Sleep Science

REVIEW ARTICLE

Sleep, neuropeptides and proteases

Sono, neuropeptídeos e proteases

Bruna Visniauskas[1], Guilherme S. Julian[1], Renato W. de Oliveira[1], Sérgio Tufik[1], Jair R. Chagas[1,2]

ABSTRACT

Neuropeptides have a fundamental role on sleep-wake cycle control and their proteolytic processing regulates their actions. Proteases are essential for the metabolism of neuropeptides, by release either from active or inactive precursors or by the inactivation of active neuropeptides. Using the Merops, a peptidase database, the Brenda Enzyme Database, the Expasy Bioinformatics Resource Portal and the published literature on normal sleep physiological regulation, we performed a review aimed to identify the proteases that can metabolize neuropeptides involved in regulating the sleep-wake cycle and could be altered during and after sleep deprivation.

Keywords: neuropeptides, proteases, sleep, sleep deprivation.

RESUMO

Neuropeptídeos têm um papel fundamental no controle do ciclo vigília-sono e as suas ações podem ser reguladas por processamento proteolítico. Proteases são essenciais para metabolização de neuropeptídeos, seja pela liberação a partir de precursores proteicos inativos ou ativos, ou pela inativação de neuropeptídeos ativos. Utilizando os bancos de dados Merops peptidases, Brenda Enzyme Databases, Portal Expasy Bioinformatics Resource e a literatura publicada sobre a regulação normal do sono fisiológico, realizamos uma revisão que tem como objetivo identificar as proteases que podem metabolizar os neuropeptídeos envolvidos na regulação do ciclo vigília-sono e que poderiam ser alteradas durante ou após a privação de sono.

Descritores: neuropeptídeos, privação do sono, proteases, sono.

SLEEP AND NEUROPEPTIDES

There are several physiological activities of peptides described in central nervous system (CNS), playing an essential role in functions such as nociception, cognition, stress, food intake, adrenergic tonus and regulation of sleep-wake states among others[1].

The peptides ACTH, corticotropin releasing factor (CRF), arginine vasopressin and substance P facilitate wakefulness, and thus play a role in modulating sleep[2].

The neuropeptide orexin (or hypocretin), synthesized exclusively in the peri-fornical tuberal region of the hypothalamus is described as a potent effector on sleep-wake cycle. In addition to stimulating feeding it promotes wakefulness[3]. Pedrazzoli et al.[4] showed that in the cerebrospinal fluid of rats deprived of sleep for 96 hours there is an increase of orexin/hypocretin, suggesting that the levels of this neuropeptide remain high due to the process of adaptation and forced wakefulness, thus leading to increased food consumption. The orexin/hypocretin system deficiencies are directly related to the sleep disorder known as narcolepsy, in humans, dogs and mice[5].

Some peptides like opioid peptides (enkephalin and β-endorphin), melanocyte stimulating hormone (MSH) and somatostatin are considered hypnogenic substances, or induce sleep[2,6,7]. These peptides co-located to function as neurotransmitters and neuromodulators[2]. Even though an hypnogenic effect is described for these peptides, there is little evidence indicating that their absence results in a prolonged insomnia[2,8].

Some preclinical and clinical studies showed that peripheral administration of several peptides results in a specific changes in sleep EEG in humans[1]. A review study by Axel Steiger, in 2007[9], demonstrated that the ICV administration of somatostatin and ghrelin caused the increase in sleep time at specific periods in rats. Both peptides showed to increase serum levels of GH, but while ghrelin increased slow-wave sleep, somatostatin increased paradoxical sleep. Vasopressin, the most important cofactor for the action of CRH in stress, increases wakefulness in rats.

Andersen and collaborators (2006)[10] have studied the involvement of the undecapeptide substance P in the sleep-wake cycle in C57BL/6J mice. Manipulating the dose of the peptide, the painful effects were avoided eliminating this potential interference. Their results demonstrated that substance P produced remarkable changes in the sleep-wake cycle of these animals, reducing sleep efficiency, increasing sleep latency and increasing the frequency of awakenings.

Administration of neurotensin in the basal forebrain cholinergic neurons reduces slow-wave sleep and paradoxical sleep and increases the frequency of awakenings[11].

A study performed with adult women shows that sleep seems to have a profound, opioid dependent, effect on the secretion of gonadotropins[12]. During the early follicular phase of the menstrual cycle there is a later secretion of luteinizing hormone (LH) and increase in amplitude of LH pulses during the sleep period. There are no evidences

[1] Departamento de Psicobiologia, Universidade Federal de São Paulo (UNIFESP).Rua Napoleão de Barros, nº 925. São Paulo - SP. Brazil. CEP: 04024-002.
[2] Ciências da Saúde, Universidade Federal de São Paulo (UNIFESP). Rua Ana Costa, nº 95. Santos - SP. Brazil. CEP: 11060-001.
Corresponding author: Jair R. Chagas. Departamento de Psicobiologia, Universidade Federal de São Paulo. Rua Napoleão de Barros, nº 925. São Paulo - SP. Brazil. CEP: 04024-002. Tel.: +55 (11) 2149-0155x189. Fax: +55 (11) 2149-0155. E-mail: jchagas1@gmail.com
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of alteration in pituitary responses to gonadotropin-releasing hormone (GnRH or LHRH) in this period. Nonetheless, when the opioid antagonist naloxone is administered, the secretion of LH is significantly reduced indicating that an increased opioid activity in the hypothalamus during sleep is modulating LH release[12].

Intravenous administration of neuropeptide Y (NPY) in young healthy male led to a reduction in sleep latency and an increase in stage II of NREM sleep[13]. Male rats deprived of sleep have increased food intake but body weight loss[14,15]. This phenomenon seems to be related to changes in NPY expression in the hypothalamus[16], specifically in the arcuate nucleus[15]. CRH levels in the arcuate nucleus were also elevated is this model of paradoxical sleep deprivation, suggesting that the increase in food consumption is related to the effects of stress during sleep deprivation.

PROTEASES

Proteases (or peptidases) are enzymes that cleave peptide bonds between amino acids of proteins. Proteases have, mainly, the function of generating new peptides, and/or metabolize it. Because of it essential role in processing, generating and inactivating bioactive peptides, the correct control of proteases activity must be strictly done[17].

Based on the mechanism of catalysis and chemical nature of amino acid residue present in their catalytic site, proteases are divided into five classes in mammals: aspartic, cysteine, serine, metallo and threonine proteases. There are multiple mechanisms of protease-activity based cell functioning control: regulation of the fate, location and activity of many proteins, modulation of protein-protein interactions, contribution to the transformation of cell information and the generation, transduction and amplification of molecular signals[17].

Neuropeptides are initially synthesized as inactive precursors, for example, the pre-pro-enkephalin, pre-pro-opiomelanocortin and pre-pro-neuropeptide Y. The generation of pro-neuropeptides and neuropeptide is classically made by intracellular proteolytic enzymes of the subtilisin family (convertases and furin) that recognize pairs of basic residues, Lys-Arg, Arg-Arg, Arg-Lys, Lys-Arg as site of cleavage to generate mature proteins[18]. Then other peptidases (aminopeptidases, carboxypeptidases and endopeptidases) may participate in the generation of the active peptide.

It has been recently shown, in cathepsin L knockout animals, that this enzyme, belonging to the cysteine protease family, plays an important role in the generation of neuropeptides[19,20]. Cathepsin L and protein convertases (PC) are endopeptidases that, from POMC, generate several active neuropeptides. For example, POMC is hydrolyzed by the enzyme prohormone convertase 1 (PC1) that generates ACTH and MSH; carboxypeptidase E then generates two types of MSH (α-MSH and γ-MSH). The conversion of POMC in several different neurotransmitters and peptide hormones is performed in a tissue specific way. For example, ACTH is produced from POMC in the anterior pituitary, but α-MSH and β-endorphin are produced from POMC in the pituitary[19].

The precursor protein may contain one or more copies of the neuropeptide, for example, the precursor pre-pro-enkephalin contains multiple copies of enkephalins, while the precursor pre-pro-neuropeptide Y contains only one copy of the active neuropeptide[19]. On the other hand mature neuropeptide Y (NPY 1-36), through the action of proteases, can generate three different forms of ligands, meaning by the two proteases: dipeptidyl peptidase IV (EC. 3.4.14.5) and aminopeptidase P (EC. 3.4.11.9), resulting in NPY3-36 and NPY2-36, respectively. The fragment NPY3-36 loses affinity for Y1 receptor, and becomes an agonist at the Y2 and Y5 receptor[21]. Recently, Abid and colleagues demonstrated that plasma kallikrein (EC. 3.4.21.34), an enzyme classically related to coagulation, inflammation and bradykinin release, might generate the inactive form NPY3-35 from the fragment NPY3-36 in human plasma. The fragment NPY3-35 has no affinity for the receptors Y1, Y2 and Y5. The intrinsic clearance suggests that the metabolic pathway of NPY1-36 for NPY3-36 is 26 times more efficient than NPY1-36 for NPY2-36. In addition, the generation of NPY2-36 from NPY1-36 is two times less efficient than NPY3-35 from the NPY3-36. These results indicate that the preferred pathway for NPY inactivation is NPY1-36 to NPY3-36 and then inactive NPY3-35 through plasma kallikrein[22].

As a general rule, which applies equally to the CNS, activities under direct control of proteases include: the liberation of active peptides from non active precursors, the generation of new ligands from already active precursors or the inactivation of endogenous or exogenously generated peptides[23,24]. Beyond this classical view of, mostly, extracellular proteolytic activities, a new level of complexity has become recently evident from the demonstration of a large intracellular peptidergic system, whose complexity has barely been touched[25].

Although examples of the various families of proteases (aspartic, serine, cysteine and metallo) are found in the CNS, the great majority of described enzymes, involved in metabolism of peptides, belongs to the class of metallopeptidases[26]. The active site of metallopeptidases has a divalent cation, Zn^{2+}, which is responsible for activating a linked water molecule, which, in turn, catalyzes the hydrolysis of peptide bond. Our group has shown alterations in the activity, expression and mRNA levels of two metallo peptidases: angiotensin I-converting enzyme (ACE, EC 3.4.15.1)[27] and thimet oligopeptidase (TOP, EC 3.4.24.15) in the CNS of male rats submitted to paradoxical sleep deprivation. ACE activity and mRNA levels were measured in hypothalamus, hippocampus, brainstem, cerebral cortex and striatum tissue extracts. In the hypothalamus, the significant decrease in activity and mRNA levels, after PSD, was only totally reversed after 96h of sleep recovery. In the brainstem and hippocampus, albeit significative, changes in mRNA do not parallel changes on ACE specific activity. Changes on ACE activity could affect angiotensin II generation, angiotensin 1-7, bradykinin and opioid peptides metabolism. ACE expression and activity modifications are probably related to some of the physiological changes (cardiovascular, stress, cognition, metabolism function, water and energy balance) observed during and after sleep deprivation[27]. We observed changes on TOP activity, in different brain tissues, after paradoxical sleep deprivation and during the sleep

recovery period, that can be related do endocrine changes like GnRH processing at the hypothalamus or pain sensitivity due to opioid peptides processing. In other tissues, like brainstem and striatum, changes in TOP are probably related to changes in the processing of neuropeptides, but there are no clear evidences in the literature to indicate the consequences of these modifications (Visniauskas, submitted). Modifications on TOP activity can potentially result in alterations in the metabolism of angiotensins, bradykinin, opioid peptides (dynorphins and enkephalins), substance P and GnRH and be related to some of the physiological changes (stress, memory and cognition, nociception and endocrine changes) observed during and after sleep deprivation.

DATABASE SEARCH

We performed a search in the databases Merops (a peptidases database (www.merops.ac.uk/), Brenda Enzyme Database (*www.brenda-enzymes.org/)* and Expasy Bioinformatics Resource Portal (www.expasy.org/). A double check was performed, looking at which enzymes are reported to hydrolyze, *in vitro* or *in vivo*, the known peptides involved in sleep regulation. Then, only proteases expressed in mammals were selected and the check performed in the other sense, meaning verifying which enzymes are reported to hydrolyze each specific substrate.

Table 1 shows the substrates and correlates to the identified proteases that can metabolize neuropeptides involved in regulating the sleep-wake cycle. These activities could, potentially, be altered during and after sleep deprivation. We have indicated the class of proteases, active or inactive fragments generated and experimental condition (*in vivo* or *in vitro*). The majority of described peptidases cleave the neuropeptides *in vitro*. Where there is no indication of the EC number it means that the International Union of Biochemistry and Molecular Biology (IUBMB) has not yet adopted the presented name of the protease as the official name.

It is a logical concept that the normal sleep depends, among many other factors, on the correct functioning of proteases, at the right time and right place, activating and inactivating neuropeptides. Many physiological changes observed in experimental models of sleep deprivation or in clinical observations, could be explained by changes in the generation or metabolism of biologically active peptides, where the role of proteases is essential. Many of these changes involve the cardiovascular system, the inflammatory responses of the immune system, food intake and energy balance, where peptides substrates of peptidases are involved. We should also keep in mind that we are far from reach the understanding of the complexity of different proteases acting on the same substrate or, sequentially, on its fragments. For example, as stated before, It has recently been shown that POMC is metabolized by convertases PC1/3 and PC2, but cathepsin L knockout mice had between 4 to 10-fold decreased production of dynorphin A[28,29]. Downstream, TOP is able to transform dynorphins in enkephalins and enkephalins can be inactivated by enkephalinases like NEP (EC 3.4.24.11), aminopeptidase N (EC. 3.4.11.2) and ACE[30,31]. This is a quite simple example, among many other possibilities, of points where the opioid metabolism can be altered.

Another level of complexity can be added to the potential importance of proteases and peptides in sleep related pathologies when sleep apnea and consequent hypoxia are considered. As pointed out in many studies neurocognitive dysfunctions can be associated to sleep quality and sleep apnea[32]. Beta-secretase (BACE-1), neprilysin, insulin-degrading enzyme (IDE) and angiotensin I-converting enzyme (ACE) are proteolytic enzymes involved in the equilibrium of soluble or insoluble beta-amyloid production[33-35] and, at least neprylisin seems to have its expression down-regulated by hypoxic conditions[36].

We have been interested in the consequences of sleep deprivation on proteolytic activity in the central nervous system, focusing on metallopeptidases like ACE, TOP and NEP. Our recent results should bring original contributions to the understanding of these dynamics in sleep deprivation and we hope this brief review, far from being exhaustive, will bring the attention of other research groups to this subject.

Table 1. Proteases that can metabolize neuropeptides involved in regulating the sleep-wake cycle.

Neuropeptides	Proteases	Catalytic type	Experimental	Fragments	References
Neuropeptide Y (NPY)	Dipeptidyl peptidase 8	Serine	*in vitro*	NPY(3-36), active	[37]
	Dipeptidyl peptidase 9	Serine	*in vitro*	NPY(3-36), active	[37]
	Plasma kallikrein (EC 3.4.21.34)	Serine	*in vitro*	NPY(3-35), inactive	[22]
	Dipeptidyl peptidase IV (EC 3.4.14.5)	Serine	*in vitro*	NPY(3-36), active	[22]
	Proprotein convertase 1 (EC 3.4.21.93)	Serine	*in vitro*	Pro-NPY (1-39), precursor of the NPY (inactive)	[38]
	Proprotein convertase 2 (EC 3.4.21.94)	Serine	*in vitro*	Pro-NPY (1-39), precursor of the NPY (inactive)	[38]
Somatostatin - 14	Calpain-1 (EC 3.4.22.52)	Cysteine	*in vitro*	peptide-Thr + Phe-peptide (inactive)	[39]
	Insulysin (EC 3.4.24.56)	Metallo	*in vitro*	peptide-Phe + Phe-peptide (inactive)	[40]
	Nardilysin (EC 3.4.24.61)	Metallo	*in vitro*	peptide-Cys^3 + Lys^4-peptide (inactive) peptide-Phe^6 + Phe^7-peptide (inactive) peptide-Phe^7 + Trp^8-peptide (inactive)	[41]
Somatostatin - 28	Nardilysin (EC 3.4.24.61)	Metallo	*in vitro*	peptide-Arg^{13} + Lys^{14}-peptide (active)	[42]
Vasopressin	Prolyl oligopeptidase (EC 3.4.21.26)	Serine	*in vitro*	Cys-Tyr-Phe-Gln-Asn-Cys-Pro + Arg-Gly-NH2 (inactive)	[43]
	Cystinyl aminopeptidase (EC 3.4.11.3)	Metallo	*in vivo*	Cys + Tyr-peptide-NH2 (Inactive)	[44]

Corticotropin (ACTH)	Angiotensin-converting enzyme-1 (EC 3.4.15.1)	Metallo	*in vitro*	peptide-His^{143} + Phe-peptide (unknown) peptide-Phe^{144} + Arg-peptide (unknown) peptide-Gly^{147} + Lys-peptide (unknown)	[45]
	Neprilysin (EC 3.4.24.11)	Metallo	*in vitro*	Ser^{138} + Tyr-peptide (active) peptide-Met^{141} + Glu-peptide (unknown) peptide-Pro^{156} + Val-peptide (unknown) peptide-Lys^{158} + Val-peptide (unknown) peptide-Arg^{154} + Arg-peptide (unknown) peptide-Pro^{149} + Val-peptide (unknown) peptide-Asn^{162} + Gly-peptide(unknown) α-MSH (active)	[45]
Prolactin	Cathepsin D (EC 3.4.23.5)	Aspartic	*in vitro*	17(active); 16 (active); 15 (active); 11 Kda Prolactin (active)	[46]
	Matrix Metallopeptidase - 1 (EC 3.4.24.7)	Metallo	*in vitro*	17; 16; 12 Kda Prolactin (active)	[47]
	Matrix Metallopeptidase - 8 (EC 3.4.24.34)	Metallo	*in vitro*	17;16 Kda Prolactin (active)	[47]
	Matrix Metallopeptidase - 2 (EC 3.4.24.24)	Metallo	*in vitro*	17; 16; 12 Kda Prolactin (active)	[47]
	Matrix Metallopeptidase - 9 (EC 3.4.24.35)	Metallo	*in vitro*	17; 16; 12 Kda Prolactin (active)	[47]
	Matrix Metallopeptidase - 3 (EC 3.4.24.17)	Metallo	*in vitro*	17; 12 Kda Prolactin (active)	[47]
	Matrix Metallopeptidase - 13	Metallo	*in vitro*	17; 16 Kda Prolactin (active)	[47]
Pro-opiomelanocortin (POMC)	Proprotein convertase 1 (EC.3.4.21.93)	Serine	*in vitro* *in vivo*	ACTH; beta-lipotropin; beta-endorphin (actives)	[48]
	Cathepsin L (EC. 3.4.22.15)	Cysteine	*in vitro*	ACTH (active)	[49]
Dynorphin A (1-8)	Angiotensin-converting enzyme-1 (EC.3.4.15.1) human somatic isoform	Metallo	*in vitro*	Leu-enkephalin (active)	[50]
	Thimet oligopeptidase (EC 3.4.24.15)	Metallo	*in vitro*	Tyr-Gly-Gly^{209} + Phe-peptide (inactive) peptide-Leu^{211} + Arg-Arg-Ile (inactive)	[51]
	Neurolysin (EC 3.4.24.16)	Metallo	*in vitro*	Gly-Gly-Phe-Leu^{211} + Arg-Arg-Ile (inactive)	[52]
Dynorphin A (1-17)	Eupitrilysin	Metallo	*in vitro*	Peptide-Pro^{216} + Lys-peptide (active)	[53]
	Proprotein convertase 2 (EC.3.4.21.94)	Serine	*in vitro*	Peptide-Ile-Arg + Pro-Lys-Peptide (active, generates Dyn1-9)	[29]
Peptide β-amiloid (Aβ1-42)	Neprilysin (EC. 3.4.24.11)	Metallo	*in vivo* *in vitro*	peptide-Val^{689} + Phe-petide (inactive)	[35]
	Eupitrilysin	Metallo	*in vitro*	peptide-Phe^{691} + Ala-peptide (inactive) peptide-Leu^{705} + Met-peptide (inactive) peptide-Gln^{686} + Lys-peptide (inactive) peptide-Lys^{687} + Leu-peptide (inactive) peptide-Phe^{690} + Phe-peptide (inactive) peptide-Ala^{701} + Ile-peptide (inactive) peptide-Gly^{704} + Leu-peptide (inactive) peptide-Gly^{700} + Ala-peptide (inactive) peptide-Gly^{709} + Val-Val (inactive)	[54]
	Angiotensin converting enzyme-I (EC. 3.4.15.1)	Metallo	*in vivo* *in vitro*	peptide-Val^{711} + Ile-Ala (active, generates Aβ1-40)	[33]
	endothelin-converting enzyme 1 (EC. 3.4.24.71)	Metallo	*in vivo* *in vitro*	peptide-Glu^{674} + Phe-peptide (inactive)	[55]
	Insulysin (EC. 3.4.24.56)	Metallo	*in vitro*	peptide-Glu^{674} + Phe-peptide (inactive)	[34]
beta-endorphin	cathepsin D (EC. 3.4.23.5)	Aspartic	*in vitro*	peptide-Leu^{253} + Phe-peptide (active)	[56]
	cathepsin E (EC. 3.4.23.34)	Aspartic	*in vitro*	peptide-Leu^{253} + Phe-peptide (active)	[56]
	Eupitrilysin	Metallo	*in vitro*	peptide-Gly^{239} + Phe-peptide (active) peptide-Phe^{240} + Met-peptide (active) peptide-Val^{251} + Thr-peptide (active) peptide-Phe^{240} + Met-peptide (active) peptide-Gly^{239} + Phe-peptide (active)	[53]

Leu- enkephalin	carboxypeptidase A6	Metallo	*in vitro*	Tyr-Gly-Gly-Phe[178] + Leu (inactive)	([57])
	dipeptidyl-peptidase I (EC. 3.4.14.1)	Cysteine	*in vitro*	Tyr-Gly[233] + Gly-Phe-Leu (inactive)	([58])
Met-enkephalin	carboxypeptidase A6	Metallo	*in vitro*	Tyr-Gly-Gly-Phe[103] + Met (inactive) Tyr-Gly-Gly-Phe + Met (inactive) Tyr-Gly-Gly-Phe[103] + Met (inactive)	([57])
	carboxypeptidase A4	Metallo	*in vitro*	Tyr-Gly-Gly-Phe + Met (inactive)	([59])
Neurotensin	Cathepsin E (EC 3.4.23.34)	Aspartic	*in vitro*	peptide-Leu[147] + Lys-peptide (active) peptide-Leu[151] + Tyr-peptide (active) peptide-Leu[162] + Lys-peptide (active)	([56])
	Thimet Oligopeptidase (EC 3.4.24.15)	Metallo	*in vitro*	pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg + Arg-Pro-Tyr-Ile-Leu (inactive)	([60])
	Neurolysin (EC 3.4.24.16)	Metallo	*in vitro*	pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro + Tyr-Ile-Leu (inactive)	([61])
Substance P	Neprilysin (EC 3.4.24.11)	Metallo	*in vitro*	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly + Leu-Met-NH2 (inactive)	([62])
	Angiotensin-converting enzyme (3.4.15.1)	Metallo	*in vitro*	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe + Gly-Leu-Met-NH2 (inactive)	([63])

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