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Short review

Sleep, genes and death: fatal familial insomnia

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Abstract

Over the past 30 years, significant progress has been made in understanding the physiologic mechanisms of sleep. Insomnia, a common complaint in general medical practice, and other sleep disorders have become increasingly recognized. In 1986, a heritable total insomnia was described and termed fatal familial insomnia; since then, the pathology of this disease has been shown to involve an accumulation of prion particles in the brains of affected patients. Prions have been more commonly associated with the transmission of spongiform encephalopathies such as scrapie (in sheep), Creutzfeldt–Jakob disease and Kuru. We briefly review the physiological and biochemical characteristics of normal sleep, describe the typical clinical characteristics of fatal familial insomnia and describe the current understanding of how prions cause neurodegenerative diseases, including fatal familial insomnia.

Keywords: Sleep; Neurodegenerative disease; Prion

Contents

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1. Introduction

The mystery of sleep has engaged minds of both literary and scientific orientation for all of history. The modern psychological study of sleep originated with Freud's publication of *The Interpretation of Dreams* in 1900. The physiologic study of sleep (reviewed in [7,8]) commenced in 1937 with the discovery of sleep stages characterized by specific electroencephalograph (EEG) patterns. In 1953, rapid-eye movement (REM) sleep was discovered, and by 1968, polysomnography (PSG), consisting of simultaneous recording of the EEG, electromyograph (EMG) and electrooculograph (EOG), was standardized for sleep scoring. These developments have revealed ways in which sleep differs from wakefulness, have led to a detailed understanding of the stages of sleep and have made it possible to explore disturbed sleep.

2. Physiologic characteristics of sleep

Neurophysiological and PSG studies have revealed a great deal about the physiology of sleep and descriptions of the basic PSG characteristics of the normal stages of non-REM (NREM) and REM sleep are available in many textbooks. Central nervous system regulation of sleep and wakefulness has been the subject of more recent investigation (reviewed in [25]). Neurons in the dorsal lateral geniculate nucleus, thalamus and brainstem are active during the stages of NREM sleep. Although forebrain mechanisms can regulate REM sleep, the neural circuits that generate it reside in the brainstem. Activity originating in the pons and moving to the lateral geniculate nucleus are found in many animals during REM sleep and have been linked to the eye movements characteristic of REM sleep. Activity of neurons in the pons, lateral geniculate nucleus, pontine reticular formation and midbrain reticular formation are important during REM sleep, while norepinephrine-containing neurons arising in the locus ceruleus, serotonin-containing neurons originating in the raphe system of the brainstem, and histamine-containing neurons located in the hypothalmus demonstrate decreased firing during REM sleep. Direct introduction of acetylcholine into the pontine reticular formation induces a state mimicking REM sleep, and destruction of cholinergic neurons projecting to the pontine reticular formation from the laterodorsal tegmental nucleus and pedunculopontine nucleus markedly reduces REM sleep. Many neurotransmitters and hormones have been implicated in the regulation of sleep and wakefulness, including uridine, interleukin 1, adenosine, prostaglandins, melatonin and γ-aminobutyric acid [8].

Despite great progress in understanding the brain regions and neurochemicals responsible for regulating sleep, the function of sleep remains unclear, although not for a lack of hypotheses [7]. Among these theories are the restorative theory that sleep is in some way essential for the restoration of body tissues, the energy conservation theory that decreased metabolism during sleep conserves limited resources, the memory reinforcement and consolidation theory that sleep is important in facilitating memory and learning, and the adaptive theory that sleep simply keeps vision-dependent creatures out of harm's way at night. Rechtschaffen and coworkers [43] showed that rats deprived of sleep for 10–30 days lost weight despite increased food intake, lost temperature regulation and eventually died. In humans, sleep deprivation of 10 days has not been shown to cause any permanent behavioral, psychological or physical problems [7].

Although such studies indicate that short term sleep deprivation does not cause any permanent damage, anecdotal reports have been made of patients with encephalitis who suffered from complete insomnia for long periods of time and whose disease process was fatal. In 1970, a French patient was documented by EEG not to sleep for 4 months; in total his insomnia lasted for 11 months and was resistant to classical sleep-inducing drugs (reported in [45]). He averaged only 35 min of sleep per day, most of it type 1 sleep, was unable to sleep even while lying on a bed in the dark (in contrast to sleep deprived subjects) and experienced nighttime hallucinations. High doses of serotonin (5 g/day) were temporarily able to induce almost 4 h of sleep per day with less than 20 min per night each of types 2, 3, 4 and REM sleep. The patient showed no decline in cognitive or behavioral function over his hospital course, which ended with his death. Although the maintenance of physical and mental health in these rare patients argues against theories that pose critical physiological or psychological functions for sleep, the terminal nature of such processes has suggested the fascinating possibility that in humans, the absence of sleep can be fatal.

3. Fatal familial insomnia

The clinical characteristics of fatal familial insomnia (FFI) appear at first glance to confirm this possibility. FFI, described by Lugaresi and coworkers in 1986 [23], is an autosomal dominant, progressive, fatal disease whose clinical course includes a set of early and late features [12,13,23,24,26,27,30,42,44]. Early symptoms and signs include insomnia, headaches, panic attacks, bizarre phobias, nocturnal somnabulance-like episodes, hallucinations, oneirism, enacted dreams, autonomic hyperactivity (impotence, sphincter impairment, fever, tachycardia, blurred vision, hyperhidrosis and increased lacrimation and salivation), ataxia, slurred speech and dysphagia. Later characteristics are insomnia, recurrence of fetal reflexes (Babinski, grasp and snout), myoclonus, tremor, breathing disorders (tachypnea, paradoxical breathing and apnea), dysarthria, astasia-abasia, apathy, weight loss, subcortical dementia, rigidity, dystonia, dyskinesia, bizarre gestures, mutism, stupor, coma and sudden death. The insomnia is

progressive and near-total and ends in death. Associated with insomnia is a disruption of circadian rhythms, evidenced by alterations in diurnal blood pressure variation [35] and secretion of hormones including cortisol [35], melatonin [36], somatotropin [37] and prolactin [37].

Two patterns of sleep abnormalities have been described in FFI patients, with a reduction in sleep spindles and K complexes, a common feature of both [46]. In patients with a disease duration of under 1 year [23,30,42,46], PSG shows total absence of normal sleep patterns but the presence of periods of under 1 min duration during which patients experienced REM sleep without atonia. In patients with a more prolonged course [30,44,46], the sleep disruption involves a progressive reduction in total sleep time, affecting both NREM and REM sleep, the latter completely absent late in the course. During NREM sleep there is a progressive decrease in spindle activity and increased θ-activity and the normal sleep cycles are completely altered, with rapid transitions between wakefulness and sleep and sudden changes in delta activity. Benzodiazepines and barbiturates fail to induce sleep in these patients, while benzodiazepine antagonists can arouse patients from the stuporous state observed late in the course [23,30]. In a recent report γ-hydroxybutyrate was shown to induce 30 min of REM sleep and 3 h of slow-wave sleep in a patient suffering from FFI; this response was associated with improvement in his mental status [44].

Neuropsychological studies of FFI patients demonstrate a progressive attention deficit and late impairment in vigilance, memory disturbances involving data manipulation but leaving semantic, retrograde and procedural memory intact, and relatively intact intellectual skills and appropriate social behavior [11,30]. Positron emission tomography demonstrates thalamic hypometabolism that is sometimes associated with generalized cortical hypometabolism [32]. This correlates with pathologic findings of thalamic degeneration and gliosis, sometimes in conjunction with cortical and cerebellar losses [13,23,24,31,44]. While these results indicate that the thalamus plays a more important role in sleep regulation than has been thought previously [22], they shed no light on the underlying mechanism and genetic basis for the disease. The first clue to the pathophysiology of FFI came from Manetto et al. [24], who reported one case in which spongiform degeneration of the cerebral cortex was noted; this finding is highly characteristic of Creutzfeldt–Jakob disease (CJD), a progressive neurodegenerative disease transmitted by the unconventional agents known as prions.

4. Prion disease and fatal familial insomnia

Prions have been implicated in the transmission of several neurodegenerative diseases in animals and humans, including bovine spongiform encephalopathy ('mad cow disease'), scrapie (a disease of sheep), CJD, Kuru (affecting women and children in New Guinea who practice ritual cannibalism) and Gerstmann–Sträussler–Scheinker syndrome (reviewed in [3,9,10,16,17,19,21,38–41,51]). Although most investigators subscribe to the prion hypothesis of transmissible spongiform encephalopathies as outlined below, some maintain, without any direct evidence, that a virus or virion is the true pathogenic agent [21].

The unusual nature of prions became clear when the scrapie agent was partially purified and found to be composed of protein with no evidence of nucleic acid; moreover, its infectivity was not altered by ultraviolet and ionizing radiation, nucleases, proteases, psoralens and hydroxylamine. Further purification of the scrapie agent led to the identification of the prion protein (PrP), a protease-resistant protein of molecular weight between twenty-seven and thirty kilodaltons (PrP 27–30) that was not present in brains of control animals. PrP 27–30 was found to be derived from a larger protein, PrP^{Sc}, by an N-terminal cleavage. A partial amino acid sequence of PrP was used to generate complimentary DNA molecular probes; molecular hybridization studies revealed that the PrP gene (*PRNP*) is present in host cells but not in the scrapie agent and that expression of the PrP gene, which is found in many cell types but is highest in neurons, is constant during scrapie infection. The *PRNP* gene product is an endogenous glycosylated protein of molecular weight between thirty-three and thirty-five kilodaltons (termed PrP^{C}), from which PrP^{Sc} is derived by post-translational modification. Identification of the *PRNP gene* led to the development of transgenic mice that provided crucial information about the nature of prion infections. Knockout mice missing both copies of *PRNP* develop normally and are resistant to scrapie, indicating that endogenous expression of PrP is critical for susceptibility to infection. In contrast, transgenic mice expressing the Syrian hamster PrP gene become susceptible to Syrian hamster prion, revealing that species-specific differences in infectivity are in some cases due to divergence of PrP sequences. Recent studies in transgenic mice have revealed that an endogenous cellular factor distinct from PrP may also play a role in species-specificity [49].

Although PrP^{C} and PrP^{Sc} share the identical amino acid sequence, there are significant differences between them: PrP^{C} is found on the cell surface and is released after treatment with phosphatidylinositol-specific phospholipase, is synthesized slowly and degraded rapidly by cells and can be completely digested by proteases, whereas PrP^{Sc} is found in endosomal vesicles and on the cell surface, is not released by phospholipase treatment, has a more rapid synthesis and slow degradation by cells and releases PrP 27–30 upon treatment with proteases [1,9,10,17,19,20,38,39,41]. In addition to being linked to the cell membrane by a glycosylphosphatidylinositol anchor, PrP also binds a cell surface proteoglycan [47]. Normal processing of PrP involves a single proteolytic cleavage with the subsequent release of an N-terminus

peptide [6,17]. The precise subcellular location at which the conversion PrP^C to PrP^{Sc} occurs is unknown, although the evidence suggests that the conversion occurs along the secretory pathway or, more likely, at the cell surface or after endocytosis of cell-surface PrP^C [17]. Additionally, the mechanism by which PrP^C is converted into PrP^{Sc} has remained unclear. The most likely model posits that exogenous PrP^{Sc} binds to endogenous PrP^C, inducing the conformational change that converts the endogenous PrP^C to PrP^{Sc} [38,51]; as PrP^C is converted, ever increasing amounts of PrP^{Sc} are formed and are able to convert still more PrP^C to PrP^{Sc}. This process appears to require the presence of an as yet unidentified cellular factor [49] (Fig. 1). The recent identification of proteoglycan binding sites for PrP [47] raises the possibility that the factor identified by Telling et al. [49] might be a cell-surface proteoglycan. The nature of the conformational change has been somewhat elucidated and involves the conversion of four α-helical regions to β-sheets (reviewed in [2,21]). Peptides containing these regions are able to induce the assumption

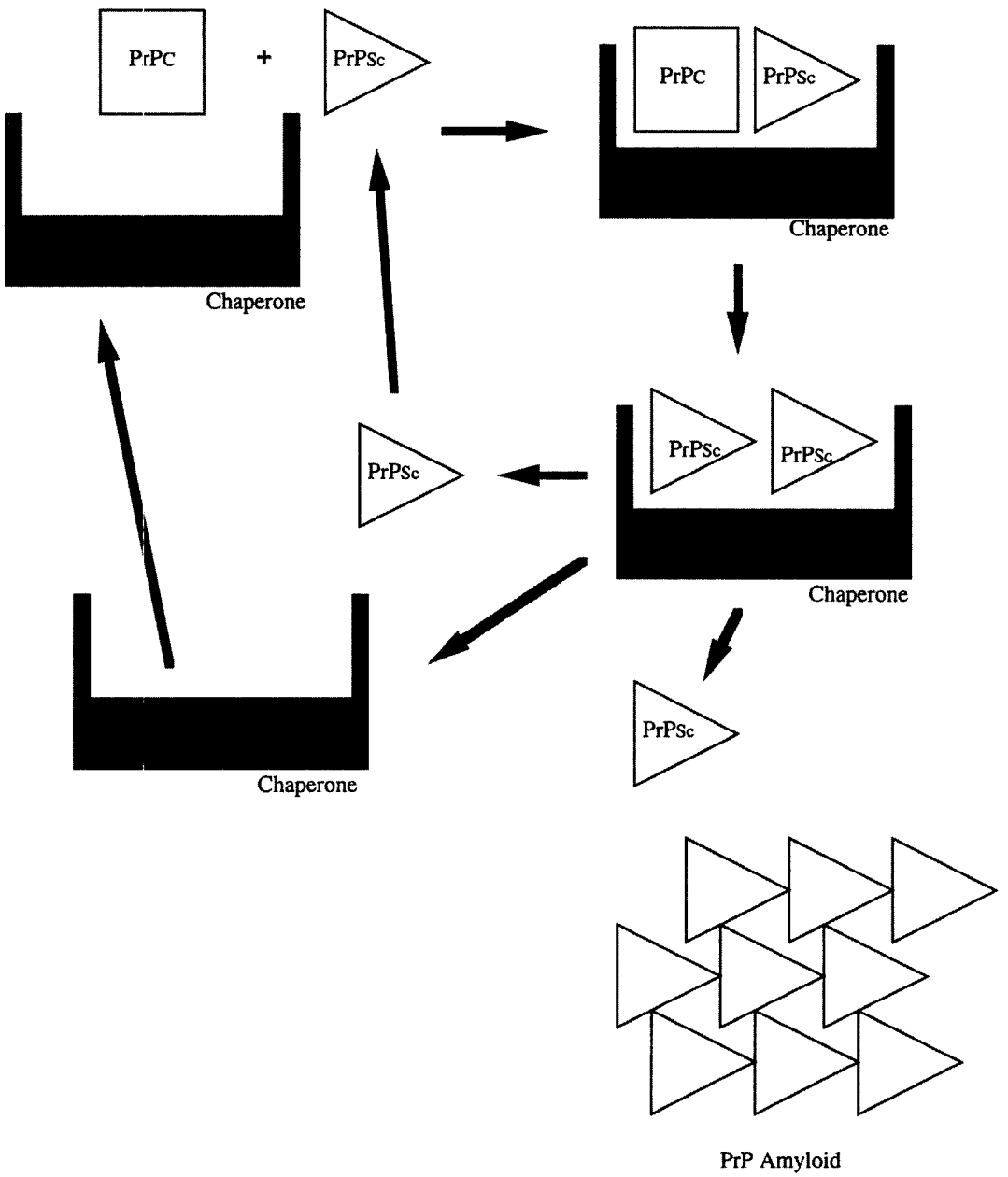

Fig. 1. Schematic diagram of proposed mechanism of prion protein deposition. Normal-conformation PrP^C is bound by the active, protease-resistant PrP^{Sc}, in addition to the proposed chaperone protein. PrP^{Sc}, in a β-sheet conformation, induces a conformation change in PrP^C in which α-helical regions become β-sheets, thus converting PrP^C to PrP^{Sc}. With each molecule of PrP^C converted to PrP^{Sc}, the effector of PrP^C conversion is increased. These events, which likely take place at the cell surface or in early endosomes, result in the deposition of a neurotoxic amyloid-like substance and in neuronal death.

of a β-sheet structure in other PrP peptides [2,18]. Interestingly, it is one of these α-helical regions that is cleaved during normal PrP processing [6,17]; thus, the degradation of PrP in non-infected cells prevents the formation of PrP^{Sc} in two ways, by blocking assumption of the β-sheet structure by PrP and by reducing the number of molecules available for conversion to PrP^{Sc}.

Because PrP^{Sc} is very slowly degraded, it likely accumulates within neurons and overwhelms them, causing cell death. By implanting PrP^{Sc}-producing tissue into the brains of PrP knockout mice, Bradner and coworkers [4] have shown that high levels of extracellular PrP^{Sc} alone, in the absence of endogenous PrP^{C}, do not induce neurodegeneration, supporting the idea that an intracellular process is responsible for neuronal death. The neuropathology characteristic of prion diseases —- spongiform degeneration, neuronal loss and reactive gliosis — usually occurs at sites of detectable PrP accumulation, although there may be areas of degeneration without detectable PrP and areas of PrP accumulation without degeneration [3,31].

The unique nature of prions as disease-causing agents is emphasized by the different forms of transmission taken by different prion diseases; for instance, CJD occurs in three distinct forms: sporadic (85%), familial (10%) and transmissible (5%) [10,38]. The isolation of the PrP gene led to the hypothesis that mutations in this gene might be responsible for inherited prion diseases, such as familial CJD and Gerstmann–Sträussler–Scheinker syndrome. Subsequently, 19 different mutations in the human PrP gene have been identified in families with inherited prion diseases [2,10,16,38,40]. These mutations in *PRNP* cause amino acid substitutions in or near the four α-helical regions [2] and make the mutant PrP inherently unstable and more likely to assume the PrP^{Sc} conformation; thus, instead of requiring exposure to exogenous PrP^{Sc} for the initiation of PrP^{C} conversion, disease-associated mutant PrP converts spontaneously, thus initiating the autocatalytic process that leads to neurodegeneration [19,39].

Against this background, Gambetti, Lugaresi and coworkers reported that the spongiform degeneration seen in some FFI patients was associated with protease-resistant PrP in the brain tissue of these patients [28]. Moreover, they identified in these patients a mutation in codon 178 of the PrP gene that results in the substitution of asparagine for aspartate (Asn^{178}). This mutation was later identified in other unrelated kindreds affected by FFI over multiple generations [24,26,27]. Others reported this mutation in unrelated families thought to be affected by familial CJD or thalamic dementia [14,29,33,44]. Some of these patients also suffered from sleep disturbances and later studies determined that FFI and familial CJD segregate with two PrP genotypes defined by the Asn^{178} mutation and a methionine/valine bimorphism at codon 129 [15]: all of the patients with FFI examined to date have a methionine at codon 129 (Met^{129}) of the *PRNP* allele containing Asn^{178}, whereas the CJD patients carry the valine mutation (Val^{129}). These and other studies raised the number of families known to be affected by FFI to near ten [12,13,44]. The two mutant PrP alleles give rise to two distinct PrP isoforms that differ both in glycosylation and in protease susceptibility, factors that are important in the differing neuropathology and clinical presentation of FFI and familial CJD [28,29]. Furthermore, homozygosity at codon 129 was associated with a more rapid disease course in both FFI and CJD patients and lower age of onset in CJD patients [15,31]. Additionally, other clinical characteristics of the two types of FFI presentations are related to the presence of homo- or heterozygosity at codon 129 [12]. Pathologic examination of homo- and heterozygotes revealed neuronal loss and gliosis in the thalamus and to a lesser extent in the brainstem and hypothalamus, to be common to both groups of FFI patients [31]. Heterozygotes, in whom a longer disease course presumably allows a greater accumulation of PrP^{Sc} at other sites, showed PrP^{Sc} accumulation in the neocortex and, if a threshold of PrP^{Sc} accumulation was exceeded, spongiosis and gliosis. A similar threshold phenomenon, without a distinction on the basis of disease duration, was observed in the limbic structures and caudate nucleus. Thus, the variable pathology in FFI patients results from regional differences in (1) the timing and rate of PrP^{Sc} accumulation and (2) the neuronal vulnerability to PrP^{Sc} [31]. Interestingly, a patient with familial CJD with a lysine mutation at codon 200 but lacking the Asn^{178} mutation whose clinical course included complete, sedative-resistant insomnia was recently reported [5], broadening the range of prion diseases associated with fatal insomnia.

5. Conclusion

The understanding of FFI, which initially seemed little more than a biological oddity but now is classed as a neurodegenerative disease transmitted by an agent part infectious and part genetic, exemplifies the elegant contributions to medicine made by molecular biology. Although the idea that sleep deprivation can be fatal is supported by the clinical picture of FFI, pathological and molecular biological studies point to prion-related thalamic neurodegeneration as the cause of insomnia. It has been suggested that PrP may normally play a role in sleep regulation because knockout mice devoid of PrP have altered circadian rhythms and sleep patterns [50]. However, insomnia is not a feature other inherited prion diseases, in which the function of PrP is likely to be similarly disrupted. Although the symptoms and progression of some FFI patients resemble those of Rechtschaffen's sleep-deprived rats, suggesting that the loss of sleep experienced by FFI patients may contribute to their inevitable demise, the fatal course of other prion diseases not distinguished by insomnia again emphasizes neurodegeneration as the deadly factor common to all the prion diseases. The study of FFI has placed

a new emphasis on the importance of the thalamus in sleep regulation, although questions regarding the relationship between thalamic degeneration and insomnia remain [34,48]. Future work may yield methods of disrupting prion replication, preventing the relentless, progressive and fatal neurodegeneration caused by these agents.

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