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Improvement in hepatic insulin sensitivity after Roux-en-Y gastric bypass in a rat model of obesity is partially mediated via hypothalamic insulin action

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Abstract

Aims/hypothesis—Roux-en-Y gastric bypass (RYGB) surgery, an effective treatment for morbid obesity, commonly leads to near complete resolution of type 2 diabetes. The underlying mechanisms, however, remain unclear and factors other than weight loss alone may be involved.

Methods—To determine whether increased hypothalamic insulin sensitivity after RYGB drives the rapid improvement in glucose metabolism, high-fat-fed rats received either an insulin receptor (IR) antisense vector or a control lentiviral vector that was microinjected into the ventromedial hypothalamus (VMH). Six weeks later, rats underwent RYGB or control gastrointestinal surgery.

Results—Four weeks after surgery, weight loss was comparable in RYGB and surgical controls. Nevertheless, only RYGB rats that received the control vector demonstrated both improved hepatic and peripheral insulin sensitivity. Insulin suppressed hepatic glucose production (HGP) by 50%

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($p < 0.05$) with RYGB, whereas the effect of insulin on HGP was completely absent in VMH IR knockdown (IRkd) rats. By contrast, both RYGB groups displayed an identical twofold increase in insulin-stimulated peripheral glucose uptake. The animals that underwent control gastro-intestinal surgery failed to show any improvement in either hepatic or peripheral insulin sensitivity; VMH IRkd did not influence the magnitude of insulin resistance.

Conclusions/interpretation—Our findings demonstrate that RYGB surgery in high-fat-fed obese rats enhances hepatic and peripheral insulin sensitivity independently of weight loss. The improved hepatic, but not the peripheral, response to insulin is mediated centrally at the level of the VMH. These data provide direct evidence that the metabolic benefits of RYGB surgery are not simply a consequence of weight loss but likely in part involve the central nervous system.

Keywords

Gastric bypass surgery; Hypothalamus; Insulin

Introduction

Previous studies have demonstrated that the hypothalamus plays a key role in the control of energy balance and metabolism. Hypothalamic nuclei [1], and the ventromedial hypothalamus (VMH) in particular, have been shown to regulate nutrient sensing and glucose homeostasis [2]. Insulin receptors (IRs) are expressed within the VMH and respond to insulin application with changes in the neuronal firing rate [3]. The important role of central nervous system insulin signalling in the development of glucose intolerance and insulin resistance was derived from studies in mouse models in which IRs were specifically deleted or suppressed acutely by knockdown (IRkd) in specific neurons in adult animals. Moreover, chronic changes in hypothalamic insulin have been shown in mice to modulate the expression of neuropeptides that regulate food intake and fat cell number and mass [4]. Taken together, these observations suggest that impaired brain insulin signalling may be an important contributor to the development of impaired glucose metabolism and obesity.

The particular brain regions participating in insulin's effects on glucose metabolism remain uncertain. However, our previous studies point to the VMH as a likely participant [2]. We demonstrated that selective chronic VMH IRkd leads to the development of hepatic insulin resistance, inappropriate glucagon secretion, glucose intolerance and, surprisingly, impaired insulin secretion [5]. These studies demonstrate that VMH IRkd can recapitulate the early stages of the development of diabetes in non-diabetes-prone rats.

With regard to treatment of impaired glucose metabolism in obesity, the Roux-en-Y gastric bypass (RYGB) is extremely effective: it results in an almost complete resolution of associated insulin resistance and frequently resolves type 2 diabetes [6]. The physiological mechanisms underlying these beneficial effects, however, remain unclear. It was originally thought that RYGB improves glucose metabolism by causing weight loss secondary to restriction of gastric volume, intestinal malabsorption or a combination of the two. However, it is now evident that the antidiabetic effect is more complex, given that the improvement of glucose and insulin levels occurs within days after RYGB; too soon to be entirely due to the weight loss, suggesting neural mechanisms might be involved [7–9]. This study was

undertaken to determine whether changes in hypothalamic insulin sensitivity contribute to the rapid improvement in glucose tolerance and insulin sensitivity that occurs after the procedure.

Methods

Animals

Adult male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC, USA) received a high-fat diet for approximately 12 weeks to induce obesity. Animals then underwent stereotaxic surgery to receive a lentiviral vector containing the IR antisense (1.5 µl/side over 15 min) to knock down the VMH IR, or a control virus using stereotaxic bilateral microinjections in the VMH (antero-posterior 2.6 mm, lateral 1 mm, ventral 8.0 mm), as previously described [5]. Six weeks later, the animals underwent either RYGB ($n=8$) or gastrointestinal control surgery ($n=8$). Surgeries were carried out at Penn State University, as described previously [10], in accordance with approved animal protocols. Briefly, all rats were fasted overnight, but allowed ad libitum water before sterile surgery was performed under anaesthesia (isoflurane 3% for induction, 1.5% for maintenance). Two surgical procedures were performed, as described below. VMH IR expression was also assessed in a separate group of RYGB ($n=6$) and control surgery rats ($n=5$) receiving a high-fat diet.

RYGB

A midline abdominal incision was made and the stomach divided to create a reduced (~20%) gastric pouch using a 45 mm GIA stapler (ETS-Flex Ethicon Endo-surgery, Blue Ash, OH, USA). Measuring from the ligament of Treitz, the small intestine was divided to create a 15 cm biliopancreatic limb and a 15 cm alimentary 'Roux' limb, with the remaining segment (65–70 cm) forming the common channel. The gastro-jejunal and jejuno-jejunal anastomoses were performed end-to-side using interrupted 5–0 polypropylene sutures, and the abdominal wall and skin were closed using 3–0 silk and 5–0 nylon.

Gastrointestinal control surgery

The control rats received a manipulation of the stomach by clearing its attachments and a transverse enterotomy at the 30 cm point on the proximal jejunum; however, this was reclosed with interrupted 5–0 polypropylene sutures without forming an anastomosis. The abdominal wall and skin were then closed, as above.

Postoperative care

Surgical incisions were treated with 0.5 ml 0.25% bupivacaine s.c. to minimise postoperative pain. Treatment with normal saline (154 mmol/l NaCl; 50 ml/kg s.c., immediately prior to and after surgery) and buprenorphine (0.5 mg/kg i.m.) was provided as needed for pain. After 24 h to allow the anastomoses to heal, the animals were maintained on a liquid diet (BOOST; Nestlé Nutrition, Minneapolis, MN, USA) and ad libitum water for 3 days. On postoperative day 3, the BOOST was removed and the animals were returned to their high-fat diet. After 2 weeks, once the animals had completely recovered, they were transported to Yale School of Medicine for metabolic assessment. Following their acclimatisation at Yale,

vascular catheters were inserted into the left carotid artery for blood sampling and the right jugular vein for infusion.

Euglycaemic glucose clamp procedures

One week following catheter implantation, hyperinsulinaemic-euglycaemic clamp studies were performed using a primed continuous infusion of [^3H]glucose, followed by an insulin ($2 \text{ mU kg}^{-1} \text{ min}^{-1}$) and variable dextrose infusion, as previously described [5].

Immunoblot analysis

Frozen tissue micropunches from the VMH were homogenised and membrane protein fractions were used for immunoblots of IR protein [5].

Statistical analysis

Treatment effects were analysed using ANOVA, followed by post hoc analysis using Prism analytical software (www.graphpad.com). $p < 0.05$ was set as the criterion for statistical significance.

Results

RYGB, VMH IRkd and body weight

The IR antisense virus diminished VMH IR expression by 30–40% in both rats receiving RYGB and those receiving control surgery compared with their respective controls normalised to α -cadherin (Fig. 1a). RYGB in the absence of viral delivery significantly increased VMH IR expression compared with surgical controls (Fig. 1b). Four weeks was not sufficient time to see greater weight loss in the RYGB compared with the control surgical group. All animals lost similar ($\sim 10\%$) body weight; VMH IRkd also altered weight loss (Fig. 1c). Euglycaemia was maintained during the hyperinsulinaemic clamp in all groups (Fig. 1d, e). In the RYGB group ($n=4$) given control viruses, glucose infusion rates were significantly increased compared with the RYGB group with VMH IRkd ($n=4$) (Fig. 1f). By contrast, none of the surgical control groups ($n=4$, each) required any glucose delivery throughout the study, reflecting their greater insulin resistance (data not shown).

Hepatic insulin sensitivity

Despite negligible differences in weight loss, RYGB significantly improved hepatic insulin sensitivity (Fig. 2a). Hepatic glucose production (HGP) during the last 30 min of the hyperinsulinaemic period was reduced by 58% in the RYGB rats (3.9 ± 0.9 in RYGB vs $9.3 \pm 1.5 \text{ mg kg}^{-1} \text{ min}^{-1}$ in sham surgical animals; $p < 0.05$). VMH IRkd significantly impaired the effectiveness of RYGB surgery. HGP in RYGB rats given control virus declined in response to insulin (7.4 ± 1 basal vs $3.9 \pm 0.9 \text{ mg kg}^{-1} \text{ min}^{-1}$ clamp; $p < 0.05$), whereas no changes occurred in IRkd rats (10.6 ± 1.6 basal vs $9.8 \pm 1.8 \text{ mg kg}^{-1} \text{ min}^{-1}$ clamp). The effect of VMH IRkd on hepatic sensitivity was specific to the RYGB group; VMH IRkd had no effect in the surgical control animals (6.9 ± 1 basal vs $7.5 \pm 0.5 \text{ mg kg}^{-1} \text{ min}^{-1}$ clamp) (Fig. 2b). Basal insulin and insulin increments during the clamp study were indistinguishable in all four treatment groups (Fig. 2e, f). The effects of VMH IRkd on HGP in RYGB-treated

rats were not due to differences in glucagon levels either at baseline (73 ± 11 control vs 70 ± 15 pg/ml IRkd) or during hyperinsulinaemia (data not shown). Plasma glucagon was also not altered by sham surgery in either surgical group (64 ± 21 control vs 75 ± 5 pg/ml IRkd).

Peripheral glucose uptake

RYGB surgery significantly improved peripheral glucose uptake (20 ± 1 RYGB vs 9.3 ± 1.5 mg kg⁻¹ min⁻¹ surgical controls; $p < 0.05$). However, VMH IRkd did not alter the increase in peripheral glucose uptake seen after RYGB (20 ± 1 control vs 21 ± 2.5 mg kg⁻¹ min⁻¹ VMH IRkd) (Fig. 2c, d).

Discussion

RYGB, a very effective treatment for morbid obesity, frequently leads to dramatic improvements in glucose metabolism, including reversal of insulin-treated type 2 diabetes [6]. While the marked weight loss that generally accompanies bariatric surgery was originally thought to be the driving force behind the dramatic improvement in glucose metabolism and insulin sensitivity, the rapidity of the effect suggests that the mechanisms responsible may be more complex [7–9]. In this study we used a targeted gene knockdown approach to diminish VMH IR expression to investigate whether changes in VMH insulin signalling pathways might contribute to some of the beneficial effects of RYGB in a rat model of obesity. Our data raise the intriguing possibility that increased VMH sensitivity to insulin may be one of the mechanisms responsible for the beneficial effects of RYGB on glucose homeostasis and that this effect is mediated at the level of the liver. Furthermore, these studies suggest that other brain regions and/or mechanisms contribute to improvements in hepatic and peripheral insulin sensitivity observed following RYGB, since VMH IRkd was incomplete and did not reverse the improvement in insulin-induced glucose disposal produced by RYGB.

The observation that neither hepatic nor peripheral insulin sensitivity improved after gastrointestinal control surgery, despite similar reductions in overall body weight, provides direct support for the suggestion that factors other than weight loss contribute to the metabolic benefits of RYGB. While changes in insulin sensitivity within the VMH are likely involved, the small cohort of animals studied and the limited hormonal measurements obtained limit definitive conclusions regarding mechanisms. Nevertheless, these studies provide evidence supporting the hypothesis that the beneficial effects of RYGB on glucose metabolism extend beyond weight loss and suggest that central nervous system mechanisms are involved.

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Abbreviations

HGP	Hepatic glucose production
IR	Insulin receptor
IRkd	Insulin receptor knockdown
RYGB	Roux-en-Y gastric bypass
VMH	Ventromedial hypothalamus

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Fig. 1.

Representative western blot showing suppression of VMH IR observed (a) after microinjection with a lentivirus containing the IR, and (b) after control or RYGB surgery. Changes in body weight (BW) in different groups of animals (c). Plasma glucose levels in RYGB (d) and gastrointestinal surgical control (e) animals during euglycaemic clamps. Glucose infusion rates (GIR) in RYGB-control and -IRkd groups (f). Squares, RYGB-control; triangles, RYGB-IRkd; inverted triangles, surgical control-control virus; diamonds, surgical control-IRkd

Fig. 2.

Comparison of metabolic variables between RYGB and surgical control groups. HGP in RYGB (a) and surgical control (b) groups. Glucose uptake was stimulated in RYGB animals (c) compared with the surgical control group (d). Basal and clamp insulin levels in all groups (e, f). Squares, RYGB-control; triangles, RYGB-IRkd; inverted triangles, surgical control-control virus; diamonds, surgical control-IRkd. $p < 0.05$, RYGB vs sham clamp