Neuroscience 247 (2013) 43–54

FOOD-INTAKE DYSREGULATION IN TYPE 2 DIABETIC GOTO-KAKIZAKI RATS: HYPOTHESIZED ROLE OF DYSFUNCTIONAL BRAINSTEM THYROTROPIN-RELEASING HORMONE AND IMPAIRED VAGAL OUTPUT

K. ZHAO,[a] Y. AO,[c] R. M. HARPER,[c] V. L. W. GO[b] AND H. YANG[a,b]*

[a] *Research & Development, Department of Veterans Affairs, Greater Los Angeles Health Care System, Los Angeles, CA 90073, USA*

[b] *Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90073, USA*

[c] *Department of Neurology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90073, USA*

Abstract—Thyrotropin-releasing hormone (TRH), a neuropeptide contained in neural terminals innervating brainstem vagal motor neurons, enhances vagal outflow to modify multisystemic visceral functions and food intake. Type 2 diabetes (T2D) and obesity are accompanied by impaired vagal functioning. We examined the possibility that impaired brainstem TRH action may contribute to the vagal dysregulation of food intake in Goto-Kakizaki (GK) rats, a T2D model with hyperglycemia and impaired central vagal activation by TRH. Food intake induced by intracisternal injection of TRH analog was reduced significantly by 50% in GK rats, compared to Wistar rats. Similarly, natural food intake in the dark phase or food intake after an overnight fast was reduced by 56–81% in GK rats. Fasting (48 h) and refeeding (2 h)-associated changes in serum ghrelin, insulin, peptide YY, pancreatic polypeptide and leptin, and the concomitant changes in orexigenic or anorexigenic peptide expression in the brainstem and hypothalamus, all apparent in Wistar rats, were absent or markedly reduced in GK rats, with hormone release stimulated by vagal activation, such as ghrelin and pancreatic polypeptide, decreased substantially. Fasting-induced Fos expression accompanying endogenous brainstem TRH action decreased by 66% and 91%, respectively, in the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMV) in GK rats, compared to Wistar rats. Refeeding abolished fasting-induced Fos-expression in the NTS, while that in the DMV remained in Wistar but not GK rats. These findings indicate that dysfunctional brainstem TRH-elicited vagal impairment contributes to the disturbed food intake in T2D GK rats, and may provide a pathophysiological mechanism which prevents further weight gain in T2D and obesity. Published by Elsevier Ltd. on behalf of IBRO.

Key words: food intake, type 2 diabetes, the vagus nerve, thyrotropin-releasing hormone, brainstem.

INTRODUCTION

Body metabolic homeostasis is tightly regulated by the central nervous system through precise adjustments of sympathetic and parasympathetic outflow to multiple visceral organs. Vagal cholinergic processes, in particular, control hunger, meal initiation, and food digestion, through cholinergic (muscarinic) regulation of gastric secretory and motility functions, especially gastric ghrelin biosynthesis and secretion (Toshinai et al., 2001; Ao et al., 2006). The vagal role can be shown by blockage of food deprivation-induced plasma ghrelin elevation following subdiaphragmatic vagotomy and atropine treatment (Williams et al., 2003). In addition, circulating ghrelin levels in humans are increased or decreased by cholinergic agonists or antagonists, respectively (Broglio et al., 2004).

Attenuated vagal motor activity and exaggerated sympathetic discharge are common in type 2 diabetes (T2D) and obese patients (Oida et al., 1999), and develop as part of the mechanisms for re-equilibration of metabolism in these conditions (Chaput et al., 2012). Vagal impairment further enhances the unbalanced sympathetic dominance. Albeit increasing energy expenditure (Straznicky et al., 2012a), sympathetic hyperactivity mediates the development of insulin resistance, hyperglycemia, reduced insulin response to glucose, lipid dysregulation, hypertension, and high cardiovascular mortality (Oida et al., 1999; Perin et al., 2001; Lindmark et al., 2003), thus is critical in the pathogenesis of obesity-associated T2D (Esler et al., 2001; Straznicky et al., 2012b). Impaired vagal function

*Corresponding author. Address: CURE: DDRC, VA GLAHS, Building 115, Room 203, 11301 Wilshire Boulevard, Los Angeles, CA 90073, USA. Tel: +1-310-478-3711x41876; fax: +1-310-268-4963.
E-mail address: hoyang@ucla.edu (H. Yang).

Abbreviations: AgRP, agouti-related peptide; Amb, nucleus ambiguus; AP, area postrema; BW, body weight; DMV, dorsal motor nucleus of the vagus; DVC, dorsal vagal complex; F48h, 48 h fasted; GHSR, ghrelin receptor; GIP, glucose-dependent insulinotropic polypeptide; GK rats, Goto-Kakizaki rats; GLAHS, Greater Los Angeles Healthcare System; GLP-1, glucagon-like peptide-1; IACUC, Institutional Animal Care and Use Committee; ic, intracisternal or intracisternal injection; LEPR, leptin receptor; MC4R, melanocortin receptor 4; NF, normally fed; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; PB, phosphate buffer; POMC, proopiomelanocortin; PP, pancreatic polypeptide; PYY, peptide YY; RF2h, 2 h refed after a 48 h fasted; T2D, type 2 diabetes; TRH, thyrotropin-releasing hormone; TRHR, TRH receptor; VA, Veterans Affairs; VMH, ventromedial hypothalamic; Y2R, neuropeptide receptor 2.

0306-4522/13 $36.00 Published by Elsevier Ltd. on behalf of IBRO.
http://dx.doi.org/10.1016/j.neuroscience.2013.05.017

underlies dysregulation of food intake and digestion in obese and T2D subjects, who commonly have gastroparesis-associated early satiety, fullness, bloating, and suppressed plasma ghrelin levels (Williams et al., 2006; Boaz et al., 2011). Understanding the mechanisms of impaired vagal function on food-intake regulation is essential to determine processes underlying T2D and obesity.

Thyrotropin-releasing hormone (TRH), a neuropeptide originally found in the hypothalamus, is synthesized also in the brainstem caudal raphe nuclei. The raphe nuclei innervate vagal and sympathetic motor neurons located in the brainstem and the spinal cord. Dense TRH-containing nerve fibers appear in the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMV), area postrema (AP), and nucleus ambiguus (Amb), all nuclei involved in vagal regulation, as well as in the spinal sympathetic intermediolateral cell column (Manaker and Rizio, 1989; Rinaman et al., 1989; Taché and Yang, 1994). TRH microinjected into the DMV, or endogenously released into the DMV after chemical stimulation of neurons in the raphe nuclei, induces potent vagal-mediated visceral responses (Ishikawa et al., 1988; Yang et al., 1993, 2002). Substantial evidence shows that TRH is the only brain peptide fulfilling all of the criteria as a neurotransmitter and/or neuromodulator activating vagal motor neurons in the DMV, where it assists vagal regulation of food digestion and nutrition metabolism by increasing gastric/intestinal secretory/motility and stimulating gut/pancreatic hormone release (Taché and Yang, 1994; Ao et al., 2005a, 2006, 2010; Yang et al., 2012). Activation of brainstem TRH receptors increases food intake by stimulating vagal cholinergic pathway-mediated gastric ghrelin release (Ao et al., 2006). Furthermore, brainstem TRH mRNA levels increase during body energy deficiencies or when energy demand is increased, such as fasting and cold exposure (Yang et al., 1994; Ao et al., 2006). The collective evidence suggests that TRH plays an essential role in mediating vagal control of energy intake and nutrition digestion, and should be a focus of attention in any examination of food regulation, especially in T2D models.

We tested the hypothesis that impaired vagal function in T2D is associated with food-intake dysregulation in T2D Goto-Kakizaki (GK) rats. The GK rat is a ''non-obese'' T2D model generated by selective breeding from non-diabetic Wistar rats with poor glucose tolerance, and shows basal hyperglycemia, hypertension, and insulin resistance (Goto et al., 1988; Yang et al., 2012). We found impaired vagal regulation of visceral organs in GK rats, a consequence of reduced vagal efferent outflow activation by brainstem TRH (Ao et al., 2005a, 2010; Yang et al., 2012). TRH analog intracisternal injection (ic), at doses that did not influence blood glucose levels and heart rate in non-diabetic Wistar rats, induced persistent sympathetic activation-mediated increases in glucose levels, blood pressure and heart rate in GK rats, concomitant with remarkably damaged vagal-counterregulation on insulin stimulation and cardiac inhibition (Ao et al., 2005a, 2010; Yang et al., 2012). The extreme and prolonged hyperglycemia and acute heart failure-induced cardiovascular mortality in GK rats indicate severely damaged TRH action on DMV vagal motor neurons (Ao et al., 2005a, 2010; Yang et al., 2012). In this study, we first established body adipose tissue content in the GK and Wistar rats and then evaluated the inappropriate management of central and peripheral signaling in response to fasting and refeeding in T2D GK rats, with focus on brainstem TRH dysfunction-associated impairment of vagal regulation.

EXPERIMENTAL PROCEDURES

Animals

Male non-diabetic Wistar rats (280–320 g, 2.5–3 months old) were purchased from Harlan Laboratories (San Diego, CA). Sex- and body weight (BW)-matched T2D GK rats (3 months old) were bred in the animal facilities of Veterans Affairs (VA) Greater Los Angeles Healthcare System (GLAHS) with an Institutional Animal Care and Use Committee (IACUC)-approved breeding protocol. Rats were housed under controlled conditions (21–23 °C, lights-on from 06:00 to 18:00) with free access to standard rat chow and tap water. The study protocol was approved by the IACUC of VA GLAHS. Experiments were performed in rats either ad lib fed (normally fed), fasted (over night, 24 h, or 48 h), or refed for 2 h after a 48-h fast. All animal studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Chemicals

The metabolically stable TRH analog RX 77368 (p-Glu-His-(3,3′-dimethyl)-Pro-NH_2, Reckitt and Colman, Kingston Upon Hull, UK) exerts potent and relatively long-lasting central effects, compared to TRH in rats and mice. RX 77368 was freshly diluted with normal saline from an aliquot (30 μg/50 μl in 0.1% bovine serum albumin/saline) stored at −80 °C.

Measurement of body lean and adipose mass quantities, blood glucose, and gut hormone levels

Lean and fat body mass quantities were measured in awake rats by non-invasive magnetic resonance imaging using EchoMRI-4in1 (EchoMedical System, Houston, Texas) and data were automatically calculated by the software installed in the equipment. Blood samples for measuring hormone levels in normally fed, fasted, and refed conditions were collected from rat right heart ventricle immediately before the transcardial perfusion. Sera (20 μl) were kept at −80 °C before hormones were measured using a Millipore Luminex with Rat Gut Hormone kit (Merck Millipore, Billerica, MA). Blood glucose levels before and during food intake were measured in other groups of rats by One Touch Ultra Blood Glucose Monitoring System (Lifescan, Milpitas, CA) using ~2 μl of whole blood collected from the tail tip after a 25-G needle puncture of unrestrained rat at each time point. There was no significant