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The circulatory and renal sympathoinhibitory effects of gastric leptin are altered by a high fat diet and obesity

Jackie M.Y. How, Talia J. Pumpa, Daniela M. Sartor *

University of Melbourne, Department of Medicine, Austin Health, Heidelberg, 3084, Victoria, Australia

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ABSTRACT

Gastric leptin elicits its cardiovascular and splanchnic sympathoinhibitory responses via a vagal afferent mechanism, however the latter are blunted/abolished in animals fed a medium high fat diet (MHFD). In a diet-induced obesity model we sought to determine whether the renal sympathetic nerve discharge (RSND) and regional vasodilator responses to gastric leptin are also affected by diet and/or obesity. The diet induced obesity model was used in 2 separate studies. After 13 weeks on a MHFD the animals were classified as either obesity prone (OP) or obesity resistant (OR) depending on their weight gain. Control animals were fed a low fat diet for an equivalent period. Arterial pressure (AP) and heart rate (HR) were monitored in isoflurane-anaesthetised, artificially ventilated animals and RSND or regional vascular responses to leptin (15 μg/kg) administered close to the coeliac artery were evaluated. OP rats had higher baseline AP compared to control/OR rats ($P < 0.05$). Close arterial leptin inhibited RSND in control animals but this response was abolished in OR and OP animals ($P < 0.01$ for both). Leptin administration increased renal vascular conductance in control animals but this response was significantly attenuated only in OP animals ($P < 0.05$). The vasodilator response in the superior mesenteric artery was not significantly different in any of the groups ($P > 0.05$). Together these results suggest that, while the renal sympathoinhibitory responses to gastric leptin are affected by diet, the vasodilator responses to leptin in the renal vascular bed are only affected in OP animals. These changes may impact on cardiovascular homeostatic mechanisms in obesity.

1. Introduction

We have previously reported that leptin is associated with acute and short-lasting sympathoinhibition, bradycardia and blood pressure lowering effects when specifically administered close to the coeliac artery in physiological doses (termed "close arterial" (Sartor and Verberne, 2010)). This latter mode of administration allows for direct infusion within the gastrointestinal region, thus mimicking the effects of naturally-released gastric leptin (Peters et al., 2005; Sartor and Verberne, 2010; Sartor, 2013). These effects of gastric leptin are very similar to those reported for cholecystokinin (CCK), a gastrointestinal hormone that has been shown to induce its cardiovascular actions via a vagally-mediated sympathetic reflex dependent on CCK_1 receptor activation (Sartor and Verberne, 2002, 2003; Verberne and Sartor, 2004; Sartor and Verberne, 2006; Sartor et al., 2006; Sartor and Verberne, 2007, 2008, 2010). However, while the cardiovascular and sympathoinhibitory effects of CCK occur immediately, those of gastric leptin develop gradually over 5 min following administration and are abolished by CCK_1 receptor blockade and bilateral cervical vagotomy (Sartor and Verberne, 2010). Leptin receptors are located on vagal afferents and on CCK-containing enteroendocrine cells lining the gastrointestinal mucosa (Wang et al., 1997; Guilmeau et al., 2003), and leptin has been shown to induce CCK release from the latter (Guilmeau et al., 2003). It was therefore proposed that the sympathoinhibitory effects observed following leptin infusion within the gastrointestinal region are likely due to the release of CCK (Sartor and Verberne, 2010) (Fig. 1). In contrast to the acute vagally-mediated sympathetic effects of gastric leptin, adipose-derived leptin is associated with sustained and long-term sympathoexcitation and blood pressure elevation via induction of transcriptional changes in the hypothalamic region (Guilmeau et al., 2004; Peters et al., 2005; Rahmouni, 2010; Sartor and Verberne, 2010; Sartor, 2013) (Fig. 1). The effects of gastric leptin may therefore be important in the cardiovascular events that occur following ingestion of a meal.

Using a diet-induced obesity (DIO) model, we recently reported that a high fat diet and obesity affects the sympathetic vasomotor responses to gastric hormones, with implications for cardiovascular homeostasis in obesity-related hypertension (How et al., 2011, 2013). In this model, animals are either fed a low fat diet (LFD; controls) or a medium high fat diet (MHFD), and animals on the latter diet can further be classified as either obesity prone (OP) or obesity resistant (OR) depending on whether they become obese on this diet or remain

* Corresponding author at: Clinical Pharmacology and Therapeutics Unit, Department of Medicine, Austin Health, Heidelberg, Victoria 3084, Australia. Tel.: +61 3 9496 3415; fax: +61 3 9459 3510.
E-mail address: dsartor@unimelb.edu.au (D.M. Sartor).

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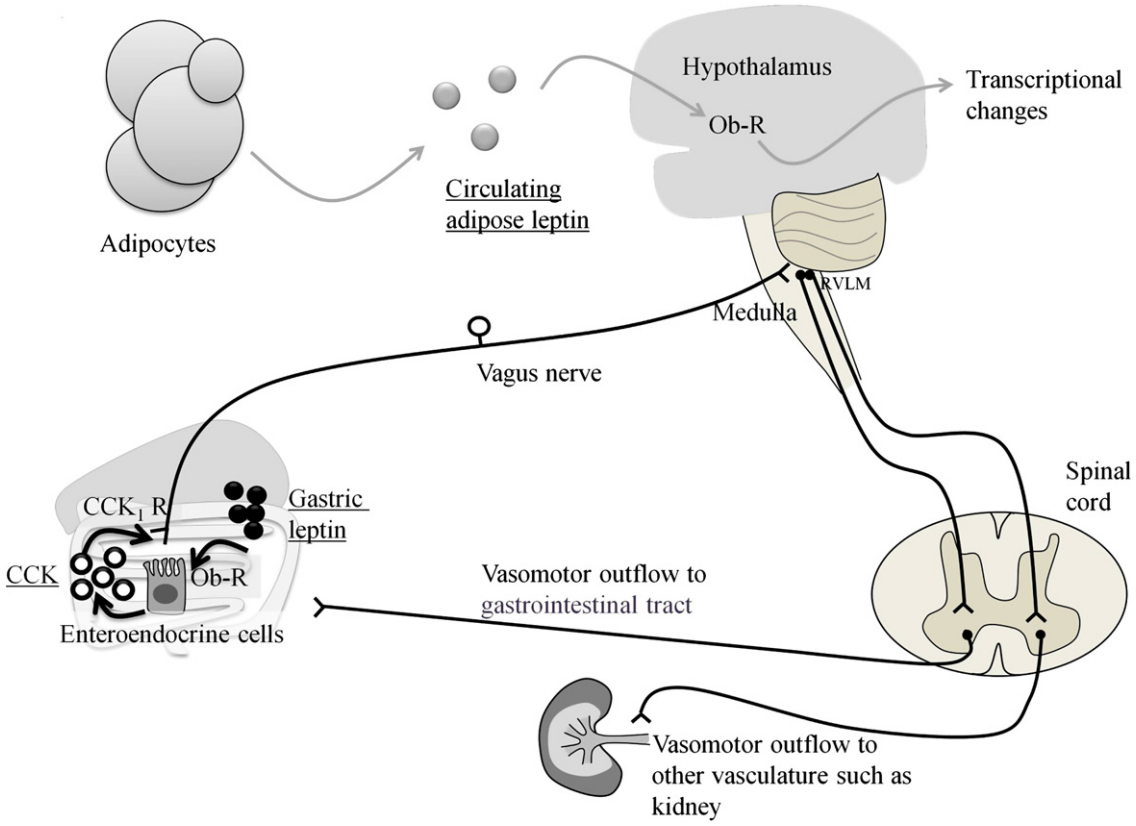

Fig. 1. Different signalling pathways proposed for the cardiovascular actions of gastric- versus adipose-derived leptin. Adipose leptin (shaded lines) is released into the circulation and is actively transported into the hypothalamus where it induces transcriptional changes involved in long-term cardiovascular regulation. Gastric leptin (solid lines) is released from the stomach and enters the small intestine where it acts on leptin receptors located on enteroendocrine cells, to induce CCK release. CCK then acts in a paracrine fashion on CCK_1 receptors located on subdiaphragmatic vagal afferents to induce a reflex response that involves inhibition of a subset of presympathetic vasomotor neurons in the rostroventrolateral medulla (Sartor and Verberne, 2010; Sartor, 2013). This results in the withdrawal of sympathetic vasomotor tone to the splanchnic and renal vascular beds. Abbreviations: CCK, cholecystokinin; CNS, central nervous system; GIT, gastrointestinal tract; Ob-R, leptin receptor; RVLM, rostroventrolateral medulla.

lean, respectively (How et al., 2011, 2013). Typically, OP rats have a higher weight gain, resting arterial pressure, adiposity and heart weight compared to either controls or OR animals (How et al., 2011, 2013). Specifically, we found that the splanchnic sympathoinhibitory response to gastric leptin in LFD control animals became sympathoexcitatory in both OP and OR animals. Since animals on the MHFD also had lower circulating CCK levels, it was suggested that the diet may be affecting CCK release thereby preventing the sympathoinhibitory response associated with gastric leptin (How et al., 2011). A large proportion of blood is directed towards the gut and kidneys after a meal, and blood flow regulation to these vascular beds is critical to cardiovascular homeostasis (Sartor, 2013), therefore our aim in the present study was to determine whether the renal sympathoinhibitory and regional vasodilator responses to leptin are similarly affected by diet and/or obesity. Furthermore, since leptin has been shown to have both sympathetically- (Rahmouni, 2010; Sartor and Verberne, 2010) and non-sympathetically-mediated (Beltowski et al., 2004a,b; Beltowski, 2006; Beltowski et al., 2010) effects on vasomotor tone, we also aimed to examine its effects on blood flow to the gut and kidney using the DIO model. We hypothesize that disruption of sympathoinhibitory and vasodilator mechanisms may impact on cardiovascular homeostasis in obesity.

2. Methods

2.1. Animals

For all experiments, male Sprague–Dawley (SD) rats were used (162–241 g; $n = 64$; Animal Resource Centre, Perth, Western Australia). This study was approved by the Austin Health Animal Ethics Committee (Heidelberg, Victoria, Australia) and all experimental procedures performed abide by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The DIO model previously described (How et al., 2011, 2013) was used in 2 separate studies (32 animals in each). Animals were housed in pairs in an animal facility with temperature-control and a 12 h light/dark cycle. In each study, 8 control animals were placed on a LFD ($n = 8$; 9% kcal from fat; AIN93M, Speciality Feeds, Western Australia) and 24 animals were placed on a MHFD ($n = 24$; 32% kcal from fat; SF04-037), for a total of 13 weeks. Animals on the MHFD were stratified according to weight gain post-hoc: those with weight gains in the upper tertile were identified as OP ($n = 8$), and those in the lower tertile OR ($n = 8$) and weight gain distribution was verified using χ^2 analysis as previously described (How et al., 2011, 2013).

2.2. General surgical procedures

At the end of the feeding period, animals were anaesthetised in an isoflurane chamber (VM Supplies, Chelsea Heights, Vic, Australia) and subsequently tracheotomised and artificially ventilated with 100% O2 (1 ml/100 g body weight, 50–60 breaths/min) containing 1.5–1.7% isoflurane. Absence of both withdrawal to firm toe pinch and eye blink response to gentle corneal probing were used at regular intervals (every 15 min) throughout the experiments to confirm adequacy of anaesthesia, and core temperature was kept within 36–38 °C using a servo-controlled heating pad linked to a rectal probe (Coherent Scientific, Hilton, SA, Australia). A cannula was inserted into the right brachial artery for measurement of AP, and heart rate (HR) was generated from the arterial pulse wave; a second cannula was inserted into the left jugular vein for intravenous (i.v.) infusion of