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REVIEW

Emerging pharmacology and physiology of neuromedin U and the structurally related peptide neuromedin S

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Neuromedin U (NMU) has been paired with the G-protein-coupled receptors (GPRs) NMU_1 (formely designated as the orphan GPR66 or FM-3) and NMU_2 (FM-4 or hTGR-1). Recently, a structurally related peptide, neuromedin S (NMS), which shares an amidated C-terminal heptapeptide motif, has been identified in both rat and human, and has been proposed as a second ligand for these receptors. Messenger RNA encoding NMU receptor subtypes shows differential expression: NMU_1 is predominantly expressed in peripheral tissues, particularly the gastrointestinal tract, whereas NMU_2 is abundant within the brain and spinal cord. NMU peptide parallels receptor distribution with highest expression in the gastrointestinal tract and specific structures within the brain, reflecting its major role in the regulation of energy balance. The *NMU* knockout mouse has an obese phenotype and, in agreement, the Arg165Trp amino acid variant of NMU-25 in humans, which is functionally inactive, co-segregated with childhood-onset obesity. Emerging physiological roles for NMU include vasoconstriction mediated predominantly via NMU_1 with nociception and bone remodelling via NMU_2. The NMU system has also been implicated in the pathogenesis of septic shock and cancers including bladder carcinoma and acute myeloid leukaemia. Intriguingly, NMS is more potent at NMU_2 receptors *in vivo* where it has similar central actions in suppression of feeding and regulation of circadian rhythms to NMU. Taken together with its vascular actions, NMU may be a functional link between energy balance and the cardiovascular system and may provide a future target for therapies directed against the disorders that comprise metabolic syndrome.
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Keywords: energy homeostasis; neuromedin S; neuromedin U; NMU_1; NMU_2; smooth muscle contraction

Abbreviations: ACTH, adrenocorticotropic hormone; AML, acute myeloid leukaemia; Arc, arcuate nucleus; CNS, central nervous system; CRH, corticotropin-releasing hormone; DIO, diet-induced obese; DMH, dorsomedial hypothalamus; DR, diet-resistant; DRG, dorsal root ganglia; FSH, follicle-stimulating hormone; GDP, guanine diphosphate; GPCR, G-protein-coupled receptor; HPA, hypothalamo–pituitary–adrenal; IL, interleukin; LH, luteinizing hormone; LOS, lower oesophageal sphincter; LPS, lipopolysaccharide; NMS, neuromedin S; NMU, neuromedin U; NMU_1, neuromedin U receptor 1; NMU_2, neuromedin U receptor 2; NSCLC, non-small cell lung cancer; NTS, nucleus tractus solitarius; NTS1, neurotensin receptor 1; OVX, ovariectomized; pontine ret. form., pontine reticular formation; PP, pancreatic polypeptide; PTX, pertussis toxin; PVN, paraventricular nucleus; SCC, squamous cell carcinoma; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VIP, vasoactive intestinal polypeptide

Introduction

Neuromedin U (NMU), first isolated from porcine spinal cord and named for its potent contractile effect on rat uterus (Minamino *et al.*, 1985), exists in two major molecular forms: an extended 23 (NMU-23) or 25 (NMU-25) amino acid peptide, or truncated 8 (NMU-8) or 9 (NMU-9) amino acid

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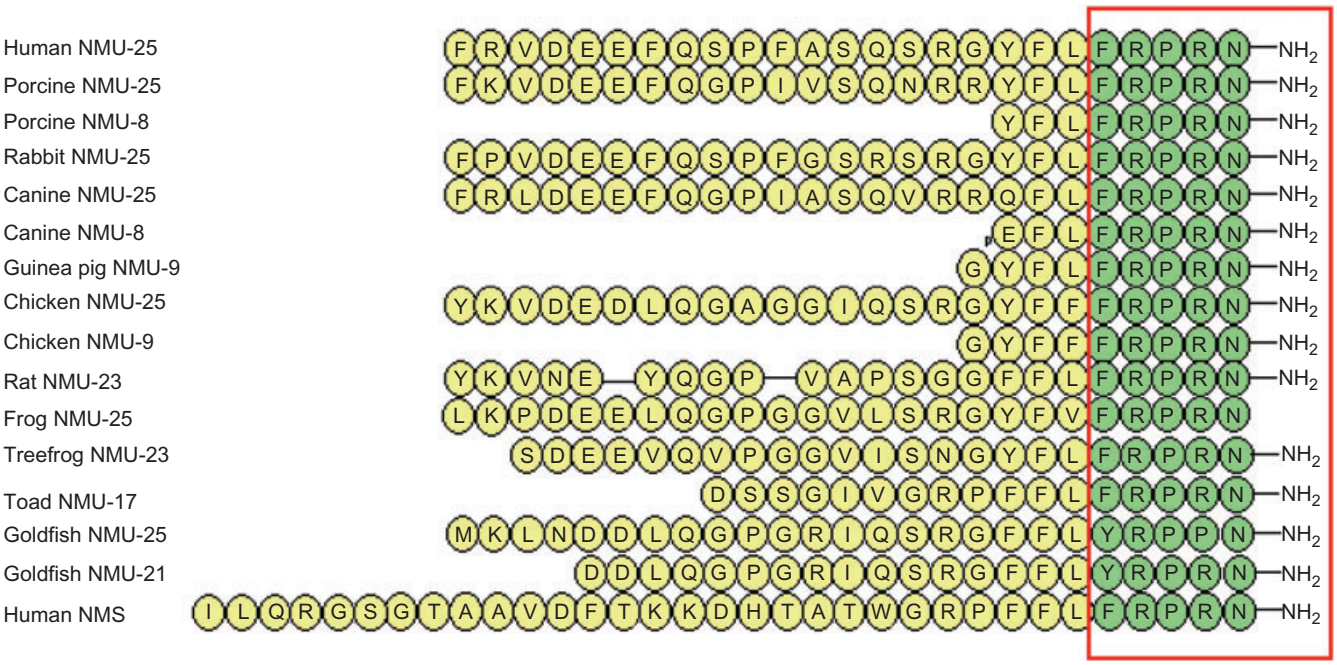

Figure 1 Amino acid sequences of neuromedin U (NMU) from mammalian (Minamino *et al.*, 1985; Minamino *et al.*, 1988; Murphy *et al.*, 1990; Kage *et al.*, 1991; O'Harte *et al.*, 1991a; Austin *et al.*, 1995), avian (O'Harte *et al.*, 1991b; Domin *et al.*, 1992), amphibian (Domin *et al.*, 1989; Salmon *et al.*, 2000; Lee *et al.*, 2005) and piscine (Maruyama *et al.*, 2008) species, and human neuromedin S (NMS). The red box, highlighting the C-terminal pentapeptide, shows conservation of this sequence in vertebrates, except goldfish. In mammalian species, the C-terminal heptapeptide is fully conserved. Amidation of the C-terminus of frog NMU-25 could not be confirmed by the study by Domin *et al.* (1989). NMU shares some structural features with pancreatic polypeptide (PP) and vasoactive intestinal polypeptide (VIP). Both NMU and PP have a C-terminal sequence of –Arg–Pro–Arg–X–$CONH_2$, whereas VIP is also amidated at the C-terminus. However, neither PP nor VIP have shown any binding or activity at NMU receptors (Hosoya *et al.*, 2000; Kojima *et al.*, 2000; Szekeres *et al.*, 2000).

C-terminal fragments. More recently NMU-17 has been isolated from the skin secretions of the Chinese red belly toad, *Bombina maxima* (Lee *et al.*, 2005), and splice variants NMU-21, NMU-25 and NMU-38 from goldfish brain (Maruyama *et al.*, 2008). NMU is therefore widely conserved throughout nature with almost absolute conservation of the amidated C-terminal pentapeptide (-Phe-Arg-Pro-Arg-Asn-NH_2; Figure 1), suggesting a 'strong evolutionary pressure' to retain this peptide (Brighton *et al.*, 2004a). This is further exemplified by the discovery of homologues in invertebrates, namely the pyrokinins (-FXPRXamide), Cap2b-like peptides (-FPRXamide) and ecdysis triggering hormones (-PRXamide); these are ligands for four receptors in *Drosophila*, which fall into the same clade as vertebrate NMU receptors (Park *et al.*, 2002). In particular, cockroach (*Periplaneta americana*) pyrokinin shares four out of five of the C-terminal pentapeptide amino acid residues with NMU (Melcher *et al.*, 2006). The focus of this review is the distribution and functions of NMU in vertebrates.

Structure-activity relationship of NMU peptides

Minimal active fragment and conservation of the C-terminus

NMU in most species has two major features: amidation of the C-terminus and a conserved C-terminal pentapeptide (Figure 1). Amidation is crucial for receptor activation; NMU-8 lacking an amide group failed to activate human and murine receptors at concentrations up to 10 $\mu mol \cdot L^{-1}$ (Hedrick *et al.*, 2000; Funes *et al.*, 2002), and had no effect on either rat uterine contractility or blood pressure (Minamino *et al.*, 1985; Sakura *et al.*, 1991) showed that residues 19–23 (FRPRN-NH_2) in rat NMU-23 retained biological activity in rat uterus but the smallest fragment with which it was possible to achieve maximum activity was residues 17–22 (FLFRPR-NH_2). Individual alanine or glycine substitution of each residue in NMU-8 resulted in reduced potency of this peptide in calcium flux (Funes *et al.*, 2002) and chicken crop smooth muscle assays (Hashimoto *et al.*, 1991), respectively; however, it became clear that Arg^7 was most important for the activity as responses were completely abolished when this residue was replaced.

Proteolytic processing of NMU

Several differences exist in the amino acid sequence of NMU between species. For instance, there is controversy surrounding whether the extended forms of NMU are intermediate precursors of the truncated forms. The dibasic cleavage site Arg^{16}–Arg^{17} is present in NMU-25 of both pig (Minamino *et al.*, 1985) and dog (O'Harte *et al.*, 1991a), preceding the sequence for NMU-8. O'Harte *et al.* (1991b) suggested that NMU-25 in chicken is a labile intermediate for NMU-9, implying Arg^{16}–Gly^{17} is also a cleavage site. Additionally, Murphy *et al.* (1990) observed a larger molecular weight NMU-like immunoreactivity (NMU-LI) than NMU-9, which has an N-terminal Gly residue, in guinea pig small intestine; although described as an extraction artefact, this could be an

extended form of NMU. Indeed, Domin *et al.* (1986) showed larger molecular weight NMU-LI in guinea pig ileum and spinal cord. In contrast, rabbit, human and frog NMU-25 possess the Arg^{16}–Gly^{17} site (Domin *et al.*, 1989; Kage *et al.*, 1991; Austin *et al.*, 1995), though only the extended form has been isolated in these species, whereas Domin *et al.* (1986) demonstrated NMU-25-like peptides predominate in pig and human. Species differences in proteolytic enzyme expression may account for these observations. In rat NMU-23, Arg^{16}–Gly^{17} is replaced by Gly^{14}–Gly^{15}, thought to be non-cleavable, indicating only the extended form is present, as confirmed by HPLC in rat brain and intestine (Domin *et al.*, 1987; Honzawa *et al.*, 1990).

N-terminus – species variation

Whereas the C-terminus is conserved between species, the N-terminus is more variable and is thought to be responsible for determining potency and duration of response to NMU. For example, rat NMU-23 is twice as potent as porcine NMU-25 (Minamino *et al.*, 1988), but three times as potent as toad NMU-17 (Lee *et al.*, 2005) in the rat uterus contraction assay. To date, the potencies of both porcine and toad NMU have not been assessed in tissue from their native species. Therefore, further studies are required to determine whether species differences in the peptide-receptor interaction also exist such that porcine and toad NMU have the same potency at their respective receptors. Nevertheless, porcine NMU-25 elicited a more potent contractile effect on rat uterus (Minamino *et al.*, 1985) and chicken crop (Okimura *et al.*, 1992) than NMU-8 and, furthermore, NMU-8 was less potent than NMU-23 at competing for [^{125}I]-NMU-23 binding from rat uterus membrane preparations (Nandha *et al.*, 1993), suggesting that the N-terminus also influences the affinity of NMU peptide-receptor interactions. Highlighting differences that can arise between tissue and cell-based assays, in the latter, porcine NMU-8 was equipotent to NMU-25 (Hosoya *et al.*, 2000; Szekeres *et al.*, 2000; Johnson *et al.*, 2004) and bound with comparable affinity (Hosoya *et al.*, 2000; Howard *et al.*, 2000; Raddatz *et al.*, 2000; Aiyar *et al.*, 2004) in functional and binding assays using human NMU receptors.

Some similarities within the N-terminus exist between species. Sakura *et al.* (1991) observed large increases in potency when the C-terminal octapeptide of rat NMU-23 was gradually extended, in particular positions 6–9 and 13–15 were important for enhancing activity. Positions 13–15 correspond to the tripeptide preceding the sequence for NMU-8; in the same way, the Asn^{15}–Arg^{16}–Arg^{17} tripeptide in porcine NMU-25 was shown to augment activity (Okimura *et al.*, 1992). Moreover, some N-terminal residues are conserved, particularly Glu^{5} (only absent from goldfish NMU-25), Gln^{8} and Pro^{10} (absent from chicken NMU-25).

Finally, canine NMU-8 contains a pyroglutamate residue at position 1; O'Harte *et al.* (1991a) demonstrated that substituting the tyrosine residue at position 1 for this entity in porcine NMU-8 resulted in a greater contractile effect on rat uterus. Similarly, substitution of Tyr^{1} with the D-form of this amino acid enhanced activity in chicken crop preparations (Hashimoto *et al.*, 1991; Sakura *et al.*, 1995) proceeded to show that this was a result of conferring resistance to aminopeptidases, reducing degradation of canine NMU-8 relative to its porcine counterpart.

NMU in humans

In humans, prepro-NMU (Figure 2) comprises 174 residues, including a 34 amino acid signal peptide, suggesting secretion of this peptide. The mature peptide, NMU-25, is located towards the C-terminus with a pair of basic residues on either side at which cleavage occurs. It has been proposed that further cleavage at sites located closer to the N-terminus results in generation of a 33 amino acid peptide, which is 95% homologous to its equivalent in rats (Austin *et al.*, 1995) and is shown to stimulate the release of prolactin (Mori *et al.*, 2005).

Peripheral distribution of NMU peptide

The distribution of NMU has been extensively investigated in human and rat (Table 1) but yet to be reported in other species. In the latter, highest levels of NMU-LI have been observed in small intestine (Domin *et al.*, 1987; Augood *et al.*, 1988; Honzawa *et al.*, 1990). In humans, all regions of the gastrointestinal tract (stomach through to rectum) had comparable levels of NMU-25 precursor mRNA expression, though NMU-LI was greatest in the jejunum (Austin *et al.*, 1995). Immunohistochemical studies have shown localization of NMU-LI to both cell bodies and nerve fibres of the myenteric and submucous plexuses within the enteric nervous system of rat small intestine (Augood *et al.*, 1988; Ballesta *et al.*, 1988; Honzawa *et al.*, 1990); interestingly, no NMU-LI was associated with the smooth muscle layers, though rapid turnover of peptide may result in levels below the limit of detection (Ballesta *et al.*, 1988). The densest area of immunoreactive nerve fibres was in the mucosa around the crypts and extending up the villi (Augood *et al.*, 1988; Ballesta *et al.*, 1988; Honzawa *et al.*, 1990).

Is NMU a locally acting or circulating peptide?

Domin *et al.* (1987) failed to detect NMU-LI in mucosal endocrine cells, which, together with low levels of NMU-LI in plasma (<0.5 pmol·L^{-1}), suggested NMU is a neuropeptide rather than circulating hormone. The localization of NMU-LI

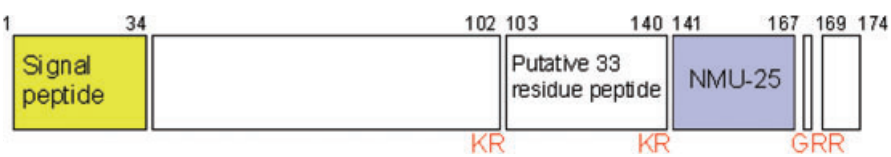

Figure 2 Schematic structure of prepro-neuromedin U (NMU) in humans. Numbers refer to residues and cleavage sites given in red.

Table 1 Distribution of neuromedin U (NMU)-like immunoreactivity (NMU-LI) and mRNA in rat and human

Species *Technique*	*Domin* et al. *(1987)*	*Fujii* et al. *(2000)*	*Szekeres* et al. *(2000)*
	Rat *RIA*	*Rat* *qRT-PCR*	*Human* *qRT-PCR*
Whole brain	+	+	+
Spinal cord	++	+	
Dorsal root ganglia	+++		
Oesophagus	+		
Stomach	+	+	++
Duodenum	+++	+++	
Jejunum	+++	+++	
Ileum	+++	++	+++
Colon	+++	++	
Rectum	+++	++	
Liver	–	–	–
Spleen		–	+
Pancreas	–	+	+
Heart		–	+
Lung		+	+
Trachea		+	
Kidney	–	–	+
Ureter	+		
Bladder	+	–	
Testis	+	+	
Epididymis	+		
Vas deferens	++		
Seminal vesicle	–	–	
Prostate	+		+
Penis	+		
Ovary	+	+	
Fallopian tube	+		
Uterus	+	+	
Urethra	+		
Vagina	+		
Pituitary	+++	+++	+++
Adrenal		+	
Thyroid		+	
Thymus		+	
Salivary gland		+	
Skeletal muscle		–	–
Adipose			++
Mammary gland		–	
Skin		–	
Bone		+	+
Bone marrow		+	+++
Costal cartilage		+	–
Lymphocytes			++
Macrophages			–

For Domin *et al.* (1987), + denotes <10 pmol·g^{-1} NMU-LI, ++ 10–50 pmol·g^{-1}, +++ >50 pmol·g^{-1}. Blank boxes indicate the tissue was not reported and – indicates below levels of detection. Highest levels of NMU-LI within rat brain were detected in nucleus accumbens, septum and hypothalamus. For Fujii *et al.* (2000), + denotes <2 copies $\times 10^{-3}$·ng^{-1} poly(A)$^+$RNA, ++ 2–4 and +++ >4. For Szekeres *et al.* (2000), + denotes <250 copies of gene's mRNA detected/ng mRNA pool, ++ 250–750 and +++ >750.
qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RIA, radioimmunoassay.

is largely the same in guinea pig small intestine, yet there are some subtle differences: NMU-LI was observed in nerve fibres around submucous arterioles, whereas in rat there was no consistent relationship between nerve fibres and blood vessels (Ballesta *et al.*, 1988), and a small population of NMU-LI positive endocrine cells in mucosal crypts were identified (Furness *et al.*, 1989), collectively suggesting that NMU could have access to the circulation. In man, low picomolar levels of NMU-25-LI were detected in plasma, suggesting NMU is a locally acting peptide rather than a circulating hormone (Mitchell *et al.*, 2009). Further sources of NMU include vascular endothelial cells, adipose tissue (Mitchell *et al.*, 2009) and keratinocytes (Moriyama *et al.*, 2005), consistent with emerging roles in vascular reactivity, energy balance and local inflammation.

Central distribution of NMU peptide

NMU-LI also has widespread distribution in the central nervous system (CNS) (Honzawa *et al.*, 1987; Ballesta *et al.*, 1988). NMU mRNA tends to be confined to the discrete regions of the brain; hence, levels in the whole brain are low. Relative to intestinal NMU mRNA expression, moderate levels were detected in the striatum, hypothalamus and medulla oblongata of rat (Fujii *et al.*, 2000), and the cingulate gyrus and medial frontal gyrus of human, with low to moderate levels in hypothalamus, locus coeruleus, thalamus, medulla oblongata and substantia nigra (Szekeres *et al.*, 2000). A high expression of NMU-LI was detected in the rat anterior pituitary gland by Domin *et al.* (1987), subsequently localized to corticotrophs (Ballesta *et al.*, 1988). In the rat spinal cord, NMU-LI levels were greater in the dorsal than ventral horn, implying a sensory role for NMU (Domin *et al.*, 1987).

NMU receptors – NMU_1 and NMU_2

NMU receptors have been designated NMU_1 and NMU_2, according to the Guide to Receptors and Channels (Alexander *et al.*, 2008), published by the *British Journal of Pharmacology*. Although high affinity, saturable and specific binding sites for [^{125}I]-NMU-23 in rat had previously been characterized (Nandha *et al.*, 1993), the molecular identity of the receptor was not known until NMU was reported by several groups to be a cognate ligand for the previously designated 'orphan' class A G-protein-coupled receptors (GPCR) NMU_1 (GPR66, FM-3; Fujii *et al.*, 2000; Hedrick *et al.*, 2000; Howard *et al.*, 2000; Kojima *et al.*, 2000; Raddatz *et al.*, 2000; Szekeres *et al.*, 2000) and NMU_2 (FM-4, TGR-1; Hosoya *et al.*, 2000; Howard *et al.*, 2000; Raddatz *et al.*, 2000; Shan *et al.*, 2000), the genes for which are located on human chromosomes 2 and 5 respectively. There are two distinct isoforms of NMU_2: a haplotype with four missense amino acid changes is found at a frequency of 15% (Bhattacharyya *et al.*, 2004); the functional significance, if any, is yet to be identified.

Nandha *et al.* (1993) had initially hinted that the NMU receptor would be coupled to a G-protein, as reduced specific binding was observed in the presence of the non-hydrolysable analogue of GTP, GTPγS. Five years later, NMU_1, then named FM-3, was cloned from a murine T-cell cDNA library and subsequently from a human P1-derived artificial chromosome library based on its homology with another GPCR, human ghrelin (previously growth hormone secretagogue) receptor (Tan *et al.*, 1998), with which it shares 33% protein sequence identity. In turn, NMU_2 was discovered because of its

sequence similarity with NMU_1 (Hosoya *et al.*, 2000; Howard *et al.*, 2000; Raddatz *et al.*, 2000; Shan *et al.*, 2000). NMU_1 and NMU_2, possibly the result of gene duplication (Shan *et al.*, 2000), share 51% protein sequence identity in human (Howard *et al.*, 2000). These receptors are 73 and 75% identical to rat NMU_1 and NMU_2, respectively (Howard *et al.*, 2000) and 79 and 81% identical to murine NMU receptors (Funes *et al.*, 2002). Much of the inter-receptor and inter-species variation arise from sequence differences in the N- and C-termini; in addition, the third intracellular loop in NMU_2 is considerably shorter than in NMU_1 (Shan *et al.*, 2000).

Using 'reverse pharmacology', these receptors were artificially expressed in cell lines and screened with libraries of ligands in conjunction with a functional assay, such as calcium mobilization or arachidonic acid release, resulting in the pairing of NMU with NMU_1 and NMU_2 (Table 2). Overwhelmingly, data suggest that NMU is equipotent at these receptors, at least in cell-based studies. Extensive radioligand binding studies have also been performed in cells expressing either receptor, demonstrating sub-nanomolar affinity (Table 2). Whether NMU receptor subtypes have similar binding and functional characteristics in native tissue is yet to be determined.

NMU receptor distribution – NMU_1 peripheral and NMU_2 central

To distinguish between the two NMU receptor subtypes, several studies have investigated their tissue distribution at the mRNA level (Table 3) in human (Hedrick *et al.*, 2000; Howard *et al.*, 2000; Raddatz *et al.*, 2000; Shan *et al.*, 2000; Szekeres *et al.*, 2000; Westfall *et al.*, 2002; Garlton *et al.*, 2004) and rat (Fujii *et al.*, 2000; Hosoya *et al.*, 2000; Guan *et al.*, 2001; Garlton *et al.*, 2004) using a range of techniques, including quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), *in situ* hybridization, Northern blot analysis and dot blot analysis.

The studies are largely in agreement: NMU_1 mRNA was more abundant in peripheral tissues, whereas NMU_2 mRNA was located more centrally. In addition, the distribution of receptors overlaps with that of NMU peptide (Table 1). At the protein level, Mangold *et al.* (2008) investigated the localization of NMU binding sites in rat brain; high densities were observed in the hypothalamus, as expected from mRNA studies, and hippocampal formation.

NMU receptor signalling

Calcium mobilization as a result of phospholipase C activation and subsequent production of inositol phosphates can occur through pertussis toxin (PTX)-insensitive $G_{q/11}$ protein and/or PTX-sensitive $G_{i/o}$ protein pathways. The overwhelming majority of studies favour the former for NMU receptors (Raddatz *et al.*, 2000; Shan *et al.*, 2000; Szekeres *et al.*, 2000; Funes *et al.*, 2002); however, Aiyar *et al.* (2004) showed that PTX caused partial inhibition of inositol phosphate accumulation in cells expressing either NMU_1 or NMU_2, though this effect was more pronounced in the latter. Lack of a PTX-sensitive component of calcium mobilization does not rule out receptor coupling to $G_{i/o}$ proteins as this pathway has other downstream effectors. Indeed, studies in cells have shown NMU receptor-mediated inhibition of forskolin-induced cAMP accumulation, prevented by pretreatment with PTX (Hosoya *et al.*, 2000; Aiyar *et al.*, 2004; Brighton *et al.*, 2004b) suggesting coupling to $G_{i/o}$ proteins, but have failed to demonstrate PTX-sensitive NMU receptor-mediated accumulation of inositol phosphates (Brighton *et al.*, 2004b). Brighton *et al.* (2004b) have further demonstrated coupling of NMU_1 and NMU_2 to both $G_{q/11}$ and G_i proteins using [^{35}S]-guanosine 5′-O-[gamma-thio]triphosphate binding and immuno-precipitation of Gα subunits, but were unable to directly compare levels of G protein activation. More recently, using chimeric G_s proteins, Hsu and Luo (2007) have demonstrated that NMU_1 primarily signals through $G_{q/11}$ proteins and NMU_2 through G_i proteins. Few studies have been performed using endogenous NMU receptors. In rat colonic smooth muscle cells, NMU receptors were shown to couple with both $G_{q/11}$ and G_i proteins (Brighton *et al.*, 2008). The NMU receptor subtype composition for rat colon is not clear; similar levels of mRNA encoding each subtype were detected by qRT-PCR (Fujii *et al.*, 2000; Hosoya *et al.*, 2000), but these do not necessarily reflect protein levels.

Agonist and antagonist studies

At present, no publications have reported the development of NMU receptor subtype-selective antagonists. However, using cell-based functional assays, natural products EUK2010, EUK2011 and EUK2012 have been described as NMU_2-specific agonists (Fang *et al.*, 2006), and icariin from *Herba epimedii* has been identified as an NMU_2-selective agonist (Zheng *et al.*, 2005), though its activity at NMU_2 was compared with the muscarinic 1 and melanocortin 4 receptors as opposed to NMU_1. Meanwhile, Meng *et al.* (2008) have discovered two synthetic low molecular weight non-selective NMU receptor agonists. Funes *et al.* (2002) showed that substitution of Arg^5 in NMU-8 with alanine resulted in a peptide with 15-fold greater potency at NMU_2 than at NMU_1. Although this alanine-substituted peptide had no antagonistic action, this information may be useful in the design of receptor subtype-specific compounds.

Functions of NMU

The focus of most research has been the role of NMU in smooth muscle contraction and regulation of feeding, reflecting the brain–gut distribution of this peptide. Like structure, function also seems to be well conserved throughout nature; pyrokinin-2, an NMU homologue encoded by the *Drosophila hugin* gene, has myostimulatory activity and has been implicated in the regulation of feeding (Melcher *et al.*, 2006). However, research into the NMU system is still in its infancy and subsequently functions continue to emerge, many of which appear disparate. This should be expected as both

Table 2 Binding and functional data from cells expressing NMU_1 or NMU_2

	Radioligand	*Cell type*	*Receptor*	*K_D nmol·L^{-1}*	*Assay*	*Ligand*	*EC_{50} nmol·L^{-1}*
Howard *et al.* (2000)	[^{125}I]-NMU-23 (rat)	HEK-293	$hNMU_1$	0.3	↑$[Ca^{2+}]_i$	NMU-25 (human)	1.0
						NMU-23 (rat)	0.75
						NMU-8 (pig)	0.11
						NMU-25 (pig)	1.36
			$hNMU_2$		↑$[Ca^{2+}]_i$	NMU-25 (human)	1.0
						NMU-23 (rat)	2.9
						NMU-8 (pig)	0.3
						NMU-25 (pig)	2.8
Fujii *et al.* (2000)	[^{125}I]-NMU-8 (pig)	CHO	$hNMU_1$	0.066	↑$[Ca^{2+}]_i$	NMU-23 (rat)	1.3
Hosoya *et al.* (2000)	[^{125}I]-NMU-8 (pig)	CHO	$hNMU_2$	0.022	↑AA release	NMU-25 (human)	4.0
						NMU-23 (rat)	
						NMU-25 (pig)	1.4–2.0
						NMU-8 (pig)	
Raddatz *et al.* (2000)	[^{125}I]-NMU-23 (rat)	COS-7	$hNMU_1$	0.61	↑$[Ca^{2+}]_i$	NMU-25 (human)	4.0
						NMU-23 (rat)	2.1
						NMU-25 (pig)	5.2
						NMU-8 (pig)	1.1
			$hNMU_2$	0.81		NMU-25 (human)	2.4
						NMU-23 (rat)	5.0
						NMU-25 (pig)	3.0
						NMU-8 (pig)	1.2
	[^{125}I]-NMU-8 (pig)		$hNMU_1$	1.2			
			$hNMU_2$	0.83			
Szekeres *et al.* (2000)		HEK-293	$hNMU_1$		↑$[Ca^{2+}]_i$	NMU-25 (pig)	0.38
						NMU-8 (pig)	0.21
						NMU-23 (rat)	0.17
					↑IP	NMU-25 (pig)	0.28
Shan *et al.* (2000)		HEK-293	$hNMU_2$		↑$[Ca^{2+}]_i$	NMU-25 (human)	5
Hedrick *et al.* (2000)		HEK-293	$hNMU_1$		↑$[Ca^{2+}]_i$	NMU-25 (human)	12
						NMU-8 (pig)	10
Kojima *et al.* (2000)		CHO	$hNMU_1$		↑$[Ca^{2+}]_i$	NMU-25 (human)	0.25
						NMU-23 (rat)	7.0
						NMU-25 (pig)	0.8
						NMU-8 (pig)	2.8
Funes *et al.* (2002)		HEK-293	$mNMU_1$		↑$[Ca^{2+}]_i$	NMU-25 (human)	20
						NMU-23 (mouse)	8.8
						NMU-8 (pig)	9.5
			$mNMU_2$			NMU-25 (human)	2.2
						NMU-23 (mouse)	1.6
						NMU-8 (pig)	3
Brighton *et al.* (2004b)	[^{125}I]-NMU-25 (human)	HEK-293	$hNMU_1$	0.14	↑$[Ca^{2+}]_i$	NMU-25 (human)	0.72
					↑IP		0.39
					↓cAMP		0.08
			$hNMU_2$	0.11	↑$[Ca^{2+}]_i$		1.07
					↑IP		0.43
					↓cAMP		0.09
Johnson *et al.* (2004)	[^{125}I]-NMU-25 (human)	T-cell clone	$mNMU_1$	0.36	↑$[Ca^{2+}]_i$	NMU-25 (human)	4.8
						NMU-23 (rat)	10.1
						NMU-25 (pig)	6.0
						NMU-8 (pig)	4.8
Aiyar *et al.* (2004)	[^{125}I]-NMU-25 (human)	HEK-293	$hNMU_1$	0.08	↑IP	NMU-25 (human)	1.6
			$hNMU_2$	0.16			1.5
			$hNMU_1$		↑$[Ca^{2+}]_i$		0.50
			$hNMU_2$				0.50
Garlton *et al.* (2004)		HEK-293	$hNMU_1$		↑$[Ca^{2+}]_i$	NMU-23 (rat)	1.25
			$hNMU_2$				1.10
Shukla *et al.* (2007)	[^{125}I]-NMU-23 (rat)	Yeast	$hNMU_2$	0.79			
		BHK		0.96			
Xia *et al.* (2008)	[^{125}I]-NMU-23 (rat)	BHK	$hNMU_1$	0.14			

'h' in front of NMU_1 or NMU_2 denotes human and 'm' murine.
AA, arachidonic acid; BHK, baby hamster kidney; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; HEK, human embryonic kidney; IP, inositol phosphates; NMU, neuromedin U.

NMU peptide and receptors have widespread dissemination in addition to their major brain–gut axis of expression. It has also been suggested that NMU may be involved in a number of pathophysiological processes, particularly in the fields of oncology and inflammation.

Smooth muscle contraction

NMU has been shown to directly contract smooth muscle of the gastrointestinal and genitourinary systems in a range of species (Table 4). However, in mouse colon (Dass *et al.*, 2007), and mouse and rat vas deferens (Prendergast *et al.*, 2006),

Table 3 Tissue distribution of mRNA for NMU1 and NMU2 in human (Raddatz *et al.*, 2000) and rat (Fujii *et al.*, 2000; Hosoya *et al.*, 2000)

	Human		*Rat*	
	NMU_1	*NMU_2*	*NMU_1*	*NMU_2*
Amygdala	+	+		
Cerebellum	++	+	+	+
Cortex	+	++	+	+
Hippocampus	+	+++	+	+
Hypothalamus	+	++	+	+++
Medulla oblongata	+	+++	+	+
Pontine ret. form.	+	+++		
Striatum			+	+
Thalamus	+	+++	+	+
Spinal cord	+	+++	+	++
DRG	+	+		
Adrenal	++	+	–	–
Pituitary	+	+	–	
Thyroid			+	–
Thymus			+	+
Skeletal muscle	+	+	–	–
Adipose			+	+
Salivary gland	+	–	–	+
Spleen	+	–	+	–
Heart	+	+	–	–
Lung	++	++	+++	+
Trachea	++	+	+	–
Liver	–	–	–	–
Pancreas	++	–	+	–
Kidney	++	+	+	–
Small intestine	+++	+	+++	+
Large intestine			+	+
Stomach	++	+	+	+
Mammary gland	+	+	+	–
Bladder			+	+
Prostate	++	+		
Seminal vesicle			–	–
Testis	+++	+++	–	+
Ovary			–	++
Uterus	++	+	+	+++
Skin			–	–
Bone			+	–
Bone marrow			–	–
Costal cartilage			+	+

For the human study, + denotes <100 copies/ng cDNA, ++ 100–300, +++ >300 and – below level of detection. For the rat study, + denotes <1 copies $\times 10^{-3}$/ng poly(A)$^+$RNA, ++ 1–2 and +++ >2. Blank boxes indicate tissue levels not reported.
DRG, dorsal root ganglia; pontine ret. form., pontine reticular formation.

NMU was only effective in potentiating electrically induced contractions, indicating an indirect neuronally-mediated action. Such actions suggest a role for NMU in the regulation of motility within these systems; indeed, NMU has been shown to elicit a prokinetic effect in mouse colon, reducing the interval between successive peristaltic waves (Dass *et al.*, 2007). Therefore, it can be speculated that the NMU system may represent a future therapeutic target for the treatment of intestinal motility disorders. Firstly, it is necessary to determine whether the role of NMU in the animal model reflects that observed in humans. NMU has been shown to cause direct contraction of human ascending colon (Jones *et al.*, 2006), though it is not reported whether NMU could also potentiate electrically induced contractions. In fact, a number of species differences relating to the contractile activity of NMU exist. For example, NMU contracted human and canine urinary bladder smooth muscle but was without effect in bladder preparations from mouse, rat, guinea pig, rabbit and ferret (Westfall *et al.*, 2002), even though NMU_1 and NMU_2 mRNA have been detected in this tissue in rat (Table 3).

Table 4 Summary of studies investigating direct contractile effect of NMU on smooth muscle preparations

Species	*Smooth muscle preparation*	
Rat	Uterus	Minamino *et al.* (1985)
	Fundus of stomach	Benito-Orfila *et al.* (1991)
	LOS	Prendergast *et al.* (2006)
	Ileum	
	Colon	Brighton *et al.* (2008)
Human	Ileum	Maggi *et al.* (1990)
	Urinary bladder	
	Gall bladder	Jones *et al.* (2006)
	Colon (ascending)	
Dog	Urinary bladder	Westfall *et al.* (2002)
	Stomach	
	Ileum	
	Colon	
Mouse	LOS	Prendergast *et al.* (2006)
	Gall bladder	
	Uterus	
	Fundus of stomach	
Guinea pig	Uterus	Prendergast *et al.* (2006)
Turtle	Small intestine	Bockman *et al.* (1989)

LOS, lower oesophageal sphincter.

Secondly, studies using NMU receptor knock-out mice have sought to determine the NMU receptor subtype responsible for smooth muscle contractile activity. Dass *et al.* (2007) demonstrated that responses to NMU in stomach and electrically-stimulated colon were similar in $NMU_2^{-/-}$ and wild-type mice, implying NMU acts through NMU_1 in these tissues. Prendergast *et al.* (2006) reported loss of responses to NMU in stomach and gall bladder of $NMU_1^{-/-}$ mice, indicating contraction was mediated by NMU_1; in contrast, responses in uterus and vas deferens were unchanged in these mice, suggesting a role for NMU_2. Alternatively, NMU_1 may be able to compensate for a loss of NMU_2 and vice versa in some tissues; precise delineation requires receptor subtype-selective antagonists.

Central energy homeostasis

Evidence from intracerebroventricular (i.c.v.) NMU administration to rats, NMU knock-out mice and mutations in man. Intracerebroventrial administration of NMU produced a reduction in food intake and body weight in both *ad libitum*-fed and fasted rats (Howard *et al.*, 2000; Kojima *et al.*, 2000; Nakazato *et al.*, 2000; Niimi *et al.*, 2001; Ivanov *et al.*, 2002; Wren *et al.*, 2002; Hanada *et al.*, 2003). In support, i.c.v. anti-NMU antisera resulted in increased feeding in rats (Kojima *et al.*, 2000; Jethwa *et al.*, 2005) whereas i.c.v. NMU also decreased food intake in Japanese quail (Shousha *et al.*, 2005), chicks (Kamisoyama *et al.*, 2007) and goldfish (Maruyama *et al.*, 2008). Furthermore, *NMU* knock-out mice were hyperphagic, resulting in an obese phenotype (Hanada *et al.*, 2004) and, as expected, i.c.v. NMU reduced their fat mass (Sato *et al.*, 2007). Transgenic mice, overexpressing the *NMU* gene, were leaner than wild types, even when fed a high-fat diet (Kowalski *et al.*, 2005). All of these studies implicate NMU in the regulation of

food intake and are consistent with its hypothalamic expression.

Consistent with this hypothesis, in humans, the Ala19Glu polymorphism correlated with an overweight or obese phenotype in middle-aged Caucasians; this amino acid change is located in the signal peptide of pre-pro-NMU and is believed to reduce export (Hainerova *et al.*, 2006). Rarer is the Arg165Trp mutation, discovered in a Czech family and associated with hypertriglyceridaemia and childhood-onset obesity (Hainerova *et al.*, 2006); the mutation is situated within the C-terminal pentapeptide of mature NMU and, from structure-activity relationship data, is expected to abolish any functional responses (Funes *et al.*, 2002). Indeed, unlike NMU-25, the Arg165Trp amino acid variant of NMU-25 was without vasoconstrictor effect in saphenous vein (Mitchell *et al.*, 2008). As well as reducing energy intake, i.c.v. NMU has also been shown to amplify energy expenditure in rats, producing increases in locomotion, core body temperature and oxygen consumption (Howard *et al.*, 2000; Nakazato *et al.*, 2000; Hanada *et al.*, 2001; Ivanov *et al.*, 2002; Wren *et al.*, 2002; Garlton *et al.*, 2004).

Upon i.c.v. administration of NMU to rats, increased c-Fos-LI, a marker for cellular activation, was observed in the paraventricular (PVN), arcuate (Arc) and supraoptic (SON) nuclei of the hypothalamus, the dorsomedial hypothalamus (DMH), the lateral hypothalamic area, the amygdala and the parabrachial nucleus, nucleus tractus solitarius (NTS) and the ventrolateral medulla of the brainstem (Niimi *et al.*, 2001; Ivanov *et al.*, 2002; Ozaki *et al.*, 2002). However, the prime candidate for the location at which NMU exerts the aforementioned effects is the PVN as direct microinjection of NMU into this area in rats resulted in reduced food intake and increased physical activity (Wren *et al.*, 2002; Novak *et al.*, 2006). NMU most likely acts through NMU_2, the gene for which is expressed in the PVN of both rats and mice (Howard *et al.*, 2000; Guan *et al.*, 2001; Graham *et al.*, 2003). Furthermore, the specific NMU_2 agonist EUK2010, a natural compound, was shown to reduce body weight of rats and mice (Fang *et al.*, 2006). However, NMU_2 knockout mice were not obese and had a similar level of motor activity relative to wild types, suggesting NMU was operating through NMU_1, even though its central expression is low, or an unidentified receptor (Zeng *et al.*, 2006). Nevertheless, these mice were refractory to i.c.v. NMU-induced reductions in food intake (Zeng *et al.*, 2006), demonstrating the importance of NMU_2 in the central regulation of feeding. Reduced food intake and increased physical activity were also observed after intra-Arc administration of NMU to rats (Wren *et al.*, 2002; Novak *et al.*, 2006); only recently have NMU receptors been localized to this area of rat brain (Graham *et al.*, 2003; Mangold *et al.*, 2008). In mice, NMU_2 mRNA was also abundant in cells located on the periphery of the ventromedial hypothalamus (Graham *et al.*, 2003), an area associated with satiety (Hoebel, 1965).

The source of NMU involved in control of energy balance is uncertain. Howard *et al.* (2000) showed a reduction in NMU mRNA in the Arc of fasted rats. In accordance, Ballesta *et al.* (1988) and Graham *et al.* (2003) demonstrated the presence of NMU-LI and NMU mRNA respectively in this structure in rat. Conflictingly, neither Honzawa *et al.* (1987) using immunohistochemistry nor Ivanov *et al.* (2002) using *in situ* hybridization could detect NMU in cell bodies of rat Arc. Other potential sources are the NTS of the brainstem and pars tuberalis of the pituitary. Ivanov *et al.* (2004) located neurones expressing the gene for NMU in rat NTS and subsequently showed that peripheral administration of cholecystokinin, a satiety hormone, increased c-*fos* expression in approximately one-third of these neurones; furthermore, it is known that a population of neurones project from the NTS to the PVN (Doyle *et al.*, 2004). NMU mRNA was detected in rat pars tuberalis (Ivanov *et al.*, 2002; Graham *et al.*, 2003), but levels increased upon fasting and decreased after central administration of leptin (Nogueiras *et al.*, 2006), which is not in accordance with an anorexigenic action for this source of NMU. In mice, NMU mRNA was most abundantly expressed in DMH and ventromedial hypothalamus; levels in the DMH were augmented in food-deprived mice compared with their *ad libitum*-fed littermates (Graham *et al.*, 2003), again at odds with the anorectic effect of NMU. In addition, a peripheral source should not be discounted, though it is yet to be determined whether NMU can cross the blood–brain barrier. It was previously believed that peptides were unable to penetrate this structure; however, it has been shown that peptides, such as vasoactive intestinal polypeptide, can cross by passive diffusion (Dogrukol-Ak *et al.*, 2004), whereas others have saturable transporter systems, for example ghrelin (Banks *et al.*, 2002). Nevertheless, current evidence suggests NMU is a locally acting rather than circulating peptide (Domin *et al.*, 1987; Mitchell *et al.*, 2008).

Interactions with corticotropin-releasing hormone (CRH) and leptin systems. Transmitter systems interact to form complicated regulatory networks. It is therefore unlikely that the NMU system operates in isolation. An understanding of such interactions allows prediction of the potential wider consequences of disrupting the NMU system should it be considered as a future therapeutic target. CRH has been shown to reduce food intake (Morley and Levine, 1982) and was released from rat hypothalamic explants upon exposure to NMU (Wren *et al.*, 2002). After central administration of NMU, c-Fos-LI was upregulated in neurones immunoreactive for CRH and CRH mRNA was increased in rat PVN (Hanada *et al.*, 2004; Yokota *et al.*, 2004). In addition, reduced expression of CRH mRNA was observed in the PVN of *NMU* knock-out mice (Hanada *et al.*, 2004) and the NMU-induced decrease in food intake and increases in locomotion, core body temperature and oxygen consumption were abolished in CRH knock-out mice (Hanada *et al.*, 2001; 2003). The reduced anorexigenic action of CRH observed with chronic central administration (Krahn *et al.*, 1990) even mirrors the effect of chronic delivery of intra-PVN NMU to rats (Thompson *et al.*, 2004). However, a study by Kowalski *et al.* (2005) casts doubt on the role of CRH in NMU-regulated feeding behaviour: mice overexpressing the NMU gene in the PVN had similar levels of hypothalamic CRH mRNA to wild types.

Several studies have investigated the relationship between NMU and the well-characterized adipostatic factor leptin (Campfield *et al.*, 1995). Leptin stimulated the release of NMU from rat hypothalamic explants (Wren *et al.*, 2002), and i.c.v. administration of anti-NMU antisera to rats reduced the

satiety effect of i.p. leptin (Jethwa *et al.*, 2005). These findings suggest NMU acts downstream of leptin. In contrast, NMU resulted in weight loss in leptin-deficient (*ob/ob*) and leptin receptor-deficient (*db/db*) mice and leptin receptor-deficient (Zucker fatty) rats equivalent to that observed in controls (Hanada *et al.*, 2004), implying leptin does not operate downstream of NMU. However, not all studies support a role for NMU in leptin-mediated inhibition of feeding (Hanada *et al.*, 2004): *NMU* knockout mice lost the same amount of weight as control mice when leptin was administered and, in rat Arc, i.c.v. leptin neither affected NMU mRNA expression nor induced c-Fos expression in NMU immunoreactive neurones. However, the major source of NMU involved in maintenance of energy homeostasis still remains unclear; such studies are required in other central structures implicated in NMU-mediated control of energy balance, e.g. NTS.

The NMU system: a novel target for anti-obesity therapy? Findings indicate that NMU has an important role in maintaining energy balance in a number of species and, therefore, suggest that NMU may be an attractive target in the fight against obesity. However, two problems can be foreseen from recent studies. Chronic administration of this peptide was without effect on the food intake of *ad libitum*-fed rats (Thompson *et al.*, 2004). This could be a result of receptor down-regulation or up-regulation of peptide degradative pathways, although effects of chronic NMU on the hypothalamo–pituitary–adrenal (HPA) axis did not differ from that when NMU is acutely administered. Most studies have shown that NMU reduces feeding in *ad libitum*-fed rats; in contrast, Ivanov *et al.* (2002) found no effect on food intake in satiated compared with fasted rats. It is suggested that the NMU system is already operating at maximum activity in the former and, therefore, exogenous NMU has no effect (Thompson *et al.*, 2004). A further explanation reflects the observation that rats did not experience a normal growth rate during the study, masking the effects of NMU, if any, on body weight (Thompson *et al.*, 2004).

The second problem is that 'central sensitivity' to NMU is reduced in obese animals: before a high-fat diet, diet-induced obese (DIO) and diet-resistant (DR) rats had similar levels of increased physical activity post-NMU administration; after 1 month, NMU produced greater physical activity in DR rats than DIO rats (Novak *et al.*, 2007). As NMU has an anorexigenic action, such a reduced functional response may contribute to the development of obesity and/or exacerbate an existing obese phenotype. A mechanism for this phenomenon is yet to be established. Although NMU receptor expression within the PVN was not investigated, receptor down-regulation or desensitization are unlikely as no difference in NMU-LI within the PVN of DR and DIO rats was detected (Novak *et al.*, 2007). Instead, reduced sensitivity to NMU may be secondary to a decrease in expression of downstream effectors. For example, in DIO relative to DR rats, lower levels of CRH mRNA were demonstrated within the brain (Michel *et al.*, 2004).

Peripheral energy homeostasis

NMU may also have peripheral actions controlling energy balance. Messenger RNA for NMU_1 has been observed in rat pancreas (Fujii *et al.*, 2000) and, more recently, this receptor has been detected at the protein level in the pancreatic islets (Kaczmarek *et al.*, 2006). Application of NMU to isolated pancreatic islets resulted in an inhibition of insulin secretion (Kaczmarek *et al.*, 2006); a reduction of this anabolic hormone is in accordance with the anorexigenic action of centrally administered NMU.

Ion transport within the gut and gastric emptying

In addition to contracting smooth muscle in the gastrointestinal system, NMU also increased electrogenic ion transport in porcine jejunum, the physiological relevance of which is unknown (Brown and Quito, 1988), and reduced gastric acid secretion and gastric emptying upon central administration (Mondal *et al.*, 2003) in keeping with its anorexigenic action. Control of gastric acid secretion was dependent on CRH and the sympathetic nervous system, as both anti-CRH antisera and yohimbine, an α_2-adrenergic receptor antagonist, prevented the inhibitory action of NMU (Mondal *et al.*, 2003).

Cardiovascular actions of NMU

Initial studies demonstrated that NMU elicited a rapid and sustained increase in systemic blood pressure in anaesthetized rats upon intravenous (i.v.) administration (Minamino *et al.*, 1985). Further studies in conscious rats (Gardiner *et al.*, 1990; Chu *et al.*, 2002) and anaesthetized dogs (Sumi *et al.*, 1987; Westfall *et al.*, 2002) supported a pressor effect for i.v. NMU but found it to be smaller and transient. More detailed experiments in chronically-instrumented rats showed NMU at lower doses could reduce superior mesenteric blood flow without changes in systemic blood pressure and renal and hindquarter blood flows, suggesting local vasoconstriction (Gardiner *et al.*, 1990). Similarly, in anaesthetized dogs, i.v. NMU decreased blood flow in the superior mesenteric artery and portal vein but had no effect on blood flow in the axillary artery (Sumi *et al.*, 1987). These results suggest NMU is an important regulator of intestinal blood flow. However, until recently, little was known about the direct effect of NMU on blood vessels. Using radioligand binding assays, [^{125}I]-NMU-25 binding sites were detected in human heart and coronary artery (dissociation constant, $K_D = 0.26$ nmol·L^{-1}). Messenger RNA encoding NMU_1 predominated in these tissues and NMU_1-LI was subsequently localized to the medial smooth muscle layer of both intra-myocardial and large conduit blood vessels (Mitchell *et al.*, 2009). Consistent with this localization and the contractile activity of NMU in other hollow organs, constrictor responses to NMU-25 have been characterized in human isolated coronary and mammary artery and saphenous vein, similar in potency and maximum contractile response to angiotensin II (Mitchell *et al.*, 2009). Human *in vivo* vascular studies in healthy volunteers and appropriate patient groups are required to determine whether the NMU system is worth pursuing as a target for the treatment of vascular diseases such as hypertension. It could be speculated that NMU released from adipose tissue could contribute to an elevated blood pressure in obesity. Meanwhile, the effect of i.v. NMU on heart rate is ambiguous. Although rat NMU-23 produced an increase in heart rate in the chronically instrumented rat model (Gardiner *et al.*, 1990), although whether this is a direct or indirect effect remains uncertain, Chu *et al.* (2002)

observed no effect on heart rate post-administration of NMU.

NMU has also been shown to exert a central influence over cardiovascular function. In both chronically-instrumented conscious and anaesthetized rats, i.c.v. administration of NMU resulted in increased systemic blood pressure and heart rate (Chu *et al.*, 2002). Furthermore, the increase in blood pressure detected in mice when the temperature of their environment is increased is not observed in *NMU* knockout mice (Nakahara *et al.*, 2004a). In contrast, microinjection of NMU into rat NTS, involved in integration of autonomic control of the cardiovascular system, produced a decrease in systemic blood pressure and heart rate (Tsubota *et al.*, 2003). Thus the central actions of NMU on the cardiovascular system are unclear at present and warrant further investigation.

Stress response

Evidence supports roles for NMU in both the central and peripheral control of the stress response. i.c.v. Administration of NMU brought about stress-related behaviour in rats such as increased grooming (Garlton *et al.*, 2004; Kojima *et al.*, 2000; Hanada *et al.*, 2001; Wren *et al.*, 2002; Zeng *et al.*, 2006), dampened by i.c.v. administration of anti-CRH antisera and the CRH receptor antagonist, α-helical CRH (Hanada *et al.*, 2001). Furthermore, intra-PVN administration of NMU to rats produced increases in plasma levels of the stress-related hormones, adrenocorticotropic hormone (ACTH) and corticosterone (Thompson *et al.*, 2004; Wren *et al.*, 2002); expectedly, low plasma corticosterone levels were present in *NMU* knockout mice (Hanada *et al.*, 2004), even when subjected to immobilization stress (Nakahara *et al.*, 2004a). Leptin has also been described as a 'stress-related peptide' (Bornstein, 1997); anti-NMU antisera reduced the release of CRH from rat hypothalamic explants exposed to leptin and, when administered centrally, lowered leptin-induced increases in plasma ACTH and corticosterone (Jethwa *et al.*, 2006). In summary, findings indicate that NMU signals through a CRH pathway in the PVN, resulting in the activation of the HPA axis, and may be a route through which leptin exerts its 'stress-related' actions. Recently, central, but not peripheral, administration of NMU has also been shown to increase secretion of the catecholamine adrenaline, which has a major role in the acute stress response, from the adrenal medulla in rat (Sasaki *et al.*, 2008).

Peripheral administration of NMU to rats resulted in increased plasma levels of ACTH and corticosterone (Malendowicz *et al.*, 1993). A direct action for NMU on the rat adrenal gland was investigated *in vitro*. Prerequisites for NMU-induced secretion of adrenal steroids were medullary chromaffin cells and the intra-medullary CRH/ACTH system; NMU had no effect on adrenal explants that lacked medullary chromaffin cells whereas exposure to both α-helical CRH and corticotropin-inhibiting peptide, antagonists of the CRH and ACTH receptors, respectively, reduced steroid secretion from cortical cells (Malendowicz *et al.*, 1994a). Intriguingly, the action of NMU on the HPA axis appears to be biphasic: low doses yielded hypertrophy of the zona fasciculata with subsequently amplified plasma levels of corticosterone and increased secretion of this steroid from adrenal explants; conversely, high doses produced hypertrophy of pituitary corticotrophs and increased plasma levels of ACTH together with a reduction in adrenal weight and no effect on corticosterone secretion (Malendowicz *et al.*, 1994b). The receptor subtype NMU_1 is most likely responsible for the direct adrenal actions of NMU. Although Fujii *et al.* (2000) found no evidence of mRNA for NMU_1 in rat adrenal gland, several studies have since detected NMU_1 in cortex and medulla at both mRNA and protein levels with no indication of NMU_2 expression (Rucinski *et al.*, 2007; Trejter *et al.*, 2008; Ziolkowska *et al.*, 2008).

Reproductive actions of NMU

Studies investigating the reproductive actions of NMU are yet to achieve clarity. In particular, it is difficult to relate the effects of NMU on ovariectomized (OVX) rats, in which sex steroids are absent, to the physiological scenario. In both pubertal and adult rats, i.c.v. NMU produced increases in serum luteinizing hormone (LH) (Vigo *et al.*, 2007a). In contrast, i.c.v. NMU reduced LH secretion in OVX rats and counteracted the increase in LH in pubertal rats receiving the pro-puberty hormone, kisspeptin-10 (KP-10) (Quan *et al.*, 2003; Vigo *et al.*, 2007a). In both OVX and KP-10-treated rats, LH levels are raised; this poses the question whether NMU has a bimodal action on LH release, suppressing secretion when LH levels are high but otherwise promoting secretion. However, in *NMU* knock-out mice, vaginal opening occurred earlier than in wild-type mice and, likewise, the LH/follicle-stimulating hormone (FSH) ratio increased at an earlier time point (Fukue *et al.*, 2006). Furthermore, NMU suppressed LH release from rat anterior pituitary cells (Fukue *et al.*, 2006). Both vaginal opening and the LH/FSH ratio are indicators for the onset of puberty and, therefore, this study suggests NMU delays puberty by suppressing gonadotropin secretion.

Factors governing the reproductive function of NMU include sex steroids and energy balance. Hypothalamic NMU mRNA levels were observed to fluctuate during post-natal maturation and the oestrous cycle, with high expression in pubertal (30 days) rats and prior to the proestrous LH surge in cycling adults, decreasing on approach to oestrus (Vigo *et al.*, 2007a). After ovariectomy, hypothalamic expression of NMU mRNA was reduced in rat; however, this effect was not observed in animals implanted with an oestrdiol and progesterone releasing device (Vigo *et al.*, 2007a). Changes in sex steroid levels may therefore be responsible for the increased NMU mRNA expression observed during puberty and the oestrous cycle. Pituitary NMU mRNA expression has also been shown to be greater during puberty in rat, decreasing towards maturity. In contrast to the hypothalamic expression of NMU mRNA, in cultured pituitary cells, such expression was reduced by oestradiol treatment. Therefore, the increase in oestradiol levels seen at puberty may inhibit NMU expression as maturity approaches and consequently prevent any suppressive effect of NMU on LH secretion (Shimizu *et al.*, 2008). Peripherally, although NMU receptor density did not alter during the oestrous cycle in rat uterus, a 60% decrease in receptor density post-ovariectomy was observed, which was rectified by treatment with oestradiol (Nandha *et al.*, 1999).

A 48 hour fasting period was shown to augment the inhibition of i.c.v. NMU on LH secretion in OVX rats (Quan *et al.*, 2003), an effect reduced by astressin, a CRH receptor antagonist, suggesting CRH, at least in part, mediates this synergistic inhibitory effect of NMU and fasting on pulsatile LH secretion (Quan *et al.*, 2004). It can be speculated that this effect may contribute to prevention of ovulation in animals whose body condition may not be suitable for pregnancy.

NMU and nociception

Domin *et al.* (1987) detected greater levels of NMU-LI in the dorsal horn of rat spinal cord than the ventral horn, suggesting a sensory function for NMU. Indeed, both electrophysiological and behavioural studies support a role for NMU in nociception. NMU increased excitability of neurones in the dorsal horn of the spinal cord *in vitro* and *in vivo* whereas animals receiving NMU intrathecally experienced thermal hyperalgesia, mechanical allodynia and greater nociceptive flexor reflexes in response to touch or pinch stimuli. In addition, a behavioural response comprising scratching, biting or licking of lower body parts was observed (Cao *et al.*, 2003; Yu *et al.*, 2003; Moriyama *et al.*, 2004; Nakahara *et al.*, 2004a). As further evidence, *NMU* knock-out mice had reduced nociceptive reflexes and increased expression of NMU mRNA in the spinal cord after injecting mice with formalin was detected (Nakahara *et al.*, 2004a).

By autoradiography, [^{125}I]-NMU-23 binding sites were localized to laminae I and II of the spinal cord in rat; more specifically, molecular studies indicated NMU_2 mRNA was present in lamina I and the outer part of lamina II of the dorsal horn (Yu *et al.*, 2003), the site of most nociceptive neurones (Light and Willcockson, 1999). NMU_1 mRNA was detected in small to medium diameter neurones of the dorsal root ganglia but not spinal cord (Yu *et al.*, 2003), even though Fujii *et al.* (2000) had previously reported its presence in this tissue. However, NMU_2 does seem the most likely candidate through which NMU exerts its nociceptive action. NMU_2 knock-out mice had reduced sensitivity to hot plate, capsaicin and formalin tests, and both NMU-induced enhanced nociceptive responses to formalin administration and increased frequency of excitatory post-synaptic currents in lamina II neurones were absent (Zeng *et al.*, 2006; Torres *et al.*, 2007). NMU_1 knock-out mice had similar sensitivity to nociceptive stimuli as wild types (Torres *et al.*, 2007), implying this receptor subtype is not involved.

NMU and the circadian 'clock'

The presence of NMU, NMU_1 and NMU_2 mRNA within the suprachiasmatic nucleus (SCN) (Nakahara *et al.*, 2004b), the location of the circadian 'clock,' and increased c-Fos-LI in the SCN when i.c.v. NMU was administered to rats (Nakahara *et al.*, 2004b) suggest a role for this peptide in regulation of circadian rhythms. Indeed, i.c.v. NMU produced a phase shift of circadian locomotor activity in rats and resulted in increased expression of mRNA for period homologue 1, a 'circadian regulator' (Albrecht *et al.*, 1997), in the SCN (Nakahara *et al.*, 2004b). In addition, re-entrainment in *NMU* knock-out mice took longer when the light–dark cycle was shifted than in wild-types (Nakahara *et al.*, 2004a). Expression of NMU itself demonstrated a circadian rhythm: mRNA content oscillated within the SCN, peaking during the light phase or subjective day in animals exposed to light–dark cycling (Graham *et al.*, 2005) or constant darkness (Nakahara *et al.*, 2004b), respectively, the latter indicating an endogenous rhythm that persists in the absence of environmental cues.

Taken together with anatomical considerations, namely that the SCN has projections to the PVN (Leak and Moore, 2001), these results suggest that NMU may exert circadian control over its PVN-mediated actions, in particular feeding. The finding that NMU mRNA within the SCN peaks during the day concords with nocturnal feeding of rodents, such that appetite would be suppressed in hours of light. However, this would also imply increased physical activity during the day, which is at odds with rodent nocturnal behaviour. In addition, the majority of evidence suggests that CRH is a downstream effector of NMU; however, no relationship between the circadian rhythms for NMU expression in the SCN and expression of CRH in the PVN was evident in mouse (Graham *et al.*, 2005). Whether the same is true in rat, which has higher levels of NMU_2 expression in the PVN (Graham *et al.*, 2003), remains to be studied.

Effects of NMU on bone

Sato *et al.* (2007) proposed that NMU acts centrally as a negative regulator of bone formation. Firstly, NMU was shown not to directly affect osteoblasts, suggesting a central action, and secondly, *NMU* knock-out mice had a higher bone mass than controls, exhibiting a larger population of osteoblasts and subsequently a greater rate of bone formation. This action of NMU is believed to occur downstream of leptin, as leptin was no longer effective at inhibiting bone formation in *NMU* knock-out mice. In contrast, Rucinski *et al.* (2008) have shown that NMU can directly stimulate proliferation of cultured rat calvarial osteoblast-like cells. Both of these effects are thought to be mediated through NMU_2.

Pathophysiological roles for NMU

Role of NMU in immunity and inflammation

Messenger RNA for NMU has been detected in monocytes and dendritic cells and NMU_1 mRNA in T cells, macrophages and natural killer cells (Hedrick *et al.*, 2000; Johnson *et al.*, 2004; Moriyama *et al.*, 2006a), suggesting an immunoregulatory role. Indeed, NMU induced secretion of interleukins (IL)-4, 5, 6, 10 and 13 from murine T cells (Johnson *et al.*, 2004); in *NMU* knock-out mice, mortality rate was lower after administration of lipopolysaccharide (LPS; endotoxin), a result of decreased production of IL-6 by macrophages and subsequent reduced septic shock (Moriyama *et al.*, 2006a). Peripherally, NMU appears to perpetuate the deleterious effects of LPS; however, NMU ameliorated LPS-induced poor performance of mice in the Y-maze test when administered centrally and reduced death of cultured hippocampal neurones when subjected to LPS. Although NMU had no effect on cytokine levels or inflammation within the brain, its up-regulation of brain-derived neurotrophic factor in the hippocampus, shown at

mRNA and protein levels, is believed to offer protection against 'neuroinflammation-induced amnesia' (Iwai *et al.*, 2008).

NMU_1 mRNA has also been observed in mast cells and eosinophils (Moriyama *et al.*, 2005; 2006b). Intraplantar injection of complete Freund's adjuvant (CFA) caused degranulation of mast cells with ensuing paw oedema and up-regulation of IL-6, tumour necrosis factor-alpha, macrophage inflammatory protein-2 and leukocyte adhesion molecules, responses not seen in *NMU* knock-out mice (Moriyama *et al.*, 2005). Similarly, subcutaneous administration of NMU produced plasma extravasation and paw oedema in wild-type and *NMU* knock-out mice but not in mast cell-deficient mice (Moriyama *et al.*, 2005), providing convincing evidence that NMU has a role in mast cell-mediated inflammation. The source of NMU in the paw was thought to be keratinocytes and, indeed, reduced NMU-LI at this site was observed after CFA administration (Moriyama *et al.*, 2005). NMU has also been shown to promote eosinophilia, allowing cell adhesion and chemotaxis *in vitro*; in addition, *NMU* knock-out mice have a reduced eosinophilic response in the ovalbumin-mediated asthma model (Moriyama *et al.*, 2006b). These findings propose that the NMU system is a potential target for the treatment of diseases characterized by mast cell or eosinophil mediated inflammation, such as inflammatory bowel disease and bronchial asthma respectively.

NMU and cancer

Changes in NMU mRNA expression have been noted in several forms of cancer (Table 5). In oesophageal squamous cell carcinoma, expression was decreased or silenced in both cell lines and primary tumours (Yamashita *et al.*, 2002). As NMU suppressed the colony-forming ability of cells derived from this cancer (Yamashita *et al.*, 2002), reduced expression results in greater tumourogenicity. In contrast, expression was increased in bladder carcinoma (Wu *et al.*, 2007), ovarian carcinoma (Euer *et al.*, 2005), non-small cell lung cancers (NSCLCs) (Takahashi *et al.*, 2006) and acute myeloid leukaemia (AML) (Shetzline *et al.*, 2004). Although the consequences of increased NMU mRNA expression in ovarian carcinoma were not reported (Euer *et al.*, 2005), NMU promoted proliferation of primary AML cells (Shetzline *et al.*, 2004) and exposure of NSCLC cells to short interfering RNA against NMU reduced cell growth (Takahashi *et al.*, 2006). In human bladder cancer cells, the NMU gene was negatively regulated by RhoGDI2, a Rho-guanine diphosphate dissociation inhibitor shown to suppress metastasis. In nude mice, injection of such cells overexpressing NMU not only increased tumour formation but also metastasis (Wu *et al.*, 2007). In bladder carcinoma, reduced expression of RhoGDI2 heralds a lower 5-year survival rate (Theodorescu *et al.*, 2004). Therefore, it can be hypothesized that reduced RhoGDI2 expression in this cancer results in increased NMU expression and subsequent metastasis. NMU was also shown to contribute to cancer-associated cachexia (Wu *et al.*, 2007), potentially through its anorectic effects but also through increased secretion of the cachectic factor IL-6 (Strassmann *et al.*, 1992; Kuroda *et al.*, 2007) from macrophages and T cells (Johnson *et al.*, 2004; Moriyama *et al.*, 2006a).

Evidence suggests that NMU has a tumourogenic action in AML, NSCLC and bladder carcinoma, suggesting the NMU system may provide a novel therapeutic target in the battle against cancer. In addition, serum samples from patients with such cancers should be analysed to determine whether NMU could be a marker of disease and diagnostic aid (Euer *et al.*, 2005).

Neuromedin S (NMS)

In 2005, a 36 amino acid peptide related to NMU was discovered in rat brain, named NMS owing to its high expression in the SCN (Mori *et al.*, 2005). These two peptides share the same amidated C-terminal heptapeptide (Figure 1) and bind to the same receptors, NMU_1 and NMU_2, though NMU_2 has a greater affinity for NMS than NMU (Mori *et al.*, 2005). However, their genes are located on different chromosomes (NMS on 2q11.2 and NMU on 4q12) and NMS mRNA has a more limited distribution, at least in rat, with the highest levels of expression in the hypothalamus, spleen and testis (Mori *et al.*, 2005). NMS has also recently been identified in the human heart (Mitchell *et al.*, 2008). Akin to the discovery of NMU analogues in the skin secretions of the treefrog, *Litoria caerulea* (Salmon *et al.*, 2000), and toad, *Bombina maxima* (Lee *et al.*, 2005), NMS analogues, NMS-17 and NMS-33, have been isolated from the dermal venoms of bombinid toads, including *B. maxima* (Chen *et al.*, 2006). Both produced functional

Table 5 Summary of cancers in which NMU mRNA has been detected

Cancer type	*Change in NMU mRNA expression*	*Action of NMU*	
Oral tumours	↓		Alevizos *et al.*, (2001)
Oesophageal SCC	↓	Reduced growth of oesophageal SCC cells	Yamashita *et al.* (2002)
Ovarian carcinoma	↑		Euer *et al.*, (2005)
Bladder carcinoma	↑, secondary to down-regulation of Rho-GDP dissociation inhibitor	Increased tumourogenicity, pulmonary metastasis and cancer-associated cachexia	Wu *et al.* (2007)
Non-small cell lung cancers	↑	Increased growth and invasion of tumour cells; NMU thought to act at a heterodimer of NTS1 and splice variant of ghrelin receptor	Takahashi *et al.* (2006)
AML	↑	Proliferation of AML cells	Shetzline *et al.*, (2004)

AML, acute myeloid leukaemia; GDP, G-protein-coupled receptors; NTS1, neurotensin receptor 1; SCC, squamous cell carcinoma.

responses in cells expressing either human NMU_1 or NMU_2 with similar potency to human and rat NMS.

Functionally, NMU and NMS have similar putative physiological actions; both cause contraction of smooth muscle preparations and increase systemic blood pressure in rats when administered i.v. (Mori *et al.*, 2005). In man, NMS elicited vasoconstriction in isolated saphenous vein with comparable potency with NMU-25 but significantly reduced maximum contractile response, suggesting NMS is a partial agonist at least in this tissue (Mitchell *et al.*, 2009). Like NMU, NMS also has roles in regulation of feeding (Ida *et al.*, 2005; Shousha *et al.*, 2006), circadian rhythms (Mori *et al.*, 2005), the HPA axis (Jaszberenyi *et al.*, 2007) and LH secretion (Vigo *et al.*, 2007b). Most recently, i.c.v. NMS has been shown to increase plasma levels of arginine-vasopressin, subsequently reducing urine production (Sakamoto *et al.*, 2007), and to stimulate oxytocin release in response to suckling (Sakamoto *et al.*, 2008) in rats.

Interestingly, although NMS induced contraction of chick rectum and elevation of rat systemic blood pressure with potencies similar to NMU (Mori *et al.*, 2005), central effects of NMS were more potent. i.c.v. Administration of NMS, compared with i.c.v. NMU, resulted in more potent phase-shifting of circadian rhythms (Mori *et al.*, 2005), suppression of feeding (Ida *et al.*, 2005), reduction of urine volume (Sakamoto *et al.*, 2007) and stimulation of oxytocin release (Sakamoto *et al.*, 2008). The mechanism behind this phenomenon is yet to be identified; it is speculated that NMU receptors in the CNS could be under the control of receptor-activity-modifying proteins (Mori *et al.*, 2005), though these primarily interact with class B GPCRs (Hay *et al.*, 2006).

Conclusion

The NMU system has a widespread distribution and diverse physiological actions, with key roles in contraction of smooth muscle and regulation of energy balance. Knock-out mice have been useful tools for elucidating functions for the NMU receptor subtypes; however, precise delineation requires the development of subtype-specific antagonists. Perhaps most intriguing are the obese phenotypes of *NMU* knock-out mice and individuals with the Arg165Trp amino acid variant of NMU-25. Taken together with its vascular actions, NMU may be a functional link between energy balance and the cardiovascular system and may provide a future target for therapies directed against the disorders that comprise metabolic syndrome.

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Conflict of interest

None declared.

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