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Evidence for a feeding related association between melanocortin in the NTS and Neuropeptide-Y in the PVN

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ABSTRACT

Melanocortin and neuropeptide-Y (NPY) are both involved in feeding and energy regulation, and they have opposite effects in the paraventricular nucleus of the hypothalamus (PVN). The present study examined an interaction between melanocortin in the nucleus of the solitary tract (NTS) and NPY in the PVN. Male Sprague-Dawley rats were implanted with cannulae in the injection sites of interest. In Experiment 1, subjects received either the melanocortin 3/4-receptor (MC3/4) antagonist SHU9119 (0, 10, 50 and 100 pmol/0.5 μl) or the MC3/4 agonist MTII (0, 10, 50, 100 and 200 pmol/0.5 μl) into the NTS. Food intake was measured at 1, 2, 4, 6 and 24-h post-injection. Administration of SHU9119 into the NTS significantly and dose-dependently increased food intake at 1, 2, 4, 6 and 6-24-h, and administration of MTII into the NTS significantly and dose-dependently decreased 24-h free feeding. In Experiment 2, subjects received the MC3/4 agonist MTII (0, 10, 50, 100 and 200 pmol/0.5 μl) into the NTS just prior to NPY (0 and 1μg/0.5 μl) in the PVN. PVN injection of NPY stimulated feeding, and administration of MTII (50, 100 and 200 pmol) into the NTS significantly and dose-dependently decreased NPY-induced feeding at 2, 4, 6 and 6-24-h. These data suggest that there could be a neuronal association between melanocortin in the NTS and NPY in the PVN, and that the melanocortin system in the NTS has an antagonistic effect on NPY-induced feeding in the PVN.

1. Introduction

A significant body of evidence indicates that the central melanocortin system is involved in the control of feeding and energy regulation (see Baldini & Phelan, 2019 for review). During the last decade it has become clear that melanocortin-3 and –4 receptors (MC3/4) are particularly important in feeding (Fan et al., 1997; Ollmann et al., 1997; Kask & Schioth, 2000; Gantz & Fong, 2003; Voisey et al., 2003). For example, it has been shown that disruption of the MC4 receptor produces hyperphagia in mice (Huszar et al., 1997), and that central administrations of MC3/4 receptor agonists, such as the endogenous ligand α-MSH (Hansen et al., 2001; Rossi et al., 1998) and the exogenous ligand MTII (Hagan et al., 2000) decrease food intake, whereas administrations of MC3/4 receptor antagonists, such as the endogenous ligand agouti-related protein (AgRP) (Williams et al., 2000; Olszewski et al., 2003; Atasoy et al., 2012) and the exogenous ligand SHU9119 (Giraudo et al., 1998) increase food intake. Consequently, the melanocortin system has been intensively studied with respect to feeding. Much of this interest has concentrated upon hypothalamic nuclei, especially pro-opiomelanocortin (POMC) neurons in the arcuate nucleus (ARC) (Huo et al., 2006), which project to the paraventricular nucleus (PVN), POMC being the precursor of the endogenous ligand α-MSH (Prichard et al., 2002) which is an endogenous MC3/4 receptor agonist (Cone et al., 1996). MC3/4 receptors in the PVN are considered important sites of action mediating the orexigenic effects of melanocortin receptor ligands (Kiss et al., 1984; Mountjoy et al., 1994). Also, injections of MC3/4 receptor agonists into the PVN decrease food intake, while injections of MC3/4 receptor antagonists into the PVN increase food intake (Giraudo et al., 1998; Kim et al., 2002; Olszewski et al., 2003).

As MC3/4 receptors and their peptides are widely distributed throughout the central nervous system, including the nucleus of the solitary tract (NTS) which is known to be involved in feeding regulation, and in other specific nuclei of the brain (Palkovis et al., 1987; Bronstein et al., 1992; Mountjoy et al., 1994; Olszewski et al., 2003), it is

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important to consider the role of the melanocortin system in brain nuclei other than the ARC and the PVN. POMC mRNA is synthesised only in two central sites, the ARC and the NTS. The NTS receives primary afferent fibres from gustatory and visceral receptors, and relays this information to ventral forebrain areas, including the PVN, and in turn receives efferent connections from these areas (Palkovis et al., 1987). Injection of MTII into the fourth ventricle (Barraco, 1994) or into the dorsal vagal complex (Grill et al., 1998) has been shown to decrease food intake, while injection of SHU9119 into the caudal NTS stimulates food intake (Zheng et al., 2005). It has also been shown that central vagal afferent endings are essential in the melanocortinergic modulation of food intake in the NTS, and that NTS MC4R activation reduces food intake, leading to the suggestion that central vagal efferent endings may be affected by signals converging in the NTS from other brain areas (Campos, Shiina & Ritter, 2014).

With respect to feeding regulation, the melanocortin system appears to be integrated with the neuropeptide-Y (NPY) system in the ARC (Cowley et al., 2001; King & Williams, 1998) and the PVN (Fan et al., 1997; Stanley, 1993). NPY is a potent stimulator of food intake, and NPY in the ARC-PVN neural circuitry is known to be involved in feeding and energy regulation (King & Williams, 1998). The MC3/4 receptor agonists α−MSH and MTII, when injected into the PVN, suppress food deprivation-induced and NPY-induced feeding (Wirth & Giraudo, 2000), and the NPY receptor antagonist 1229U91 attenuates the MC4 receptor antagonist HS014-induced feeding (Kask et al., 1998). That is, increased food intake following HS104 injection (a melanocortin antagonist), is attenuated by co-injection of 1229U901 (a NPY antagonist). These findings suggest that the melanocortin system in the ARC-PVN circuitry has a modulatory action on NPY in energy regulation. Morphological investigations have demonstrated that NPY terminals form connections directly onto neighbouring melanorcortinergic cell bodies and dendrites (Csiffary et al., 1990; Hovarth et al., 1992), and that the NPY receptor is co-expressed with POMC (Broberger et al., 1997; Fuxe et al., 1997) and AgRP (Hahn et al., 1998; Kim et al., 2002). The overall implication is that melanocortin inhibits the expression of NPY in the hypothalamus, and that this effect is mediated by MC3/4 receptors.

The action of NPY in feeding (Clark et al., 1984; Levine & Morley, 1984; Sutton, Patterson, & Berthoud, 2004) appears to be largely in the ARC and the PVN (Allen et al., 1983; Chronwall et al., 1985), but POMC and AgRP projections are not limited to these regions, and innervated areas have been found along the neuraxis as far caudally as the NTS (Barraco, 1994; Alessi et al., 1988). Also, a retrograde tracing study has shown that some POMC neurons from the ARC project to the NTS (Wirth et al., 2001). Therefore, it is possible that there is an interaction between melanocortin and NPY in the ARC-PVN neural circuitry, at least with respect to feeding, and that this might be mediated through the activation, or deactivation, of melanocortin systems in the NTS.

The current study examined whether the melanocortin system in the NTS was involved in feeding, and whether the melanocortin system in the NTS interacted with NPY in the PVN. In the current study, injections were aimed at the rostro-medial NTS for the investigation of melanocortin mediated effects on feeding. This was because the caudal region of the NTS has been shown to affect cardiovascular function, especially respiratory control (Boscan et al., 2002), whereas the rostro-medial NTS is known to influence neurotransmitter mediated feeding (Edwards et al., 1986; Kotz et al., 1995; Kotz et al., 2000).

2. Methods and materials

2.1. Experiment 1: effect of MC3/4 antagonist SHU9119 and agonist MTII injection into the NTS on food intake

2.1.1. Animals

Fifteen male Sprague-Dawley rats (Harlan, UK), weighing 225–250 g, were housed individually in stainless steel hanging cages, with the temperature in the vivarium controlled at 22 °C, under a 12-h light/12-h dark cycle with lights on at 0700-h. Prior to experimental manipulations, subjects were given ad libitum access to water and standard laboratory chow diet (Harlan, UK).

2.1.2. Surgery

Subjects were anaesthetised with sodium pentobarbital (60 mg/kg) and fitted with 23-gauge stainless steel cannulae (Plastics One, Austin, TX) in the NTS. Stereotaxic co-ordinates (Paxinos & Watson, 2013) with the incisor bar set at 3.3 mm below the interaural line were 12.7 mm posterior and −1.4 mm lateral to the bregma, and 6.9 mm below the surface of the skull. The injector extended 1 mm beyond the tip of the guide cannula. All animals were allowed to recover for one week before experimental testing.

2.1.3. Experimental design and injections

During experimental trials, immediately before drug injection, food was removed from the cages. Following injection, pre-weighed diets were placed into the cages. The NTS injection was administered in a 0.5 μl volume over 30-s. All subjects received the MC3/4 antagonist SHU9119 (Phoenix, Pharmaceuticals, CA, USA) at 0, 10, 50 and 100 pmol/0.5 μl into the NTS. Subjects were randomly assigned to treatment, and at least 4 days elapsed between each experimental trial. Each subject received all treatments in a counter-balanced fashion. Food intake was measured at 1, 2, 4, 6 and 24-h post injection and corrected for spillage. After the SHU9119 trials, subjects received the MC3/4 agonist MTII (Phoenix, Pharmaceuticals, CA, USA) at 0, 10, 50, 100, and 200 pmol/0.5 μl into the NTS. At least 4 days elapsed between each experimental trial and each subject received all treatments in a counter-balanced fashion. Food intake was measured at 24-h post injection and corrected for spillage.

2.2. Experiment 2: effect of MC3/4 agonist MTII injection into the NTS on NPY-induced feeding in the PVN

2.2.1. Animals

Eighteen male Sprague-Dawley rats (Harlan, UK), weighing 225–250 g, were housed individually in stainless steel hanging cages, with the temperature in the vivarium controlled at 22 °C, under a 12-h light/12-h dark cycle with light on at 0700-h. Prior to experimental manipulations, subjects were given ad libitum access to water and standard laboratory chow diet (Harlan, UK).

2.2.2. Surgery

Subjects were anaesthetised with sodium pentobarbital (60 mg/kg) and fitted with 23-gauge stainless steel cannulae (Plastics One, Austin, TX). One cannula was placed into the PVN and one was placed into the NTS. Stereotaxic co-ordinates (Paxinos & Watson, 2013) with the incisor bar set at 3.3 mm below the interaural line were, PVN, 1.9 mm posterior and −0.5 mm lateral to the bregma, and 7.3 mm below the surface of the skull and, NTS, 12.7 mm posterior and −1.4 mm lateral to the bregma, and 6.9 mm below the surface of the skull. The injector extended 1 mm beyond the tip of the guide cannula. All animals were allowed to recover for one week before experimental testing.

2.2.3. Experimental design and injections

During experimental trials, immediately before drug injection, food was removed from the cages. Following injection, pre-weighed diets were placed into the cages. The PVN and the NTS injections were administered in a 0.5 μl volume over 30-s. There was at least a 30-s interval time between injections. Subjects were injected with NPY (Sigma, UK) into the PVN and MTII (Phoenix Pharmaceuticals, CA, USA) into the NTS. Subjects were randomly assigned to treatment group and received either, (a) saline (0.5 μl) into the PVN preceded by saline (0.5 μl) into the NTS, (b) NPY (1 μg/0.5 μl) into the PVN preceded by saline (0.5 μl) into the NTS, (c) NPY into the PVN preceded by MTII (10 pmol/0.5 μl) into the NTS, (d) NPY into the PVN preceded by MTII (50 pmol/

0.5 μl) into the NTS, (e) NPY into the PVN preceded by MTII (100 pmol/0.5 μl) into the NTS, and NPY into the PVN preceded by MTII (200 pmol/0.5 μl) into the NTS. At least 4 days elapsed between each experimental trial and each subject received all treatments in a counter-balanced fashion. Food intake was measured at 1, 2, 4, 6 and 24-h post injection and corrected for spillage.

All procedures involving animals were conducted under UK Home Office License [Animals (Scientific Procedures) Act 1986] and were approved by the Institutional Animal Ethics Committee in accordance with the guidelines on laboratory experimentation involving the use of animals.

2.3. Histology

At the completion of Experiments 1 and 2, all subjects received an overdose of sodium pentobarbital and were perfused through the ascending aorta with 300 ml of 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde (w/v) in 0.1 PBS. The brain was removed and stored in a 10% sucrose (w/v) solution in 0.1 M PBS. For cannula placement verification, coronal sections (50 μm) were cut on a cryostat and mounted on gelatin-coated glass microscope slides. The sections were stained with a 0.1% thionin solution (Sigma, UK), dried and cover slipped. Data from subjects with incorrectly placed cannulae were excluded from the final analysis.

2.4. Data analysis and statistics

Food intake data at each time point and cumulative food intake in each Experiment were analysed by one-way repeated measures analysis of variance. Post hoc analysis for each experiment was performed using the Bonferroni correction. F-values for the ANOVAs are presented with subscripted degrees of freedom of the treatments and total sample sizes.

3. Results

3.1. Cannula placement

Fig. 1 shows representative cannulae placements with a map of cannula termination points in the PVN (A) and in the NTS (B), and that PVN cannulae were placed dorsal to the PVN. All NTS cannulae terminated in a medial region from −11.6 mm to – 13.24 mm posterior to bregma (Paxinos & Watson, 2013).

3.2. Experiment 1

Injection of SHU9119 (10, 50 and 100 pmol/0.5 μl) into the NTS significantly and dose dependently increased food intake at 1, 2, 4, and 6-h post-injection. This finding is shown in Fig. 2 (1-h; $F_{3, 36} = 5.79$, $P = 0.0025$, 2-h; $F_{3, 36} = 10.45$, $P = 0.0001$, 4-h $F_{3, 36} = 11.566$, $P = 0.0001$ and 6-h; $F_{3, 36} = 12.525$, $P = 0.0001$). Also, injection of SHU9119 (100 pmol/0.5 μl) into the NTS significantly increased food intake at 6-24-h and 0-24-h post-injection. This finding is shown in Fig. 3 (6-24-h; $F_{3, 36} = 5.778$, $P = 0.0025$ and 0-24-h; $F_{3, 36} = 16.616$, $P = 0.0001$). Injection of MTII (10, 50, 100 and 200 pmol/0.5 μl) into the NTS significantly and dose dependently decreased 24-h food intake. This is finding shown in Fig. 4 ($F_{4, 48} = 12.356$, $P = 0.0001$).

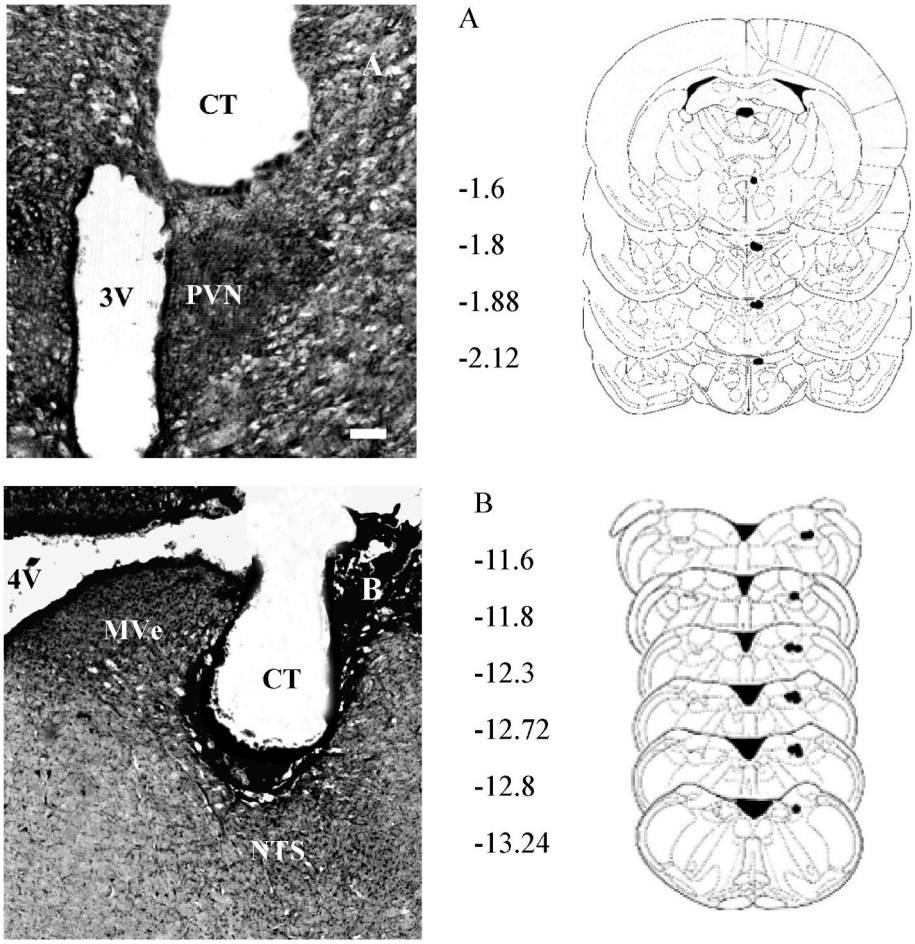

Fig. 1. Photomicrographs showing representative cannula placement in the PVN (A) and in the NTS (B). CT; cannula tract, 3V; third ventricle, 4V; fourth ventricle, MVe; medial vestibular nucleus. Scale bar = 100 μm.

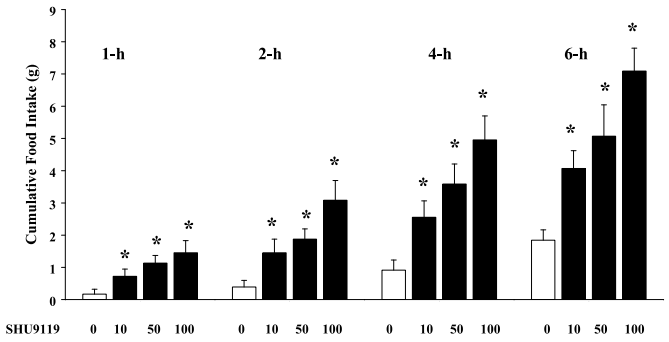

Fig. 2. Effects of SHU9119 (0, 10, 50 and 100 pmol/0.5 μl) injections into the NTS on food intake at 1, 2, 4 and 6-h post-injection. Values are means ± S.E.M. *P < 0.05 as compared to saline injection into the NTS.

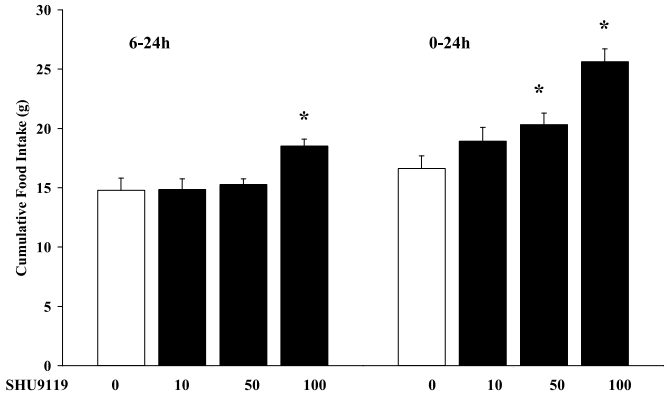

Fig. 3. Effects of SHU9119 (0, 10, 50 and 100 pmol/0.5 μl) injections into the NTS on food intake at 6-24-h and 0-24-h post-injection. Values are means ± S.E.M. *P < 0.05 as compared to saline injection into the NTS.

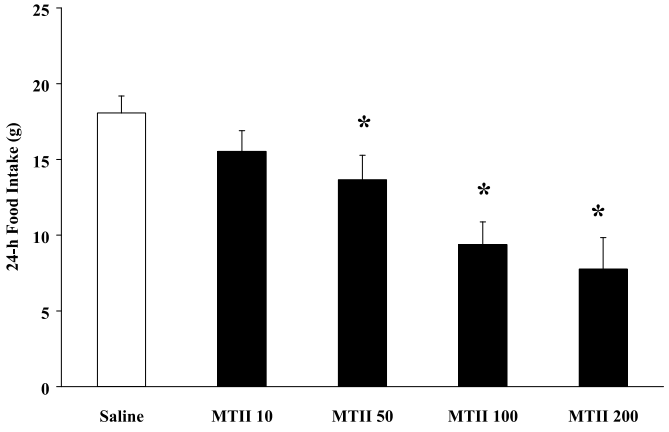

Fig. 4. Effects of MTII (0, 10, 50, 100, 200 and 400 pmol/0.5 μl) injections into the NTS on food intake at 24-h post-injection. Values are means ± S.E.M. *P < 0.05 as compared to saline injection into the NTS.

3.3. Experiment 2

Injection of NPY (1 μg/0.5 μl) in the PVN increased food intake at 1, 2, 4, and 6-h post-injection. Injection of MTII (50, 100 and 200 pmol/0.5 μl) in the NTS significantly and dose dependently decreased food intake that was induced at 2, 4, and 6-h post-injection by NPY injection in the PVN. This finding is shown in Fig. 5 (2-h; $F_{4,\ 48} = 7.354$, $P = 0.0001$, 4-h; $F_{4,\ 48} = 9.262$, $P = 0.0001$ and 6-h; $F_{4,\ 48} = 13.452$, $P = 0.0001$). Also, injection of MTII (50, 100 and 200 pmol/0.5 μl) significantly and dose dependently decreased food intake at 6-24-h and 0-24-h post-injection, as compared to the NPY/saline injection group. This finding is shown in Fig. 6 (6-24-h; $F_{4,\ 48} = 4.146$, $P = 0.0025$, 0-24-h; $F_{4,\ 48} = 7.408$, $P = 0.0001$).

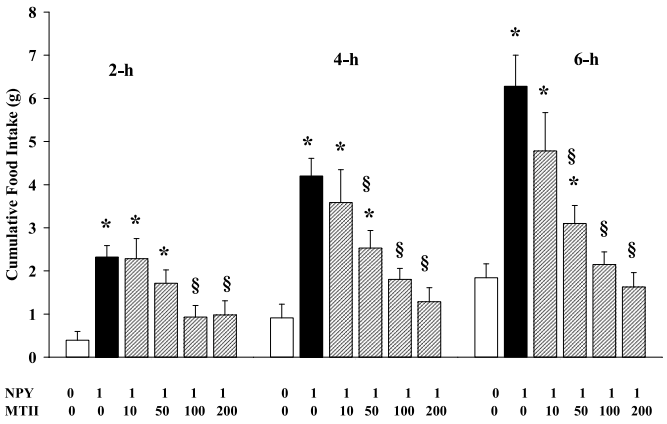

Fig. 5. Effects of MTII (0, 10, 50, 100 and 200 pmol/0.5 μl) injections into the NTS on NPY (1 μg/0.5 μl) injection induced food intake into the PVN at 1, 2, 4 and 6-h post-injection. Values are means ± S.E.M. *P < 0.05 as compared to saline injection into both areas and §P < 0.05 as compared with NPY injection into the PVN and saline injection into the NTS.

4. Discussion

With respect to food intake, the NTS is known to be involved in the integration of forebrain descending melanocortinergic signals, in conjunction with leptin signals and gut satiety signals (Blevins et al., 2004; Sutton, Patterson, & Berthoud, 2004; Sutton et al., 2005; Blevins et al., 2009; Zhao et al., 2012). Also, MC3/4 receptor binding and MC4 receptor mRNA expression studies have shown that an MC3/4 receptor exists in the NTS, and the endogenous peptides α-MSH and agouti-related peptide (AgRP) for MC3/4 receptors exist in the NTS (Mountjoy et al., 1994; Roselli-Rehfuss et al., 1993). Experiment 1 of this study investigated the effect on food intake of the melanocortin antagonist SHU9119 (Cowley et al., 2001; Grieco et al., 2007) and of the melanocortin agonist MTII (Wikberg, 1999) following injection into the NTS. Direct injection of SHU9119 into the NTS increased short-term and long-term feeding, while direct injection of MTII into the NTS decreased feeding dose dependently.

It has been shown that injection of single dose of SHU9119 (62.5 pmol) into the nucleus of the vagus nerve, that includes the dorsal motor

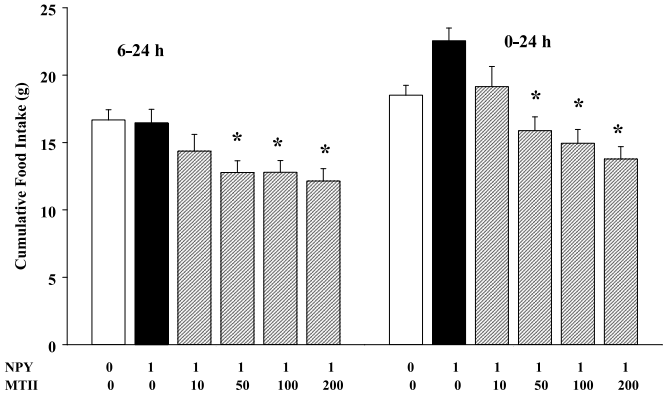

Fig. 6. Effects of MTII (0, 10, 50, 100 and 200 pmol/0.5 μl) injections into the NTS on NPY (1 μg/0.5 μl) injection induced food intake into the PVN. Values are means ± S.E.M. *P < 0.05 as compared to saline injection into both areas and §P < 0.05 as compared with the NPY injection into the PVN and saline injection into the NTS.

nucleus of vagus nerve which contains the highest density of MCR4 mRNA in the brain, and into the caudal NTS, increases 24-h food intake (Williams et al., 2000). And that a single dose of SHU 9119 (0.065 nmol) into the caudal NTS increases 14-h food intake and meal size, and increases the satiety ratio (Zheng et al., 2005). The current study shows that injection of SHU9119 into the medial NTS stimulated feeding and that the feeding induced lasted up to 24-h post injection, which similar to the effect of SHU9119 injected directly into the PVN (Giraudo et al., 1998). The MC3/4 agonist MTII has been consistently shown to decrease food intake (Williams et al., 2000; Grill et al., 1998), this includes following injection into the NTS, where a rapid and sustained reduction of food intake was reported (Campos et al., 2014). In Experiment 1 of the current study, injection of MTII into the NTS significantly and dose dependently decreased 24-h free feeding.

Experiment 2 investigated a possible interaction between MTII in the NTS and NPY in the PVN in relation to feeding. NPY plays an important role in energy homeostasis. Injection of NPY into the central nervous system increases food intake (Clark et al., 1984; Levine & Morley, 1984; Stanley & Liebowitz, 1984), the PVN (Abe et al., 1989; Billington et al., 1994; Stanley et al., 1985) is a primary site of action for NPY, and injection of NPY into the PVN produces a dose-dependent increase in interdigestive gastric acid output (Humphreys et al., 1988) Also, immunoreactivity for NPY is highest in the PVN (Allen et al., 1983; Chronwall et al., 1985; Sawchenko et al., 1985; Stanley et al., 1993), and injection of the opioid antagonist naltrexone into the NTS, prior to injection of NPY into the PVN, blocks the feeding and thermogenic effects seen after injection of NPY into the PVN (Kotz et al., 1995). Consequently, the NTS is implicated as being part of the circuitry involved in the feeding and other effects of NPY in the PVN.

The results of Experiment 2 demonstrated that MTII injection into the NTS blocked NPY-induced feeding in the PVN. This antagonism of NPY-induced feeding implies that the melanocortin system in the NTS might have an antagonistic effect on feeding induced by NPY in the PVN, and it suggests that there might be a neuronal relationship between melanocortin in the NTS and NPY in the PVN. It is known that NTS neurons project directly to the PVN (Kirchgessner & Sclafani, 1988). The central melanocortin system is involved in the regulation of energy homeostasis (Girardet & Butler, 2014), including food intake (Baldini & Phelan, 2019), through projections to melanocortin receptors in hypothalamic nuclei and extrahypothalamic nuclei (Li et al., 2019), and much of the research on melanocortin receptors has focused on the PVN (Hill & Faulkner, 2017). Interestingly, AgRP which was originally identified as a peptide expressed by neurons in the mediobasal hypothalamus, acts as an antagonist of MC3/4 receptors (Ellacott & Cone, 2004). AgRP/NPY neurons have projections that converge with those from POMC neurons to MC4R neurons in the PVN and seem to be involved in the control of food intake and energy expenditure (Aponte et al., 2011; Atasoy et al., 2012; Atasoy et al., 2014; Cowley et al., 1999; Cowley et al., 2001). In the hypothalamus, AgRP-expressing neurons co-express NPY, and react to orexigenic and anorexigenic signals from the periphery to regulate food intake (Hahn et al., 1998). AgRP/NPY neurons also project to POMC neurons of the ARC, to neurons in the dorsomedial nucleus of the hypothalamus, and to other brain regions to control food intake (Bagnol et al., 1999; Broberger et al., 1998; Haskell-Luevano et al., 1999; Legradi & Lechan, 1999; Singru et al., 2007). Subsequently, a relationship involving feeding between melanocortin in the NTS and NPY in the PVN might be expected. However, there may be alternative explanations. For example, Balthasar et al. (2005) found that MC4R expression in Sim1 neurons that project to the NTS can counter the reduced feeding effects of MTII administration, thus suggesting that MC4R in the NTS could mediate the feeding effects of MTII or SHU9119 following injection into the NTS. Also, it is possible that some of the injectate might have migrated away from the targeted area of the NTS and brought about an effect as a result of diffusion into the 4th ventricle. So, the findings of the current study do not definitively demonstrate that two opposing systems are at work.

In the current study, injection of SHU9119 into the NTS significantly and dose dependently increased food intake, and injection of MTII into the NTS significantly and dose dependently decreased 24-h food intake. Injection of NPY into the PVN increased food intake, and injection of MTII into the NTS significantly and dose dependently decreased the food intake that was induced at 2, 4, and 6-h post-injection by NPY injection in the PVN. This suggests that there could be a direct or indirect functional relationship between the melanocortin system in the NTS and the NPY system in the PVN in relation to food intake.

Ethical statement

All procedures involving animals were conducted under UK Home Office License [Animals (Scientific Procedures) Act 1986] and were approved by the Institutional Animal Ethics Committee in accordance with the guidelines on laboratory experimentation involving the use of animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

None.

Data availability

Data will be made available on request.

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None.

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