Alterations in hypothalamic gene expression following Roux-en-Y gastric bypass

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ABSTRACT

Objective: The role of the central nervous system in mediating metabolic effects of Roux-en-Y gastric bypass (RYGB) surgery is poorly understood. Using a rat model of RYGB, we aimed to identify changes in gene expression of key hypothalamic neuropeptides known to be involved in the regulation of energy balance.

Methods: Lean male Sprague-Dawley rats underwent either RYGB or sham surgery. Body weight and food intake were monitored bi-weekly for 60 days post-surgery. *In situ* hybridization mRNA analysis of hypothalamic AgRP, NPY, CART, POMC and MCH was applied to RYGB and sham animals and compared with *ad libitum* fed and food-restricted rats. Furthermore, *in situ* hybridization mRNA analysis of dopaminergic transmission markers (TH and DAT) was applied in the midbrain.

Results: RYGB surgery significantly reduced body weight and intake of a highly palatable diet but increased chow consumption compared with sham operated controls. In the arcuate nucleus, RYGB surgery increased mRNA levels of orexigenic AgRP and NPY, whereas no change was observed in anorexigenic CART and POMC mRNA levels. A similar pattern was seen in food-restricted versus *ad libitum* fed rats. In contrast to a significant increase of orexigenic MCH mRNA levels in food-restricted animals, RYGB did not change MCH expression in the lateral hypothalamus. In the VTA, RYGB surgery induced a reduction in mRNA levels of TH and DAT, whereas no changes were observed in the substantia nigra relative to sham surgery.

Conclusion: RYGB surgery increases the mRNA levels of hunger-associated signaling markers in the rat arcuate nucleus without concomitantly increasing downstream MCH expression in the lateral hypothalamus, suggesting that RYGB surgery puts a brake on orexigenic hypothalamic output signals. In addition, down-regulation of midbrain TH and DAT expression suggests that altered dopaminergic activity also contributes to the reduced intake of palatable food in RYGB rats.

1. INTRODUCTION

Obesity, diabetes and the metabolic syndrome are major health problems worldwide. The World Health Organization estimates that more than 1.9 billion adults and over 42 million children under the age of five are overweight or obese [1]. With today's epidemic proportions of obesity, more deaths are related to overweight than underweight. Lifestyle intervention and pharmacological treatment have had limited success in weight management in obese patients [2,3]. Hence, to date bariatric surgery procedures, including Roux-en-Y gastric bypass (RYGB), are considered the most effective treatment of obesity and its co-morbidities, such as type-2 diabetes mellitus (T2DM), hypertension and stroke [4,5].

The exact mechanisms underlying the weight loss and anti-diabetic effects of gastric bypass are not fully understood, and substantial efforts are being made to identify novel targets for more efficacious anti-obesity drug therapies. One of the consistent findings in animal models as well as in humans after RYGB is elevated plasma levels of gut-

derived hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), and it is believed that these hormones are involved in the beneficial effects of surgery [6—9]. However, despite the intense research into the underlying mechanisms of RYGB-induced weight loss, currently, there is a lack of studies specifically investigating the changes in central signaling pathways involved in metabolic control. It is well-known that long-term energy balance is regulated by a complex network of central signaling pathways that regulate food intake, energy expenditure and metabolic efficacy [10]. Essential for weight homeostasis is the integration of orexigenic and anorexigenic signaling by the leptin and melanocortin dependent pathways within the central nervous system (CNS) [11]. The hypothalamic arcuate nucleus (Arc) is of special importance, as this region contains two distinct leptin responsive neuronal populations, i.e. orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) expressing neurons as well as anorexigenic cocaine and amphetamine related transcript (CART) and proopiomelanocortin (POMC) expressing neurons [12—14]. These first order neurons in the Arc project to the paraventricular nucleus (PVN)

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and the lateral hypothalamus (LHA), which, in combination, determine the balance of behavioral and autonomic outputs involved in eating behavior and metabolism (For review see [14]). Present information on transcriptional changes in the Arc following bariatric surgery is limited to a few studies assessing the effects of biliopancreatic and duodenal-jejunal bypass in rats. Interestingly, both procedures were shown to significantly increase the arcuate NPY expression, indicating that orexigenic pathways were stimulated without neutralizing the surgically induced reductions in food intake and body weight [15,16]. This counterintuitive response indicates that other downstream signaling cascades may be blocked, potentially MCH expression in the LHA, which is known to be strongly modulated by POMC (inhibition) and NPY (activation) application [12].

Besides the homeostatic regulation of energy balance, central hedonic signaling is also able to influence whole body energy homeostasis [17]. In accordance, the mesolimbic dopaminergic pathway is closely associated with the regulation of food intake where elevated levels of dopamine in the ventral tegmental area (VTA) causes increased motivation to consume particularly energy-dense food [18]. This may also be relevant for the weight lowering effect of gastric bypass procedures, as rodent RYGB models are reported to change food preference from high to low sweet and fat food stimuli [19—21], indicating that RYGB modifies the hedonic value of energy-dense food. To date, however, very few studies have investigated the central component of this altered behavior on the transcriptional level.

The present study was undertaken to get further insight into the central signaling pathways potentially influenced by RYGB surgery. A special emphasis was put on key relay structures conveying orexigenic and anorexigenic signaling in the hypothalamus, including the Arc and LHA. In addition, to investigate neural circuitries underlying the interaction between nutritional status and reward, the impact of RYGB on transcriptional markers of midbrain dopaminergic neurotransmission was assessed.

2. MATERIALS AND METHODS

2.1. Experimental animals

All animal experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animals, and in compliance with personal animal license (2013-15-2934-00784) issued by the Danish Committee for Animal Research.

Male Sprague Dawley rats 12 weeks of age underwent either RYGB (n = 9; body weight 409 ± 16 g) or sham surgery (n = 9; body weight 360 ± 7 g). Four weeks prior to surgery, animals had ad libitum access to a two-choice diet consisting of chow (altromin 1324, Brogaarden, Denmark) and the Gubra high palatable high fat diet (HPHF diet; Nutella (Ferrero, Italy), peanut butter (pcd, Netherlands) and powdered chow [22]). The rats were single-housed throughout the study under controlled environmental conditions (12 h light/12 h dark cycle; 22 ± 1 °C; 50 ± 10% relative humidity). Body weight, food and water intake were monitored bi-weekly during the study.

In addition, male Sprague Dawley rats were included as food restricted (n = 8) and free-fed (n = 8) controls for the analysis of hunger vs. satiety associated hypothalamic gene expression. Free-fed animals had ad libitum access to the two-choice diet as described above, whereas food restricted animals had access to approximately 50% of the food ingested by free-fed animals. Animals were terminated by decapitation under CO_2/O_2 anesthesia after 48 h, and brains were quickly removed and snap frozen on crushed dry ice and stored at −80 °C until further processing.

2.2. Surgery

Three days prior to surgery, animals were put on liquid diet (Osmolite 1 Cal.; Abbott Nutrition). On the day of surgery, animals underwent whole body composition analysis by non-invasive EchoMRI scanning (EchoMRI-900 Analyzer, EchoMRI, USA), and a wire grate was placed in the bottom of the cage to prevent coprophagia. Prior to surgery, animals were subcutaneously administered with warm saline (20 ml/kg), buprenorphine (0.03 mg/kg), enrofloxacin (0.5 mg/kg) and carprofen (0.5 mg/kg) to prevent post-operative infection, lethargy and pain relief.

The RYGB surgical procedure was performed according to Chambers et al. [23]. In brief, the abdomen was exposed using a midline laparotomy after induction of surgical anesthesia with an isoflurane/O_2 mixture. The jejunum was transected 30 cm distal to the ligament of Treitz. A longitudinal anti-mesenteric incision was made 10 cm distal to the transected bowel and connected to the afferent limb of the jejunum with a running 8-0 Vicryl absorbable suture (Ethicon, Somerville, NJ, USA). The stomach was exposed and the fundus was excised by making a vertical cut along the line separating the corpus from the fundus with an ETS-FLEX 35-mm staple gun (Ethicon). A second staple line was placed across the waist of the stomach, creating a gastric pouch approximately 10% the size of the normal stomach. The distal remnant was returned to the peritoneal cavity and an incision was made on one side of the gastric pouch considering the vascular architecture. The efferent limb of the transected jejunum was connected to the gastric pouch with a running 8-0 nylon non-absorbable suture (Ethicon). After repositioning the gastric pouch into the peritoneal cavity, the abdominal wall was closed.

The sham procedure followed the steps of the RYGB but gastrointestinal surgery only included a transection of the jejunum 30 cm distal to the ligament of Treitz, which was immediately re-sutured.

2.3. Post-surgery care

The wire grate was kept in place for five days post-operatively, and only liquid diet was offered in this period. Subcutaneous injections of warm saline BID (20 ml/kg), carprofen QD (5 mg/kg) and enrofloxacin QD (5 mg/kg) were administered for five days after surgery. On day six, the wire grate was removed and ad libitum access to the two-choice diet was re-introduced.

2.4. Termination

60 days post-surgery, animals were euthanized by decapitation under CO_2/O_2 anesthesia. All animals were euthanized in the morning, 2—3 h after lights on. RYGB and sham operated animals were terminated in a randomized order. Prior to termination, animals underwent an additional whole-body echo-MRI scan. Following termination, brains were quickly removed, snap frozen on crushed dry ice, and stored at −80 °C until further processing.

2.5. Tissue processing

12 μm thick coronal sections of the hypothalamus and midbrain were cut on a cryostat (CM 1850, Leica, Germany) using systematic uniform random sampling. Serial sections were sampled at every 180 μm (i.e. every 15th section) and mounted on super-frost plus slides. Sections from the hypothalamus were sampled from the rostral part of the hypothalamic PVN to the caudal part of the Arc. Sections from the midbrain spanned the full rostro-caudal extension of the VTA and the substantia nigra (SN). Sections were allowed to dry at room temperature for an hour and then kept at −80 °C until hybridization was carried out.