Journal of Hepatology 1998; **29**: 832–835
Printed in Denmark · All rights reserved
Munksgaard · Copenhagen

Journal of Hepatology
ISSN 0168-8278

Special Article

Abnormalities of circadian rhythmicity in liver disease

Andres T. Blei and Phyllis Zee

Departments of Medicine and Neurology, Lakeside VAMC and Northwestern University, Chicago, IL, USA

Under normal conditions, a wide range of biological processes exhibit a diurnal rhythm of approximately 24 h (the term *circadian* arises from the Latin, *circa*=approximately, *diem*=day). The most obvious rhythm is the one related to variations in the sleep/wake cycle, but almost all aspects of the internal environment undergo such changes over a 24-h period. This oscillation occurs in parallel to the daily alteration in external conditions that our rotatory planet imposes on all species. Thus, a teleological perspective of circadian function would posit that such internal organization allows adequate adaptation to the changing external environment. However, there is ample evidence to indicate that circadian rhythms arise from an internal time-keeping system that functions even in the absence of external cues (1). The existence of a "biological clock" with its afferent/efferent connections allows the organism to foresee and anticipate the modifications in the external environment that occur during the day/night cycle.

Abnormal rhythms of several biological parameters have been described in patients with cirrhosis. The robustness of these findings will be influenced by the frequency of recording, and few studies have utilized continuous or very frequent samples in patients with liver disease. Furthermore, a diurnal variation cannot be truly termed circadian until it is shown that the rhythm persists under conditions of a constant external environment. Still, most biological oscillations are shown to persist under controlled circumstances, and diurnal variations under natural conditions can be viewed as reflecting circadian processes. Reports of abnormal rhythmicity in liver disease include such diverse processes as the lack of diurnal variation of arterial pressure in continuous 24-h recordings (2), the blunted rhythm of several hormones, such as angiotensin and aldosterone (3), the nocturnal rise in portal pressure (4) and alterations of the rhythm of plasma melatonin (5) and sleep patterns (6) in patients with compensated cirrhosis. The clinical observations suggest a generalized abnormality of circadian function in cirrhosis. Our experimental observations suggest a complex pathogenesis for these abnormalities.

Correspondence: Andres T. Blei, Department of Medicine/111E, Lakeside VAMC, 333 E Huron St, Chicago, IL 60611, USA. Fax: 312–908–5998.

Circadian Dysfunction and Hepatic Encephalopathy

A common symptom of patients with early hepatic encephalopathy is the inability to sleep during the night, while exhibiting sleepiness during the daytime. It was logical then to examine whether experimental models of hepatic encephalopathy exhibit changes in circadian rhythmicity. It had been previously shown that rats after portacaval anastomosis, a model that reproduces some features of hepatic encephalopathy, exhibit alterations in the distribution of activity during the 24-h day (7). In our studies, we went one step further by choosing the rhythm of circadian locomotor activity to characterize potential abnormalities in circadian function. In this paradigm, animal cages include an attached wheel where rodents will initiate running activity during the nighttime hours. This behavioral rhythm can be easily measured for many weeks without disturbing the animal's environment via a computerized analysis of wheel revolutions.

Experiments in this model were performed under strict conditions of entrainment to the light/dark cycle and in a setting that allowed expression of "free-running" circadian rhythms (constant low level illumination). These animals exhibited a spectrum of abnormalities in circadian function, ranging from a blunted capacity to entrain to the light/dark (LD) stimulus to an absence of endogenous circadian rhythm of locomotor activity (8,9). We also observed that the abnormal entrainment to the L/D cycle is affected by the extent of portal-sytemic shunting (9) and is ameliorated by a low-protein diet (10), by the administration of neomycin (11) and is less conspicuous with a stenosed portacaval shunt (12). These interventions, known to improve an abnormal mental state in human liver disease, suggest that abnormalities of circadian func-

tion in liver disease may arise from neurochemical alterations seen in hepatic encephalopathy. Preliminary observations indicate that hyperammonemia in the absence of liver disease can result in abnormalities of circadian locomotor activity in the rat (13). Under this light, the derangement of circadian function could arise from the effects of neurotoxins implicated in hepatic encephalopathy on specialized areas of the brain.

Anatomical Considerations

The anatomical pathways that subserve the circadian timekeeping system are well defined (14) (Fig. 1): (a) The suprachiasmatic nucleus (SCN) of the anterior hypothalamus is the pacemaker, a "biological clock" that can generate "endogenous" self-sustained rhythms in the absence of external clues. (b) Afferent pathways, related to photic and non-photic stimuli, travel (in the case of the former) from the retina via the retinohypothalamic tract to the SCN. (c) Efferent pathways, a complex array of connections to other structures in the hypothalamus, thalamus and basal forebrain, link the SCN to different effector systems and neurohumoral connections.

Where could the abnormality lie in patients with liver disease?

Afferent pathways

Anatomical changes in the retina have been described in patients with cirrhosis (15). These are characterized by abnormalities in Müller cells, glial cells of the retina, that parallel the morphologic alterations in astrocytes seen throughout the brain (16). It has been postulated that these changes could add to deficiencies in vitamin A (17) and translate into functional abnormalities of phototransduction (18). However, it is unknown whether the subset of retinal cones that serve as photoreceptors for the circadian system may be affected.

Non-photic signals can influence circadian function in several vertebrate species. Changes in ambient temperature, periodic presentation of food and changes in physical activity/inactivity will affect the "biological clock" of rodents (19). In the case of humans, factors such as social cues, the sleep/wake state and physical exercise are important synchronizing agents (20). It is possible that the impact of chronic disease on general well-being may affect such non-photic stimuli in liver disease. However, when we compared the sleep patterns of patients with cirrhosis to those of another chronic ailment, chronic renal failure, we noted differences in the choice of bedtime and awakening between the two diseases (6). Nonetheless, the role of chronic disease in circadian dysfunction is still a distinct possibility and studies are needed to explore the role of non-photic signals in chronic liver disease.

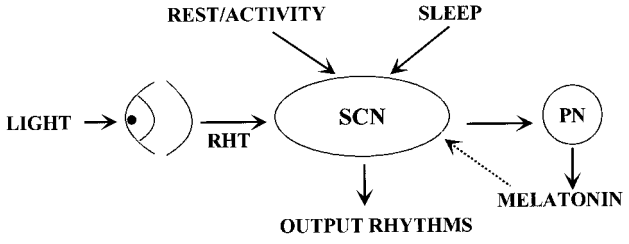

Fig. 1. A schematic depiction of circadian organization. SCN=suprachiasmatic nucleus. RHT=retinohypothalamic tract. PN=pineal gland.

Suprachiasmatic nucleus

Translation of the photic signal utilizes neurotransmitter systems known to be affected in the brain of patients with liver disease. Exposure to light results in glutamate release from the endings of the retinohypothalamic tract, acting through both N-methyl-D-aspartic acid (NMDA) and non-NMDA glutamate receptors on neurons of the SCN (21). Inhibitory influences on the SCN arise among others from the intergeniculate leaflet, a process that involves GABA neurotransmission and the mid-brain raphe nucleus, via serotoninergic neurotransmission. However, the mechanisms that generate circadian rhythms are still incompletely understood. The recent isolation of the *Clock* gene, whose mutation results in a complete loss of the rhythm of locomotor activity in the mouse (22), points to molecular mechanisms that control the generation of circadian rhythmicity.

Individual neurons from the SCN independently express circadian firing rates (23), suggesting that an integrated response occurs in the SCN itself. Astrocytes, whose multiple end-foot processes establish a dense connection with neurons and endothelial cells, may play an important role in this integration (24). The rich glial content of the SCN can be identified using stains for glial-fibrillary acidic protein. Current views of the pathogenesis of hepatic encephalopathy point not only to abnormalities in astrocyte anatomy and function but to a deranged astrocyte-neuronal interaction (25,26). A working hypothesis is that such abnormalities in the SCN may underlie the genesis of abnormal circadian function in liver disease.

Efferent pathways

The complexity of pathways that link the SCN to the multiple biological processes that undergo circadian variability is starting to be unraveled. The best char-

acterized efferent connection is the multisynaptic pathway that directs the synthesis and release of melatonin from the pineal gland during darkness (27). Melatonin is derived from tryptophan → serotonin and, once synthesized, is released into the circulation, as the hormone is not stored in the pineal gland. In plasma, melatonin has a short elimination half-life (ca. 30 min) and undergoes hepatic elimination via cytochrome P-450-mediated hydroxylation, with subsequent sulphation. Not unexpectedly, the plasma clearance of melatonin is reduced in cirrhosis (28).

The biological role of melatonin is still being defined (29). One effect is on the circadian system itself. The presence of melatonin receptors within the SCN of mammals points to a feedback loop where melatonin influences the activity of the circadian pacemaker (30). Thus, two factors are important in the control of the output from the SCN: light and melatonin. While the endogenous pacemaker may cycle with a circadian period of more than 24 h, light and melatonin provide synchronization to the external conditions and entrain the SCN to a 24-h day. The effects of light and melatonin on the SCN are in opposite directions and occur within a window of time, around dawn or dusk, where phase advances or delays occur. Thus, shining bright light in the evening and administering melatonin in the morning delay the output from the SCN; morning light and evening melatonin result in a phase advance (31).

When measured at frequent intervals, the rhythm of plasma melatonin is markedly altered in patients with compensated cirrhosis, (Fig. 2) (5). These abnormalities can be summarized as a delayed onset of nocturnal rise (21:20 vs 19:45 in well-matched controls), a delayed peak nocturnal time (00:30 vs 05:30) and elevated levels during the day. The latter is of particular interest as administration of exogenous melatonin in the morning hours will delay the phase of circadian rhythms. Thus high morning melatonin levels could explain the delayed phase of the melatonin rhythm in liver disease. Our more recent evaluation of sleep patterns of compensated cirrhosis also indicates a preference for later bedtime and later awakening, consistent with a shift towards later hours of diurnal activity (6).

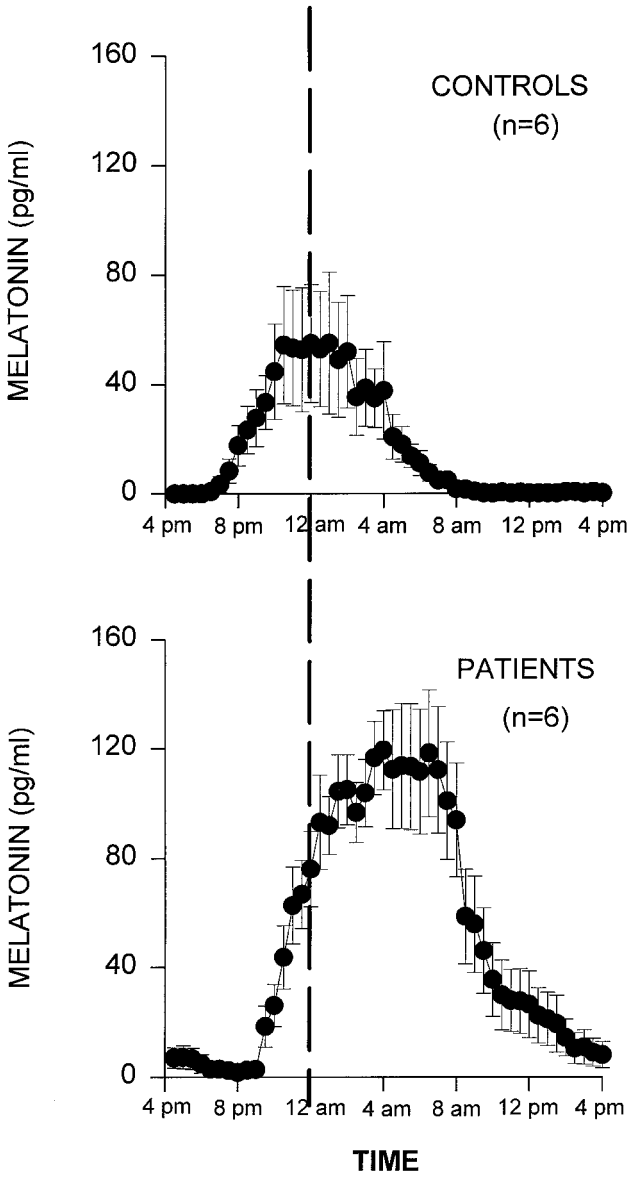

Fig. 2. Melatonin was measured every 30 min in stable cirrhotic subjects and age/sex matched controls. Reproduced from reference 5 with permission.

Therapeutic Implications

In essence, we have proposed two explanations for the alterations in circadian function seen in chronic liver disease. First, abnormalities of circadian rhythms arise from the effects on the SCN and/or its afferent/efferent connections of neurotoxins implicated in the pathogenesis of hepatic encephalopathy. Second, the impaired hepatic metabolism of melatonin results in elevated morning plasma levels that cause a phase shift of the output from the circadian clock. It is possible that both explanations, that combine the effects of hepatocellular dysfunction and portal-systemic shunting, are responsible for the circadian abnormality in liver disease. A better understanding of the pathogenetic mechanisms should lead to novel therapeutic interventions aimed at ameliorating the disturbance in circadian function.

On one hand, the impact of long-term therapy of hepatic encephalopathy needs to be re-examined. Non-absorbable disaccharides are administered over a prolonged period to patients with chronic or recurrent

encephalopathy; if long-term therapy improves circadian parameters, a rationale for a more widespread use for these agents would arise. On the other hand, treatment with bright light, administered in the morning hours, may provide a method to phase advance the output from the circadian clock and correct abnormalities that arise from this shift (31). Treatment of circadian dysfunction is not an attempt to correct a biological curiosity but a measure that may impact on areas of daily functioning that profoundly affect the quality of life of these patients, including the poor sleep pattern and chronic fatigue that plague many individuals with chronic liver disease.

References

1. Pittendrigh CS. Circadian systems: general perspective. In: Aschoff J, editor. Biological Rhythmus. New York: Plenum; 1981. p. 57–80.
2. Moller S, Winberg N, Henriksen JH. Non-invasive 24-hour ambulatory arterial blood pressure monitoring in cirrhosis. Hepatology 1995; 22: 88–95.
3. Trevisani F, Bernardi M, DePalma R, Pancione L, Capani F, Baraldini M, et al. Circadian variation in renal sodium and potassium handling in cirrhosis. The role of aldosterone, cortisol and sympathoadrenergic tone and intratubular factors. Gastroenterology 1989; 96: 1187–98.
4. Alvarez D, Golombek D, Lopez P, de las Heras M, Viola L, Sanchez S, et al. Diurnal fluctuations of portal and systemic hemodynamic parameters in patient with cirrhosis. Hepatology 1994; 20: 1198–203.
5. Steindl PE, Finn B, Bendok B, Rothke S, Zee PC, Blei AT. Disruption of the diurnal rhythm of plasma melatonin in cirrhosis. Ann Intern Med 1995; 123: 274–7.
6. Córdoba J, Cabrera J, Lataif L, Penev P, Zee PC, Blei AT. High prevalence of sleep disturbance in cirrhosis. Hepatology 1998; 27: 339–45.
7. Warbritton JD, Geyer MA, Leppson BW, Fischer JE. Behavioral model of early hepatic encephalopathy in rats. Surg Forum 1979; 30: 394–6.
8. Zee PC, Mehta R, Turek FW, Blei AT. Portacaval anastomosis disrupts circadian locomotor activity and pineal melatonin rhythms in rats. Brain Res 1991; 560: 17–22.
9. Coy DL, Mehta R, Zee P, Salchli F, Turek FW, Blei AT. Portal-systemic shunting and the disruption of circadian locomotor activity in the rat. Gastroenterology 1992; 103: 222–8.
10. Steindl PE, Coy DL, Finn B, Zee PC, Blei AT. A low protein diet ameliorates disrupted diurnal locomotor activity in rats after portacaval anastomosis. Am J Physiol 1996; 271: G555–60.
11. Blei AT. Circadian rhythms and liver disease. In: Haussinger D, Jingermann K, editors. Liver and Nervous System. Lancaster: Kluwer; 1998 (in press).
12. Córdoba J, Dupuis J, Gottstein J, Blei AT. Stenosis across a portacaval anastomosis affects circadian locomotor activity in the rat. A multivariable analysis. Am J Physiol 1997; 273: G1218–25.
13. Romero M, Barrenechea C, Insausti R, Felipo V, Gaztelu JM. Chronic hyperammonemia induced changes in the motor activity circadian rhythm of the rat. In: Record C, Al-Mardini H, editors. Advances in Hepatic Encephalopathy and Metabolism in Liver Disease. Newcastle-upon-Tyne: Ipswich Book Co., Ltd.; 1997. p. 319–24.
14. Moore RY. Circadian rhythms: basic neurobiology and clinical applications. Ann Rev Med 1997; 48: 253–66.
15. Reichenbach A, Fuchs U, Kasper M, el-Hifnawi E, Eckstein AK. Hepatic retinopathy: morphological features of retinal glial (Muller) cells accompanying hepatic failure. Acta Neuropath 1995; 90: 273–81.
16. Norenberg MD. Astrocytic-ammonia interactions in hepatic encephalopathy. Semin Liver Dis 1996; 16: 245–53.
17. Newman NJ, Capone A, Leeper HF, O'Day DG, Mandell B, Lambert SR, et al. Clinical and subclinical ophthalmic findings with retinol deficiency. Ophthalmology 1994; 101: 1077–83.
18. Eckstein AK, Reichenbach A, Jacobi P, Weber P, Gregor M, Zrenner E. Hepatic retinopathia. Changes in retinal function. Vision Res 1997; 37: 1699–706.
19. Van Reeth O, Hinch D, Tecco JM, Turek FW. The effects of short periods of immobilization on the hamster circadian clock. Brain Res 1991; 545: 208–14.
20. Van Reeth O, Sturis J, Byrne MM, Blackman JD, L'Hermite-Baleriaux M, Leproult R, et al. Nocturnal exercise phase delays the circadian rhythms of melatonin and thyrotropin secretion in normal men. Am J Physiol 1994; 266: E964–74.
21. Moore RY. Entrainment pathways and the functional organization of the circadian system. Progr Brain Res 1996; 111: 103–20.
22. King DP. Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, et al. Positional cloning of the mouse circadian CLOCK gene. Cell 1997; 89: 641–53.
23. Welch DK, Logatehis DE, Meister M, Reppert SM. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing patterns. Neuron 1995; 14: 697–706.
24. Servière J, Lavialle M. Astrocytes in the mammalian circadian clock: putative roles. Progr Brain Res 1996; 111: 57–73.
25. Norenberg MD, Neary JT, Bender AS, Dombro RS. Hepatic encephalopathy: a disorder in glial-neuronal communication. Progr Brain Res 1992; 94: 261–9.
26. Butterworth RF. Portal-systemic encephalopathy; a disorder of neuro-astrocytic metabolic trafficking. Develop Neurosci 1993; 15: 313–9.
27. Cassone VM. Melatonin and suprachiasmatic nucleus. In: Klein DC, Moore RY, Reppert RM, editors. Suprachiasmatic Nucleus: the Mind's Clock. London: Oxford University Press; 1991. p. 309–23.
28. Iguchi H, Kato KI, Ibayashi H. Melatonin serum levels and metabolic clearance rate in liver cirrhosis. J Clin Endocrinol Metab 1982; 54: 1025–7.
29. Turek FW. Melatonin: pathway from obscure molecule to international fame. Perspect Bio Med 1997; 41: 8–20.
30. McArthur AJ, Gilette MY, Prosser RA. Melatonin directly resets the rat suprachiasmatic clock *in vitro*. Brain Res 1991; 565: 158–61.
31. Lewy AJ, Sack RL, The role of melatonin and light in the human circadian system. Progr Brain Res 1996; 111: 205–16.