Pharmacomodulation of brain neuromedin U (NMU) signaling as a potential therapeutic strategy

Artur Pałasz[1], John J. Worthington[2], Łukasz Filipczyk[1], Karolina Saganiak[3]

[1] Department of Histology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, ul. Medyków 18, 40-752, Katowice, Poland

[2] Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, LA1 4YG, UK

[3] Department of Anatomy, Collegium Medicum, Jagiellonian University, ul. Kopernika 12, 31-034, Kraków, Poland

MINI - REVIEW

ORCID IDs

Artur Pałasz; 0000-0002-2632-1211

John J. Worthington; 0000-0002-1429-5669

Łukasz Filipczyk; 0000-0001-9352-0715

Corresponding author: Artur Pałasz, Medyków Street 18, 40-752 Katowice, Poland
apalasz@sum.edu.pl, +48 32 208 83 77

Abstract

Neuromedin U (NMU) belongs to a family of multifunctional neuropeptides that modulate the activity of several neural networks of the brain. Acting via metabotrobic receptor NMUR2, NMU plays a role in the regulation of multiple systems, including energy homeostasis, stress responses, circadian rhythms and endocrine signaling. The involvement of NMU signaling in the central regulation of important neurophysiological processes and its disturbances is a potential target for pharmacological modulation. Number of preclinical studies have proven that both modified NMU analogues such as PASR8-NMU or F4R8-NMU and designed NMUR2 agonists e.g. CPN-116, CPN-124 exhibit a distinct pharmacological activity especially when delivered transnasally. Their application can potentially be useful in the more convenient and safe treatment of obesity, eating disorders, Alzheimer diseases-related memory impairment, alcohol addiction and sleep disturbances. Accumulating findings suggest that pharmacomodulation of the central NMU signaling may be a promising strategy in the treatment of several neuropsychiatric disorders.

Key words: neuromedin U, NMUR2, neuropeptides

Significance

Brain neuromedin U (NMU) signalling seems to be involved in the origin of several central pathologies including disturbed energy homeostasis, drug addiction and memory impairment. Intranasal administration of selective NMU receptors agonists could be taken into consideration as a promising and more effective treatment option for aforementioned disorders.

Introduction

Neuromedin U (NMU) is a multifunctional neuropeptide involved in the regulation of pleiotropic neurophysiological processes such as energy balance and food intake, sleep, circadian rhythm, stress response, blood pressure and pain perception (Teranishi and Hanada 2021, Martinez and O'Driscoll 2015, Ahnaou and Drinkenburg 2011). NMU was discovered and isolated for the first time in 1985 from pig spinal cord (Minamino et al., 1985) as a novel member of the smooth muscle-contracting factor family. In humans, a 25-amino acid molecule (NMU-25) has been identified, while in rats a shorter (NMU-23) form occurs (Malendowicz and Rucinski 2021). Two types of metabotropic NMU receptors (NMUR) are currently known: NMUR1 and NMUR2, both coupled predominantly with $G_{q/11}$ protein (You et al., 2022), although possible coupling of $G_{i/0}$ has also been suggested (Hsu and Luo 2007). NMUR1 immunoreactivity is widely distributed in the gastrointestinal epithelia. In contrast, NMUR2 expressing cells are present exclusively in the several brain regions in particular in the magnocellular hypothalamic (especially in the paraventricular nucleus, PVN), thalamus, hippocampus (area CA1), substantia nigra, brainstem nuclei, spinal cord and some neocortical regions (Howard et al., 2000, Shan 2000). A population of NMU-immunoexpressing neurons is found in the rat nucleus accumbens (NAc), hypothalamus, septum, amygdala, globus pallidus and brainstem (Brighton et al., 2008). Distribution of NMU perikarya in the human brain is so far not sufficiently mapped, however NMU precursor protein was identified in the hypothalamus, NAc, thalamus, locus coeruleus (LC), cingulate and medial frontal gyri (Szekeres et al., 2000). NMU mRNA expression was detected in the rat hypothalamus, especially in the arcuate (ARC) and ventromedial nuclei (VMH) and median eminence as well as in the brainstem nuclei. (Howard et al., 2000). A population of NMU-positive cells were also found peripherally, in the whole gastrointestinal tract, especially in its submucosal and myenteric plexi (Nakashima et al. 2010). These regulatory neurons belong to the enteral nervous system (ENS) and may affect several functions of local cholinergic and peptidergic neurons (Ballesta et al. 1988, Honzawa et al., 1987).

NMU-related regulatory mechanisms seem to occur in the entire body (e.g. in the reproductive, endocrine, gastrointestinal and lymphatic systems) and are associated with the functioning of several tissues and cells (Raddatz et al., 2000, Domin et al. 1990, Hedrick et al., 2000, Moriyama et al., 2005). Accumulating reports have highlighted the pathophysiological role of NMU in the origin of in immune responses, inflammatory processes and cancer biology (Ye et al., 2021, Przygodzka et al., 2019). There is therefore a promising justification for attempts to use NMU and its analogues in the treatment of various disorders (Malendowicz and Rucinski 2021). A role of NMU signaling in the central regulation of many neurophysiological processes and its disturbances may therefore be a potential target for safer and effective pharmacological modulation. This minireview aims at reporting and critically discussing recent findings suggesting a potential usefulness of NMU and its analogues in the treatment of some neuropsychiatric or metabolic disturbances that are functionally related to some impairments of hypothalamic regulatory circuits.

Mechanisms of NMUR2 agonists action at the level of hypothalamic centres

The mechanism of action of NMUR2 agonists on the central regulation of energy balance and consumatory behaviour has not yet been completely explained. Two separate population of hypothalamic neurons are considered to play a key role in this complex process; proopiomelanocortin/cocaine-amphetamine regulated transcript (POMC/CART) neurons in the arcuate nucleus (ARC) and corticotropin releasing factor (CRF)-releasing cells in the parvocellular part of paraventricular nucleus (PVN) (Fig.1). Activation of their NMUR2 receptors causes exocytosis of anorexigenic factors α-melanocortin (α-MSH) and CRF respectively (Nagai et al. 2018). The main effect of α-MSH release is a stimulation of melanocortin 4 receptors (MC4R) in the ventromedial hypothalamus (VMH) that entails a subsequent release of brain-derived neurotrophic factor (BDNF) and inhibition of orexigenic 26RFa exocytosis. A simultaneous activation of MC4R-expressing CRF neurons in the PVN is an alternative way, which also causes an enhanced CRF transmission both synaptic and neurosecretory. CRF may also affect VMH neurons directly through the activation of CRF receptor 2 (CRFR2) that exert feeding suppression. Alternatively, NMU and probably its active derivatives may directly depolarize CRH-expressing

parvocellular PVN but not POMC/CART and magnocellular neurons via the opening of hyperpolarization-activated cyclic, nucleotide-gated cationic channels (HCNs). The fundamental physiological role of CRF signaling is the generation of stress responses by the activation of the hypothalamic-pituitary-adrenal (HPA) axis and triggering of the peripheral sympathetic activity. However, CRF is also distinctly involved in other regulatory mechanisms e.g. it has anorexigenic and hypermetabolic properties and it may play a role in the pathogenesis of depression and anxiety acting as an agonist of the CRFR2. (Tenk et al., 2016). Comparative studies report that both NMU and neuromedin S (NMS) manifest anorexigenic effects in rodents via activation of MC4R-dependent melanocortin signaling (Nakahara et al., 2010).

It is also worth mentioning, that several neuronal populations of the brain reward centres including nucleus accumbens (NAc) and dorsal raphe nucleus (DRN) exhibit NMU2 receptor expression (Anan et al., 2020, McCue et al., 2017) suggesting its modulatory role within the regulatory circuit DRN – NAc – ventral pallidum) involved in the mechanisms of drug addiction (Kasper et al., 2018). In a recent study by Vallöf *et al*., (2020) reported that interebroventricular infusion of NMU significantly decreased alcohol intake in high, but not low, ethanol-consuming rat and, attenuated induced locomotor stimulation. Alcohol-induced dopamine release in the NAc has also been highly attenuated after NMU treatment but the levels of circulating alcohol and corticosterone remained unchanged. This may indicate that NMU administration does not evoke or reinforce general stress responses and may therefore be taken into account as a novel, relatively safe therapeutic option. Importantly, a reduced water intake has not been reported after discontinuation of NMU administration (Vallöf et al., 2020). Having regarded the fact that alcohol use disorder (AUD) belongs to the main social and medical problems and novel more effective therapeutic strategies are required, the aforementioned results suggest cautiously that NMU analogues can be considered as potential treatment of AUD in forthcoming clinical practice. However, an efficacy of intranasal drug administration should also be estimated in animal models of alcohol addiction.

Pharmacological perspectives of intranasal NMUR2 agonists application

The aforementioned multifaceted involvement of NMU signaling in brain regulatory pathways may also imply the potential roles of the neuropeptide in the origin of pathogenetic background of some neuropsychiatric and metabolic disorders. Moreover, NMURs, especially NMUR2, have gathered attention as novel and potentially promising targets for pharmaceutical agents. Both NMU analogues, e.g. CPN and several flavonoid derivatives, were found to be potent and selective agonists of NMUR2 in animal models (Nagai et al., 2018, Ma et al., 2014). Highly invasive intracerebroventricular drug administration, while commonly used in basic animal models, must not be applied as a clinical treatment strategy, thus more safe, practical and non-invasive intranasal delivery of such NMU analogues should be considered. The blood-brain barrier (BBB) disables the penetration of exogenous neuropeptides and their macromolecular derivatives to almost all brain structures via cerebral circulation, therefore studies on alternative and convenient intranasal routes are undergoing intensive development. There are two known, parallel mechanisms of transnasal drug transport into the brain: 1) straight influx into the cerebrospinal fluid (CSF) via epithelial cell layer and submucosa and/or entry to neuropilus using extracellular diffusion within perineuronal spaces; 2) intracellular transmission of the pharmaceutical molecules across the olfactory neurons to cortical areas (Borroto-Escuela et al., 2015). Of note, the exchange of molecules between two parts of the recently described glymphatic system: CSF and brain extracellular fluid (BECF) may also facilitate the efficient central distribution of intranasally delivered medications (Tanaka et al., 2020, Fig. 1).

Number of peptide medications are currently available in the intranasal form. Most of them are on the market, but some are still in clinical development or in laboratory in research use only. Some current clinical examples of intranasally applied peptides with their main pharmacological properties are mentioned below. Synthetic gonadoliberin (GnRH) agonists such as buserelin and nafarelin are currently used in the treatment of prostate and breast cancer and endometriosis as well as polycystic ovary syndrome, PCOS) (Harada 2008 et al., Brogden et al., 1990). Vasopressin analogues e.g. desmopressin are often used in the treatment of nocturnal polyuria, hemophilia A, diabetes insipidus, Willebrand disease or uremic bleeding (Fein and Herschkowitz 2017). Oxytocin may also be helpful for selected group of patients

suffered from postratumatic stress disorder (PTSD) (Szafoni and Piegza 2022), postpartum depression (Lindley-Baron-Cohen et al., 2022), autism spectrum disorder (ASD) (Plemeniti et al., 2021), memory impairments (Zhao et al., 2019). Transnasally applied insulin not merely decrease blood glucose level, but it can also improves memory in healthy individuals and those suffered from Alzheimer disease (AD) (Gaddam 2021). Exendin, a long-acting glucagon-like peptide-1 receptor agonist, leading to increased insulin release is approved for use in adults with type-2 diabetes as an adjunctive therapy for those taking metformin and/or a sulfonylurea (Zhai et al., 2020). GALP (Galanin-like peptide) is a neuropeptide which regulate feeding and causing weight loss (Kageyama et al., 2016). Leptin is a potent anorexigenic satiety factor, also decreases body weight and food intake (Novakovic et al., 2009). Administration of PACAP, the regulatory peptide that stimulate adenylate cyclase in the pituitary may improves cognititive processes (Rat et al., 2011). Davunetide (CP201) does exhibit efficacy in prodromal Alzheimer's disease patients (Ivashko-Pachima et al., 2019). These days, every single attempt helping defeat Covid-19 pandemic is particularly important. Recent preliminary study reports, that the intranasally delivered TAT-peptides might significantly prevent entry of SARS-CoV-2 molecules into the both lung and olfactory bulb cells (Su et al., 2022).

Effect of NMUR2 agonists on eating behaviour and energy expenditure

NMU signaling is also considered an important part of the hypothalamic circuits controlling energy homeostasis and consumatory behaviour (Teranishi and Hanada 2021, Niimi et al., 2001, Nakazato et al. 2000). Functional disturbances in the neurochemistry of this complex regulatory system may lead to distinct food intake changes manifested by the onset of eating disorders or increased obesity-promoting adipose tissue storage. It has been widely proven that NMU is a potent anorexigenic factor in rodent species, its icv administration and targeted infusion into the PVN highly decreases food intake and body weight. (Howard et al. 2000, Wren et al. 2002) An overexpression of the NMU gene causes feeding suppression but its silencing has in turn an orexigenic effect in animal models (Kowalski et al., 2005, Hanada et al., 2004). The anorexigenic action of NMU does not occur in NMUR2 knockout mice, exposing the key role of this receptor in the neuropeptide activity at the level of

hypothalamic pathways (Zeng et al., 2006). Psychiatric pharmacotherapy, particularly schizophrenia treatment with both classical and atypical antipsychotics highly increases the risk of serious and relatively fast weight gain, e.g. in case of long-term clozapine and olanzapine administration (Garriga et al., 2022). The possible prophylaxis and therapy of this neuroleptic-related obesity is therefore an important topic in current psychopharmacology. Intranasal delivery of anorexigenic NMU analogues can possibly be a promising option as a relatively safe and convenient adjuvant treatment in clinical neuropsychiatry and general medicine. A number of recent preclinical studies attempt to meet some of these expectations. Structural analysis of synthetic newly-designed pentapeptide-type NMURs agonists showed that a hexapeptide (e.g. CPN-223) is a minimum active molecule with both NMUR1-selectivity and serum stability (Takayama et al., 2020). A novel, highly specific NMUR2 agonist: CPN-116 (3-cyclohexylpropionyl-Leu-Leu-A2pr-Pro-Arg-Asn-NH2-A2pr-L-2,3-diaminopropionic acid) does exhibit a higher stability in the CSF than in the blood. A complex pharmacological study on this novel molecule by Tanaka et al., (2020) reports that CPN-116 concentration in the rat brain after intranasal application (at dose 1mg/animal) was higher than those after i.p./i.v. administration and sufficient to suppress food intake and to decrease body weight in examined animals. Pharmacokinetic analysis revealed a distinct bioavailability of CPN-116(24,2%) after transnasal administration, which turned out to be much better than anticipated. The anorexigenic activity of CPN-116 was considered dose-dependent and it was achieved via direct specific stimulation of brain NMUR2 and subsequent elevation of blood corticosterone levels (Tanaka et al., 2020).

Interestingly, subcutaneous administration of NMU analogues acting as nonselective NMUR1/2 and NMUR2 specific agonists (NMU-0002 and NMU-2084 respectively) caused anorexigenic effects and led to a decrease of body weight in mice with diet-induced obesity (DIO), however in this case several side effects related to unwanted activation of peripheral NMUR1 receptors occurred e.g. elevated intestinal motility and diarrhoea (Nagai et al., 2018). Analogous, potent anti-obesity effects have been reported after injection of other NMUR2 selective agonist NMU-7005 to DIO mice. Interestingly, a NMU-7005 administered concomitantly with a glucagon-like peptide-1 receptor (GLP-1R) agonist; liraglutide exposed even more efficacious anorectic effect, suggesting that NMUR2-related physiological action is independent from GLP signaling stimulation (Kaisho et al., 2017). The NMU-8

molecule conjugated to polyethylene glycol (PEGylated) exhibit extended and potent anorexigenic effects in DIO mice when administered subcutaneously as once-daily injections (Masuda et al., 2017). A potential modification of the above described NMU peptides by addition of CPP domains may theoretically enable their intranasal delivery to avoid several peripheral side effects. Another PEG-ylated NMU analogue and selective NMUR2 agonist, named Compound 37 manifested a strong, dose dependent anorexigenic effect in mice: with a weight loss of more than 12% in two weeks (Kanematsu-Yamaki et al., 2017). Intranasally delivered oxytocin did not meet its therapeutic expectations in the treatment of obesity and eating disorders (Russel and Hunt 2023, McCormack et al., 2023). Given all aforementioned data, an administration of NMURs agonists seems to be a promising and safe alternative for the treatment of obesity and other metabolic disturbances with oxytocin or leptin. However, there are a lot of important questions related to the NMU analogues pharmacology and many more further studies are urgently needed to prove their potential usefulness in clinical practice.

NMU derivatives in the treatment of memory impairment

It has been previously shown that NMU exhibited protective effects on LPS-induced inflammatory-dependent neuronal cell death in the mice hippocampus via increased release of BNDF from neuroglia (Iwai et al., 2008). A recent behavioural study performed on mice with use of Y-maze test, reports that the intranasal administration of two NMU derivatives, namely PASR8-NMU and F4R8-NMU (at a dose 5.6μg/animal) significantly reduces lipopolysaccharide (LPS)-induced memory impairment (Sasaki-Hamada et al., 2018). Modified NMU molecules were enriched with cell-penetrating peptides (CPPs) to facilitate their cellular, actin-dependent, uptake by fluid-phase endocytosis (Nakase et al. 2010). Therefore, all designed NMU analogues contain CPPs: octaarginine (R8) and the following synthetic penetration-accelerating sequence (PAS): FFLIPKG and FFFFG (for PASR8-NMU and F4R8-NMU respectively). Importantly only intracerebroventricular infusion but not transnasal administration of NMU have the above-described effects suggesting that both PASR8 and F4R8 molecular domains are insufficient for the appropriate

intranasal delivery of NMU into the brain. Moreover, the more stable PASR8-NMU molecule turned out to be more effectively delivered into the mouse brain than F4R8-NMU. Structural analysis revealed, that hippocampal neurons showed immunofluorescence 30 minutes after intranasal administration of indocyanine green (ICG)-labeled PASR8-NMU, but not F4R8-NMU or vehicle. The ICG-labeled PASR8-NMU cellular populations exhibited denser fluorescence than the vehicle group in the hippocampus and PVN but not in other brain regions (Sasaki-Hamada et al., 2018). It was recently suggested, that the activation of NMUR2 affects GABAergic inhibitory tone in the hippocampal CA1 area (Sasaki-Hamada et al., 2021). Furthermore, NMU acting as an antagonist of L-type voltage gated calcium channels may block Ca^{2+} influx into the hippocampal neurons (Zhang et al., 2010). Neurodegnerative changes occurred in AD are related to augmented phosphorylation of L-type channels, subsequent excess of Ca^{2+} level in the neuroplasm and finally inhibition of long term potentiation (LTP). Both aforementioned reports my suggest cautiously that intranasal application of NMUR agonists can potentially be useful in the safe treatment of some memory deficits also in the course of AD. Nevertheless, too little is currently known about the putative memory protective mechanism of NMU analogue action in the brain and all clinically oriented expectations, while intriguing, are so far highly precocious.

Concluding remarks

Although NMUR2 agonists exposed several promising pharmacological advantages, a number of important questions has to be addressed before the beginning of more advanced clinical trials. All potential side effects of NMU signaling modulators should particularly be taken into account when developing new pharmacological strategies. Firstly, some aforementioned concerns regarding their stressogenic stimulatory effects on HPA axis have been raised (Nagai et al., 2018). Indeed, an activation of brain NMUR2 has been found to modulate anxiety-like behaviour and trigger stress-related molecular events by CRH exocytosis in animal models (Hanada et al., 2001, Telegdy and Adamik 2013; Zeng et al. 2006). However, it was also suggested that NMU-23, may alternatively exert antidepressant-like behavioural effects in mice (Tanaka and Telegdy 2014). Furthermore, a wide distribution NMUR2 receptors in

the brain suggest that NMU signaling is also involved in a relatively broad spectrum of neurophysiological processes including autonomic and mental functions. Therefore, it should not be excluded that intranasally administered NMU analogues may activate NMUR2 receptors located in the limbic system and necortical regions or even receptors of other neuropeptides evoking pharmacological effects that would have been difficult to predict. Moreover, brain derived NMU, and probably its intranasally delivered analogues, are able to pass the BBB backwardly, reach the NMUR1-expressing peripheral organs and affect their functions (Gevaert et al., 2016). Intranasal NMUR2 agonists administration may often be used as an adjuvant side effect therapy in the treatment of schizophrenia and other neuropsychiatric disorders. This raises the risk of possible undiserable pharmacological interactions between NMU analogues and antipsychotic or antidepressants/anxiolytic medications. Recent studies reporting changes of NMU and NMUR2 expression in the rat brain after both acute and long-term treatment with escitalopram and clonazepam (Piwowarczyk-Nowak et al., 2022) seems to support this assumption. A new, more appropriate way for postneuroleptic weight gain treatment has been revealed and may also be a potential alternative in the design of new therapeutic strategies for other NMU mediated pathologies, such as drug addiction, eating and neuropsychiatric disorders. Nevertheless, there are still many questions related to the pharmacological effects of NMURs agonists and many more detailed basic and clinical studies are needed to prove their potential usefulness for forthcoming clinical medicine.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are openly available in PubMed Database.

CONFLICT OF INTEREST

The authors have no conflict to disclose

AUTHOR CONTRIBUTIONS

Conceptualization, Analysis, Writing – Original draft preparation, A.P.; Writing-reviewing; J.W.; Data acquisition, Ł. F.; Visualization, K.S.

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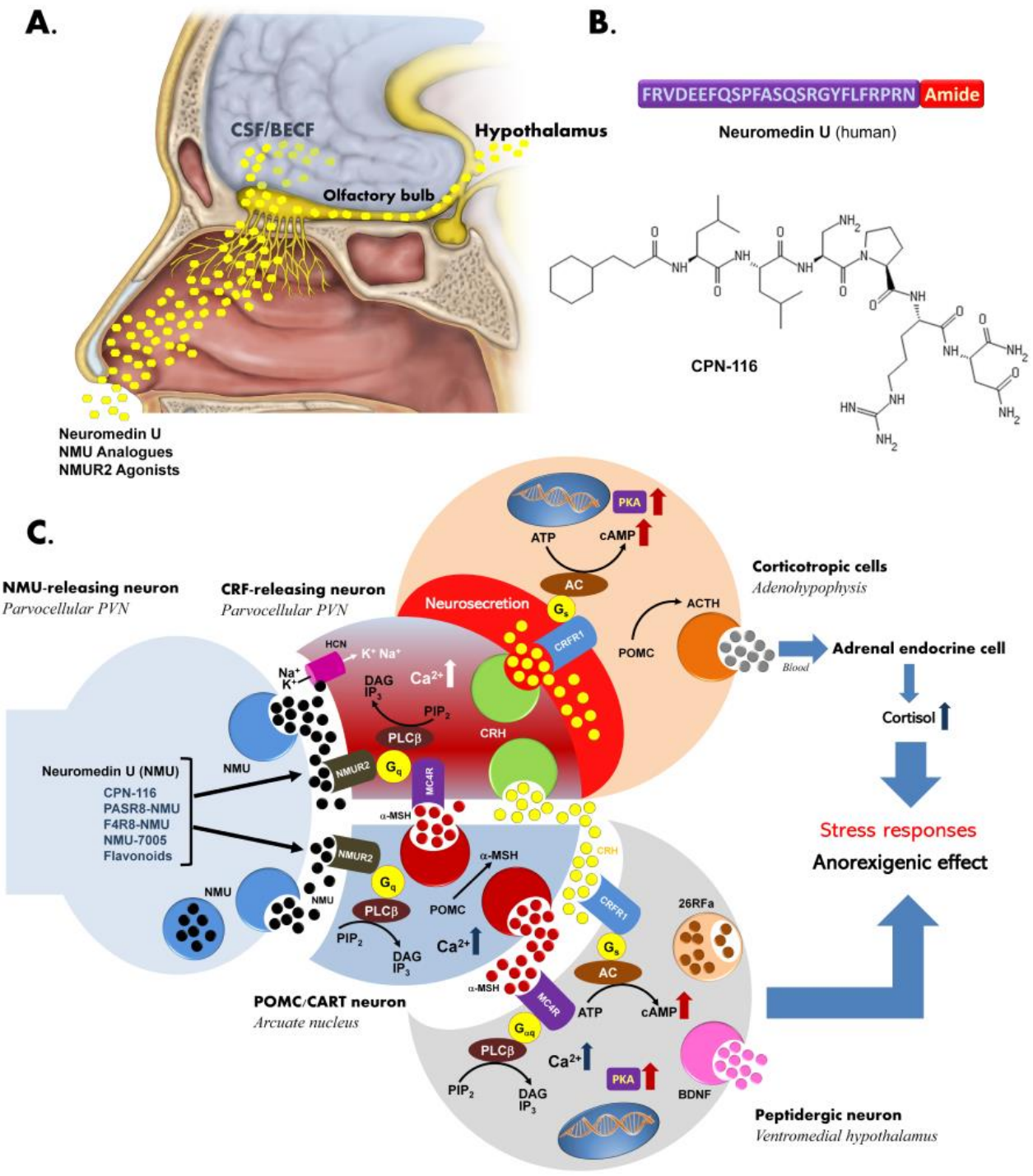
A.
CSF/BECF
Hypothalamus
Olfactory bulb
Neuromedin U
NMU Analogues
NMUR2 Agonists
B.
FRVDEEFQSPFASQSRGYFLFRPRN Amide
Neuromedin U (human)
CPN-116
C.
NMU-releasing neuron
Parvocellular PVN
CRF-releasing neuron
Parvocellular PVN
Corticotropic cells
Adenohypophysis
Neurosecretion
ATP
cAMP
PKA
AC
ACTH
POMC
Blood
Adrenal endocrine cell
Cortisol
Stress responses
Anorexigenic effect
HCN
K+ Na+
Na+ K+
DAG
IP3
Ca2+
PIP2
PLCβ
CRH
CRFR1
Neuromedin U (NMU)
CPN-116
PASR8-NMU
F4R8-NMU
NMU-7005
Flavonoids
NMU
NMUR2
Gq
MC4R
α-MSH
26RFa
Gs
Gαq
BDNF
POMC/CART neuron
Arcuate nucleus
Peptidergic neuron
Ventromedial hypothalamus

Figure 1. A) Intranasally administered NMU analogues or designed NMUR2 agonists may enter into the brain directly through the diffusion across nasal mucosa into glymphatic spaces filled with cerebrospinal (CSF) and brain extracellular fluid (BECF). Alternatively their molecules undergo intracellular transmission across the chemoreceptive neurons and olfactory bulb to several brain structures. B) Chemical structures of the human NMU and NMUR2 analogue; CPN-116. C) Neuromolecular mechanisms of NMU analogues action at the level of hypothalamus in the context of their possible pharmacological effects. Transnasally delivered NMUR2 agonists directly activate postsynaptic G-coupled NMUR2 receptors of the hypothalamic POMC/CART and CRH neurons that activate phospholipase Cβ (PLCβ) signaling pathway, increase inositol triphosphate (IP3) concentration, trigger calcium efflux from the endoplasmic reticulum and finally promote αMSH and CRH release respectively. Alternatively, NMU and probably its active derivatives may directly depolarize CRH-expressing parvocellular neurons in an exclusive manner via opening of hyperpolarization-activated cyclic, nucleotide-gated cationic channels (HCNs). α-MSH release stimulates melanocortin MC4R receptors in the ventromedial hypothalamus (VMH) causing a subsequent release of anorexigenic factor BDNF and inhibition of orexigenic 26RFa exocytosis. Peptidergic neurons of the ventromedial hypothalamus and hypophyseal corticotrope cells exhibit CRFRs expression. Activation of CRFRs increases the adenylate cyclase (AD) activity and cAMP synthesis. A simultaneous activation of MC4R-expressing CRF neurons in the PVN is an alternative way which supports CRF transmission both synaptic and neurosecretory. A CRF signaling pathway is responsible for the stress response generation by the activation of the hypothalamic-pituitary-adrenal (HPA) axis and triggering of the peripheral sympathetic activity.