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Implications of diet modification on sympathoinhibitory mechanisms and hypertension in obesity

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ABSTRACT

We have previously demonstrated that a number of rats fed a moderately high-fat diet (MHFD) become obese and hypertensive and had compromised sympathoinhibitory and vasodilator responses to the gut hormones cholecystokinin (CCK) and gastric leptin. This has implications for increased resistance in vascular beds that attract a large proportion of cardiac output after a meal and may be an important mechanism underlying the development of hypertension in obesity in which food consumption is greatly increased. The aim of this study was to determine whether swapping a MHFD for a low-fat diet (LFD) would induce weight loss in obese animals, reverse the signs of hypertension and restore sympathoinhibitory reflexes. Male Sprague–Dawley rats were placed on a LFD (controls; $n = 8$) or a MHFD ($n = 24$) for 11 weeks after which the latter displayed either an obesity-prone (OP) or obesity-resistant (OR) phenotype. All animals were fed a LFD for a further 6 weeks after which they were anaesthetised with isoflurane and artificially ventilated for evaluation of resting arterial pressure (AP) and renal sympathetic nerve responses to CCK (0.1–4 μg/kg) and leptin (15 μg/kg). Weight gain in OP animals remained higher than OR or controls following diet switch ($P < 0.05$ for both). Resting AP was not significantly different between OP (103 ± 4 mmHg), OR (102 ± 3 mmHg) or control (104 ± 3 mmHg) animals and sympathoinhibitory responses to CCK or leptin were not different between the groups ($P > 0.05$). These results demonstrate that diet modification can have beneficial effects on sympathetic function and restore normotension without the need for weight reduction.

1. Introduction

The majority of newly diagnosed cases of hypertension can be attributed to obesity (Moore et al., 2005), and globally, hypertension remains the leading risk factor for mortality (World Health Organization, 2013). However, the exact pathophysiology of obesity-related hypertension remains vague. In light of the obesity epidemic that shows no sign of relenting, it is imperative that we advance our knowledge in this area, particularly given that a significant proportion of patients remain unresponsive to hypertensive medications (Esler, 2010; Lambert et al., 2010; Plump, 2010). Weight loss has been demonstrated to be beneficial in the treatment of obesity-related hypertension in humans, although this remains an unrealistic goal for many obese subjects, with most regaining weight within 3 years (Straznicky et al., 2010; Sumithran et al., 2011). Many diets have proven successful with respect to weight reduction, although these have had varying success in lowering blood pressure (Straznicky et al., 2010; Landsberg et al., 2013). Even the Dietary Approaches to Stop Hypertension (DASH) diet, which has been lauded as a more sustainable approach for obese subjects, has shown variable success rates (Landsberg et al., 2013). Therefore, increased knowledge regarding the mechanisms involved in the aetiology of hypertension in obesity is essential and urgently required in order to improve dietary and therapeutic approaches to treat obesity-related hypertension.

It is well established that the gut hormones cholecystokinin (CCK) and gastric leptin participate in cardiovascular regulation via a vagally mediated tri-synaptic central reflex similar to that of the baroreflex (Sartor and Verberne, 2002, 2003, 2006, 2007, 2008, 2010; Sartor et al., 2006; Sartor, 2013). These gut hormones specifically inhibit renal and splanchnic sympathetic nerve discharge, thereby promoting vasodilation in the renal and splanchnic vascular beds (Sartor, 2013). Our laboratory has successfully established a Sprague–Dawley diet-induced obesity (DIO) model to study obesity-related hypertension and was the first to demonstrate that sympathoinhibitory reflexes induced by gastrointestinal hormones are compromised in obese hypertensive rats (How et al., 2011, 2013a,b; Sartor, 2013). These reflexes are important since they govern blood flow to the gut and kidney that collectively attract a major proportion of cardiac output after a meal (Sartor, 2013).

To date, there are few studies in the literature that have examined the effects of weight loss and/or caloric restriction in animal models. Enriori et al. found that transferring diet-induced obese mice from a

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high fat diet (HFD) to a low-fat diet (LFD) for 7 weeks induced significant weight loss and reversed central leptin resistance (Enriori et al., 2007). However, this study was focussed on metabolic parameters and cardiovascular effects were not examined. In the obese spontaneously hypertensive rats, weight cycling (i.e. weight loss and regain) has been shown to exacerbate hypertension (Ernsberger et al., 1996). However, to date, evaluations of the cardiovascular and sympathetic effects of dietary restriction have not been conducted in a DIO model of hypertension. Our previous studies have demonstrated that hypertension is positively correlated with adiposity and weight gain (How et al., 2014). Arterial pressure (AP), weight gain and adiposity were all inversely correlated with vascular conductance responses to CCK in both the renal and splanchnic vascular beds, consistent with obese hypertensive animals having blunted vasodilator responses to gut hormones (How et al., 2013a,b). Furthermore, we also demonstrated that only obese hypertensive animals had compromised sympathoinhibitory responses to gut hormones in both the splanchnic and renal nerves (How et al., 2011, 2013b). Reduced renal and splanchnic sympathoinhibition and vasodilation postprandially may therefore lead to increased regional vascular resistance. This may be an important mechanism underlying the development of hypertension in obese patients who consume larger quantities of high-energy and high-fat foods that may increase and prolong the haemodynamic demand of the renal and splanchnic circulations (Sartor, 2013). To determine the contribution that diet and/or obesity has on aberrant sympathoinhibitory responses and to evaluate the importance of the latter in the aetiology of hypertension in obesity, the aim of the current study was to examine the effects of swapping a HFD for a LFD on body weight, adiposity, AP and renal sympathoinhibitory responsiveness to gut hormones. We hypothesised that weight loss in obese animals would restore normotension in conjunction with normalisation of gut-related sympathoinhibitory reflexes.

1.1. Animals

All experiments were performed using male Sprague–Dawley rats (180–210 g, $n = 32$; Animal Resource Center, Perth, Western Australia). Animals were housed in a temperature-controlled animal facility with a 12-h light and 12-h dark cycle. This study was approved by the Austin Health Ethical Review Committee (Heidelberg, Victoria, Australia) and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

1.2. Generation of obese rats

Six-week-old animals were acclimatised for 4 days before being placed on their specialised diets, purchased from Specialty Feeds (Glen Forrest, Western Australia). Animals were placed on either a moderately high-fat diet (MHFD; 15.5% total fat; 31% kcal from fat; SF04-037; $n = 24$) or a LFD (4% total fat; 9% kcal from fat; AIN93M; control animals; $n = 8$), as described previously (How et al., 2011, 2013a,b, 2014). Animals had unrestricted access to food and water, and all rats remained on their designated diets for 11 weeks. After this time, all animals on the MHFD were placed on the LFD for a further 6 weeks, whilst the control animals remained on their LFD for the entire 17-week period. To accommodate the maximum of 4 electrophysiological experiments that could be performed each week, the animals were staggered so that each week only 4 rats were placed onto their respective diets as previously described (How et al., 2011, 2013a,b, 2014). Rats had access to unlimited food and water. Body weights were recorded daily, whilst food consumption was measured twice weekly. Animals on the MHFD were stratified into the obesity-prone (OP) or obesity-resistant (OR) group post hoc, based on their weight gain (final weight – initial weight) at week 11 before diet switch and validated using the χ^2 analysis as previously described (How et al., 2011, 2013a,b, 2014). Briefly, animals within the MHFD group falling within the upper or lower tertile of weight gain were deemed OP or OR, respectively. Animals not falling into either group were excluded from the analyses. LFD animals served as controls.

1.3. General surgical procedures

Following the 17-week feeding cycle, on each experimental day, either a MHFD or a LFD control rat was chosen at random to avoid bias. Animals were anaesthetised in a chamber with isoflurane vapour (VM Supplies, Chelsea Heights, VIC, Australia). The rats were subsequently tracheotomised to enable artificial ventilation with 100% O_2 (1 mL/100 g body weight, 60 breaths/min) containing 1.5–1.7% isoflurane. The adequacy of anaesthetic depth was assessed throughout the procedure, and was verified on a 15-min basis through the absence of withdrawal to both firm toe pinch and the absence of an eye blink response to gentle corneal probing. Core temperature was maintained between 36 and 38 °C using a rectal probe with input to a servo-controlled heating pad (Coherent Scientific, Hilton, South Australia). The left jugular vein was cannulated for intravenous drug administration, and the right brachial artery for AP and heart rate (HR) measurement as previously described (How et al., 2011). A third cannula was inserted into the left carotid artery and fed down the abdominal aorta, and a midline laparotomy was made to enable placement of the cannula just upstream from the coeliac artery. This was used for 'close arterial' infusion of leptin and CCK into the gastrointestinal circulation as previously described (Sartor and Verberne, 2010; How et al., 2011, 2013a,b). At the conclusion of cannulations, all incisions were closed.

1.4. Renal nerve recording

A retroperitoneal incision was made for isolation of the left renal nerve that was placed onto the bared tips of a pair of Teflon-coated silver wires (bare diameter 250 μm; AM Systems, Chatswood, NSW, Australia). This was then embedded in Kwik-Cast silicone sealant (Coherent Scientific, Hilton, SA, Australia). The retroperitoneal incision was closed and the electrode externalised. The animal was then placed onto its stomach before the commencement of recording. Renal sympathetic nerve discharge (RSND) was recorded using a differential AC amplifier (band pass 30–3000 Hz; gain ×10k) as previously described (How et al., 2011, 2013a,b).

1.5. Excision of fat pads

At the conclusion of the experiments, animals were euthanized through the administration of an overdose of isoflurane. The abdominal area was then opened for the excision of the following fat pads: epididymal, infrarenal and subcutaneous fat pads were collected as previously described and used to calculate adiposity index [total fat content/(final weight-total fat content) ×100] (How et al., 2011, 2013a,b).

1.6. Data analysis and statistics

All data are expressed as mean ± standard error of mean (SEM), with $P < 0.05$ set as the level of significance. Statistical analyses were conducted using GraphPad Instat (version 3.05 for windows 95 GraphPad Software, San Diego, CA, USA). Data between OP, OR and control (LFD) groups were analysed using the one-way analysis of variance followed by the Tukey–Kramer multiple comparison test.

Baseline HR, AP and RSND was recorded and analysed using a Cambridge Electronic Design, computer-assisted data acquisition system and Spike2 software (version 5.13, Cambridge, UK). Following the completion of all surgical procedures, a minimum of 15 min was allowed for the stabilisation and post-surgical recovery of all conditions and parameters before subsequent experimental protocols were initiated. AP and HR were monitored following close arterial infusion of CCK (0.1, 0.5, 1, 2 and 4 μg/kg) as a single bolus in random order, and its effects on RSND measured during the nadir of the depressor response. Leptin