Original Paper

Neuro
-endocrinology

Neuroendocrinology
DOI: 10.1159/000341406

Received: December 13, 2011
Accepted after revision: June 14, 2012
Published online: ■■■

Intranasal Insulin Modulates Intrinsic Reward and Prefrontal Circuitry of the Human Brain in Lean Women

Stephanie Kullmann[a–c] Sabine Frank[a, c] Martin Heni[d] Caroline Ketterer[d]
Ralf Veit[a, b] Hans-Ulrich Häring[d] Andreas Fritsche[d] Hubert Preissl[a, e]

[a]MEG Center, University of Tübingen, [b]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Paul Langerhans Institute Tübingen, [c]Graduate School for Neural and Behavioural Sciences, University of Tübingen, and [d]Department of Internal Medicine IV, University Hospital, Tübingen, Germany; [e]Department of Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, Ark., USA

Key Words
Resting-state functional magnetic resonance imaging · Insulin · Obesity · Food reward

Abstract
Aim: There is accumulating evidence that food consumption is controlled by a wide range of brain circuits outside of the homeostatic system. Activation in these brain circuits may override the homeostatic system and also contribute to the enormous increase of obesity. However, little is known about the influence of hormonal signals on the brain's non-homeostatic system. Thus, selective insulin action in the brain was investigated by using intranasal application. ***Methods:*** We performed 'resting-state' functional magnetic resonance imaging in 17 healthy lean female subjects to assess intrinsic brain activity by fractional amplitude of low-frequency fluctuations (fALFF) before, 30 and 90 min after application of intranasal insulin. ***Results:*** Here, we showed that insulin modulates intrinsic brain activity in the hypothalamus and orbitofrontal cortex. Furthermore, we could show that the prefrontal and anterior cingulate cortex response to insulin is associated with body mass index. ***Conclusion:*** This demonstrates that hormonal signals as insulin may reduce food intake by modifying the reward and prefrontal circuitry of the human brain, thereby potentially decreasing the rewarding properties of food. Due to the alarming increase in obesity worldwide, it is of great importance to identify neural mechanisms of interaction between the homeostatic and non-homeostatic system to generate new targets for obesity therapy.

Introduction

Obesity has extensive consequences due to the strong associations with numerous health conditions (e.g. diabetes) and heightened mortality [1, 2]. It has been proposed that the alarming increase in obesity is mostly caused by the ready availability of highly palatable food. Therefore, most conceptualizations of human eating behavior propose an interaction between brain areas controlling metabolic homeostasis and those dealing with cognitive and emotional processing to influence food intake [3, 4]. The brain homeostatic system (i.e. hypothalamus) perceives and integrates circulating metabolic and hormonal cues reflecting available fuel sources, such as ghrelin, leptin

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0028–3835/12/0000–0000$38.00/0

Accessible online at:
www.karger.com/nen

Stephanie Kullmann
MEG Center, University of Tübingen
Otfried Müller Strasse 47, DE–72076 Tübingen (Germany)
Tel. +49 7071 298 7703
E-Mail stephanie.kullmann@med.uni-tuebingen.de

and insulin, to stimulate or inhibit feeding in order to maintain appropriate levels of energy balance. Nonetheless, recent research suggests that in addition to the homeostatic system, higher cognitive functions also play an important role in energy regulation [for reviews, see 4, 5]. In fact, human brain imaging studies have revealed that food-related stimuli can activate brain circuits implicated in reward, particularly the orbitofrontal cortex (OFC), insula, amygdala, hypothalamus, striatum and midbrain regions [4] and prefrontal areas essential for executive function including inhibitory control of feeding behavior [6, 7]. Furthermore, the central nervous system (CNS) circuitry can be modified by hormonal signals, like insulin, which act in the CNS as regulators of whole-body energy homeostasis through their receptors expressed in the limbic forebrain [see 8 for review, 9, 10]. Rodent studies suggest that insulin acts on the homeostatic and non-homeostatic system of the brain thereby accessing reward-related brain areas. For example, insulin modulates the excitability of neurons not just in the hypothalamus but also in the hippocampus, influencing learning and memory processes [11, 12] and decreasing the rewarding value of food through the 'reward circuitry', such as by decreasing dopamine signaling [13, 14]. Hence, it is possible, in addition to its role as a homeostatic signal, that insulin may influence the reward circuitry of the human brain, thereby modulating the hedonic aspects of eating behavior. To date, the central nervous effects of insulin in humans still remain mostly unclear. Since the effects of insulin and glucose in human studies are difficult to differentiate, we used the intranasal administration of insulin. This technique allowed us to study central insulin action by selective delivery of the hormone into the brain, without relevant effects on peripheral glucose concentrations [15]. After intranasal insulin administration, insulin enters the cerebrospinal fluid compartment and influences brain function, demonstrating for example beneficial effects on memory functions and promoting weight loss [16–19]. In a recent functional magnetic resonance imaging (fMRI) study, we found that intranasal insulin attenuates visual processing of food images [20]. However, little is known about the effect of insulin on intrinsic brain activity. Recent research has provided increasing evidence that intrinsic brain activity is important for healthy brain function, revealing changes during 'resting state' related to several different medical conditions such as Alzheimer's disease [21] and obesity [22].

In our study, we performed 'resting-state' fMRI in healthy lean female subjects to assess intrinsic brain activity before, 30 and 90 min after intranasal insulin application. For this purpose, we used a voxel-wise frequency-domain measure of BOLD signal dynamics called fractional amplitude of low-frequency fluctuations (fALFF) [23, 24]. fALFF is strongest in gray matter and has been shown to be a reliable and consistent index [24]. Previous studies have observed that fALFF is associated with differences in behavior [25] and emotional cognition [26].

We present evidence that insulin influences intrinsic brain activity beyond the homeostatic systems of the brain modulating the OFC, anterior cingulate cortex (ACC), prefrontal cortex (PFC) and hypothalamus.

Materials and Methods

Subjects and Study Design

Seventeen female subjects (body mass index (BMI) 21.16 ± 1.64 kg/m^2, age 24.47 ± 2.21 years) were recruited. All subjects were healthy as ascertained by a physician. Particularly, they did not suffer from psychiatric, neurological or metabolic diseases. Any volunteer treated for chronic disease or taking any kind of medication other than oral contraceptives was excluded at screening. Eating behavior of the subjects was assessed by the German Three-Factor Eating Questionnaire [27]. All subjects were normal-sighted or had corrected-to-normal vision. Informed written consent was obtained from all subjects and the local ethics committee approved the protocol.

All subjects participated in two conditions, insulin and placebo, on two different days in randomized order with a time lag of 7–28 days. The subjects were blinded to the order of the conditions. Experiments were conducted after an overnight fast of at least 10 h and started at 07:00 h with a 'resting-state' fMRI measurement under basal conditions. After the basal measurement, an insulin/placebo spray was administered intranasally as described below. After 30 and 90 min, the second and third 'resting-state' fMRI measurements were performed. Before the experiment, subjects confirmed their fasting state. Subjective feeling of hunger was rated on a visual analogue scale from 0 to 10 (0: not hungry at all; 10: very hungry) at time points 0, 30 and 90 min. However, we only have a complete hunger rating of both measurement days for 11 subjects. Venous blood samples were obtained at 0, 30, 60, 90, and 120 min and plasma glucose and plasma insulin concentrations were determined (fig. 1).

Intranasal Insulin and Placebo Spray and Analytical Procedures

The insulin and placebo spray were prepared in nasal sprays as previously described [15]. Each puff consisted of 0.1 ml solution containing 40 IU insulin (400 IU/ml; Insulin Actrapid; Novo Nordisk, Mainz, Germany) or 0.1 ml vehicle for placebo. Each subject received four doses of 0.1 ml insulin or placebo spray within 5 min. Two doses were applied in the left and two doses in the right nostril resulting in a total insulin dose of 160 IU insulin on the insulin condition day. Blood glucose was determined using the glucose-oxidase method (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Plasma insulin was measured by commercial chemiluminescence assays for ADVIA Centaur (Siemens Medical Solutions, Fernwald, Germany).

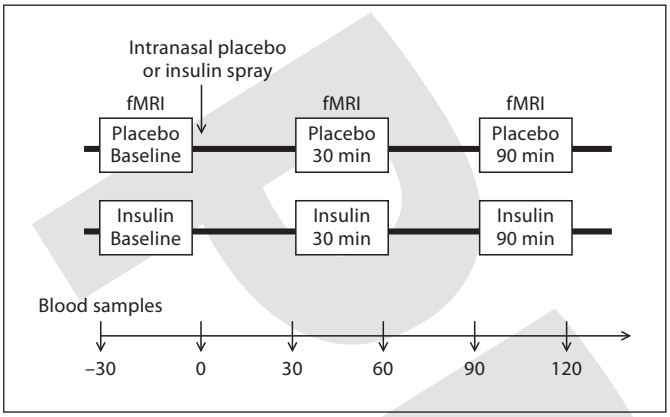

Fig. 1. Schema of test procedure. The order of placebo day and insulin day were balanced over subjects.

Table 1. Metabolic parameters during fMRI experiment

Time min	Glucose, mmol/l		Insulin, pmol/l	
	placebo spray	insulin spray	placebo spray	insulin spray
0	4.60 ± 0.26	4.72 ± 0.36	41 ± 20	43 ± 19
30	4.45 ± 0.49	4.41 ± 0.42	52 ± 24*	67 ± 28*
60	3.42 ± 1.97	3.53 ± 1.83	46 ± 19	47 ± 15
90	4.53 ± 0.54	4.35 ± 0.40	48 ± 22	41 ± 15
120	4.61 ± 0.41	4.46 ± 0.71	43 ± 23	27 ± 12

All data are given as mean ± SD.
* Significant differences between groups (t test).

Data Acquisition

Whole-brain functional fMRI data was obtained by using a 3.0 T scanner (Siemens Tim Trio, Erlangen, Germany). Functional data were collected by using echo-planar imaging sequence: TR = 1.8 s, TE = 30 ms, FOV = 210 mm^2, matrix 64 × 64, flip angle 90°, voxel size 3 × 3 × 4 mm^3, slice thickness 3 mm, 1 mm gap and the images were acquired in ascending order (where TR is repetition time, TE is echo time, FOV is field of view). Each brain volume comprised 28 axial slices and each functional run contained 160 image volumes, resulting in a total scan time of 4.52 min. In addition, high-resolution T_1-weighted anatomical images (MPRage: 176 slices, matrix: 256 × 224, 1 × 1 × 1 mm^3) of the brain were obtained. All subjects were instructed not to focus their thoughts on anything in particular and to keep their eyes closed during the resting-state MR acquisition.

Data Preprocessing

Functional image preprocessing and statistical analysis were carried out by using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Images were realigned to the first image. Unwarping of geometrically distorted EPIs was performed using the FieldMap Toolbox available for SPM5 to account for susceptibility by movement artifacts. A mean image was created and co-registered to the T_1 structural image. The anatomical image was normalized to the Montreal Neurological Institute (MNI) template, and the resulting parameter file was used to normalize the functional images (voxel size: 3 × 3 × 3 mm^3). Finally the normalized images were smoothed with a three-dimensional isotropic gaussian kernel (FWHM: 6 mm). FMRI data were high-pass (cut-off period 128 s) and ~~low-pass filtered (autoregression model AR(1))~~.

Fractional Amplitude of Low-Frequency Fluctuation

Low-frequency (0.01–0.08 Hz) fluctuations (LFF) of the blood oxygenation level dependent (BOLD) signal in resting-state fMRI data are thought to reflect intrinsic neural activity in non-humans [28] and humans [29]. We carried out the fALFF analysis on the preprocessed functional data using REST (http://resting-fmri.sourceforge.net), as recently described [23, 30, 31]. fALFF is determined based on the analysis of the temporal data at each voxel. The regional intensity of spontaneous BOLD fluctuations is quantified by the power spectrum in the low-frequency range (0.009–0.08 Hz) and regularized by the power in the whole frequency range (0–0.25 Hz) [23]. The resulting activity maps are normalized and further analyzed by standard procedures. fALFF is a reliable and consistent index exhibiting moderate to high test-retest reliability [24].

Statistical Analysis

To control for daytime variations in cerebrocortical activity and fatigue or repetition effects, we performed a placebo experiment in random order and interpreted the change after insulin application in relation to placebo-derived changes. For each subject, the fALFF maps of the basal fMRI measurement were subtracted from the fALFF maps of the 30- and 90-min fMRI measurement. The fALFF maps of each subject, corrected for basal fMRI measurement, were entered into a second-level analysis in SPM5 using a full-factorial model to determine the effect of insulin versus placebo (factors: condition and time) 30 and 90 min after applying the spray. Age and BMI were used as confounding covariates, which means that the effect of age and BMI were removed from the data. Only activations exceeding a whole-brain false discovery rate (FDR) of $p < 0.05$ were considered as significant. Furthermore, we used a region-of-interest approach by using the WFU PickAtlas tool (http://www.fmri.wfubmc.edu/download.htm) for the hypothalamus, due to its fundamental role in energy homeostasis and expression of insulin receptors [10, 32–34]. SPM5's small volume correction to correct for multiple comparisons was used. To evaluate BMI-associated activity, we performed a multiple regression analyses in SPM5 using the baseline corrected fALFF maps for insulin and placebo day separately. Only activations exceeding a whole-brain FDR of $p < 0.05$ were considered as significant.

Hunger ratings were evaluated using a repeated measurement ANOVA in SPSS (version 19; SPSS Inc.). Results with values of $p < 0.05$ were considered statistically significant. The statistical analysis of metabolic parameters was performed with JMP 7.0 (SAS Institute, Cary, N.C., USA). All data are given as unadjusted mean ± SD. The parameters were log transformed to approximate normal distribution prior to statistical analysis. Paired t tests were used to test for significant differences between insulin and placebo. Results with values of $p < 0.05$ were considered statistically significant (table 1).

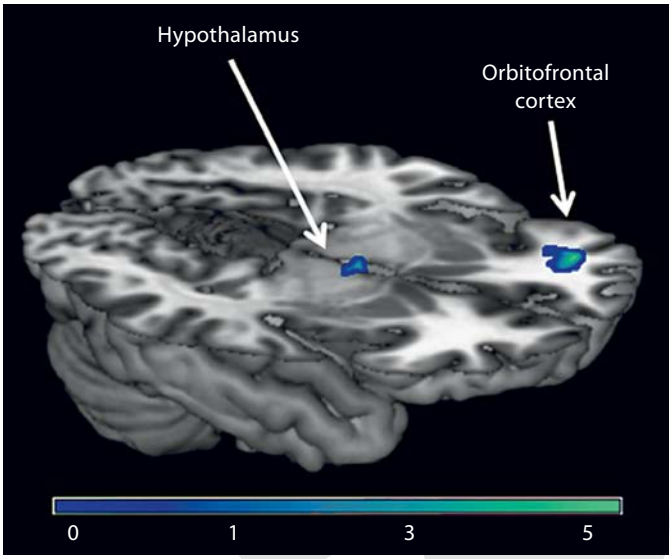

Fig. 2. Main effect of condition: insulin < placebo. Intranasal insulin-induced fALFF decrease in the hypothalamus and OFC. Color-coded z-value map represents significant ($p_{FDR} < 0.05$, corrected for multiple comparisons) voxels of *decreased* fALFF 30 and 90 min after insulin application corrected for basal measurement.

Table 2. Effect of intranasal insulin on LFF

Region	MNI coordinates[a]			z values[b]	Cluster size
	x	y	z		
Main effect of condition: insulin < placebo					
OFC left	−39	39	−9	4.04	34
Hypothalamus left	−3	−9	−9	3.47[c]	3

[a] Montreal Neurological Institute (mm).
[b] $p_{FDR} < 0.05$ corrected for multiple comparisons.
[c] Small volume corrected.

Results

Effect of Intranasal Insulin on Low-Frequency BOLD Fluctuations

We investigated the effect of intranasal insulin 30 and 90 min after spray application on fALFF. As shown in table 2, we observed a significant main effect of condition in the left OFC and left hypothalamus ($p_{FDR} < 0.05$, corrected for multiple comparison). There was no main effect of time or significant interaction. The OFC and hypothalamus showed a decrease in fALFF 30 and 90 min after intranasal insulin application (fig. 2).

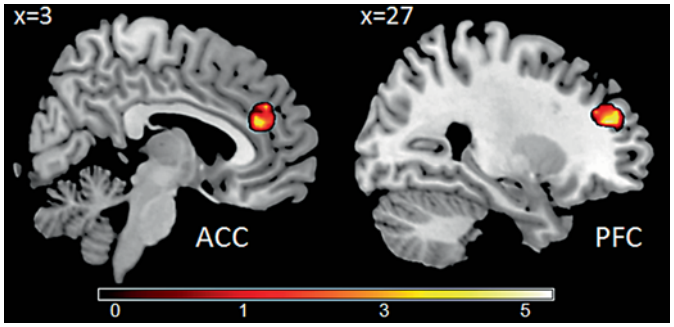

Fig. 3. BMI-associated low-frequency BOLD fluctuations. The intranasal insulin-induced fALFF increase in the PFC ($r = 0.61$; $p = 0.001$) and ACC ($r = 0.56$; $p = 0.02$) showed a positive correlation with BMI 30 and 90 min after insulin application, respectively. Color-coded z-value map represents significant ($p_{FDR} < 0.05$, corrected for multiple comparisons) voxels showing a significant correlation with BMI after insulin application corrected for basal measurement.

BMI-Associated Effect of Intranasal Insulin on Low-Frequency BOLD Fluctuations

We investigated the effect of insulin and placebo after spray application on fALFF dependent on BMI. We found a positive correlation between BMI and fALFF 30 min after intranasal insulin application in the superior frontal gyrus (BA10) (peak voxel x: 27, y: 42, z: 21; $r = 0.61$, $p_{FDR} = 0.001$, corrected for multiple comparison) (fig. 3). Furthermore, we found a positive correlation between BMI and fALFF 90 min after intranasal insulin application in the anterior cingulate gyrus bilaterally (peak voxel x: ±3, y: 33, z: 15; $r = 0.56$, $p_{FDR} = 0.02$, corrected for multiple comparison) (fig. 3). No significant correlation was observed after placebo spray administration.

Hunger Rating

Hunger rating 30 and 90 min after spray application were corrected for hunger rating under basal conditions (mean hunger score (±SE) under basal conditions 5.6 ± 0.73 on a 10-point VAS scale). The repeated measurement ANOVA revealed no significant main effect of time (30 vs. 90 min) or condition (placebo vs. insulin) and no significant interaction ($p > 0.05$).

Metabolic Parameters

Fasting plasma levels of glucose did not differ between insulin and placebo day (4.8 ± 0.1 vs. 4.6 ± 0.1 mmol/l). Fasting plasma insulin levels also did not differ between insulin and placebo day (45 ± 6 vs. 46 ± 6 pmol/l). Thirty minutes after intranasal insulin application, plasma

insulin levels showed a slight increase ($p < 0.05$), but this was not accompanied by a decrease in plasma glucose levels ($p > 0.05$). For the other time points, no differences in insulin levels between insulin and placebo application were observed.

Discussion

To date, effects of the important anorexic hormone insulin on the human brain still remain mostly unclear. Therefore we investigated, in this present study, the effect of insulin on the brains' homeostatic and non-homeostatic system. Intranasal application of insulin allowed us to selectively monitor insulin action in the brain without relevant effects on peripheral glucose metabolism. We used a reliable and consistent index (fALFF), reflecting the temporal dynamics of the BOLD signal at each voxel, to study intrinsic neuronal activity [24]. We were able to show that intrinsic brain activity is modulated by intranasal insulin 30 and 90 min after application. Specifically, we found that insulin was associated with a decrease in fALFF in the left OFC and hypothalamus 30 and 90 min after application compared to placebo. Furthermore, we observed BMI-associated activity in response to insulin in the PFC and ACC 30 and 90 min after application, respectively. The above-mentioned brain areas, including the striatum and amygdala, are central elements in the control of food intake acting in concert to regulate the learning of rewarding aspects of food [35]. In a recent magnetoencephalography (MEG) study, we found that insulin modifies the dynamics of the global brain network during resting state leading to increased global communication efficiency in lean adults [36]. Since insulin acts as an anorexic signal reducing food intake [37], we hypothesized that the increase in global efficiency could be connected to an augmented signaling in neural systems implicated in food intake.

The insulin-induced decrease in intrinsic activity was observed in the OFC and hypothalamus. The latter belongs to the brain's homeostatic system maintaining appropriate levels of energy balance, which is fundamental in the control of peripheral homeostatic activity. In accordance with that, we found the LFF in the hypothalamus to decrease up to 90 min after intranasal insulin application, which is probably related to decreases in neuronal activity in the lateral hypothalamic area known as the hunger center [33, 38]. The inhibition of hypothalamic activity might lead to an increase in satiety and potentially to an attenuated response to food stimuli. Hypothalamic dysfunction could potentially compromise the induction of satiety and indeed, altered hypothalamic processing after glucose ingestion was observed in obese adults and patients with diabetes type 2 [33, 39].

In this study, intranasal insulin modulated central elements of the reward system by a decrease in LFF in the OFC. Several studies have illustrated that the OFC is involved in the integration of different food modalities and in reward evaluation [40–42]. Furthermore, in a recent study we showed that the OFC is sensitive to fasting insulin levels [22], which is in accordance with the present study revealing that the OFC responds to cerebral changes in insulin directly independent of meal related sensory experience. The hypothalamus is also linked to the 'motivational circuitry' of the CNS both anatomically and functionally [8]. In particular, the hypothalamus orexin system drives midbrain neural activity modulating dopamine release [43]. Since insulin is known to impact orexin activity in the hypothalamus [44], it could be possible that intranasal insulin administration may decrease food intake and food reward through reduced overall orexin tone subsequently modulating dopamine release in the midbrain. Alterations in this circuitry could potentially lead to overeating; in fact, previous imaging studies have shown a hyperresponsiveness of the reward circuitry in obese [45–48] and diabetic subjects [49]. However, further studies with obese subjects and patients with diabetes are needed to evaluate this hypothesis.

The insulin-induced response in intrinsic ACC and prefrontal activity was associated with the subjects' BMI. The ACC is involved in emotion and cognition. The affective division of the ACC, as seen in this study, is primarily linked to paralimbic and subcortical regions associated with affective and autonomic processes including OFC, nucleus accumbens and hypothalamus. Hence the ACC is implicated in modulating, among other things, endocrine responses [50].

Cognitive influences on appetite are mediated by the PFC. Particularly, the DLPFC plays a critical role in the deployment of self-control monitoring behavioral consequences [7]. Hence the PFC is an important component involved in the termination of feeding. Indeed altered PFC and ACC activity has been observed in obese individuals during baseline [51] and while viewing food pictures [52]. Concurrently, in this study insulin modulated neural activity in the ACC and PFC correlated with BMI. Taken together, we can speculate that the insulin-induced increase in intrinsic activity in the ACC modifies the reward circuitry, which is important for the hedonic evaluation of food stimuli, while the PFC exerts inhibiting ef-

fects on eating by modulating the neural activity of the reward circuitry and the hypothalamus probably due to the efferent inhibitory projections. Our results further indicate a possible effect of BMI on cerebral insulin sensitivity. However, since all our subjects were of normal weight further studies are needed to evaluate the effect of BMI on insulin action in the brain in obese individuals.

Finally, we want to acknowledge some limitations of this study. First, we only investigated lean female subjects, therefore our results cannot be generalized to men or obese subjects based on this study alone. Similar studies in male subjects should be pursued as there may be gender differences in insulin action in the brain [53]. In addition, it has to be investigated which brain areas show a central insulin resistance in obese subjects. Second, we did not analyze the effect of insulin action on food intake. However, the results of the hunger ratings showed no differences between insulin and placebo administration, which concurs with a recent study showing that intranasal insulin has no short-term effect on appetite and food intake in lean women during the fasting state, as in our study [54]. Nevertheless, further studies are needed to evaluate the relationship between insulin action in the brain and behavioral correlates in order to generate new therapies for obesity and insulin resistance. Lastly, even though intranasal insulin did not induce changes in peripheral glucose metabolism, we did observe subtle rises in insulin levels. Since very high insulin levels are present in the brain after nasal delivery of the hormone [15], the small changes in plasma insulin are therefore very unlikely to induce additional changes in brain activity.

In conclusion, the intranasal application of insulin allowed us to stimulate cerebral insulin signaling without affecting peripheral glucose levels; hence, we were able to observe selective insulin action in the brain. In previous studies, using MEG, we were able to show that insulin modifies the dynamics of the global brain network; here, we were able to localize insulin action in the brain. Our data suggest that the hypothalamus and most notably, the reward and prefrontal circuitry are involved in the integration of insulin action, an important anorexic hormonal signal, thereby potentially decreasing the rewarding properties of food and reducing food intake.

Acknowledgements

This study was supported by the 'Kompetenznetz Adipositas' (Competence Network for Adiposity) (FKZ: 01GI0837 and 01GI0849) funded by the German Federal Ministry of Education and Research (BMBF) and the German Center for Diabetes Research (DZD e.V.).

Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Flegal KM, Carroll MD, Ogden CL, Johnson CL: Prevalence and trends in obesity among US adults, 1999–2000. JAMA 2002;288:1723–1727.

2 Flegal KM, Graubard BI, Williamson DF, Gail MH: Excess deaths associated with underweight, overweight, and obesity. JAMA 2005;293:1861–1867.

3 Berthoud HR: Neural systems controlling food intake and energy balance in the modern world. Curr Opin Clin Nutr Metab Care 2003;6:615–620.

4 Kenny PJ: Reward mechanisms in obesity: new insights and future directions. Neuron 2011;69:664–679.

5 Volkow ND, Wang GJ, Baler RD: Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci 2011;15:37–46.

6 Appelhans BM: Neurobehavioral inhibition of reward-driven feeding: implications for dieting and obesity. Obesity (Silver Spring) 2009;17:640–647.

7 Hare TA, Camerer CF, Rangel A: Self-control in decision-making involves modulation of the VMPFC valuation system. Science 2009;324:646–648.

8 Figlewicz DP, Sipols AJ: Energy regulatory signals and food reward. Pharmacol Biochem Behav 2010;97:15–24.

9 Marks JL, King MG, Baskin DG: Localization of insulin and type 1 IGF receptors in rat brain by in vitro autoradiography and in situ hybridization. Adv Exp Med Biol 1991;293:459–470.

10 Unger JW, Livingston JN, Moss AM: Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. Prog Neurobiol 1991;36:343–362.

11 Lathe R: Hormones and the hippocampus. J Endocrinol 2001;169:205–231.

12 Zhao WQ, Chen H, Quon MJ, Alkon DL: Insulin and the insulin receptor in experimental models of learning and memory. Eur J Pharmacol 2004;490:71–81.

13 Figlewicz DP, Szot P, Chavez M, Woods SC, Veith RC: Intraventricular insulin increases dopamine transporter MRNA in rat VTA/substantia nigra. Brain Res 1994;644:331–334.

14 Patterson TA, Brot MD, Zavosh A, Schenk JO, Szot P, Figlewicz DP: Food deprivation decreases MRNA and activity of the rat dopamine transporter. Neuroendocrinology 1998;68:11–20.

15 Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL: Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 2002;5:514–516.

16 Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B: Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol 2012;69:29-38.

17 Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, Kern W: Intranasal insulin improves memory in humans. Psychoneuroendocrinology 2004; 29: 1326–1334.
18 Benedict C, Frey WH 2nd, Schioth HB, Schultes B, Born J, Hallschmid M: Intranasal insulin as a therapeutic option in the treatment of cognitive impairments. Exp Gerontol 2010;46:112–115.
19 Hallschmid M, Benedict C, Schultes B, Fehm HL, Born J, Kern W: Intranasal insulin reduces body fat in men but not in women. Diabetes 2004;53:3024–3029.
20 Guthoff M, Grichisch Y, Canova C, Tschritter O, Veit R, Hallschmid M, Haring HU, Preissl H, Hennige AM, Fritsche A: Insulin modulates food-related activity in the central nervous system. J Clin Endocrinol Metab 2010;95:748–755.
21 Greicius MD, Srivastava G, Reiss AL, Menon V: Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci USA 2004;101:4637–4642.
22 Kullmann S, Heni M, Veit R, Ketterer C, Schick F, Haring HU, Fritsche A, Preissl H: The obese brain: association of body mass index and insulin sensitivity with resting-state network functional connectivity. Hum Brain Mapp 2012;33:1052–1061.
23 Zou QH, Zhu CZ, Yang Y, Zuo XN, Long XY, Cao QJ, Wang YF, Zang YF: An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state FMRI: fractional ALFF. J Neurosci Methods 2008;172:137–141.
24 Zuo XN, Di Martino A, Kelly C, Shehzad ZE, Gee DG, Klein DF, Castellanos FX, Biswal BB, Milham MP: The oscillating brain: complex and reliable. Neuroimage 2010;49:1432–1445.
25 Mennes M, Zuo XN, Kelly C, Di Martino A, Zang YF, Biswal B, Castellanos FX, Milham MP: Linking inter-individual differences in neural activation and behavior to intrinsic brain dynamics. Neuroimage 2011;54:2950–2959.
26 Cox CL, Uddin LQ, Di Martino A, Castellanos FX, Milham MP, Kelly C: The balance between feeling and knowing: affective and cognitive empathy are reflected in the brain's intrinsic functional dynamics. Soc Cogn Affect Neurosci 2011.
27 Pudel D, Westenhöfer J: Fragebogen zum Essverhalten (FEV). Handanweisung. Göttingen, Hogrefe, 1989.
28 Shmuel A, Leopold DA: Neuronal correlates of spontaneous fluctuations in FMRI signals in monkey visual cortex: implications for functional connectivity at rest. Hum Brain Mapp 2008;29:751–761.
29 Laufs H, Krakow K, Sterzer P, Eger E, Beyerle A, Salek-Haddadi A, Kleinschmidt A: Electroencephalographic signatures of attentional and cognitive default modes in spontaneous brain activity fluctuations at rest. Proc Natl Acad Sci USA 2003;100:11053–11058.
30 Yang H, Long XY, Yang Y, Yan H, Zhu CZ, Zhou XP, Zang YF, Gong QY: Amplitude of low-frequency fluctuation within visual areas revealed by resting-state functional MRI. Neuroimage 2007;36:144–152.
31 Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, Tian LX, Jiang TZ, Wang YF: Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev 2007;29:83–91.
32 Oomura Y, Kita H: Insulin acting as a modulator of feeding through the hypothalamus. Diabetologia 1981;20(suppl):290–298.
33 Matsuda M, Liu Y, Mahankali S, Pu Y, Mahankali A, Wang J, DeFronzo RA, Fox PT, Gao JH: Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes 1999;48:1801–1806.
34 Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J: Functional MRI of human hypothalamic responses following glucose ingestion. Neuroimage 2005;24:363–368.
35 Dagher A: The neurobiology of appetite: hunger as addiction. Int J Obes (Lond) 2009; 33(suppl 2):S30–S33.
36 Stingl KT, Kullmann S, Guthoff M, Heni M, Fritsche A, Preissl H: Insulin modulation of magnetoencephalographic resting-state dynamics in lean and obese subjects. Front Syst Neurosci 2010;4:157.
37 Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG: Central nervous system control of food intake. Nature 2000;404:661–671.
38 Oomura Y: Central mechanism of feeding. Adv Biophys 1973;5:65–142.
39 Vidarsdottir S, Smeets PA, Eichelsheim DL, van Osch MJ, Viergever MA, Romijn JA, van der Grond J, Pijl H: Glucose ingestion fails to inhibit hypothalamic neuronal activity in patients with type 2 diabetes. Diabetes 2007; 56:2547–2550.
40 Kringelbach ML, Rolls ET: The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog Neurobiol 2004;72: 341–372.
41 Rolls ET, Baylis LL: Gustatory, olfactory, and visual convergence within the primate orbitofrontal cortex. J Neurosci 1994;14:5437–5452.
42 Small DM, Zatorre RJ, Dagher A, Evans AC, Jones-Gotman M: Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain 2001;124:1720–1733.
43 Davis JF, Choi DL, Benoit SC: Insulin, leptin and reward. Trends Endocrinol Metab 2010; 21:68–74.
44 Moriguchi T, Sakurai T, Nambu T, Yanagisawa M, Goto K: Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia. Neurosci Lett 1999; 264:101–104.
45 Gautier JF, Chen K, Salbe AD, Bandy D, Pratley RE, Heiman M, Ravussin E, Reiman EM, Tataranni PA: Differential brain responses to satiation in obese and lean men. Diabetes 2000;49:838–846.
46 Gautier JF, Del Parigi A, Chen K, Salbe AD, Bandy D, Pratley RE, Ravussin E, Reiman EM, Tataranni PA: Effect of satiation on brain activity in obese and lean women. Obes Res 2001;9:676–684.
47 Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM: Relation of reward from food intake and anticipated food intake to obesity: A functional magnetic resonance imaging study. J Abnorm Psychol 2008;117:924–935.
48 Rothemund Y, Preuschhof C, Bohner G, Bauknecht HC, Klingebiel R, Flor H, Klapp BF: Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. Neuroimage 2007;37: 410–421.
49 Chechlacz M, Rotshtein P, Klamer S, Porubska K, Higgs S, Booth D, Fritsche A, Preissl H, Abele H, Birbaumer N, Nouwen A: Diabetes dietary management alters responses to food pictures in brain regions associated with motivation and emotion: a functional magnetic resonance imaging study. Diabetologia 2009;52:524–533.
50 Bush G, Luu P, Posner MI: Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 2000;4:215–222.
51 Volkow ND, Wang GJ, Telang F, Fowler JS, Goldstein RZ, Alia-Klein N, Logan J, Wong C, Thanos PK, Ma Y, Pradhan K: Inverse association between BMI and prefrontal metabolic activity in healthy adults. Obesity (Silver Spring) 2009;17:60–65.
52 Martin LE, Holsen LM, Chambers RJ, Bruce AS, Brooks WM, Zarcone JR, Butler MG, Savage CR: Neural mechanisms associated with food motivation in obese and healthy weight adults. Obesity (Silver Spring) 2009.
53 Benedict C, Kern W, Schultes B, Born J, Hallschmid M: Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. J Clin Endocrinol Metab 2008;93:1339–1344.
54 Hallschmid M, Higgs S, Thienel M, Ott V, Lehnert H: Postprandial administration of intranasal insulin intensifies satiety and reduces intake of palatable snacks in women. Diabetes 2012;61:782–789.