cor-
porate, Veendam, Netherlands) in water to a volume of 300 mL,
the equally sweet aspartame solution was obtained by dissolving
880 mg pure aspartame (Aspartamum; BUFA Corporate, Uit-
geest, Netherlands) in 300 mL water, and the equicaloric malto-
dextrin solution was obtained by dissolving 78.9 g Fantomalt
(Nutricia, Netherlands) in water to a volume of 300 mL (300
kcal). The amount of aspartame that was needed to match the
sweetness of the 75-g glucose solution was estimated from the
data of the PhD dissertations of de Graaf (17) and Schifferstein
(18). A pilot test, in which 5 volunteers were asked to assess the
sweetness of the 2 solutions in a blind fashion, confirmed that this
determination was correct. The subjects were blinded as much as
possible; all stimuli looked identical and the subjects did not
know in advance the stimulus they would receive. When asked
afterward, the subjects were unable to discern between the glu-
cose and aspartame solutions. The maltodextrin solution was
unknown to the subjects, and most of the subjects reported a

slight starch taste, which made them guess it was maltodextrin.
Water was recognized by the subjects as such.

The subjects fasted (no food or beverages, except water) over-
night from 2200 and were scanned the next morning, starting
between 0930 and 1000. The scans were performed on 4 separate
days ≥1 wk apart with a 1.5-T Philips Gyroscan ACS-NT system
(Philips Medical Systems, Best, Netherlands). The subjects were
placed in a supine position and their heads were immobilized
with a vacuum cushion that was designed for use in a MRI head
coil (Medical Intelligence, Schwabmuenchen, Germany). The
functional scan consisted of a T2'-weighted gradient-echo, seg-
mented echo-planar imaging sequence [repetition time = 120
ms, echo time = 40 ms, flip angle = 30 °, image matrix = 198 ×
256, field of view = 208 × 208 mm, 12 signal averages/scan, 33
k-lines acquired/excitation pulse (14)] with which a 10-mm thick
mid sagittal slice was scanned. The images were reconstructed
onto 256 × 256 pixels. The subjects were scanned for 37 min (256
scans). After a reference period (baseline) of 7.2 min (50 scans),
the subjects ingested one of the test solutions through a peroral tube.
After the functional scan, a T1-weighted anatomical scan was made
of the same slice (repetition time= 600 ms, echo time = 18 ms,
field of view = 230 × 230 mm).

Blood sampling and analysis

On every test day, 10 blood samples were drawn with 5-mL
syringes in combination with a canula that was placed in an
antecubital vein; one sample was taken before scanning and 9
samples were taken during scanning at −5 and −3 min (before
stimulus ingestion) and at 1, 3, 5, 7, 10, 20, and 29 min after the
onset of drinking. The samples were immediately injected into
2-mL K-Oxalate/Na-Fl tubes. The tubes were kept on ice until the
experiment ended and were then centrifuged at 1730 × g for 10
min at 4 °C. Blood plasma was stored in aliquots at −80 °C until
analyzed. Sample handling and analysis were performed by
U-diagnostics Corporate (Utrecht, Netherlands; an ISO 9002-
certified laboratory). Glucose concentrations were measured
with the glucose-oxidase method on a VITROS 250 Chemistry
System (Johnson & Johnson Clinical Diagnostics, Rochester,
NY). Insulin concentrations were measured with an immunoass-
ay (AxSYM insulin assay; Abbott, Abbott Park, IL).

Data preprocessing

fMRI data of the hypothalamus were preprocessed and ana-
alyzed as described previously (14). All 256 functional images of
each time series were motion-corrected with the Multimodi-
ality Image Registration using Information Theory software for image
registration by maximization of mutual information (19). The
images were aligned to the middle image and the anatomical
T1-weighted image was also coregistered with this image.

Data analysis

For every subject, the hypothalamus was manually segmented
with the use of the anatomical image and divided into 4 regions
by 2 orthogonal axes according to predefined criteria (20): the
upper anterior hypothalamus (UAH), lower anterior hypothala-
mus, upper posterior hypothalamus (UPH), and lower posterior
hypothalamus. The anterior–posterior axis was defined by the
line passing through the centers of the anterior commissure and
the mammillary body. The upper–lower axis was defined by the
line passing through the optic chiasm, which is perpendicular to
the anterior–posterior axis (Figure 1). The UAH and UPH were the regions of interest (ROIs) because these regions respond to glucose (14). In addition, a square reference area (10 × 10 pixels) of about the same size as the hypothalamus was delineated in the frontal cortex, anterior of the genu of the corpus callosum. At every time point, the mean gray value in the hypothalamus as a whole and in each ROI was calculated. Next, these mean gray values were normalized to the mean of their 7-min baseline value, which yielded the percentage signal change from mean baseline. The global signal drift (scanner drift) was corrected by subtracting the mean signal in the reference area from those in the ROIs. We tested for an effect of every stimulus using differential regression analysis (21), in which the mean signal changes per minute were compared with the mean signal change during the 7-min reference period using Student’s t tests. Because drinking causes artifacts (14), data from the first 2 min after the onset of drinking were excluded from the analysis. A Bonferroni-corrected threshold of $P = 0.0018$ was employed because we performed 28 t tests per condition.

To compare treatment effects, the area under the curves (AUCs) were calculated for every subject from 2 to 30 min after stimulus ingestion in the UAH and the UPH (193 time points). For the blood data, the AUCs above baseline (ie, the first measurement) were calculated for every subject. The AUCs were calculated with a trapezoidal integration. With the use of randomized block designs (analysis of variance), the AUCs of the

![FIGURE 1. Segmentation and subdivision of the hypothalamus into 2 regions of interest (20). Ac, anterior commissure; mm, mammillary body; oc, optic chiasm; UA, upper anterior hypothalamus; UP, upper posterior hypothalamus.]

![FIGURE 2. Mean (±SEM) functional magnetic resonance imaging (fMRI) responses in 5 men in the fasting state and after ingestion of four 300-mL test substances. □, water; ■, water + 75 g glucose (G); ●, water + aspartame (A); □, water + maltodextrin (M). Top: signal changes from baseline in the upper anterior hypothalamus (left panel) and the upper posterior hypothalamus (right panel) during the 7-min reference period and in the 28 min after stimulus ingestion. The vertical bars labeled “drinking” indicate the approximate period of stimulus ingestion (2 min). Bottom: P values of the Student’s t tests that compared the mean signal per minute with the mean signal during the reference period. Signal changes during the reference period are near zero. Mean (±SEM) reference in the upper anterior hypothalamus: −0.032 ± 0.050 (water), −0.003 ± 0.045 (G), −0.011 ± 0.065 (A), −0.033 ± 0.048 (M). Mean (±SEM) reference in the upper posterior hypothalamus: 0.025 ± 0.055 (water), −0.003 ± 0.046 (G), −0.009 ± 0.066 (A), −0.020 ± 0.069 (M). The dashed lines indicate the Bonferroni-corrected threshold of $P = 0.0018$. After glucose ingestion, significant decreases in fMRI signal in the upper anterior hypothalamus and upper posterior hypothalamus were observed.]
and corrected for multiple comparisons when appropriate) was
sion 12.0.1; SPSS Inc, Chicago, IL). A
pairwise using Tukey's honestly significant difference test. Sta-
tistical analyses were done with SPSS statistical software (ver-
For the glucose and insulin data, we compared all treatments
Figure 2
Hypothalamic fMRI signal
RESULTS

The fMRI data and the blood glucose and insulin data were tested for
an overall effect of treatment. The AUCs of the fMRI data were
also tested for an effect of ROI. If a treatment effect was ob-
ered, post hoc tests were performed to compare treatments. For
the fMRI data, we used Dunnett’s t test to compare all other
treatments with water (control condition) and Tukey’s honestly
different significance test to compare the treatments pairwise.
For the glucose and insulin data, we compared all treatments
pairwise using Tukey’s honestly significant difference test. Sta-
tistical analyses were done with SPSS statistical software (ver-
ion 12.0.1; SPSS Inc, Chicago, IL). A P value < 0.05 (two-sided
and corrected for multiple comparisons when appropriate) was
considered significant.

FIGURE 3. Mean (±SEM) area under the curve (AUC) of the fMRI
response in the upper hypothalamus (UH) of 5 men. The AUCs of the 2
hypothalamic regions of interest were combined because an analysis of
variance showed no significant effect of the regions of interest on AUC. G,
glucose; A, aspartame; M, maltodextrin. A significant effect of treatment was
observed on the AUC, P = 0.005. Bars with different letters are significantly
different, P < 0.05 (Dunnett’s t test in a comparison with water and Tukey’s
honestly significant difference in pairwise comparisons of treatments).

Blood measurements

Plasma concentrations and AUCs of glucose and insulin are shown in Figure 4. Energy intake (glucose and maltodextrin)
resulted in increased glucose and insulin concentrations starting
5–10 min after the onset of ingestion. Similar to water, aspartame
(ie, sweet taste) did not affect glucose and insulin concentrations.
An early rise in insulin concentration was seen only after glucose
ingestion, 5 min after the onset of drinking. At this time, no rise
in plasma glucose is yet observed. Analyses of variance showed
that significant effects of treatment on glucose and insulin con-
centrations existed. The AUCs of glucose and maltodextrin were
not significantly different, but were significantly greater than
those of water and aspartame.

DISCUSSION

We investigated the effects of sweet taste and energy content
on the hypothalamic response to glucose ingestion and on the
concomitant changes in blood glucose and insulin concentra-
tions. Glucose ingestion resulted in a prolonged signal decrease
in the upper hypothalamus. Water, aspartame, and maltodextrin
had no such effect. Ingestion of either glucose or maltodextrin
resulted in similar increases in blood glucose and insulin con-
centrations. However, only glucose triggered an early rise in
plasma insulin. The sweet taste of aspartame did not trigger any
insulin response.

The prolonged signal decrease we observed in the UAH and
UPH after the ingestion of a 75-g glucose solution was similar to
the response we had previously reported but with a smaller am-
plitude (14). Both the aspartame and maltodextrin ingestions
were not associated with significant changes in the hypothalamic
signal. Apparently, neither sweet taste nor energy content was
sufficient to trigger a response. This suggests that a hypothalamic
response requires both sweet taste and energy content.

The aspartame and maltodextrin ingestions did not result in
early changes in the insulin concentration. Our finding that swal-
lowing an aspartame-sweetened liquid does not elicit CPIR adds
to reports of the absence of CPIR in response to tasting (not
swallowing) aspartame-sweetened liquids (8, 9). In the study by
Teff et al (9), tasting saccharine as well as sucrose solutions also
did not induce CPIR. Moreover, Bruce et al (8) showed that only
the combination of sweet taste with the presentation of a meal
induced CPIR. Thus, although one would expect the existence of
conditioned adaptive responses after the ingestion of sweet stim-
uli, these studies suggest that, in general, sweet liquids do not
induce CPIR, presumably because they do not cause sufficient
sensory stimulation. CPIR was not observed in our study, and
although this agrees with the findings of other studies, it could
have been due to our small sample size (n = 5) and to the high
variability in glucose and insulin concentrations, especially after
the ingestion of carbohydrates. Interestingly, Grill et al (22)
found a preeminent role of glucose in triggering CPIR in rats;
other sugars (ie, sucrose and fructose) and a nonnutritive sweet-
ener (sodium saccharine) did not elicit CPIR, whereas glucose
did. They note that this is particularly striking, because glucose
is neither the most intense nor the most palatable substance that
was studied.

The maltodextrin solution, a nonsweet energy-rich liquid, did
not elicit CPIR or a hypothalamic response. The absence of CPIR
is not surprising given the sort of sensory stimulation it caused (a
nonsweet watery taste). Also, the subjects were unfamiliar with
this particular substance and thus were not conditioned to this
combination of taste and energy content.

We had previously hypothesized that the early decrease in the
hypothalamic signal, which was observed <5 min after glucose
ingestion, was associated with CPIR (14). Our insulin data showed that only glucose was associated with an early rise in insulin concentration, and only glucose triggered a decrease in fMRI signal in the upper hypothalamus. This supports our hypothesis. In addition, we hypothesized that the additional decrease in fMRI signal was associated with a rise in insulin concentration. However, because maltodextrin causes an insulin response similar to that of glucose but no significant change in fMRI signal, this hypothesis is not supported. Taken together, our data show no direct relation between changes in the hypothalamic fMRI signal and the plasma insulin concentration. It is striking, however, that only glucose ingestion was associated with a hypothalamic response as well as an early insulin response. This raises the question whether this hypothalamic response is specifically triggered by glucose. Our present findings in humans and the findings of Grill et al (22) in rats suggest a particular sensitivity of the digestive system to glucose. It remains to be investigated whether other sweet carbohydrates, such as sucrose, induce a hypothalamic response.

We found a similar but smaller hypothalamic response after glucose ingestion than was previously reported (14). A possible reason for this is the stress caused by the blood sampling; otherwise, the experimental setup of both studies was the same. Stress could influence BOLD measurements of the hypothalamus in 2 ways. First, stress can affect responses of the hypothalamus because its paraventricular nucleus plays a major role in generating adaptive autonomic, behavioral, and hormonal responses to stress (23, 24). Second, stress could affect the BOLD signal. Acute stress has been shown to cause changes in hematocrit (25), which affects the source of the BOLD signal. It has been suggested that the stress caused by MRIs is a source of variation in hematocrit and therefore of variation in the BOLD signal (26); in our case, however, both experiments involved the same MRI protocol. Still, the added stress of the blood sampling procedures could have affected hematocrit. In addition, the effect of venipuncture on cortisol concentrations, a measure of stress, has been shown to be highly variable (27, 28), which suggests that the effects of stress on the fMRI response will vary between subjects, too. Therefore, the assessment of stress in future MRI experiments is warranted, especially when the experiments involve blood sampling; stress should be measured with salivary cortisol measurements, which allow for an unstressed reference measurement, and with a stress questionnaire to assess the subjective stress of each person. This also suggests that future studies could benefit from a larger sample size, given the small effect size seen in the present study.

In conclusion, we found that sweet taste and energy content on their own did not trigger a prolonged decrease in hypothalamic signal similar to that observed after glucose ingestion. Also, the sweet taste of aspartame was not sufficient to trigger an early insulin response. An early rise in plasma insulin concentration was observed after glucose ingestion, but not after maltodextrin ingestion. It is of interest whether the fMRI signal decrease we observed in the hypothalamus is specifically triggered by glucose. Our findings suggest that the combination of taste (sweetness) and energy content might be important in triggering adaptive physiologic responses to liquid stimuli, such as soft drinks. Moreover, a matching combination of sweet taste and energy...
content could be necessary for an adequate regulation of energy intake.

PAMS contributed to the experimental design, conducting the experiment, data analysis and interpretation, and writing of the manuscript. CdG contributed to the experimental design, interpretation of the data, and reviewing of the manuscript. AS contributed to the experimental design and reviewing of the manuscript. MJPvO contributed to the experimental design, gave advice with regard to the practical experimental setup, and reviewed the manuscript. JvdG contributed to the experimental design, conducting the experiment, and reviewing of the manuscript. None of the authors has any conflicts of interest.

REFERENCES