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Review

CSF markers in sleep neurobiology

Jose E. Martínez-Rodríguez *, Joan Santamaria

Neurology Service, Hospital Clínic de Barcelona and Institut d'Investigació Biomédica August Pi i Sunyer (IDIBAPS), C/Villarroel 170, Barcelona 08036, Spain

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Abstract

The cerebrospinal fluid has been used in the study of normal and pathological conditions of the central nervous system for more than a century. CSF analysis has also been applied to the study of sleep and its disorders but methodological aspects have often limited the results. The discovery of the hypocretin system (also known as orexin system) and its involvement in the pathophysiology of narcolepsy has opened a new field in the diagnosis of hypersomnia by CSF analysis and has revived the interest on this subject in sleep medicine. Older and new lines of research involving CSF measurement of hypocretin and other neurotransmitters in sleep and its disorders are reviewed.

Keywords: Cerebrospinal fluid; Sleep; Hypocretin; Orexin

Contents

* Corresponding author. Tel.: +34 93 227 5413; fax: +34 93 227 5783.
E-mail address: 33029jmr@comb.es (J.E. Martínez-Rodríguez).

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1. Introduction

Sleep is a physiologic state of the brain characterized by a periodic and reversible loss of consciousness. Sleep is not merely a passive state but requires a complex and fine regulation performed mainly by the brainstem and diencephalic structures [1]. The function of normal sleep is of crucial importance for normal life and disorders of sleep may cause an important morbidity and decrease in the quality of life in humans. However, at the beginning of the XXI century, the exact function of sleep remains unknown.

The cerebrospinal fluid (CSF) has several physiologic functions such as physical support of the central nervous system (CNS) structures, regulation of the intracranial pressure, control of the chemical CNS environment, and transport of nutrients, neurotransmitters and neuromodulators along the neuroaxis [2]. The CSF can be considered as a mirror of the external neuronal environment due to the anatomical continuity with the brain extracellular space, providing information of the functionality of many neuronal systems [3]. All these properties make the CSF analysis and interesting research field in the neurobiology of several neurological conditions and a useful tool in diagnosis of CNS disorders such as infections, inflammatory and neurodegenerative processes. Chemical CSF analysis has also been applied to the field of sleep and its disorders, and recently, CSF hypocretin measurement is becoming a useful test in the differential diagnosis of hypersomnias. In this work, we will review the neurobiology of specific CSF markers that can play a role in sleep physiology and their dysfunction in human sleep disorders.

2. The sleep/wake cycle

Human sleep is consolidated in a monophasic form in accordance to the decrease of solar light exposure at night. The behavioral state of sleep is composed by two different phases regulated by an ultradian cycle along the sleep: NREM and REM sleep. NREM is characterized by synchronization of the thalamocortical projections resulting in delta EEG activity, and REM sleep by EEG desynchronization, rapid eye movements and a decrease in EMG activity [4].

Sleep is regulated by homeostatic and circadian factors. The two-process model of sleep regulation described by Borbély [5] proposes that the sleep/wake dependent homeostatic process (process S) increases sleepiness exponentially along the wake period and decrease progressively during sleep. The sleep/wake independent circadian process (process C) counteracts the increasing propensity to sleep of process S along the wake period. At night, process C decreases and allows the beginning of sleep. The suprachiasmatic nucleus, the brain clock-master, is entrained by the light/dark cycle and, by positive and negative feedbacks in the protein expression of circadian genes, accounts for the main circadian control that orchestrate the sleep/wake cycle [4]. Control of sleep requires integration between the circadian and homeostatic factors. This integration is performed in a “bipolar” model by the hypothalamus to give origin to the sleep/wake cycle [1]. Since the first descriptions of van Economo of patients with encephalitis lethargica epidemica and hypothalamic lesions [6], it has been observed that the anterior hypothalamus has a sleep-promoting function and the posterior hypothalamus a wake-promoting function.

Several groups of neurons with different neurotransmitters distributed in the brainstem and diencephalus are playing a role in the sleep/wake control [4]. The main neural systems are placed in the ventrolateral posterior area (VLPA) of the preoptic hypothalamus (GABA and galanin), the tuberomammillary region of the posterior hypothalamus (histamine), the perifornical area in the posterior hypothalamus (hypocretin), the dorsal raphe nuclei of the brainstem (serotonine), midbrain dopaminergic neurons, the locus coeruleus in the pons (noradrenaline), and the laterodorsal tegmental area and pedunculopontine nuclei in the pons (acetylcholine). Briefly, during wakefulness, noradrenergic, serotoninergic, histaminergic and cholinergic activity are high and decrease progressively during NREM sleep. In REM sleep, noradrenergic, histaminergic and serotoninergic activity are almost silent but cholinergic tone is high [4,7]. VLPA neurons, using inhibitory GABA and galanin, are sleep-active and project to wake-promoting regions of the pos-

terior hypothalamus, such as the tuberomammilary nuclei (histamine) and perifornical area (hypocretin), and brainstem [8,9]. The hypocretinergic activity could work in the consolidation of the wake period avoiding sudden transitions between wakefulness and sleep [8].

3. Anatomy and physiology of the CSF system

CSF is mainly produced in the choroid plexus of the ventricles by passive diffusion from the blood and also by active transport. Tight junctions in the apical borders of choroidal epithelium make a controlled

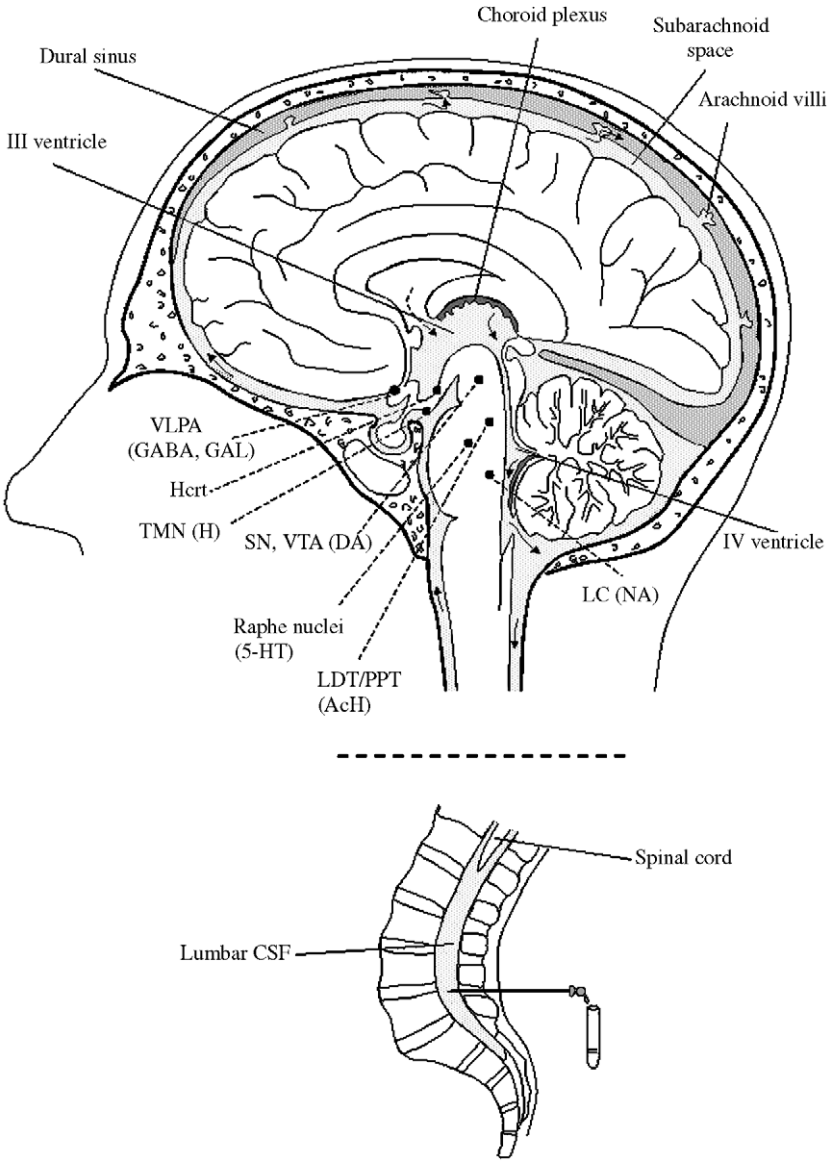

Fig. 1. Main neurotransmitters systems involved in sleep/wake regulation and CSF. Arrows show CSF circulation along ventricular and subarachnoid space. Lumbar cistern CSF and usual place of a lumbar puncture performance are indicated at the bottom of the figure. VLPA: ventrolateral posterior area, GABA: gamma-animo butyric acid, GAL: galanina, Hcrt: hypocretin system, TMN: tuberomammilary nuclei, H: histamine, SN: substantia nigra, VTA: ventral tegmental area, DA: dopamine, 5-HT: serotonine, LDT/PPT: laterodorsal tegmental area and pedunculopontine nuclei, Ach: acetylcholine, LC: locus coeruleus, NA: noradrenaline.

barrier between the CSF and the blood. CSF is 99% water, relatively acellular and, in comparison with plasma, has a higher concentration of chloride and magnesium ions and a lower concentration of glucose, proteins, potassium and urea. The total volume of CSF in a normal adult human is about 140 mL (30 mL in the ventricular cavities and 110 mL in the subarachnoid space). 30 mL of the subarachnoid CSF surrounds the spinal cord. The average rate of CSF production is 500 mL/day, or 0.35 mL/min. Thus, CSF is completely renewed every 5–7 h [2].

The CSF flows from the lateral ventricles through the third by the foramina of Monro and to the fourth ventricle by the aqueduct of Sylvius. The lateral foramens of Luschka and the medial of Magendie in the fourth ventricle allow CSF to access the basal cisterns and the subarachnoid space over the cerebral hemispheres and spinal cord. The CSF leaves the CNS by drainage into the venous blood of the dural sinuses across the arachnoid villi (Fig. 1). The circulation of CSF has been termed as the "third circulation", comparable with the blood and lymph. CSF circulation is promoted by hydrostatic pressure gradients between ventricular and subarachnoid space and the dural sinus and by arterial pulsations through the choroidal vasculature.

The CSF is in a protected compartment in relatively free equilibrium with the interstitial fluid that baths the parenchymal cells of the CNS through the ependyme in the ventricles and the pia-glial membrane in the subarachnoid system. Thus, CSF analysis may proportionate a reproducible, relatively non-invasive and easy to perform assessment of the CNS and its environment [2,3], providing useful information of the functionality of many neuronal systems in normal and pathological sleep. In this way, a neurotransmitter/neuromodulator release from the brain to the CSF might not have a merely passive presence. The characteristics of CSF physiology might proportionate to some neurotransmitters the way to work in intercellular communication in an endocrine-like volume transmission [2,3,10–13]. The hypothesis of the CSF working as a medium for neuroendocrine transmission was first postulated by Cushing and Goetsch in 1910, suggesting a release of active substances from the posterior hypothalamus to the third ventricle and its distribution through the CNS [14]. Many neuronal system terminals, such as the serotoninergic and hypocretinergic, are in close proximity with ependymal cells and the CSF [10,15]. Hypothetically, this kind of neurotransmission might have a role in the regulation of the sleep/wake cycle since it can work in a slow and long-term modulation over diverse and widespread systems producing a sustained function that may collaborate in the maintenance of the wake and sleep phases [3].

4. CSF and sleep

4.1. History of CSF analysis

Classic anatomists believed that the CSF was derived from a vaporous humor that was produced in the cerebral ventricles and, after death or in some pathological states, this fluid condensed in water. The work by Cotugno in 1761 proposed that CSF filled ventricular and subarachnoid spaces in life organisms [16]. Approaches to obtain CSF started with Magendie in 1825 that performed the first cisternal puncture, and Quincke in 1891 carried out a lumbar puncture to obtain CSF [17]. Mestrezat in 1912 made the first correlations between chemical changes in CSF and pathological processes [18]. CSF became a usual analysis in clinical practice after the reference work by Merritt and Fremont-Smith in 1937 establishing the CSF changes in disease [19].

The presence of a CSF hypnogenic factor was first hypothesized at the beginning of the XX century [20,21] and later revaluated by Schnedorf and Ivy [22]. CSF from sleep-deprived dogs was administered by ventricular infusion to wake animals causing sleep in the following hours. Pappenheimer was the first to obtain convincing evidences for a transferable factor obtained from sleep-deprived goats that was called factor S [23]. A REM-promoting factor was also investigated by the group of Jouvet. CSF obtained of REM-sleep deprived donors restored REM sleep in insomniac-animals due to pretreatment with *p*-chlorophenylalanine, a potent suppressor of serotonin biosynthesis [24]. Interest in the search for a sleep-promoting factor was concomitantly expanded to the extraction from brain extracts, blood and urine. However, identification of sleep-promoting substances is difficult and controversial and the exact physiologic

role in sleep of many constituents isolated from these experiments is not well determined [5].

Since the 1960s, newly developed assays allowed to measure CSF levels of monoamine metabolites as a reflection of central metabolism. These approaches were applied to the field of psychiatric and neurological disorders and, later, used in the research of the pathophysiology of hypersomnias. Methodological aspects of these experiments often limited the interpretation of the results. However, after the discovery of the hypocretin system in 1998 and the finding by Nishino in 2000 of undetectable CSF Hypocretin-1 (Hcrt-1) levels in human narcolepsy [25], CSF analysis has acquired a new role in sleep clinical practice as a helpful tool in the diagnosis of this disorder.

4.2. General aspects of CSF analysis

In clinical practice, the most common method of CSF analysis is to obtain CSF from the spinal subarachnoid space by a lumbar puncture. However, the most frequent adverse effect of this method is the post-lumbar puncture headache. This headache is due to intracranial hypotension and it is mainly related with CSF leakage into the extradural space through lumbar dural defects after the puncture. The use of atraumatic needles, with a small diameter, a lateral opening and a closed end, separate the dural fibers rather than cutting them and may decrease the incidence of headache by reducing the damage in the dura mater [26].

CSF analysis is not free of methodological limitations. The complex processes involved in the sleep/wake cycle can not be simplified in a quantitative analysis of the measurement of a particular substance in the CSF and multiple variables should be considered when designing the study [27]. There are some possible "pitfalls" that should be taken into account when a CSF study is performed.

First of all, it is important in any assay to precise what is being biologically and chemically measured. Quantization of substances may be subjected to nonspecific factors in the assay that can alter the binding of the ligand to be measured and give a nonspecific result. Range of detection and correlation with other quantitative methods should be kept in mind. In the particular case of hypocretin analysis, the current method of measurement by radioimmunoassay has a high variability interassay that makes necessary to include reference CSF samples with different known concentrations as internal controls to obtain a reliable CSF value [28].

Clinical aspects are crucial when performing the study. The example of monoamines illustrates how many factors may be involved in the correct interpretation of an experiment. Age, sex, height and weight, diet, prior behavior and motor activity, body position at lumbar puncture, site of CSF obtaining, tapping time, sample contamination (i.e., blood), storage procedure, and even atmospheric pressure have been showed to interfere with CSF values [29–31]. Patients should be free from drugs that could alter neurotransmitter metabolism such as psychotropic, sedatives and stimulants.

The measurement of a neurotransmitter/neuromodulator in CSF does not mean that its value is a marker of the biological function of the system. CSF measurements are not useful in neurotransmitters working almost exclusively in the concrete spatiotemporal synaptic neurotransmission whose biological activity is not reflected in CSF concentrations. CSF measurements would be more useful in substances that could work in the modulatory volume transmission.

The concentration of a particular substance in the CSF may not reflect its concentration in the specific brain area of interest where the substance makes its function. Measurements of neurotransmitters and metabolites may be the result of an average output of multiple sources or predominate from a specific source, i.e. the spinal cord, making erroneous a lumbar CSF value if considered as a reflection of brain concentration. A partial or scarcely involvement of a neural system may not be reflected in CSF levels of its neurotransmitters which may remain normal. Additionally, normality of a substance or its metabolites does not mean that the system is not affected since the hypothetic dysfunction can be due to alteration in receptors.

CSF concentrations of a specific substance may be in relation to a specific sleep state or behavior. However, there can be a delay in the liberation of the substance from the brain tissue to the CSF that mitigates the correlation of the CSF level with a specific sleep state at the time of evaluation [32]. Additionally, circadian variations are usual in the CSF levels of

many substances requiring a control of the time of CSF obtaining [32].

The highest CSF concentrations of a substance are close to the brain structures where it is mainly produced [2,27]. This may create a rostrocaudal gradient between ventricular, cisternal and lumbar CSF that can be observed in some substances such as monoamines [33,34] and may explain different CSF concentration from the first to the last sample obtained by a lumbar puncture. Additionally, changes in the permeability of the blood–brain barrier might also influence CSF levels. Knowledge of the normal CSF circulation, distribution and metabolism of a substance in the CSF should be interpreted in the results.

4.3. CSF and neuronal systems involved in sleep regulation

Some of the neurotransmitters involved in sleep regulation may be identified in CSF. However, at present, CSF Hcrt-1 measurement is the only CSF marker that has shown a clinical utility in sleep medicine. Other CSF markers of neuronal systems remain in the research field of sleep physiology and its disorders.

4.3.1. The hypocretin system

The discovery of the hypocretin system [35,36] has expanded the knowledge of the hypothalamic function in sleep regulation and has provided a useful marker for narcolepsy, an emblematic disease in sleep medicine. The hypocretinergic activity has been related with wakefulness and motor activity [37–40]. The system is located in the perifornical area of the posterior hypothalamus and is formed by a group of neurons with widespread excitatory projections through the CNS, mainly to areas involved in sleep/wake regulation such as monoaminergic and cholinergic systems. There are two neurotransmitters, hypocretin 1 and 2 (Hcrt-1 and Hcrt-2), derived from a common precursor, the preprohypocretin. Hcrt-1 is a peptide of 33 amino acids and two intra-chain disulphide bonds, and Hcrt-2 is a 28 amino acid peptide [35,36]. Two receptors, Hcrtr1 and Hcrtr2, are superposed by the CNS in an overlapped form [41].

Hcrt-1 is more stable than Hcrt-2 in CSF and can be reliably measured in crude or extracted CSF by radioimmunoassay, and also in brain tissue, but it is not consistently measured in plasma [42]. CSF Hcrt-1 levels are independent of age and gender, stable during long storage periods and after repeatedly sample thawing and freezing, and have no evidence of CSF concentration gradient in normal human subjects [43,44]. CSF Hcrt-1 levels were correlated with the hypocretinergic neural population in the rat hypothalamus showing a 50% decrease in CSF Hcrt-1 when hypocretinergic neurons were reduced to 73% [45]. Based on this study, it can be argued that normal levels of CSF hypocretin do not imply a normal hypocretinergic population since residual surviving neurons could maintain normal levels by increasing the hypocretinergic production. However, in cases of undetectable CSF Hcrt-1 such as in narcoleptic patients, it is hypothesized that all hypocretinergic neurons are lost or minimally present.

Hypocretinergic activity has strong circadian variations in the rat hypothalamus [37,46]. Circadian variations may also be observed in CSF hypocretin levels across a 24-h period. Hypocretin levels were high in rat CSF during the active period (the dark phase) and decrease by 40% at the end of the rest period (light phase) [47]. In squirrel monkeys, primates with a consolidated sleep in a single episode like humans, CSF hypocretin-1 peaks in the latter third of the active period, with lowest levels at the wake time that progressively increased through the active period [48]. In humans, a similar diurnal variation is suggested with a CSF Hcrt-1 increase around 10% in the late evening [49]. Direct connections between the supraquiasmatic nuclei and hypocretin neurons in the posterior hypothalamus account for the main circadian variation in their activity [50]. Suprachiasmatic lesions in rats eliminate the daily fluctuation of CSF hypocretin-1 levels [51,52]. CSF levels can increase by sleep deprivation [37,52,53] and by forced wakefulness [48], explaining an additional homeostatic component helping in wakefulness consolidation. Furthermore, locomotor activity can have an important influence on hypocretinergic activity [38–40,51].

4.3.1.1. CSF hypocretin measurements in sleep disorders

Narcolepsy and hypersomnias. The main clinical application of CSF Hcrt-1 determination is in the differential diagnosis of narcolepsy and hypersom-

nias. Narcolepsy is characterised by excessive diurnal somnolence (EDS) and abnormal manifestations of REM sleep such as cataplexy, sleep paralysis and hypnagogic hallucinations [54]. Cataplexy is a sudden loss of muscle tone usually evoked by emotions that is almost pathognomonic of this disorder. Narcolepsy is usually a sporadic disease, although there are familial cases and a single report of atypical narcolepsy with a mutation in the preprohypocretin gene [55]. Patients with narcolepsy have a strong association with the HLA DQB1*0602 suggesting a probable autoimmune mechanism [54]. At present, narcolepsy is diagnosed based on the presence of EDS plus cataplexy or at least 2 REM sleep onsets (SOREMs) in the Mean Sleep Latency Test (MSLT) [56]. However, the presence of cataplexy is often not easily recognized in clinical practice. Up to 15% of narcoleptic subjects do not present SOREMs and, moreover, the presence of SOREMs may be found in other sleep disorders [57].

The hypocretin system was first shown to be altered in narcolepsy in the canine and after in the murine model of the disease [58,59]. In a few pathologic studies in narcoleptic humans, a selective loss of hypocretinergic neurons in the hypothalamus is found [55,60]. In addition, undetectable CSF hypocretin-1 levels (under 40 pg/mL) occur in most narcoleptic patients [25,61]. Levels are not significantly influenced by the duration of the disease or by psychotropic medications [61], making this test useful in patients taking drugs that can alter the MSLT results. Most narcoleptic patients with typical cataplexy and HLA positive are hypocretin-deficient. Usually, patients with narcolepsy without cataplexy and idiopathic hypersomnia have normal CSF Hcrt-1 levels. However, some cases of typical narcolepsy-cataplexy, HLA-negative patients and many familial cases have normal levels [61,62], suggesting that other factors may be implicated in the pathophysiology of the disease. The view of narcolepsy as a syndrome with different pathophysiologies is also suggested by CSF Hcrt-1 studies in canine narcolepsy. Sporadic narcoleptic dogs have undetectable hypocretin levels in a similar way to sporadic human narcolepsy, but levels were normal in the narcoleptic canine model due to mutations in the hypocretin receptor 2 [63].

In an appropriate clinical setting, CSF Hcrt-1 measurement can be considered as a biological marker of narcolepsy with a specificity of 99% and a sensitivity of 87% [61]. CSF Hcrt-1 levels lower than 110 pg/mL are considered in the low range and suggestive of narcolepsy. Above 200 pg/mL are in the normal range and values between 110 and 200 pg/mL are considered intermediate and usually of undetermined clinical significance [61]. Clinical indications for CSF Hcrt-1 determination in the diagnosis of hypersomnias has been recently reviewed [64].

Neurodegenerative disorders. CSF Hcrt-1 levels have been found decreased in some neurodegenerative diseases. This fact should be interpreted with caution before to assume a direct hypothalamic involvement in the absence of neuropathological studies that confirm the involvement of the hypocretin system since decreased CSF levels, mainly in the intermediate range, may be a nonspecific finding [65].

***Alpha-synucleopathies**: Parkinson's disease (PD), diffuse Lewy body disease and multiple system atrophy form the spectrum of the alpha-synucleopathies, sharing in common abnormalities in the citoplasmatic protein alpha-synucleine. The hypothalamus and brainstem may be affected in the degenerative process of these disorders [66] and, hypothetically, might account for some of the sleep complains observed with a different prevalence in these diseases. PD is characterized by a main degeneration of the brainstem dopaminergic system in the substantia nigra. Patients complain of EDS very often in the course of the disease and in relation with the use of levodopa and dopaminergic agonist [67]. In diffuse Lewy body disease there are fluctuations of the level of consciousness and hallucinations. No abnormalities were found in CSF Hcrt-1 levels of patients with PD [44,68,69] and dementia with diffuse Lewy body disease [28]. The description of some patients with advanced PD and low CSF ventricular Hcrt-1 levels suggests a possible involvement of the system along the progression of the disease but needs further evaluation [70].

***Myotonic dystrophy**: this is an autosomal dominant disorder characterized by a progressive multisystem involvement with myotonia, muscle weakness, intellectual impairment, heart abnormalities and endocrine disturbances. ESD is very often reported and its physiopathology remains poorly understood. CSF Hcrt-1 levels were measured in six patients with myotonic dystrophy type I and ESD and values

were found in the low-moderate range [71]. However, levels did not correlate with disease severity and polysomnographic sleep features.

***Niemann-Pick disease type C** is an autosomal recessive lipid storage disorder due to a mutation in the Niemann-Pick C1 (NPC1) gene, less often NPC2, that it is hypothesized to regulate the intracellular transport of low density lipoproteins. Many tissues are altered by accumulation of unesterified cholesterol and sphingolipids resulting in a variable clinical phenotype. This disorder shares with narcolepsy the presence of cataplexy in their clinical picture. CSF hypocretin-1 levels were found in the intermediate range in some patients with and without cataplexy [72,73].

Autoinmune disorders. ***Guillain-Barre syndrome (GBS)**: GBS is an acute demyelinating polyradiculitis with an autoimmune etiology that has been related with previous viral and campylobacter jejuni infections. EDS has been reported in some cases of GBS [74,75] that could be explained in the setting of CNS involvement. In fact, CNS involvement in GBS has been reported as inappropriate secretion of antidiuretic hormone [76], descriptions of visual hallucinations [77] and brainstem white matter inflammation [78,79]. Interestingly, a few Japanese cases of severe GBS have been described with undetectable or low-moderate CSF Hcrt-1 levels at disease onset [75]. In some cases, CSF Hcrt-1 levels returned to normal values after a few months. At present, there are no neuropathological studies of the hypocretin system in GBS and it is not known if the low levels are due to a direct injury on the hypocretinergic neurons or to other factors such as blood–brain barrier dysfunction during the course of the process [44].

***Paraneoplastic disorders:** the anti-Ma2 encephalitis is a paraneoplastic syndrome usually associated with germ-cell tumor of the testis that shares with narcolepsy the presence of ESD and cataplexy in the clinical picture. CSF Hcrt-1 levels have been found undetectable in four of six patients [80]. An immune-mediated hypocretinergic dysfunction has been suggested in this syndrome that could resemble the hypothetic autoimmune damage of narcolepsy.

Hypocretin system in other diseases. ***Restless legs syndrome** (RLS) is one of the most prevalent sleep disorders (5–15% in the general population) [81]. RLS is characterized by an unpleasant feeling in the legs with a circadian worsening at night that characteristically is induced by rest and alleviated with movement. Insomnia and sleep disruption may occur secondarily in these patients. RLS pathophysiology has been related to iron deficiency and a dopaminergic hypofunction. Typically, clinical complains improved with levodopa and dopaminergic agonist. CSF analysis has contributed to the knowledge of the RLS pathophysiology showing abnormalities in the central iron metabolism. Despite the normal serum levels of iron-related proteins, CSF ferritin (an iron storage protein) is decrease and CSF transferrin (an iron transport protein) is increased in RLS, suggesting an alteration in the blood–brain barrier iron transport mechanism and a reduction in the brain iron stores [82,83]. Tyrosine hydroxylase, the rate-limiting enzyme in the production of dopamine, requires iron as a cofactor and its deficiency may impair the dopamine production. A possible relation to the circadian variation in the activity of this enzyme might account for the clinical worsening at night [67,82].

The hypocretin system has been also related with RLS in a hypothetic interaction with the dopaminergic system. A hypocretinergic hyperfunction was suggested by the finding of elevated CSF Hcrt-1 levels at late evening in patients with early-onset RLS, who usually have a familial aggregation in an autosomal dominant inherited pattern [81]. However, this result could not be replicated in a subsequent study, although CSF was obtained in the evening some hours before those in the previous study [84].

The involvement of the hypocretin system has been studied in other diseases in the last years. Undetectable levels have additionally been reported in single cases of Hashimoto's encephalopathy [85] and progressive supranuclear palsy [86]. There are reports of low-intermediate CSF Hcrt-1 levels in patients with Prader-Willi syndrome [61], the autosomal dominant cerebellar ataxia, deafness and narcolepsy syndrome [87], cranial trauma [44], CNS infections [44], stroke [61], some cases of multiple sclerosis in the setting of a probable hypothalamic involvement [88], acute disseminated encephalomyelitis [89], the Kleine-levin syndrome in the somnolence period [69], and in post-traumatic hypersomnia [69]. CSF levels were found normal in obstructive sleep apnea [61], fatal familial insomnia and Creutzfeldt-Jakob disease

[69,90], amyotrophic lateral sclerosis [44] and Alzheimer disease [44,69].

4.3.2. The monoaminergic systems

4.3.2.1. The serotoninergic system. Serotoninergic neurons are located in the brainstem raphe nuclei with a diffuse projection through the CNS. The observations reporting the effects of serotonin on sleep are often contradictories [91]. It has been hypothesized that serotonin has a possible role in the wake period as a component of the homeostatic process S, and also promotes the liberation of sleep-induced substances facilitating the initiation of slow-wave sleep [91]. In a single report, serotonin was reported to change in the CSF of human lateral ventricle along the ultradian cycle of REM and NREM sleep [92]. Serotonin showed a rapid decrease at the beginning of the REM sleep and a rapid increase with the subsequent NREM sleep. 5-Hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, may also be measured in CSF as a marker of the serotoninergic system activity [34], but lumbar CSF 5-HIAA levels have a dual origin from brain and spinal cord [27,33]. High cisternal CSF 5-HIAA levels have been related in primates with shorter sleep latency [93].

4.3.2.2. The noradrenergic system. The locus coeruleus is the main source of noradrenaline in the CNS with widespread projections along the brain. One of the main projections of the hypocretinergic system is the locus coeruleus, whose neurons bear HcrtR1. Modulation of noradrenergic activity is crucial for the maintenance of muscle tone in wakefulness. The activity of the locus coeruleus is almost absent in REM sleep and in cataplexy [7]. The anticataplexy drugs used in narcolepsy (tricyclic antidepressants and serotonin reuptake inhibitors) act by inhibition of the noradrenalin reuptake [54].

Ventricular CSF noradrenaline levels experiment circadian variations in primates with high concentrations during the light hours and low during the dark hours, but the main metabolites of noradrenaline, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and 3-methoxy-4-hydroxymandelic acid (VMA), did not show circadian variations [32,94]. The utility of lumbar CSF MHPG as a reflection of brain noradrenergic function is limited by its important synthesis in the spinal cord [33]. CSF noradrenaline levels can be increased by amphetamines for as long as 36 h after intake [94].

4.3.2.3. The dopaminergic system. Dopamine is playing an important but poorly understood role in the modulation of the sleep/wake cycle [67]. The main central sources of dopamine are the substantia nigra and the midbrain ventral tegmental area with projections to basal ganglia, limbic system, prefrontal cortex and thalamus. A rostral extension of the ventral tegmental area, the ventral periaqueductal gray area, was found to increase c-fos cell expression in the wake period and biochemical lesions increased sleep time [95]. Midbrain dopaminergic neurons might modulate the thalamocortical activity [96] and mediate part of the arousal behavioral components of hypocretin system [97]. Parkinson's disease, the hallmark of the neurological disorders with central dopamine system degeneration, developed ESD along the course of the disease. The common drugs used in the treatment of primary hypersomnias, such as amphetamines and modafinil, increase the synaptic levels of dopamine by their affinity for the dopamine active transporter and their wake-promoting effect is abolished in knock-out mice for the dopamine transporter gene [98]. In CSF, the dopaminergic system has been measured in sleep disorders predominantly by its metabolites, homovanillic acid (HVA), which has a considerable CSF caudocranial concentration gradient [27,33], and 3,4-dihydroxyphenylacetic (DOPAC).

4.3.2.4. CSF monoamines in sleep disorders. Most studies of monoamines and metabolites in sleep disorders come from the 70–80 s, sometimes with contradictories results. After the hypocretin discovery and revival of CSF analysis in sleep medicine, new studies are being performed on this subject. Readers should interpret the results taking into account the general aspects of CSF studies commented above.

Hypersomnias. The monoaminergic system is also implicated in the physiopathology of narcolepsy. Neuropathological studies show an altered brain monoaminergic neurotransmission in narcolepsy [99] and, as commented above, usual treatments used for this disease improve hypersomnia and cataplexy by

decreasing the reuptake of dopamine and noradrenaline. Low CSF levels of dopamine and serotonin metabolites were found in human narcolepsy and idiopathic hypersomnia [100,101]. In canine narcolepsy, dopamine, serotonin and its metabolites were low compared with normal dogs [102]. An increased dopamine turnover was suggested by oral probenecid administration, a weak organic acid that competitively inhibits the active transport of acidic metabolites from CSF, increasing the CSF accumulation of dopamine metabolites [103]. However, similar results can be found in posttraumatic and idiopathic hypersomnia suggesting that this may be a non-specific observation [101,103,104].

Restless legs syndrome. Involvement of monoamine systems were investigated by CSF analysis in patients with RLS showing normal levels of HVA and low levels of 5-HIAA [105]. However, a subsequent work did not show any significant difference in CSF concentrations of dopaminergic or serotoninergic metabolites [106]. The later study were performed at early evening, when symptoms are usually present, leading to conclude that circadian variations do not influence these results.

4.3.3. Prostaglandins

Prostaglandins are eicosanoids with several biologic actions including inmunological and neuromodulatory properties. The role of PGD_2 as a sleep-promoting substance has been extensively studied by Urade et al. [107]. PGD_2 is the most abundant prostaglandin in the central nervous system and it is produced in the brain by the enzyme lipocalin-type PGD_2 synthase localized in the rat in leptomeninges and choroids plexus, from where it is secreted to CSF to become the beta-trace protein, the second most abundant protein in CSF after albumin. Ventricular infusion of PGD_2 induces both NREM and REM sleep, and inhibition of L-PGDS by selenium compounds produce insomnia in rats in a time- and dose-dependent form [107]. PGD_2 act as a sleep-promoting substance predominantly at the ventral surface of the rostral basal forebrain in close proximity to the sleep-promoting VLPO area. After PGD_2 infusion into the subarachnoid space below the rostral basal forebrain, c-fos expression increases in the adjacent leptomeningeal cells and in neurons of the VLPA and decreases in the wake-active neurons of the tuberomammilari nuclei [108]. The somnogenic effect of PGD_2 could be mediated by adenosine by inducing its liberation from meningeal cells as a paracrin signaling molecule [109].

CSF PGD_2 and PGE_2 levels in rats show circadian variations across a 24-h period closely related to the sleep/wake state and higher levels have been correlated with a high sleep propensity [110]. In humans, high CSF PGD_2 levels were described in sleeping sickness [111]. Other clinical and CSF studies do not support a role of prostaglandins in sleep disorders such as that found in schizophrenics patients [112] or in narcoleptic dogs [113]. However, some considerations about the value of the measurement of prostaglandins in CSF should be taken in mind. Detection of CSF prostaglandin levels is subjected to individual variability and may differ between species and experimental conditions [113–115]. In normal human subjects, CSF prostaglandin levels are usually low and sometimes unstable, particularly PGD_2 [110, 112]. Moreover, CNS pathological process such as CNS injuries or infections may increase the CSF prostaglandin levels and modifies the results when trying to correlate it to a specific behavior or sleep/wake state [114].

5. Conclusions

CSF analysis is an accessible way of investigating the neurobiology of many disease of the CNS, and particularly, in sleep disorders. In the clinical setting, CSF analysis is becoming to be an additional exploratory assay in some patients with sleep disorders such as narcolepsy and hypersomnias. However, a correct selection of patients and potential methodological problems should be kept in mind when performing often time consuming and expensive studies. It is feasible that in the future new neurotransmitters and neuromodulators will be detected in the CSF and this will help to explain their role in normal and pathologic sleep.

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