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Review

Neuromedin U: A Multifunctional Neuropeptide with Pleiotropic Roles

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BACKGROUND: Neuromedin U (NmU) belongs to the neuromedin family, comprising a series of neuropeptides involved in the gut–brain axis and including neuromedins B and C (bombesin-like), K (neurokinin B), L (neurokinin A or neurotensin), N, S, and U.

CONTENT: Although initially isolated from porcine spinal cord on the basis of their ability to induce uterine smooth muscle contraction, these peptides have now been found to be expressed in several different tissues and have been ascribed numerous functions, from appetite regulation and energy balance control to muscle contraction and tumor progression. NmU has been detected in several species to date, particularly in mammals (pig, rat, rabbit, dog, guinea pig, human), but also in amphibian, avian, and fish species. The NmU sequence is highly conserved across different species, indicating that this peptide is ancient and plays an important biological role. Here, we summarize the main structural and functional characteristics of NmU and describe its many roles, highlighting the jack-of-all-trades nature of this neuropeptide.

SUMMARY: NmU involvement in key processes has outlined the possibility that this neuropeptide could be a novel target for the treatment of obesity and cancer, among other disorders. Although the potential for NmU as a therapeutic target is obvious, the multiple functions of this molecule should be taken into account when designing an approach to targeting NmU and/or its receptors.

Neuromedin U (NmU)[2] is a peptide involved in a myriad of different processes in the body. The fact that its sequence is highly conserved among different species suggests that it is an ancient molecule with key roles that has been preserved throughout evolution. Here we attempt to summarize these roles and examine the possibilities of using NmU as a therapeutic target for various disorders.

Structure

NmU is usually present in different species as peptides 8 or 25 amino acids long (NmU-8 and NmU-25, respectively), although other forms also exist *(1–4)*. These forms are generated from a longer precursor protein, cleaved by as-yet-unknown proteases *(5)*. The human and rat precursor protein is 174 amino acids long and includes a 34–amino acid signal peptide, indicating it is secreted. The existence of alternative forms was predicted from the study of putative proteolytic sites along the NmU precursor sequence, which revealed a second peptide derived from the neuromedin U (*NMU*)[3] gene, termed proNMU$_{104–136}$. This longer form has shown activity that affects feeding behavior, body weight, and metabolic activity in mice *(6)*. Interestingly, the effect of proNMU$_{104–136}$ does not appear to be mediated by NmU receptor 2 (NMUR2), the NmU receptor expressed preferentially in the brain (see later discussion of energy homeostasis and feeding).

The NmU sequence is highly conserved across species (Fig. 1), suggesting that NmU structure closely correlates with its function. The best-conserved region is the C terminus, particularly the last 5 amino acids; disruption or replacement of residues in this region results in reduced ability of NmU to induce smooth muscle contraction *(7)*. On the other hand, modifications in the N-terminal region also appear to induce changes in NmU function, although these modifications appear to be related to potency and stability of the molecule. In several species, the longer NmU-25 form is more potent than the NmU-8 form *(8–10)*. Presumably, changes in the N-terminal region alter the 3-dimensional structure of the peptide and favor a conformation with enhanced activity. Conversely, modification of the N terminus may

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[2] Nonstandard abbreviations: NmU, neuromedin U; NMUR2, NmU receptor 2; NmU-LI, NmU-like immunoreactivity; ACTH, corticotropin; GPCR, G-protein–coupled receptor; ERK, extracellular signal-regulated kinase; GSHR1b, growth hormone secretagogue receptor 1; NTSR1, neurotensin receptor 1; i.c.v., intracerebroventricular; PVN, paraventricular nuclei; CRH, corticotropin releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; IL-4, interleukin-4; VHL, Von Hippel Lindau; hypoxia-inducible factor 1-α; FOXM1, forkhead box protein M1; mdm2, mouse double minute 2 homolog.

[3] Human genes: *NMU,* neuromedin U; *ARHGDIB,* Rho GDP dissociation inhibitor (GDI) beta; also known as *RhoGDI2*.

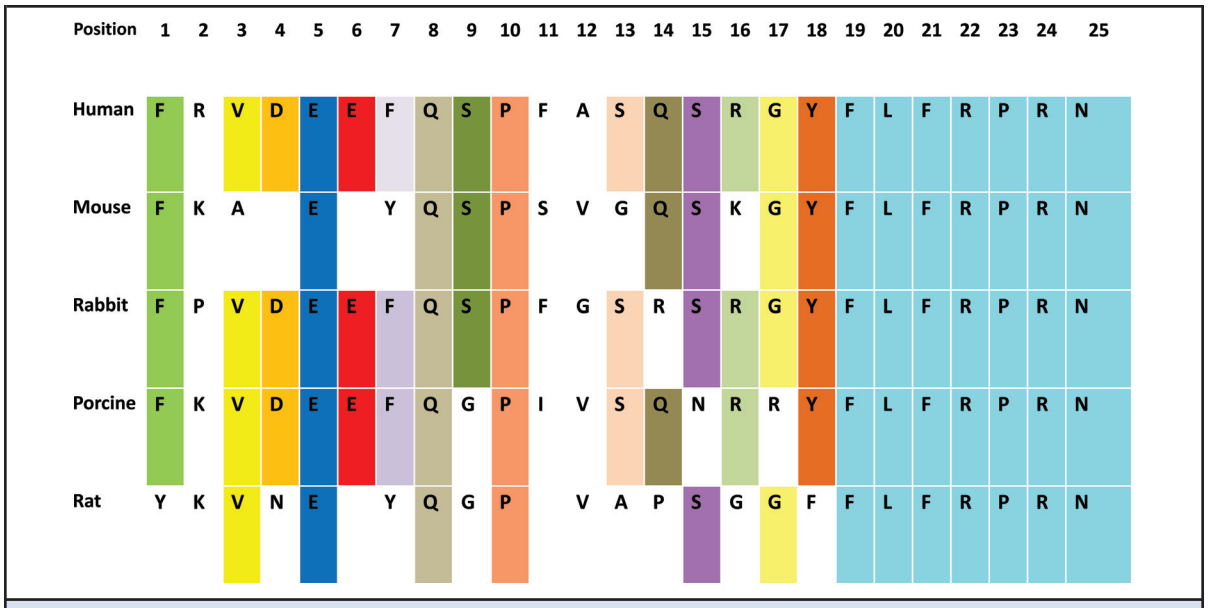

Fig. 1. Alignment of NmU sequences from different species.

Conserved residues among species are highlighted in color, and the highly conserved C-terminus region is also highlighted as a whole. To show conserved residues more clearly, mouse and rat sequences–which are shorter–are shown with gaps in between residues.

result in increased resistance to degradation by peptidases, thereby increasing the peptide half-life *(11, 12)*. Lastly, posttranslational amidation of the C-terminal amino acid is also essential for NmU activity, because deamidated forms are unable to induce smooth muscle contraction or intracellular Ca^{2+} influx *(9, 13, 14)*.

Point-mutation studies have revealed that changes in amino acid sequence may alter binding of NmU to its receptors, NMUR1 and NMUR2; for example, substitution of certain residues may not affect NmU activity at NMUR2, but reduces the response mediated by NMUR1 *(13)*. These data indicate that NmU specificity may be altered by small changes in its sequence. Recently, NMUR1- and NMUR2-selective hexapeptide agonists were discovered by a structure–activity relationship study using respective human receptor-expressing cells *(15)*.

A peptide closely related to NmU, neuromedin S (NmS), was isolated from rat brain *(16)*. This human peptide was shown to be 33 amino acids long (36 amino acids long in rat), considerably longer than NmU, but it shares substantial sequence homology. Both NmU and NmS share the 7 residues located in the C terminus of their sequence, including the amidation of the C-terminal amino acid. Moreover, NmS binds to NmU receptors NMUR1 and NMUR2, with some data suggesting that NMUR2 has higher affinity for NmS than for NmU *(16)*.

Organ and Tissue Distribution

NmU displays a widespread distribution throughout the body, as shown by RIA and immunohistochemical analysis to detect NmU-like immunoreactivity (NmU-LI). The highest concentrations of NmU-LI can be found in the gastrointestinal tract, from esophagus to rectum, with peak concentrations in the small intestine, particularly the duodenum and jejunum *(17–21)*, and usually detected within the enteric nervous system *(17, 21, 22)*. NmU-LI is not found in nervous fibers supplying smooth muscle layers, although this could be due to high turnover of the peptide *(22, 23)*. Along the gastrointestinal tract, NmU-LI colocalizes with calcitonin gene-related peptide, substance P, vasoactive intestinal peptide, and neuropeptide Y *(22–24)*. These data demonstrate that NmU is present in different types of neurons (cholinergic, noncholinergic, and sensory), suggesting multiple different roles for this peptide along the gastrointestinal tract.

Lower, but still substantial, concentrations of NmU-LI are found in the central nervous system *(25)*. Expression in the brain is actually localized in discrete regions, which means that expression levels in the whole brain are quite low *(20, 22, 26, 27)*. The regions with highest NmU-LI are the anterior pituitary gland, striatum, hypothalamus, medulla oblongata, cingulate gyrus, and medial frontal gyrus, with lower expression levels in hypothalamus, locus coeruleus, thalamus, and substantia

nigra *(20, 28, 29)*. In the rat, NmU-LI colocalizes with corticotropin (ACTH) *(20, 22, 30, 31)*. These results show that NmU expression in the brain is restricted to regions implicated in somatosensory, motor, and auditory functions.

Meanwhile, NmU-LI concentrations in the spinal cord are higher in the dorsal horn than in the ventral horn, suggesting that NmU may play a sensory role; this is further supported by high NmU-LI levels in the dorsal root ganglia *(20)*.

Elsewhere, NmU-LI can be found along the genitourinary tract, with the highest concentrations detected in the ureter, vas deferens, prostate, fallopian tubes, and urethra *(20)*. Other studies have shown expression of NmU in the testis, ovary, thyroid gland, spleen, lymphocytes, adipose tissue, endothelial cells, keratinocytes, and placenta *(14, 28, 29, 32–34)*. Interestingly, circulating concentrations of NmU in plasma or serum are very low *(20, 34)*, suggesting that this peptide acts locally in an autocrine or paracrine manner, rather than as a circulating hormone.

NmU Receptors

Two different receptors exist for NmU, termed NMUR1 (also known as GPR66 and FM-3) and NMUR2 (also known as TGR-1 and FM-4), encoded by genes located in human chromosomes 2 and 5, respectively *(14, 28, 29, 35–39)*. These receptors were identified from orphan class A G-protein–coupled receptors (GPCRs), possessing 7 transmembrane domains. In a similar way to NmU, the C-terminal regions of NMUR1 and NMUR2 are highly conserved across different species and appear to be determinant for biological activity *(40)*. In fact, NMUR1 and NMUR2 share significant sequence homology, suggesting that they arose as a duplication of an ancestral gene *(39)*. Both receptors appear to bind with similar affinity to different forms of NmU.

There is certain controversy regarding the tissue distribution of NmU receptors. Different techniques have given rise to dissimilar results, and studies in different species have added to these discrepancies. Most studies agree that NMUR1 is preferentially expressed in the periphery, particularly in the gastrointestinal tract, while NMUR2 is predominantly expressed in the central nervous system *(41)*. Human NMUR1 mRNA has been detected at the highest concentrations in stomach and small intestine, while it is also present in pancreas, adrenal cortex, heart, lung, trachea, mammary gland, bone marrow, peripheral lymphocytes, genitourinary system, placenta, mammary gland, spleen, and adipose tissue *(14, 29, 36, 38, 42)*. Some studies have also detected low concentrations of NMUR1 expression in human brain, but this finding has not been consistent. As for NMUR2, expression is restricted to discrete regions in the brain, especially in the substantia nigra, medulla oblongata, pontine reticular formation, spinal cord, and thalamus, but also in the hippocampus, hypothalamus, and cerebral cortex *(36, 38, 39)*. In a similar way to NMUR1, NMUR2 expression has been detected in peripheral tissues such as testis, gastrointestinal and genitourinary tract, liver, pancreas, adrenal gland, thyroid gland, lung, trachea, spleen, and thymus, with highest expression levels in the testis *(35, 38, 39, 42)*.

It should be noted that NmU has also been shown to act independently of NMUR1 and NMUR2. For example, in a model of arthritis the NmU-mediated Ca^{2+} influx and proinflammatory effect was unaffected by NMUR1/2 deficiency *(43)*; these results are consistent with the fact that complete Freund adjuvant-induced inflammation mediated by NmU is maintained in Nmur1- and Nmur2-deficient mice *(44)*. However, the only other NmU receptor reported in the literature is a heterodimer formed by the growth hormone secretagogue receptor 1b (GHSR1b) and the neurotensin receptor 1 (NTSR1) *(45)*. Because it has been shown that knockout mice for Ntsr1 showed a similar phenotype to wild-type mice in inflammation-mediated disease *(43)*, it is reasonable to suggest that other, as yet undiscovered receptor/s for NmU exist. As deduced from reported interactions and also by the NmU structure, it is likely that this receptor would also belong to the GPCR family.

Signaling Mediated by NmU

NmU binding to NMUR1 and NMUR2 results in increased intracellular Ca^{2+} mediated by phospholipase C activation *(13, 14, 28, 29, 35–39)*. This phenomenon occurs following coupling of receptors to Gα proteins, particularly $G\alpha_{q/11}$ and $G\alpha_i$ subunits *(46)*. There is certain specificity for each receptor's pathway, since NMUR1 signals mainly through $G\alpha_{q/11}$ and NMUR2 through $G\alpha_i$ *(47)*. The increase in intracellular Ca^{2+} also results in release of arachidonic acid, presumably through Ca^{2+}-dependent activation of phospholipase A_2 *(28, 35)*.

NmU binding to its receptors also results in inhibition of forskolin-mediated cAMP accumulation *(35, 40, 46)*. Ultimately, and in a similar way to other GPCR-mediated signaling, NmU activates the extracellular signal-regulated kinase (ERK) pathway, although the exact mediators have not yet been identified *(46)*.

Functions

A myriad of different functions have been ascribed to NmU (Table 1, Fig. 2), although most reports reflect its role in feeding, energy balance, and smooth muscle contraction, in agreement with its high levels of expression along the brain–gut axis. However, its widespread distri-

Table 1. Effects of NmU receptor activation.

	NMUR1	NMUR2
Smooth muscle contraction	+	+
Stress response	+	–
Food intake	+ (peripheral)	+ (central)
Intestinal motility	+	–
Nociception	–	+
Impaired bone formation	Not tested	+
Cytokine secretion	+	–

bution, together with evidence of colocalization with markers of diverse cellular function, suggests that NmU is involved in multiple processes in the body.

SMOOTH MUSCLE CONTRACTION

NmU-8 and NmU-25 were first identified on the basis of their ability to induce uterine muscle contraction *(48)*. A direct, dose-dependent contractile effect of NmU has been detected for different organs and tissues of the gastrointestinal and genitourinary tracts, and an indirect, enhancing effect of electrically induced contraction has also been identified, suggesting that NmU can act as a direct contraction inducer or as a potentiator of another contraction stimulus *(49–54)*. Studies with mice deficient in NmU receptors have shown that gastrointestinal smooth muscle contraction is likely mediated by NMUR1, because contraction is unaffected in Nmur2$^{-/-}$ mice but compromised in Nmur1$^{-/-}$ mice *(53, 54)*. On the contrary, genitourinary tract contraction appears to involve both receptors, although compensatory effects in the absence of one receptor cannot be ruled out.

Smooth muscle contraction in the gastrointestinal tract has consequences for gastric emptying and peristalsis. Indeed, NmU induces NMUR1-mediated kinetic activity in mouse colon *(53)*. The effects of NmU in gastric acid secretion and gastric emptying, however, appear to be mediated from the central nervous system (see section on gastric secretion and motility).

BLOOD PRESSURE AND BLOOD FLOW

NmU-LI can be localized in the smooth muscle layer of intramyocardial and large conduit blood vessels; this is in agreement with NmU-25 displaying constrictor effects on isolated human arteries and veins *(34)*. Several reports have shown that intravenous administration of NmU increased blood pressure without affecting heart rate, suggesting an increase in peripheral resistance due to local vasoconstriction *(8, 42, 48, 55, 56)*. Interestingly, NmU released from adipose tissue could contribute to increased blood pressure in obese patients, although this remains to be proven.

Conflicting evidence has been obtained about the central effect of NmU in heart rate and regulation of blood pressure following intracerebroventricular (i.c.v.)

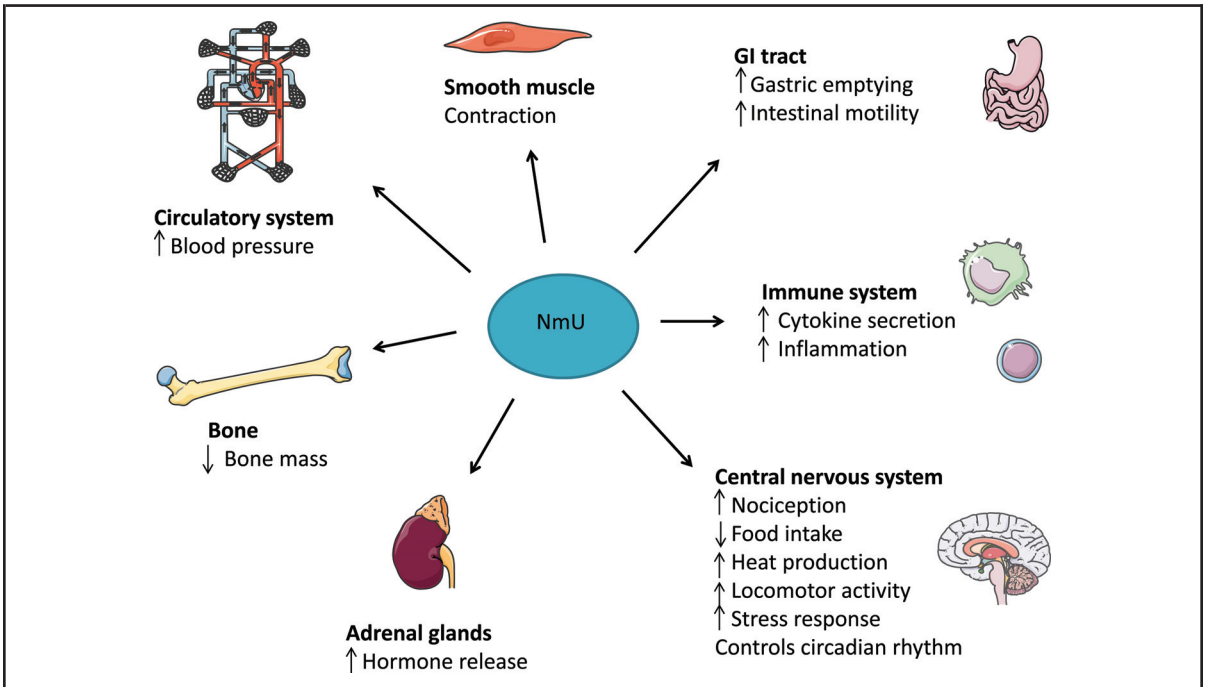

Fig. 2. Summary of the multiple functions of NmU in different organs and tissue types. GI, gastrointestinal.

administration *(8, 55, 57, 58)*, suggesting that although NmU might indeed regulate these phenomena centrally, further work is needed before its true role is elucidated. However, more recent reports appear to indicate that the prevalent effect of central NmU administration is to increase blood pressure through regulation of sympathetic activity *(59–62)*.

STRESS RESPONSE

The paraventricular nuclei (PVN) of the hypothalamus secrete corticotropin-releasing hormone (CRH), which in turn stimulates the pituitary gland to secrete ACTH; ACTH then induces production and release of cortisol by the adrenal glands *(41)*. Thus, the PVN are central in regulating the hypothalamus–pituitary–adrenal gland axis and the stress response, and relatively high levels of NmU expression at this site suggest a potential role for NmU in stress response regulation.

Indeed, i.c.v. administration of NmU to rats induced stress-related behavior *(37, 63–66)*. This was coupled to an increase in c-*Fos* expression, increased depolarization, and neuronal firing by cells of the PVN following NmU administration *(67–71)*. Moreover, significant increases in plasma concentrations of stress-related hormones (ACTH, corticosterone, adrenalin) were also detected following central or subcutaneous administration of NmU; these results are in agreement with low ACTH plasma concentrations detected in $NmU^{-/-}$ mice *(57, 65, 69, 72–75)*. The effects of NmU can be blocked by treatment with anti-CRH antibodies or CRH antagonists and, conversely, treatment with anti-NmU antibodies inhibits CRH released from hypothalamic explants. Furthermore, NmU does not induce stress-related behavior in mice deficient in CRH *(63, 76)*. These results suggest that NmU participates in the hypothalamus–pituitary axis, and that its effects are mediated by CRH. Mice deficient in NMUR2 do not display differences in their response to stress compared to wild-type mice, suggesting that this receptor does not mediate NmU effects on the stress response *(66)*.

As well as a central effect in the hypothalamus, NmU can also stimulate release of steroids from adrenal medullary chromaffin cells in the adrenal cortex *(77, 78)*; this effect can be prevented by treatment with CRH or ACTH peptide inhibitors, suggesting the existence of a paracrine NmU/CRH/ACTH axis in the adrenal cortex.

ENERGY HOMEOSTASIS AND FEEDING

Expression of NmU along the brain–gut axis suggested a role for this peptide in feeding. As expected, i.c.v. administration of NmU or its alternative, longer-form $proNMU_{104-136}$, decreased food intake and feeding-associated behavior in rats *(6, 36, 37, 65, 68, 79–81)* and also in other animals *(82–84)*. Supporting these reports, i.c.v. administration of NmU antiserum caused the opposite effects, with increased food intake in rats *(37, 85)*, a phenomenon also observed in $NmU^{-/-}$ mice, which are hyperphagic and obese *(73)*. Interestingly, treatment of $NmU^{-/-}$ mice with i.c.v. NmU reduced their fat mass *(73)*, and transgenic mice overexpressing NmU displayed reduced body weight and fat storage compared to their wild-type littermates, even on a high-fat diet *(86)*. Moreover, certain genetic variants of NmU—which according to structure/function correlation appear to be devoid of or have reduced functional activity—are associated with overweight and obesity *(87)*. NmU expression appears to be regulated by feeding behavior as well, because NmU concentrations in the hypothalamus are reduced by fasting *(36)*. Altogether, these results highlight the role of NmU in feeding-associated behavior.

The effect of NmU on feeding-related behavior is diminished or lost in $Nmur2^{-/-}$ mice, suggesting that it is predominantly mediated by this receptor *(6, 88)*. However, mice deficient in NMUR2 do not reproduce the phenotype of $NmU^{-/-}$ mice, showing no obesity, hyperphagia, or reduced energy expenditure; this was explained by suggesting that $proNMU_{104-136}$ mediates this effect through a different receptor, either NMUR1 or a yet unidentified receptor *(6)*. A recent report shows that knockdown of NMUR2 resulted in changes in food intake only when rats were fed a high-fat diet *(89)*, which might explain discrepancies. Experiments with these animals also revealed that NMUR2 might be involved in determining preference for high-fat foods. More recently, it has been reported that peripheral (i.e., not i.c.v.) administration of NmU also results in reduced food intake, lower body weight, and increased core body temperature and metabolic rate, but these effects are mediated by NMUR1, consistent with the expression pattern of this receptor *(90)*. These results indicate a more complex pathway for NmU signaling, although both central and peripheral effects, mediated by NMUR1, NMUR2, and/or still unknown receptors, appear to be similar.

The effects of NmU in the brain and the periphery, however, are not restricted to feeding: i.c.v. administration of NmU also increased gross-locomotor activity, body temperature, heat production, and oxygen consumption in rats *(36, 63, 65, 79–81, 91)*, suggesting a wider effect of NmU on energy homeostasis. The effect of NmU on body temperature occurs without inducing shivering, suggesting that it is exerted through chemical, not physical, means; this is usually mediated by sympathetic activity, which is connected to NmU secretion. Moreover, NmU can increase body temperature in rats without affecting their feeding behavior, suggesting that regulation of both effects by NmU occurs independently *(80)*. It appears that the lower body weight induced by NmU is also independent, at least in part, from the increase in locomotor activity *(86)*. On the other hand,

peripheral administration of NmU improved glucose tolerance *(90)*; these results are in stark contrast with the inhibitory effects of NmU in pancreatic insulin secretion, which appears to be mediated by somatostatin and NMUR1 *(92, 93)*, and the observed increase in insulin levels in $NmU^{-/-}$ mice *(73)*. This discrepancy might be explained by different effects of NmU depending on the administration route (i.e., central or peripheral), although further studies are needed to clarify this.

The mechanism of NmU-mediated reduced feeding and increased metabolic rate has not yet been completely elucidated, although evidence suggests links between effects of CRH, sympathetic activity, and the leptin pathway. NmU exerts at least some of its functions in a CRH-dependent manner, and indeed reduced food intake and increased oxygen consumption and core temperature in response to i.c.v. administration of NmU do not occur in $CRH^{-/-}$ mice *(81)*. Moreover, i.c.v. administration of CRH also results in reduced feeding in rats *(94)*, while CRH mRNA levels are reduced in $NmU^{-/-}$ mice *(73)*. In a similar way, central administration of oxytocin also inhibits feeding, and c-Fos expression is increased in oxytocin-producing neurons following stimulation with NmU *(68)*. This peptide is also able to induce release of corticosteroid hormones which, as part of the stress response, also reduce food intake *(41)*; this mechanism could also explain the alteration in feeding-associated behavior stimulated by NmU.

The anorectic effect of NmU might also be related to leptin, a hormone with similar effects on food intake. Leptin is released from adipose tissue and exerts its effect on the hypothalamus, where it can induce the release of NmU *(65)*. Furthermore, NmU mRNA levels were reduced in leptin-deficient mouse strains *(36)* and the effects of leptin were also reduced following administration of anti-NmU antisera *(85)*. However, weight loss was induced in $NmU^{-/-}$ mice following administration of leptin, indicating that this hormone only partially mediates NmU effects *(73, 85)*.

Recently, new NmU analogs have been developed for the treatment of obesity, based on the beneficial effects of NmU in food intake, locomotor activity, metabolic rate, and glucose tolerance. Polyethyleneglycol- and human serum albumin–conjugated NmU forms have recently been developed, which exert a potent anorexigenic effect and induce glucose tolerance in vivo with a much longer half-life compared to that of NmU *(95, 96)*. However, the effectiveness of this type of therapy in obese patients is under question, because chronic administration of NmU to rats showed no effect on food intake *(74)*. Complex mechanisms for desensitization, compensation, and regulation that are still not fully understood may be at play for NmU, and further studies are needed before these therapies can reach patients.

GASTRIC SECRETION AND MOTILITY

Reduced food intake is associated with reduced gastric emptying, so it is consistent with the anorexigenic role of NmU that i.c.v. administration of this peptide decreases and delays gastric emptying *(97)*. This effect appears to be mediated by CRH and the sympathetic nervous system, similar to stress responses. Although these effects seem to be central, it cannot be ruled out that NmU secreted along the gastrointestinal tract plays a role in local regulation of secretion and motility. This is shown by the fact that NmU enhances colon motility in vivo and in isolated electrically stimulated organs in an NMUR1-mediated pathway *(53)*; furthermore, intraperitoneal administration of NmU increased small intestine transit in mice, whereas intestinal motility is decreased in $NmU^{-/-}$ mice *(98)*.

CIRCADIAN RHYTHM

NmU is expressed in the suprachiasmatic nucleus of the hypothalamus, involved in the regulation of circadian rhythm. Increased c-*Fos* expression in the suprachiasmatic nucleus was observed following i.c.v. administration of NmU, as well as a phase shift in circadian locomotor activity coupled to increased expression of period homolog 1, a component of the circadian clock mechanism *(99, 100)*. Melatonin, a hormone known to be expressed under circadian control, also regulated NmU mRNA expression *(101)*. Moreover, the *NmU* gene is located close to the clock gene, a transcription factor that regulates circadian rhythm in mice *(102)*. These results were supported by studies confirming that NmU mRNA expression levels follow a circadian rhythm, with fluctuation throughout the day in animals subjected to light/darkness cycles *(101, 103)*. Similarly oscillating mRNA concentrations were detected in animals housed in complete darkness, suggesting that NmU expression, although affected by circadian rhythm, is at least partly independent of environmental light stimuli *(100)*. Circadian regulation of NmU is in agreement with its role in feeding, a behavior associated with circadian cycle.

HORMONE RELEASE

As well as the above-mentioned effects of NmU on the release of hormones from the adrenal gland, this peptide also appears to induce the secretion of oxytocin and vasopressin *(69)*. In the thyroid gland, NmU increases mRNA concentrations of type II deiodinase, an enzyme responsible for converting inactive thyroid hormone into its active form, in a similar way and to a similar extent as does thyroid-stimulating hormone *(104)*. A role for NmU in modulating thyroid gland function could also explain some of the general effects of this peptide in metabolic rate and core body temperature, among others.

The relevance of NmU in sex hormone–related phenomena is confirmed by the fact that NmU expression

varies from infancy through puberty and adulthood and along the female cycle, modulated by ovarian steroids *(105)*; NmU receptor expression has also been shown to be estrogen dependent *(106)*. Furthermore, NmU treatment induced ERK activation and progesterone release mediated by NMUR2 in cultured theca/interstitial cells of the ovary *(107)*.

NmU, however, was shown to inhibit the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) following i.c.v. administration in ovariectomized rats *(108)*. This effect was mediated by CRH *(109)*, and it was further shown that NmU inhibited the release of LH and FSH from pituitary cells in vitro, while $NmU^{-/-}$ mice display signs of puberty at an earlier age than their wild-type counterparts *(110)*. It should be noted that these experiments were performed in conditions that differ considerably from physiological ones, and in animals producing no endogenous sex steroids or NmU. In contrast, i.c.v. administration of NmU to nonovariectomized rats, a model that more closely resembles physiological conditions, suggests a role for this peptide as a positive regulator of LH and FSH release, with an overall effect of inducing early puberty signs *(105)*. This is in agreement with developmentally regulated expression of NmU, the concentrations of which decrease in rats going from puberty to mature adulthood *(111)*. More studies are needed to support this role for NmU as a stimulator of sex hormone release.

NOCICEPTION–PAIN SENSING

As previously mentioned, NmU is expressed in spinal cord regions associated with sensory functions. In vitro experiments showed increased sensory neuron excitability following NmU treatment in spinal cord explants *(112)*. This was confirmed in rats and mice, which experience hyperalgesia and reduced pain thresholds following central administration of NmU *(113, 114)*; on the contrary, $NmU^{-/-}$ mice showed decreased pain-associated behavior under different stimuli *(57)*. Furthermore, NmU enhancement of inflammation could also contribute to nociceptive signaling (see discussion of immune regulation below).

Nociceptive effects of NmU appear to be mediated by NMUR2, because facilitated excitatory synaptic transmission in spinal dorsal horn neurons, a mechanism by which NmU stimulates pain, was abolished in spinal cord slices from $Nmur2^{-/-}$ mice; moreover, mice deficient in this receptor showed reduced nociceptive responses to NmU administration, as well as reduced responses to a variety of painful stimuli *(44, 66)*. On the contrary, $Nmur1^{-/-}$ mice did not show any difference in pain response compared to their wild-type littermates, suggesting that this receptor is dispensable for nociceptive signaling mediated by NmU.

BONE REMODELLING

Leptin has been shown to regulate bone mass formation. Leptin-deficient and leptin receptor–deficient mice show an overall increase in bone mass due to enhanced bone formation *(115–118)*. The interaction between NmU and leptin regarding feeding behavior suggests these 2 molecules may also act together in bone formation. $NmU^{-/-}$ mice were found to display increased bone mass *(119)*; furthermore, treatment of wild-type mice with an NMUR2 agonist decreased their bone mass through impaired bone formation. Leptin- and sympathetic nervous system–induced bone formation was abolished in $NmU^{-/-}$ mice, suggesting that NmU acts downstream of these effectors. Reduced bone formation in $NmU^{-/-}$ mice appeared to be a consequence of a central effect of NmU in the hypothalamus, as no direct effects on osteoblasts were observed *(119)*. However, another report showed direct stimulation of osteoblast-like rat cell proliferation by NmU, an effect that was mediated by NMUR2 *(120)*.

A recent observational study showed that NmU polymorphisms are linked to bone density and quality in children, particularly when associated with polymorphisms in genes related to the sympathetic nervous system *(121)*.

IMMUNE REGULATION

NmU mRNA has been detected in antigen-presenting cells, particularly monocytes and dendritic cells, and NMUR1 mRNA has been detected in T and natural killer cells, and also at lower concentrations in other immune and hematopoietic cells such as eosinophils and mast cells *(14, 122)*, suggesting a role in the immune response. NmU was first shown to induce synthesis and release of several different cytokines [interleukin-4 (IL-4), IL-5, IL-6, IL-10, and IL-13) from a mouse T-helper 2 cell line in an NMUR1-dependent fashion *(123)*. It was later shown that NmU is required for IL-6 production, as shown by impaired IL-6 secretion by macrophages in $NmU^{-/-}$ mice *(124, 125)*. However, the exact role of NmU in regulating cytokine production is still unknown, as are the signaling pathways where this peptide is involved, although phospholipase C, calcineurin, and the MEK and PI3K (phosphatidylinositol 3-kinase) pathways are known to be required for this effect *(123)*. It is clear, though, that NmU has the ability to modulate the immune response.

The fact that NmU is involved in secretion of proinflammatory cytokines explains its role as an inflammation promoter: local NmU administration induces progressive vasodilation and edema, while NmU-deficient mice show decreased extravasation mediated by substance P *(122)*. Aside from localized inflammatory phenomena, mice deficient in NmU showed decreased mortality following lipopolysaccharide-induced septic shock,

and this was due to reduced circulating IL-6 concentrations *(124)*. Consistent with this result, $NmU^{-/-}$ mice were shown to develop a less severe form of antibody-mediated arthritis *(43)*. Binding of NmU to NMUR1 present in mast cells induced their degranulation, resulting in vasodilation, edema, and neutrophil infiltration *(33)*. NmU was also shown to promote eosinophil activation, migration, and adhesion to inflammatory sites in a dose-dependent and NMUR1-mediated manner, thus suggesting a role for this peptide in allergic reactions *(122)*. In spite of these effects being mediated by NMUR1, NmU-mediated inflammation has been shown to be unaffected in $Nmur1^{-/-}$ mice *(125)*. Moreover, NmU-mediated inflammation and arthritis were also unaffected in $Nmur2^{-/-}$ and double Nmur1/Nmur2 knockout mice, suggesting that at least some inflammatory effects might be mediated by an as yet unknown receptor *(43, 44)*.

CANCER

Pharmacological unmasking of esophageal squamous cell carcinoma and head and neck squamous cell carcinoma cell lines revealed that NmU expression was epigenetically silenced in these tumor types *(126, 127)*. These results were confirmed by analysis of clinical tumor and healthy patient samples, which showed that the NmU promoter is frequently hypermethylated in tumor tissue. Treatment of esophageal tumor cell lines with NmU reduced their ability to form colonies, confirming the tumor suppressor function of NmU in these cells *(126)*.

However, many studies have now shown that NmU is overexpressed in several different types of cancer *(45, 128–133)*. Moreover, overexpression of NmU was associated with worse prognosis *(45, 133)*. NMUR2 has been detected in human pancreatic cancer samples and canine peripheral nerve sheath tumors, suggesting that the autocrine/paracrine NmU-NMUR2 axis might be important for the development and/or progression of these tumors *(131, 134)*. Strikingly, tumor growth and migration-promoting effects of NmU in non–small cell lung carcinoma were not mediated by NMUR1 or NMUR2, but by a novel heterodimer formed by GSHR1b and NTSR1 *(45)*.

Several functional assays have revealed that overexpression or treatment of cancer cells with exogenous NmU increases cell proliferation, migration, invasion, and resistance to loss of anchorage-induced apoptosis, while knockdown of NmU expression has the opposite effects *(128, 131–133)*. These results were confirmed in in vivo assays, where NmU overexpression was shown to enhance tumor formation and metastasis, as well as possibly inducing cachexia *(130)*. Interestingly, although NmU-transfected T24 bladder carcinoma cells grew slower in vitro than empty vector–transfected cells, they were more efficient at tumor formation in vivo, suggesting that the growth-promoting effect of NmU requires the tumor microenvironment.

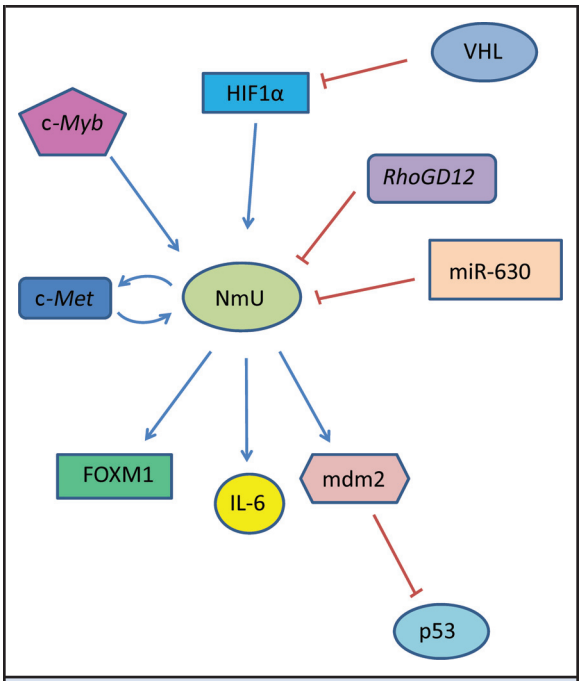

Fig. 3. Regulation of NmU expression and signaling pathways influenced by NmU. miR-630, microRNA 630.

In spite of all of the evidence toward a role for NmU in cancer formation, progression, and metastasis, the mechanism of action remains elusive. The expression of NmU appears to be regulated positively by several oncogenes and negatively by certain tumor suppressor genes (Fig. 3). For example, Von Hippel Lindau (VHL) protein downregulates NmU expression in renal cell carcinoma cells, likely due to downregulation of the hypoxia-related transcription factor hypoxia-inducible factor 1-α (HIF1α) *(132)*. Moreover, the metastasis-suppressing gene *ARHGDIB* [Rho GDP dissociation inhibitor (GDI) beta; also known as *RhoGDI2*] negatively regulates NmU expression *(130)*. On the other hand, the oncogenes c-*Myb* and c-*Met* appear to stimulate NmU expression *(128, 131)*. Interestingly, c-*Met* also appears to be upregulated following NmU treatment, suggesting a positive loop between these 2 molecules *(131)*. Regarding other genes that are regulated by NmU, this peptide appears to be required for secretion of IL-6, a cytokine involved in cancer cell growth and cachexia *(124)*, although it is not clear whether NmU affects IL-6 mRNA concentrations *(123, 135)*. NmU was also found to positively regulate Forkhead box protein M1 (FOXM1), a transcription factor and human protooncogene *(45)*. Finally, treatment of pancreatic cancer cells with NmU also upregulated mouse double minute 2 homolog (mdm2), a known p53 inhibitor,

suggesting another mechanism through which NmU may exert its tumor-promoting effects *(131)*.

Concluding Remarks

NmU is a multifunctional neuropeptide with multiple roles in different cell and tissue types, relaying central nervous system signals and stimulating organ functions, but also directly affecting certain cell types in many ways, from increasing proliferation and migration to inducing release of hormones and autocrine/paracrine factors. Most of its functions appear to be carried out through receptors NMUR1 and NMUR2, although alternative receptors have been described. It is also possible that NmU may bind and signal through other, yet undescribed, receptors as well. Perhaps NmU will become an ideal target for the therapy of certain disorders, particularly obesity and cancer, although the multiple roles of this neuropeptide should be taken into account when attempting to block its functions for therapeutic uses.

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