Central Pro-Opiomelanocortin but not Neuropeptide Y mediates sympatho-excitation and hypertension in fat fed conscious rabbits.

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Objective: High fat diet (HFD)-induced hypertension in rabbits is neurogenic due to the central sympatho-excitatory actions of leptin. Hypothalamic melanocortin and neuropeptide Y (NPY) neurons are recognized as the major signalling pathways through which leptin exerts its central effects. In this study we assessed the effects of specific antagonists and agonists to melanocortin and NPY receptors on HFD-induced sympatho-excitation and hypertension.

Methods: Rabbits were instrumented with intracerebroventricular cannula, renal sympathetic nerve activity (RSNA) electrode and blood pressure telemetry transmitter.

Results: After 3 weeks HFD (13.5% fat, n=12) conscious rabbits had higher RSNA (+3.8nu, P=0.02), blood pressure (+8.6mmHg, P<0.001) and heart rate (+15b/min, P=0.01) and brain derived neurotrophic factor (BDNF) levels in the hypothalamus compared with rabbits fed a control diet (4.2% fat, n=11). Intracerebroventricular administration of the melanocortin receptor antagonist SHU9119 reduced RSNA (-2.7nu) and blood pressure (-8.5mmHg) in HFD but not control rabbits thus reversing 100% of the hypertension and 70% of the sympatho-excitation induced by a HFD. By contrast, blocking central NPY Y1 receptors with BVD10 increased RSNA only in HFD rabbits. Intracerebroventricular α-MSH increased RSNA and heart rate (P<0.001) in HFD rabbits but had no effect in control rabbits.

Conclusion: These findings suggest that obesity-induced hypertension and increased RSNA are dependent on the balance between greater activation of melanocortin signalling through melanocortin receptors and lesser activation of NPY sympatho-inhibitory signalling. The amplification of the sympatho-excitatory effects of α-MSH also indicates that the underlying mechanism is related to facilitation of leptin-melanocortin signalling, possibly involving chronic activation of BDNF.

Abbreviations: ARC, arcuate nucleus of the hypothalamus; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DMH, dorsomedial hypothalamus; ERK, extracellular signal-regulated kinase; HFD, high-fat diet; HR, heart rate; ICV, intracerebroventricular; MAP, mean arterial pressure; MC3/4, melanocortin 3 and 4; MEK, mitogen-activated proteinkinase; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVH, paraventricular hypothalamus; RSNA, renal sympathetic nerve activity; SNS, sympathetic nerve system; VMH, ventromedial hypothalamus; WAT, white adipose tissue; a-MSH, a-melanocortin stimulating hormone

INTRODUCTION

Obesity is a precursor to serious cardiovascular and metabolic diseases [1] and relatively modest reductions in body weight are associated with reduced incidence of cardiovascular events [2]. Evidence suggests that increased sympathetic nerve activity (SNA) to the kidneys and skeletal muscle vasculature occurs secondary to the accumulation of body fat and is a major mechanism of obesity induced hypertension [3-5]. In animal studies, bilateral renal denervation in dogs reverses diet-induced hypertension [6]. Antic and colleagues abolished high fat diet (HFD) induced hypertension in conscious rabbits with and β-adrenoceptor blockade [7]. Increasingly the focus has been on the circulating adipokine leptin which has long been known to regulate appetite and hence energy intake and influence metabolism [4].

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Leptin is secreted primarily by adipocytes and is present in serum in direct proportion to the percentage of adipose tissue [8, 9]. Chronic systemic or acute central infusions of leptin increase blood pressure in control rats or rabbits via stimulation of the SNS [10-14]. These effects appear to be mediated by leptin receptors located on alpha-melanocortin stimulating hormone (α-MSH) and neuropeptide Y (NPY) containing neurons in the arcuate nucleus of the hypothalamus (ARC) [15]. The interaction involves leptin mediated inhibition of NPY and stimulation of α-MSH positive cells as well as reciprocal connections between the two neuronal populations, resulting in a push-pull mechanism through which the effects of leptin ensue [16].

We have previously demonstrated that feeding a HFD for 3 weeks leads to increased mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) in conscious rabbits [5]. Importantly we have shown that ganglion blockade completely abolishes the increase in MAP suggesting that this model of obesity hypertension is neurogenic [17]. Circulating plasma levels of leptin were increased in the first week of the diet and strongly correlated with increased visceral adiposity, MAP and RSNA [5, 17]. The surprising discovery was that the central responses to leptin were not only preserved but substantially augmented in HFD rabbits following only 3 weeks on the diet [5]. Other studies have suggested that while the appetite inhibitory effects of leptin were reduced, the SNA effects were preserved, a phenomenon termed selective leptin resistance [18]. We suggested that the mechanism of the hypertension involved sympathetic activation and increased responsiveness to central sympathoexcitatory effects of leptin due to increased plasma leptin arising from visceral fat accumulation [5, 17].

To investigate the contribution of insulin and leptin, we used specific peptide antagonists administered acutely after 1 or 3 weeks of a HFD in rabbits [19]. The insulin antagonist had only a small effect on blood pressure and no effect on RSNA. By contrast the leptin antagonist after 3 weeks (and not after 1 week) reduced blood pressure and RSNA to levels close to those observed in rabbits on a normal fat control diet (CD) [19]. The contemporaneous occurrence of the amplified sympatho-excitatory effects of leptin and the marked hypotensive effect of the leptin antagonist suggests there is not a simple maintenance of leptin signalling affecting sympathetic activity but a marked amplification of leptin's effect by a HFD. This HFD-induced change in leptin function could arise from either changes in leptin signalling at its receptor or possibly through leptin effects downstream involving changes in the sensitivity of NPY and α-MSH signalling. In the present study we assessed whether the cardiovascular effects of a HFD are dependent on activation of NPY or MC3/4 receptors and whether there is a change in the sensitivity to either the α-MSH or NPY receptors. Changes in sensitivity can also be mediated by synaptic plasticity. Chronic activation of MC4 receptors is associated with increased levels of brain derived neurotrophic factor (BDNF) mediated by a mitogen-activated protein kinase (MEK) which activates extracellular signal-regulated kinase (ERK) pathways. Todo and colleagues recently found that activation by leptin of a MEK-ERK pathway in the ventromedial hypothalamus (VMH) results in the enhancement of melanocortin receptor signalling and consequently increased muscle insulin sensitivity [20]. Thus we also measured levels of BDNF in regions of the hypothalamus in CD and HFD rabbits.

METHODS

Animals

Experiments were conducted in 68 male New Zealand White rabbits (2.25-2.75kg). Rabbits were housed under controlled light (6:00 to 18:00) and temperature (22°c ± 2°c) conditions. Experiments were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for Scientific Use of Animals.

Experimental Procedures and Protocol for haemodynamic experiments

Rabbits underwent preliminary surgery under isoflurane anaesthesia and carprofen (3 mg / kg) was given 24 hours before and after surgery for analgesia. Rabbits were implanted with an intracerebroventricular (ICV) cannula into the lateral ventricle, as described previously [21]. MAP and HR were measured either from a catheter placed acutely into the ear artery under local anaesthesia or by using a blood pressure telemetry transmitter (TA11PA-D70, Data Sciences International, St. Paul, MN, USA) which was implanted into the aorta via a branch of the left iliac artery a week earlier [22]. Baseline MAP and HR were measured in conscious rabbits over a 1-hour period prior to the diet. Rabbits then either continued on a CD (standard rabbit chow of 2.63 kcal/g containing 4.2 % total fat, Specialty Feeds, Glen Forrest, Australia) or were placed on a HFD (modified rabbit chow with 5% pork fat and 5% soya oil, 13.3 % total fat, Specialty Feeds) *ad libitum* for 3 weeks [5].

Two weeks after the initiation of the diet, a recording electrode was implanted on the left renal nerve, under isoflurane anaesthesia [5, 23]. One week later, in conscious rabbits, after a 1-hour period for recovery from handling, resting MAP, HR and RSNA were recorded for 1 hour. This period is sufficient to provide stable data similar to that in the home cage [24]. A 50 µl ICV injection of the vehicle (Ringer's solution, Baxter, Toongabbie, NSW, Australia) was then given followed by increasing doses of either α-MSH or NPY (1, 3 and 10 nmol or 0.5, 1.5, 5 nmol, respectively, Tocris, Ellisville, USA) and their respective antagonists, SHU9119 (MC3/4 receptor antagonist; 0.038, 0.075, 0.188 nmol, Tocris) or

BVD10 (Y_1 receptor antagonist; 3, 10, 30 nmol, Tocris) delivered in 50 µl vehicle ICV at 30 minute intervals. A time control study involved the injection of 4 vehicle doses. Experiments were conducted in randomised order on separate days.

Assessment of Plasma Leptin, Body Fat, Organ Weights and c-Fos and BDNF immune-histochemistry.

After 3 weeks of dietary intervention, rabbits were euthanized by anaesthetic overdose (Lethabarb, 100 mg / Kg, i.v, Virbac Animal Health, Woolpit, UK). Lean body mass, total fat and total body mass, bone mineral density and bone mineral content at week 3 were measured using a dual energy absorptiometry X-ray (DEXA) machine (Discovery A-QDR series, Hologic Inc. MA, USA). White adipose tissue (WAT) pads from mesenteric viscera, retroperitoneal area, testes and bladder were dissected and weighed. Brains (CD, n = 7, HFD, n = 3) were perfusion fixed (4 % paraformaldehyde), sectioned in the coronal plane and processed for Fos immunohistochemistry [5, 25]. Analysis of c-Fos immunoreactivity following the top dose of ICV α-MSH or the vehicle was carried out as previously described in a blinded manner [5]. BDNF was determined using image intensity from staining using a BDNF antibody (raised in goat) from Novus Biologicals diluted 1 in 300. Some of the rabbits were fasted for 4 hours before blood samples were taken and plasma stored at −80°C. Leptin concentrations were assessed using a radioimmunoassay multispecies kit (LINCO Research, St Charles, MO, USA).

Data Analysis

MAP, HR derived from the arterial pressure pulse, and the RSNA raw signal, rectified and integrated with a 20 ms time constant, were digitized online at 500 Hz and averaged over 2 seconds. In order to allow for between animal comparisons, RSNA was normalized to the maximum RSNA burst height recorded during the nasopharyngeal response evoked by smoke, taken to be 100 normalized units [26-28]. The peak was estimated using a 100 ms window. RSNA measured in raw microvolts varies markedly among individual rabbits depending on the recording conditions. Thus scaling to the maximum response to smoke is baroreceptor-independent, removes artificial differences between groups and reveals differences which are undetectable when RSNA is expressed in microvolts [26]

Values averaged over 30 minutes were expressed as mean ± SEM or mean difference ± SE of the difference (SED). Data were analyzed by split plot repeated-measures ANOVA, which allowed for within- animal and between-animal (group) contrasts and adjusted for multiple testing using the Bonferroni method [22]. For all statistics shown we refer to the main effect as a subscript, e.g P_{diet} refers to the effect of diet. One-way ANOVA was used for data collected at a single time point. Type 1 error was controlled using Bonferoni and Greenhouse Geisser corrections. A probability of $P < 0.05$ was considered significant.

RESULTS

Effect of 3 Week Fat-feeding on Body Weight, WAT and Organ Weights and Plasma Leptin Concentrations

Initial body weights were not different between the dietary groups (2.94 ± 0.06 kg before HFD and 2.91±0.06 kg before CD, $P_{group} = 0.7$). At the end of the 3 week feeding protocol, total body mass as measured by DEXA, was 9 % greater in HFD fed rabbits compared with CD fed rabbits due in the main to a 59 % greater total WAT mass (Table 1, $P_{diet} < 0.01$ for both). The retroperitoneal, visceral, cardiac and testicular fat pads were 31 – 40 % heavier in HFD rabbits compared with controls even after being expressed as percentage of body weight (Table 1, $P_{diet} < 0.05$). The weights of other organs such as liver, kidney, spleen and left ventricle heart were similar in both groups ($P_{diet} > 0.05$). Plasma leptin concentrations after 3 weeks HFD were more than double those in CD rabbits (Table 2, P = 0.02).

Effect of HFD on Cardiovascular Variables and RSNA

Baseline MAP and HR were 68 ± 1 mmHg and 172 ± 3 bpm, respectively, averaged over 23 rabbits. At the end of 3 weeks of HFD, MAP had increased by +15 ± 1 % and HR had increased by +21 ± 2 % whilst CD animals showed no change in MAP from baseline and a smaller increase in HR (+9 ± 2 %, $P_{diet} < 0.001$, Table 2). After 3 weeks of HFD, normalized total RSNA was +30 ± 8 % higher in HFD rabbits compared with CD rabbits ($P_{diet} = 0.02$, Table 2) which was due to a +31 ± 6 % greater RSNA burst amplitude in HFD rabbits compared with controls ($P_{diet} < 0.001$, Table 2). RSNA frequency did not differ between the two groups even when the difference in HR was taken into account ($P_{diet} = 0.1$; Table 2). RSNA expressed in microvolts and the average nasopharyngeal response (µV), which was used to normalize the RSNA signal, were similar between the CD and HFD groups (Table 2).

Effect of SHU9119 and α-MSH on Cardiovascular Variables and RSNA

Central administration of the MC3/4 receptor antagonist SHU9119 attenuated RSNA (expressed in normalised units and µV) and MAP at the highest dose by -26 ± 7 %, -23 ± 5 % and -11 ± 3 %, respectively in HFD (n = 6, $P_{drug} < 0.01$) but not CD-fed rabbits (n = 4, $P_{drug} > 0.05$, Figure 1). By contrast, increasing doses of SHU9119 had no effect on HR in either dietary group ($P_{drug} > 0.05$, Figure 1).

ICV administration of the agonist α-MSH resulted in an increase in normalised RSNA in HFD rabbits (+66 ± 11 % for 10 nmol dose, $P_{drug} < 0.001$, n = 11, Figure 2) that was dose dependent ($P_{lin} = 0.02$). A similar pattern was observed when RSNA was uncorrected and expressed in microvolts (+80 ± 13 % at the highest dose, $P_{drug} < 0.001$). By contrast α-MSH had little effect in CD rabbits (n = 11; $P_{diet} = 0.02$, Figure 2). HR also increased in a dose dependent manner in the HFD-fed rabbits (+24 ± 3 % for 10 nmol dose, $P_{drug} < 0.001$, $P_{lin} < 0.001$) whilst CD rabbits only responded to the top dose (+10 % for 10 nmol

dose, P_{drug} = 0.01; Figure 2). These doses of α-MSH administered ICV produced a small reduction in MAP in the CD (-3.7±1.6 mmHg, *P*=0.003) and in the HFD group (-3.2±1.4 mmHg, P_{drug} = 0.001; P_{diet} = 0.9, Figure 2).
Injection of vehicle had no effect on cardiovascular parameters or RSNA in either dietary group ($P > 0.05$, Figure 3).

Effect of BVD10 and NPY on Cardiovascular Variables and RSNA

Central administration of the NPY Y_1 receptor antagonist BVD10 (3-30 nmol) resulted in a marked elevation of RSNA (expressed as normalised units or μV) in HFD animals alone that was dose dependent (P_{diet} = 0.02, P_{lin} = 0.03, n = 5; Figure 4). There were no significant effects on MAP and HR in either groups.
ICV administration of 0.5-5 nmol of NPY to HFD rabbits dose-dependently reduced MAP, with a 5 ± 2 % reduction at the highest dose (P_{lin} = 0.04, n = 10) but had no effect in CD rabbits (n = 11, Figure 5). HR increased slightly after NPY in CD rabbits but had no effect in HFD rabbits (+7 ± 2 % at 5 nmol dose in CD rabbits, P_{drug} = 0.001, Figure 5). NPY had no dose-dependent effect on RSNA in either group. However, overall, RSNA after NPY treatment was lower in HFD than CD rabbits ($P_{diet} < 0.01$, Figure 5).

Effect of ICV α-MSH on Hypothalamic c-Fos Expression Levels

Following administration of ICV α-MSH to CD-fed rabbits, c-Fos expression was detected in all hypothalamic nuclei examined, with very high levels in the organum vasculosum of the lamina terminalis and strong activation in the medial preoptic nucleus, ARC, paraventricular hypothalamus (PVH), dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH) and supraoptic nucleus (Figure 6). There was noticeably less activation of the median preoptic nucleus (Figure 6). By contrast, HFD rabbits had approximately 80 % fewer c-Fos positive cells compared with CD controls in the PVH, DMH and VMH nuclei (P_{diet}<0.001, Figure 6). In addition, c-Fos expression was 30-40 % lower in the ARC, medial preoptic, and organum vasculosum of the lamina terminalis nuclei of HFD-fed rabbits compared with controls (*P*<0.05 for all three, Figure 6). There was no effect of a HFD on the activation of the supraoptic and median preoptic nuclei (P_{diet}>0.05, Figure 6). There was markedly less activation in CD-fed rabbits following ICV administration of vehicle compared with α-MSH (Figure 6).

Effect of a HFD on hypothalamic BDNF

The intensity of immunostaining for BDNF was 46% greater in the VMH (P_{diet}<0.001, n=3 per group, Figure 7) and 40% greater in the DMH (P_{diet}<0.01) of 3 week HFD rabbits compared to CD rabbits. By contrast there was no difference detected between groups in the staining in the PVN (HFD 15% greater than CD, P_{diet}=0.16, Figure 7).

Discussion

The major finding of the current study was that administration of the MC3/4 receptor antagonist, SHU9119, produced a marked sympatho-inhibition and fall in blood pressure in rabbits given a HFD for 3 weeks but no effect in CD rabbits. The extent of the fall essentially reversed all the cardiovascular effects of the HFD. We have previously demonstrated that the hypertension and sympatho-excitation induced by a HFD is also reversed by central administration of a leptin antagonist [19]. This is consistent with the activation of leptin receptors in the ARC by circulating leptin, leading to activation of projections to other regions of the hypothalamus that release α-MSH. Furthermore, the hypertension induced by a HFD is completely abolished by sympathetic inhibition using a ganglion blocking agent, suggesting that the hypertension in this model is predominantly neurogenic [17]. An important finding was that central α-MSH administration results in a 4 fold greater activation of RSNA and tachycardia in the HFD rabbits compared to CD rabbits suggesting there is marked facilitation of the capacity of the melanocortin to increase sympathetic activity. We also examined the contribution of NPY signalling. The NPY Y_1 receptor antagonist BVD10 increased RSNA in HFD rabbits but had no effect in CD rabbits. Thus a HFD also increased a sympatho-inhibitory contribution from NPY pathways but this was not sufficient to prevent the excitatory effects coming from the melanocortin pathway. ICV injection of NPY produced a hypotensive response in HFD rabbits, accompanied by an inhibition of the activation of RSNA (i.e. reduced RSNA levels) compared with those on a CD suggesting there is also facilitation of NPY and that there is additional capacity of this pathway to counter the effects of the melanocortin system. Taken together, we suggest that both melanocortin and NPY pathways become sensitised within 3 weeks of exposure to an obesogenic diet but the dominant effect is the sympatho-excitatory action of melanocortin (see schema Figure 8). Our finding of greater levels of the neurotrophic factor BDNF in the VMH and DMH suggests that it may be involved in the process that leads to amplification of the leptin-melanocortin signalling underlying obesity-induced hypertension. These findings do not preclude the possibility that there is also an additional contribution of increased leptin signalling (Figure 8).

Central α-MSH regulates specific sympathoexcitatory responses

Da Silva and colleagues have shown that the pressor, tachycardic and anorexic effects of chronic infusion of an MC3/4 agonist are maintained in HFD rats [29]. Furthermore, chronic infusion of SHU9119, the MC3/4 antagonist, for 11 days lowered blood pressure in Zucker obese rats that had a defective leptin receptor, but had only a transient effect in normal rats [30]. The same MC3/4 antagonist infused chronically for several days decreased blood pressure to a greater extent in

diet-induced obese rats than rats on a normal fat diet and completely abolished the diet-induced hypertension [31]. Our findings are consistent with the above studies except that we observed a reduction in blood pressure with acute ICV administration of SHU9119. Our findings are therefore unlikely to be influenced by down or up-regulation of receptor signalling which may occur with chronic administration. Further, we measured RSNA in conscious animals reflecting the direct output from the central nervous system independent of any diet induced changes in neuroeffector function [32]. Our observation that α-MSH administration produces a marked facilitation of RSNA and heart rate parallels the previously reported augmented RSNA and tachycardia responses to ICV leptin in HFD rabbits [5]. These findings suggest that the α-MSH signalling which is down-stream from the leptin receptor is not only intact but is also tonically activated to the extent of maintaining the obesity-induced hypertension. The increased blood pressure is likely due to the pressor effects of leptin and also insulin as we have shown that an acute injection of specific antagonists reverses the hypertension [19]. We suggest that increased circulating leptin activates leptin receptors at the level of the ARC which are then amplified by the α-MSH signalling pathway which acts as a second order pathway downstream from leptin [33]. In support of this we have observed a very similar pattern of activation of specific hypothalamic nuclei in CD rabbits by ICV α-MSH (in the present study) and ICV leptin [5] and α-MSH in CD rabbits. By contrast, HFD-fed rabbits exhibited reduced Fos protein accumulation, likely due to neurons already being chronically activated by the HFD [34]. Thus, HFD induced activation of sympathetic output to renal beds might have its origins in augmented α-MSH activity. However, we cannot rule out a contribution of increased leptin signalling as well.

We suggest that leptin acts in the ARC to stimulate POMC neurons but also acts within the VMH to release α-MSH to activate MC4R that over time strengthens synaptic transmission (Figure 8). This process involving MC4R has been recently described in a hippocampal long term potentiation process as extensively reviewed by Caruso and colleagues [35]. The mechanism involves increased cyclic AMP–protein kinase A activity which induces the association of repressor/activator protein 1 homologue (RAP1), in turn activating MEK and ERK through phosphorylation [35]. This activates transcriptional factor cAMP-responsive element-binding protein and the transcription of plasticity-associated genes and synthesis of secretory BDNF [35]. Our current findings show an activation of BDNF in the hypothalamus after 3 weeks of a HFD suggesting that the MC4R-BDNF synaptic plasticity may also occur within the hypothalamus in response to a HFD and may be responsible for obesity induced hypertension. Interestingly, acute administration of glucose increases BDNF message in the VMH and mice with depleted BDNF in the VMH and DMH become obese [36].

Lack of pressor effect to ICV α-MSH

One unexpected result was the lack of pressor response to α-MSH in either dietary group. Hypothalamic α-MSH is a known modulator of sympathetic outflow to skeletal muscle, brown adipose tissue and renal beds as these are increased with the administration of an MC3/4R agonist [37, 38]. Matsumura and colleagues have been able to demonstrate pressor effects and increases in RSNA of α-MSH given ICV to Japanese white rabbits on a normal diet at doses as low as 0.1 nmol. Also α-MSH increases blood pressure in fasted normal and obese female anaesthetised rats [39]. Thus it is surprising in our study that α-MSH had little effect on blood pressure, also given that the MC3/4R antagonist SHU9119 markedly reduced both blood pressure and RSNA. We have previously shown that ICV leptin causes a pressor effect in 3 week HFD rabbits [19] which we suggest is upstream from the α-MSH effect. Taken together we can see little reason for a lack of pressor effect of α-MSH in the present study and in this regard α-MSH stands alone. We are very confident of our finding as none of the 22 rabbits given the drug increased blood pressure by more than 2 mmHg. One possibility is that while α-MSH is able to further increase RSNA, the maximum effect has been reached by the endogenous activation of POMC signalling in non-renal beds such that no further doses are effective.

Response to Central NPY

In the current study, rabbits fed a HFD for 3 weeks showed a modest reduction in MAP of approximately 4 mmHg following ICV NPY administration representing a 50 % reduction from post-diet values but no change in RSNA. The same doses in CD rabbits had no effect on blood pressure but increased RSNA. Thus a HFD amplifies the hypotensive and sympatho-inhibitory effects of NPY. Further, ICV administration of the NPY antagonist BVD10 increased MAP and RSNA in fat-fed rabbits suggesting that tonic NPY activity is increased. Indeed, NPY mRNA is known to increase in the DMH and VMH following high-fat feeding [40]. Despite the functional relevance of increased NPY expression remaining unclear, it is likely to be pivotal in maintaining a positive energy balance [41]. Combined, these observations suggest NPY signalling may be increased in our HFD rabbit model and may also have driven the observed weight-gain and increase in WAT observed in HFD rabbits. Given that leptin directly inhibits NPY at the ARC [42] and NPY activity is increased in the DMH and VMH [40], it is likely that in obesity the hyperphagic and cardiovascular effects of NPY are region-specific. POMC neurons are known to express the Y_1 receptor, through which they may be inhibited by NPY [43]. Indeed recent studies suggest that NPY inputs to the PVH inhibit presympathetic activity and converge with α-MSH sympatho-excitatory inputs [44]. A more recent study from the same group found that inhibition of NPY receptors in the PVH is required to unmask the hypertensive effects of activation of MC3/4 receptors at

the level of the PVH [45]. Thus, the observed increase in RSNA following administration of BVD10 may reflect decreased inhibition of the POMC system and subsequent increase in RSNA (Figure 8).

The strength of the current study is in our ability to quantify sympathetic output to renal vasculature following central infusion of α-MSH. This parameter is a direct output from the central nervous system and is a more accurate measure of hypothalamic function than blood pressure. We conclude that the enhanced responsiveness to central α-MSH observed in HFD-fed rabbits is indicative of a change in the activity of the POMC system. Importantly, this change involves NPY containing neurons as well and may underlie the hypersensitivity to leptin previously observed in this model. However, one of the limitations of the current approach using ICV administration is that the drugs may act at various areas of the brain and therefore we cannot be specific about which areas are affected. Furthermore, MC3 and MC4 receptors are located in various brain regions such as the cortex, thalamus, brainstem and spinal cord as well as the well characterised location in the hypothalamus [46].

Perspectives

It has been assumed that impaired leptin signalling in the CNS is the cause of selective leptin resistance, a state in which the sympathoexcitatory effect of leptin is maintained despite a loss of its anorectic property. Indeed, we have shown that both inulin and leptinergic pathways are dysregulated early on in high fat feeding. Irrespective of the ligand, ARC neurons transduce their information via secondary messenger signalling pathways and constitute a likely mechanism through which obesity related hypertension may occur. Here we demonstrate that consumption of a HFD for a relatively short period of time results in hypersensitivity of both the NPY and α-MSH signalling pathways. Importantly we suggest that activation of BDNF due to chronic leptin-α-MSH stimulation may change strength of signalling specifically related to presympathetic neurons. The increase in BDNF in the DMH and the VMH which are known locations of presympathetic vasomotor neurons [47] suggests these regions may be the key to the understanding of obesity-related hypertension.

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Conflicts of interest

There are no conflicts of interest.

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Table 1: Body Composition in CD and HFD-fed rabbits after 3 weeks of diet.

	CD	HFD	P_{diet}
Post-Mortem Body composition	n=22	n=24	
Bodyweight (Kg)	3.29 ± 0.06	3.48 ± 0.07	0.03
Retroperitoneal WAT(g)	53.0 ± 5.3	78.6 ± 6.4	0.004
Retroperitoneal WAT (% BWT)	1.6 ± 0.1	2.2 ± 0.2	0.004
Visceral WAT (g)	40.1 ± 6.3	67.0 ± 5.9	0.003
Visceral WAT (% BWT)	1.2 ± 0.2	1.9 ± 0.1	0.002
Cardiac WAT (g)	3.7 ± 0.6	5.4 ± 0.6	0.03
Cardiac WAT (% BWT)	0.11 ± 0.02	0.16 ± 0.02	0.04
Testicular & Bladder WAT (g)	5.1 ± 0.5	8.0 ± 0.7	0.003
Testicular & Bladder WAT (% BWT)	0.15 ± 0.01	0.22 ± 0.02	0.003
Total WAT (g)	97.7 ± 13.2	154.9 ± 12.4	0.003
Total WAT (% BWT)	2.9 ± 0.3	4.4 ± 0.3	0.002
Estimates from DEXA	n=8	n=9	
Lean Body Mass (g)	3085 ± 41	3229 ± 50	0.04
Fat Mass (g)	103 ± 14	263 ± 26	<0.001
Total Body Mass (g)	3193 ± 38	3492 ± 59	<0.001
% Fat	3.2 ± 0.5	7.4 ± 0.7	<0.001
Bone Mineral Content (g)	56.0 ± 0.95	60.5 ± 1.07	0.006
Bone Mineral Density (g/cm^2)	0.223 ± 0.004	0.230 ± 0.003	0.19

Values are mean ± SEM. BWT = bodyweight. P is the probability for the comparison between groups.

Table 2: Haemodynamics, sympathetic nerve activity and leptin concentration in CD and HFD-fed rabbits after 3 weeks of diet.

	CD	HFD	P_{diet}
Haemodynamics and RSNA	n=11	n=12	
MAP (mmHg) at baseline	70.3 ± 1.1	66.3 ± 1.0	0.02
MAP (mmHg) at 3 weeks	69.3 ± 1.3	77.9 ± 1.0	<0.001
Δ MAP from baseline (mmHg)	-1.0 ± 1.6	11.6 ± 1.5	<0.001
HR (bpm) at baseline	177 ± 4	167 ± 4	0.11
HR (bpm) at 3 weeks	195 ± 4	211 ± 4	0.01
Δ HR from baseline (bpm)	17.8 ± 5.3	43.6 ± 3.7	<0.001
RSNA (nu)	9.0 ± 1.0	12.8 ± 1.0	0.02
RSNA Burst Amplitude (nu)	24.3 ± 0	35.1 ± 2	<0.001
RSNA Frequency (bursts per second)	7.1 ± 0.6	6.5 ± 0.3	0.30
RSNA Frequency (bursts per heart beat)	2.2 ± 0.2	1.9 ± 0.1	0.13
RSNA (μV)	31.3 ± 5.6	30.3 ± 1.6	0.84
Nasopharyngeal Response (μV)	299 ± 38	256 ± 23	0.36
Plasma Leptin (ng/ml, n=4-5)	0.95 ± 0.09	2.20 ± 0.39	0.02

Values are mean ± SEM. P is the probability for the comparison between groups.

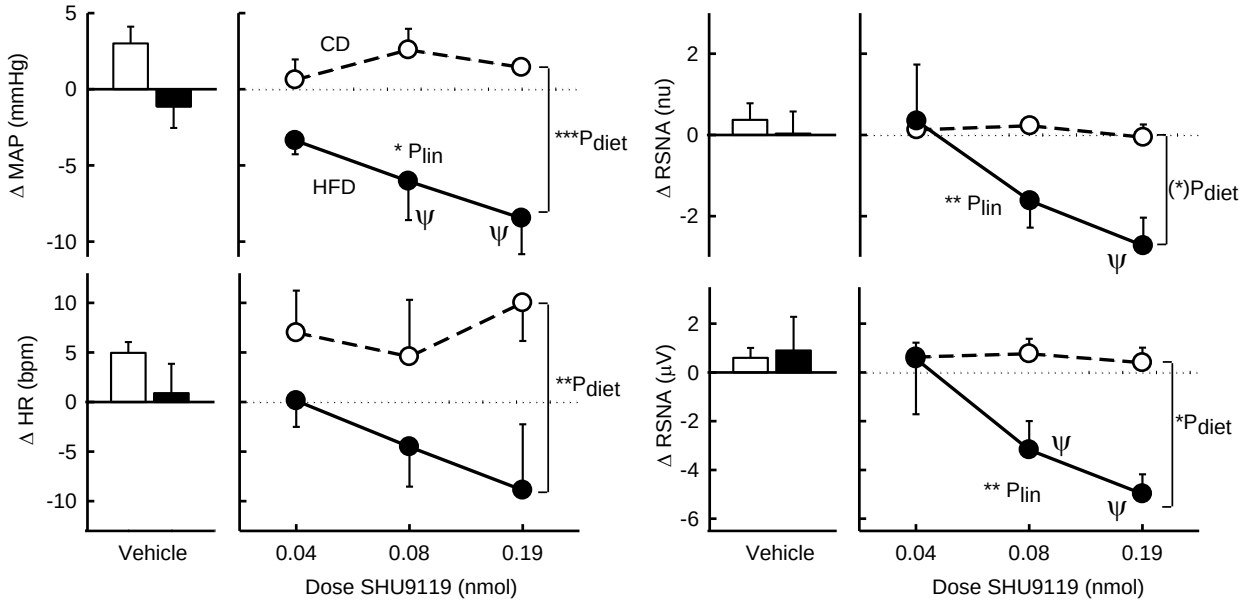


Figure 1: Left panels: changes from baseline of mean arterial pressure (MAP), heart rate (HR) and total renal sympathetic nerve activity (RSNA, expressed as normalised units, NU and raw μvolts, μV) in response to vehicle injection (Ringer's Solution, 50 μl) in rabbits fed a control diet (CD; unfilled; n=4) or a high fat diet (HFD; filled; n=6) for 3 weeks. Right panels: 30-minute averages of changes from vehicle in MAP, HR and RSNA in response to increasing doses of ICV SHU9119 in both CD (unfilled circles) and HFD (filled circles) rabbits. Data are mean ± SED indicating variance between animals. Ψ for effect of individual doses (P<0.05). 0.05< (*)P_{diet}<0.1, *P_{diet}<0.05, **P_{diet}<0.0, ***P_{diet}<0.001 for effect of diet; *P_{lin}<0.05, **P_{lin}<0.01 for significance of linear trend effect of SHU9119.

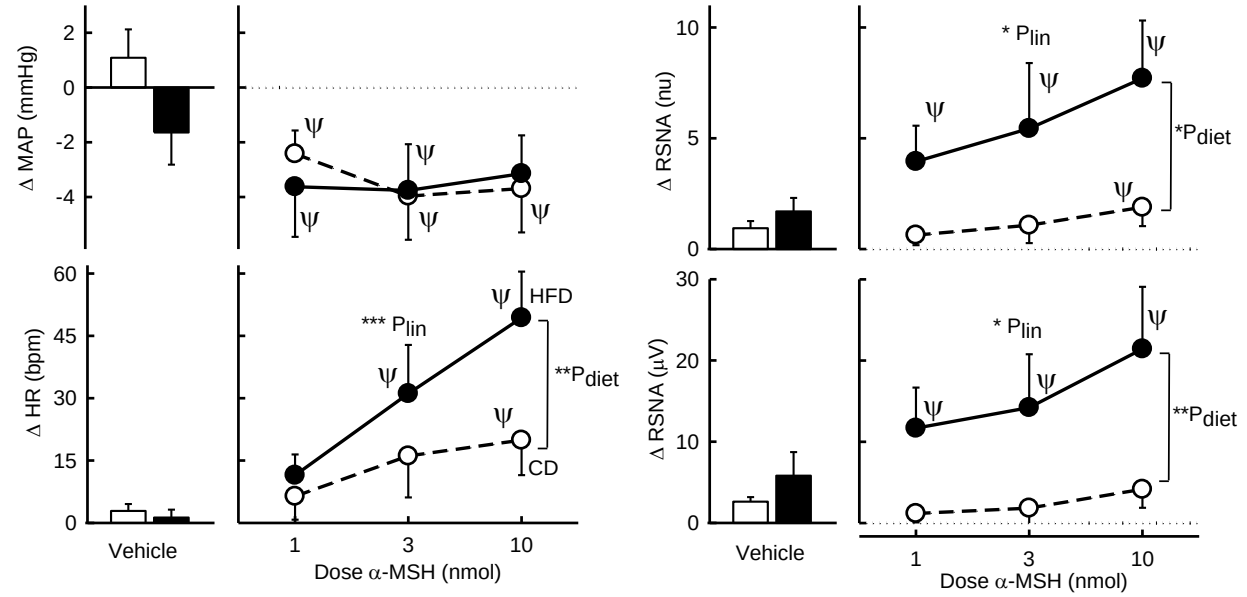


Figure 2: Left: changes from baseline of mean arterial pressure (MAP), heart rate (HR) and total renal sympathetic nerve activity (RSNA, expressed as normalised units, NU and raw μvolts, μV) in response to vehicle injection (Ringer's Solution, 50 μl) in rabbits fed a control diet (CD; unfilled bars; n=11) or a high fat diet (HFD; filled bars; n=11) for 3 weeks. Right: 30-minute averages of changes from vehicle in MAP, HR and RSNA in response to increasing doses of ICV α-MSH in both CD (unfilled circles) and HFD (filled circles) rabbits. Data are mean ± SED indicating variance between animals. Ψ for effect of individual doses (P<0.05). **P_{diet}<0.01 for for effect of diet. *P_{lin}<0.05, ***P_{lin}<0.001 for significance of linear trend effect of α-MSH.

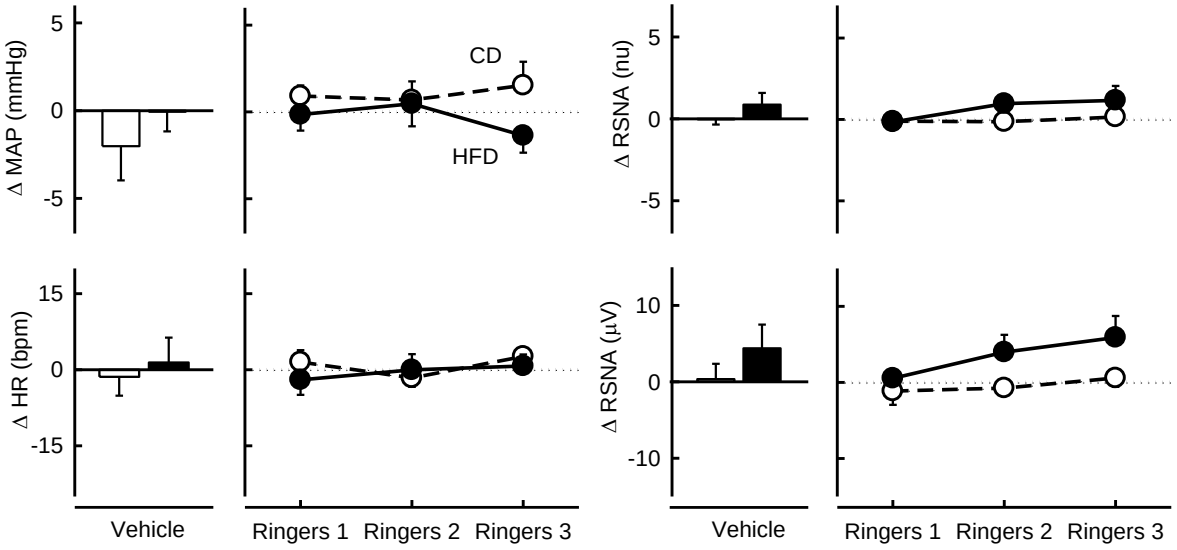


Figure 3: Left: changes from baseline of mean arterial pressure (MAP), heart rate (HR) and total renal sympathetic nerve activity (RSNA, expressed as normalised units, NU and raw μvolts, μV) in response to vehicle injection (Ringer's Solution, 50 μl) in rabbits fed a control diet (CD; unfilled bars; n=4) or a high fat diet (HFD; filled bars; n=5) for 3 weeks. Right: 30-minute averages of changes from vehicle in MAP, HR and RSNA in response to increasing doses of ICV vehicle in both CD (unfilled circles) and HFD (filled circles) rabbits. Data are mean ± SED indicating variance between animals.

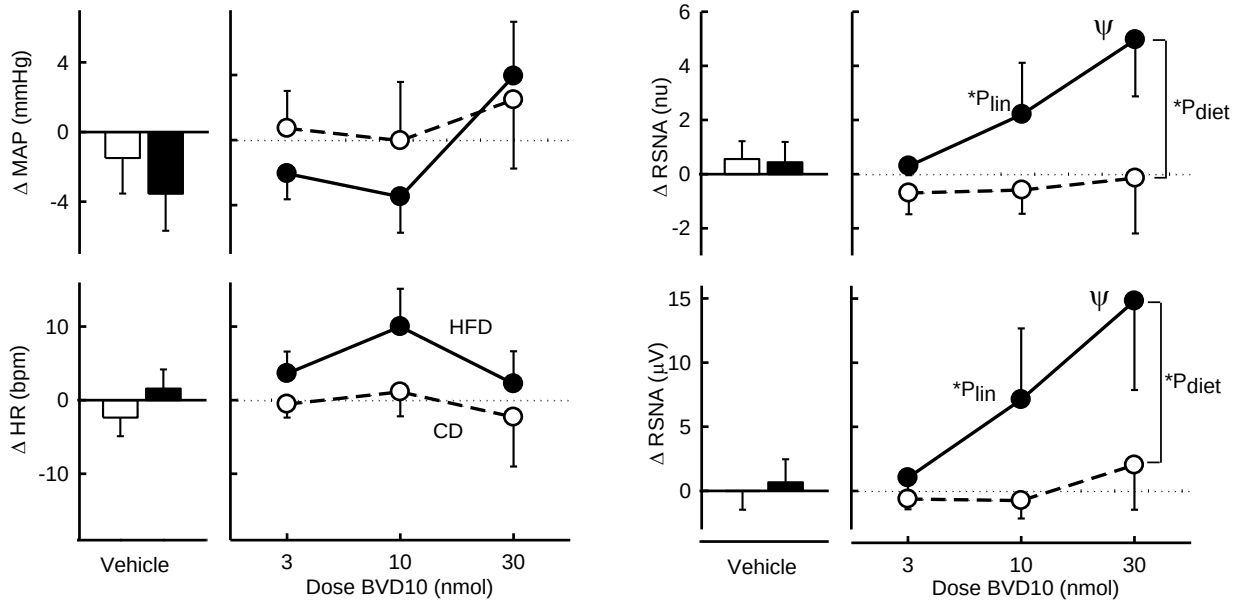


Figure 4: Left panels: changes from baseline of mean arterial pressure (MAP), heart rate (HR) and total renal sympathetic nerve activity (RSNA, expressed as normalised units, NU and raw μvolts, μV) in response to vehicle injection (Ringer's Solution, 50 μl) in rabbits fed a control diet (CD; unfilled; n=5) or a high fat diet (HFD; filled; n=5) for 3 weeks. Right panels: 30-minute averages of changes from vehicle in MAP HR and RSNA in response to increasing doses of ICV BVD10 in both CD (unfilled circles) and HFD (filled circles) rabbits. Data are mean ± SED indicating variance between animals. Ψ for effect of individual doses (P<0.05). *P_{diet}<0.05 for effect of diet; *P_{lin}<0.05 for significance of linear trend effect of BVD10.

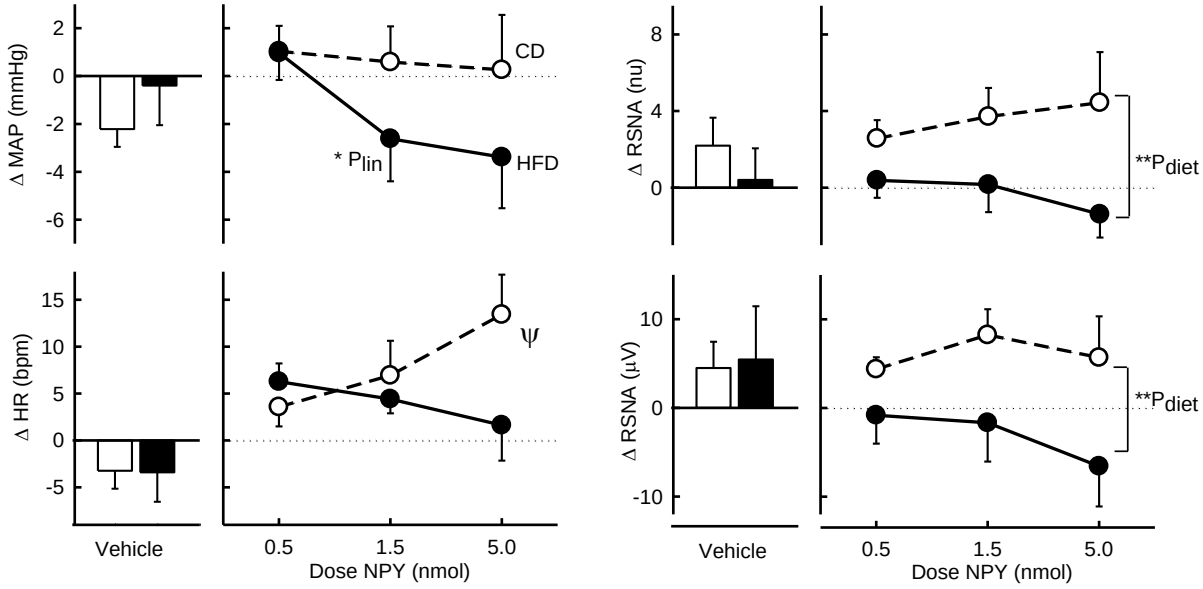


Figure 5: Left panels: changes from baseline of mean arterial pressure (MAP), heart rate (HR) and total renal sympathetic nerve activity (RSNA, expressed as normalised units, NU and raw μvolts, μV) in response to vehicle injection (Ringer's Solution, 50 μl) in rabbits fed a control diet (CD; unfilled; n=10) or a high fat diet (HFD; filled; n=11) for 3 weeks. Right panels: 30-minute averages of changes from vehicle in MAP HR and RSNA in response to increasing doses of ICV NPY in both CD (unfilled circles) and HFD (filled circles) rabbits. Data are mean ± SED indicating variance between animals. Ψ for effect of individual dose (P<0.05) . **P_{diet}<0.01for for effect of diet. *P_{lin}<0. 05, **P_{lin}<0.01for significance of linear trend effect of NPY.

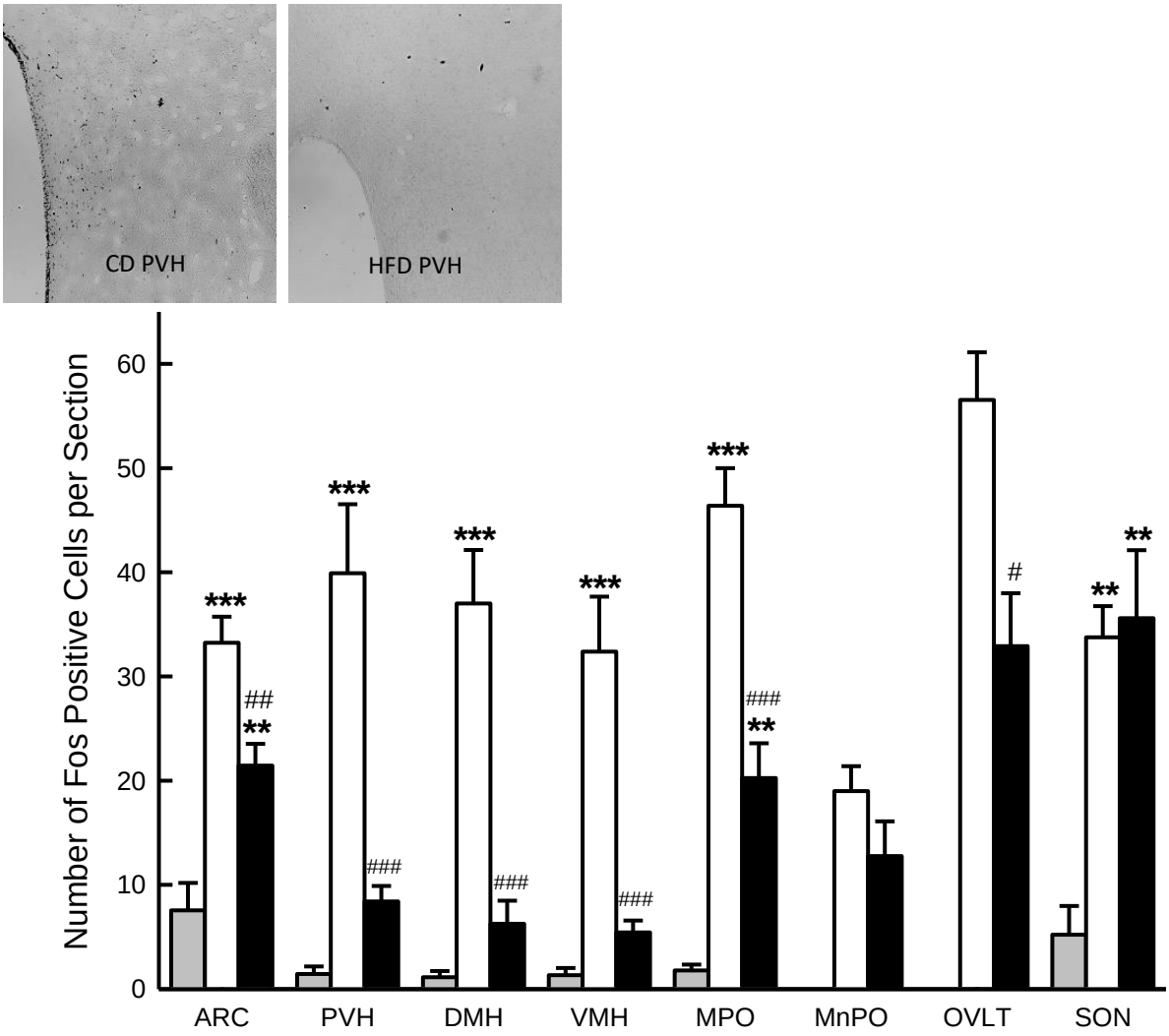
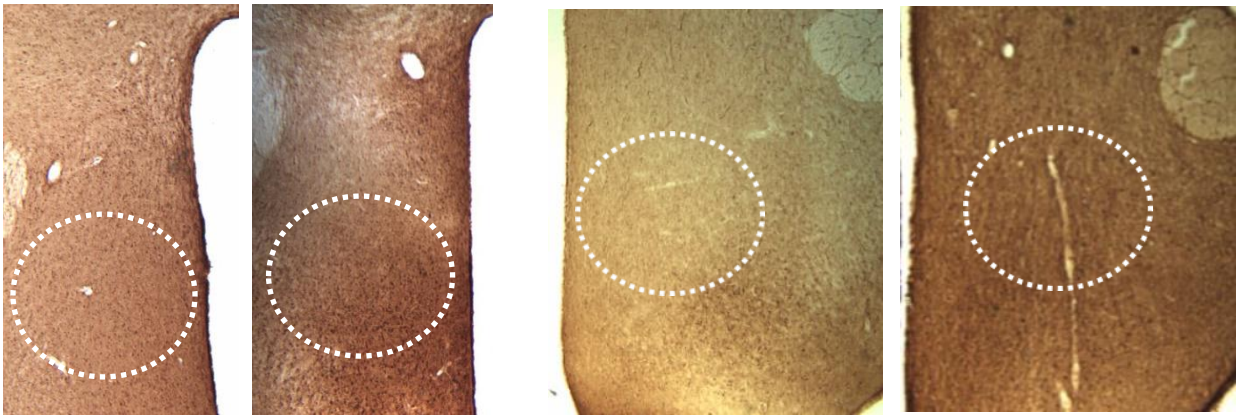


Figure 6: Mean number of c-Fos positive neurons in the hypothalamus of normal fat diet (CD; n=4; white bars) and high fat diet (HFD; n=3; black bars) fed rabbits as detected by c-Fos immunoreactivity induced by ICV α-MSH or ICV Ringer's solution (grey bars, n=3). Top right corner, coronal micrographs of the PVN in CD and HFD rabbits used as representative images of Fos immunoreactivity. Data are mean ± SEM indicating variance between animals. #*P*<0.05, ##*P*<0.01 and ###*P*<0.001 for α-MSH in CD vs α-MSH in HFD; **P<0.01, ***P<0.001 for α-MSH vs vehicle in CD. ARC, arcuate nucleus; PVH, paraventricular hypothalamus; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; MPO, medial preoptic nucleus; MnPO, median preoptic nucleus; OVLT, organum vasculosum of the lamina terminalis; SON, supraoptic nucleus.



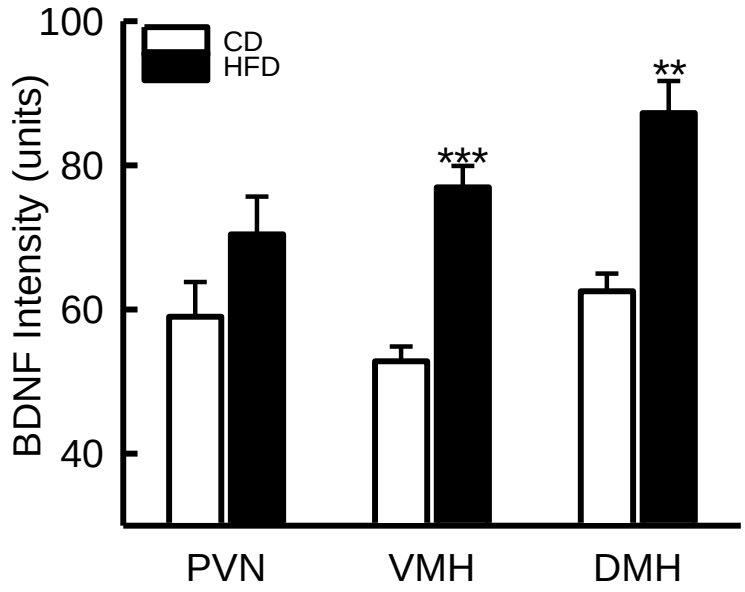


Figure 7: Upper: Example of coronal sections from rabbit hypothalamus stained with an antibody for BDNF. Circle indicates region quantified representing the dorsomedial hypothalamus (DMH) left and ventromedial hypothalamus (Right). Lower: Quantification of the levels (arbitrary units) of BDNF staining in the paraventricular nucleus (PVN), ventromedial hypothalamus (VMH) and DMH of the hypothalamus of CD (open bars, n = 3) and HFD (filled bars, n = 3) rabbits. ***P_{diet}<0.001 **P_{diet}<0.01for CD vs HFD.

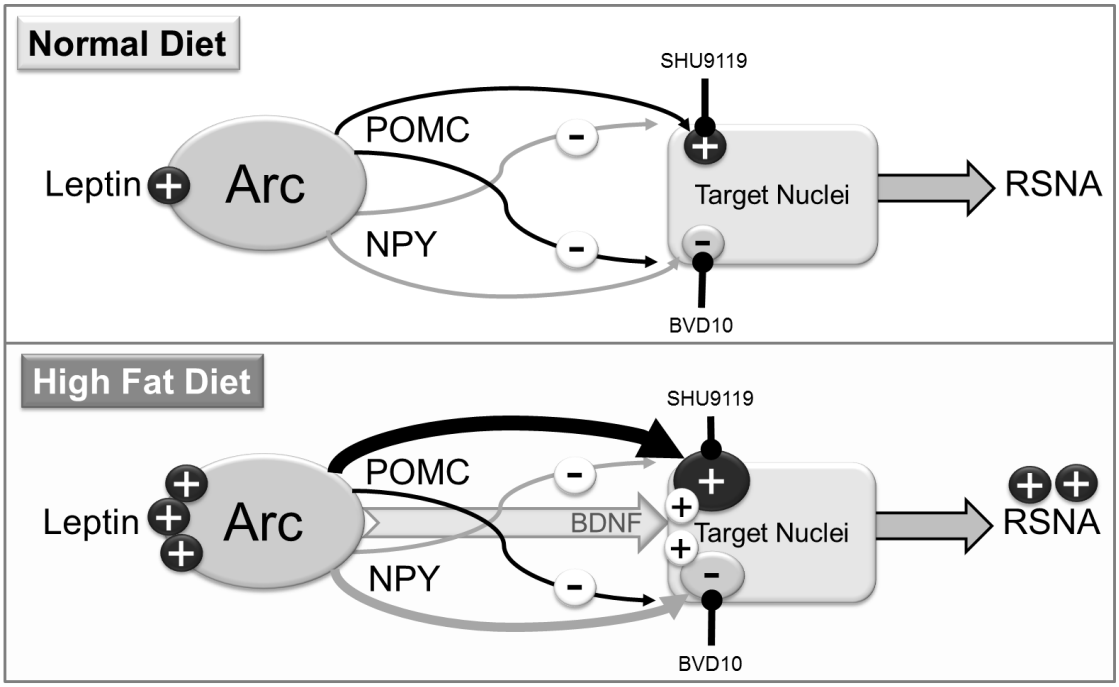


Figure 8: Schema representing the possible central pro-opiomelanocortin (POMC) and neuropeptide Y (NPY) signaling mechanisms regulating RSNA during a normal (upper panel) and high fat (lower panel) diet. Under normal conditions, leptin activation of both POMC and NPY pathways, but mutual inhibition [16], results in little or no activation of renal sympathetic nerve activity (RSNA). Thus blockade of melanocortin receptors with SHU9119 or NPY receptors with BVD10 has little effect on RSNA. With a high fat diet, chronic activation of leptin signaling due to elevated levels of leptin in plasma results in increased signaling of both sympatho-excitatory POMC neurons releasing α-MSH and sympatho-inhibitory NPY projections releasing NPY. However the sympatho-excitatory effects of α-MSH dominate leading to increased RSNA. The increased signaling is thought to be due to a synaptic plasticity induced by greater production of BDNF in the ventromedial hypothalamus [20] and as demonstrated in the current study. This model explains why SH9119 reduced and BVD10 increased RSNA but only during a high fat diet.