Regulation of behavioral activity by the suprachiasmatic nuclei

Houben, M.B.

Chapter 8

Chronic renal failure does not affect the mouse locomotor activity in darkness conditions

Matthieu Monge[a,b,#], Thijs Houben[c,#], Hetty de Boer[a], Marina Aleksinskaya[a], Ziad Massy[b,d], Ton Rabelink[a], Johanna H. Meijer[c] and Anton Jan van Zonneveld[a]

Departments of [a]Nephrology and the Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands; [b]INSERM U-1088, Jules Verne University of Picardie, Amiens, France; [c]Department of Molecular Cell Biology - Laboratory for Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands; [d]Department of Nephrology, Ambroise Pare Hospital, Paris, France

[#]The two first authors contributed equally to this study

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Abstract

Circadian rhythms regulate blood pressure, hormonal release, and sleeping patterns. Chronic renal failure (CRF) is associated with a loss of nocturnal blood pressure decrease, and dialyzed patients experience sleep disturbances. We induced CRF in mice (thermocauterization-nephrectomy) and compared their rest-activity rhythm with sham-operated animals over a 12-week period. Mice were housed individually in constant darkness. An infrared motion sensor continuously recorded their behavioral activity. Actograms were generated and subsequent periodogram analysis quantified the free-running period (FRP) and the strength of the circadian rhythm. Chronic renal failure mice compared to sham had high levels of serum urea, anemia, and a slight increase in the rhythm strength. However, the FRP was comparable in both groups. Twelve weeks of CRF do not decrease the strength or alter the period of the endogenous circadian rest-activity rhythm in mice. Our results suggest that uremic toxins do not impair the central circadian pacemaker.

Introduction

Rhythms that follow the daily 24-h light/dark (LD) cycle are present in many biological parameters such as hormonal release, body temperature, blood pressure, activity, and sleep. The major pacemaker driving 24-h rhythms is located in the suprachiasmatic nuclei (SCN) of the hypothalamus (1). Patients under conventional hemodialysis (HD) experience sleep-wake alternation disorders (2-4), which can be improved by switching to nocturnal HD or automated peritoneal dialysis (5). As chronic renal failure (CRF) by itself can harm the circadian profile of some parameters, such as blood pressure (6-8) and melatonin secretion (9), it remains unclear if the sleep disorders found in HD patients are associated with the dialysis technique or CRF per se.

Therefore, we used a surgical mouse model of stable CRF (10) to study the impact of chronic uremia on the circadian pattern of behavior, using an actogram-periodogram method to quantify the rhythm.

Experimental Procedures

Animals and surgery

Four 8-weeks-old male C57black6/J mice (Charles River, Inc) underwent surgery to induce renal failure (CRF group) and were followed for the next 12 weeks. Briefly, under inhaled isoflurane anesthesia, mice underwent a thermocauterization of the right kidney cortex (week 2), followed two weeks later (week 0) by a contralateral nephrectomy. Both procedures were preceded by the removal of the kidney capsule in order to preserve the adrenal gland (11). The nephrectomy step (week 0) is considered as the beginning of renal failure. Four age- and sex-matched controls were used as sham-operated controls (Sham group). Animals were housed individually, and fed with standard diet. The study received the agreement of the animals' local ethical committee (DEC 07020-03).

Behavioral activity recordings

Behavioral activity of the animals was recorded using passive infrared motion detection sensors (Hygrosens®, Loffingen, Germany) mounted underneath the lid of

the cage and connected to a ClockLab data collection system (Actimetrics®, Wilmette, IL, USA) that recorded the amount of sensor activation in 1 min bins. After four days in a 12 h light–12 h dark cycle, animals were exposed to constant darkness (DD) for the remainder of the experiment, to record their free-running behavioral activity rhythm. While behavioral activity can be recorded using running wheels, we used passive infrared motion detectors to avoid indirect effects that CRF may have on wheel running as a consequence of the resulting anemia (12).

Behavioral activity data were plotted and analyzed using the Clocklab analysis software. For the periodogram analysis (13), three 10-day time windows were selected during which no experimental procedures were performed and the behavioral activity recordings were continuous (early period A, middle period B, and late period C; see the timeline in Figure 1). The free-running period (FRP) (t) was quantified as the period corresponding to the peak of the circadian component in the periodogram (Figure 2). The strength of the circadian component was quantified by calculating the difference between the peak and the corresponding 99% confidence limit (14).

Assessment of renal failure

Blood was taken from the tail vein under dim red light at baseline (week 72) and weeks 1, 6, and 12 after the nephrectomy, and 5 h after the onset of rest (see Figure 1). Serum urea was measured using Cobas Integra® appartus (Roche, Switzerland), and hemoglobin levels from EDTA-anticoagulated blood using a Sysmex® apparatus.

Statistics

Blood parameters (urea and hemoglobin), free running periods, and rhythm strength at different time points were expressed as mean + SD, and compared by repeated measures ANOVA ($p < 0.05$ was considered as significant).

Results

Renal function measurements

In the CRF group, urea values were significantly higher at weeks 1, 6, and 12 than in the control group and at baseline, but were not different between the weeks, validating the existence of chronic but non-progressive kidney failure in the model. Anemia is a known parameter of chronic renal insufficiency. Hemoglobin levels were significantly lower in the CRF group at week 12. Data are summarized in Table 1.

Table 1. Assessment of kidney function.

	Urea (mmol/L)				Hemoglobin (g/dL)
	Baseline	Week 1	Week 6	Week 12	Week 12
Sham	10.4 ± 1.5	10.5 ± 2.2	11.7 ± 1.0	9.8 ± 1.0	14.9 ± 1.1
CRF	10.4 ± 1.2	34.6 ± 4.9*/**	29.8 ± 2.9*/**	30.8 ± 5.0*/**	12.1 ± 2.3***

Notes: Serum urea (mmol/L) values are significantly higher in the CRF group compared to baseline and to the sham-operated group. Hemoglobin levels are decreased in the CRF group 12 weeks after renal failure induction. Data are mean ± SD. *$p < 0.002$ vs sham, **$p < 0.02$ vs baseline, ***$p < 0.03$ vs sham.

The impact CRF on circadian activity

The actograms show that behavioral activity remains rhythmic in both CRF and control animals throughout the experiment (Figure 1). Periodogram analysis confirmed the presence of a significant circadian rhythm in the behavioral data in all CRF and Sham animals during the early, middle, and late periods (Figure 2). The average FRP became shorter over time, but this was not significantly different between CRF versus control animals. The extent to which the data show an oscillatory pattern in time versus a random distribution is quantified in the rhythm strength, which declined in the control group between periods B and C, but not in the CRF group (Table 2). Repeated measures ANOVA over the three time windows revealed no difference between sham and CRF animals in their FRPs.

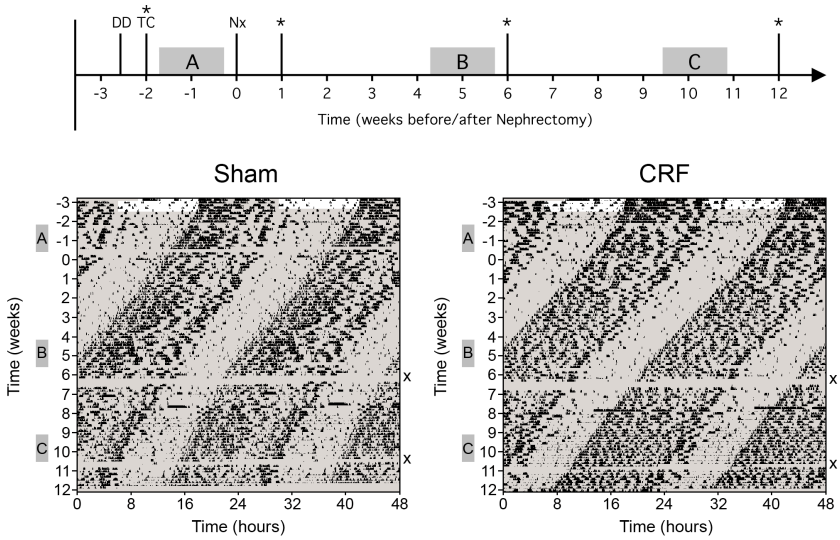

Figure 1. Time-line for the experiment and representative actograms. Animals were housed individually upon arrival (week 73), and placed under a 12 h light–12 h dark cycle (LD) for four days, then under constant darkness for the rest of the experiment (DD). Throughout the experiment, activity was recorded by a passive infra-red sensor. Thermocauterization of the right kidney cortex (TC) was performed after animals had been in DD for three days. Nephrectomy (Nx) was performed two weeks after TC (week 0). Blood samples were taken at four time points (*) and animals were sacrificed after the last sampling in week 12. Periodogram analysis was performed on behavioral data from 10 consecutive days during three time windows in which animals were not exposed to any experimental procedures: the first window was between TC and Nx (early period A, grey block); the second window was in weeks 4 and 5 (middle period B); and the third in weeks 9 and 10 (late period C). Below, representative actograms are shown for a sham-operated and a CRF animal. Each line shows the amount of activation of the passive infrared sensor during 48 h. Consecutive days are plotted below each other, so that data in the right half of each line are repeated on the left half on the next line (that is, the first line shows days 1 and 2, the second shows days 2 and 3, and so on for a total of 109 days. The background color indicates the lighting status, with the white background representing lights on and grey background indicating lights off. Computer failure interrupted the recording on two occasions, marked by an x and shown as grey bands.

Table 2. Free-running period (h) and rhythm strength (arbitrary units) during the three periods of the study (for details of calculating these parameters, see legend to Figure 1).

	Free-running period		Rhythm strength	
	Sham	CRF	Sham	CRF
Period A	23.3 ± 0.4	23.3 ± 0.4	0.19 ± 0.05	0.21 ± 0.05
Period B	22.9 ± 1.0	23.0 ± 0.8	0.22 ± 0.06	0.22 ± 0.05
Period C	22.9 ± 1.0	23.1 ± 0.9	0.17 ± 0.04*	0.23 ± 0.07

Notes: The free-running periods remain stable and comparable between the groups. The rhythm strength decreases non-significantly in the sham-operated group, whereas it remains stable in the CRF group. Data are mean ± SD. *$p < 0.05$ vs CRF.

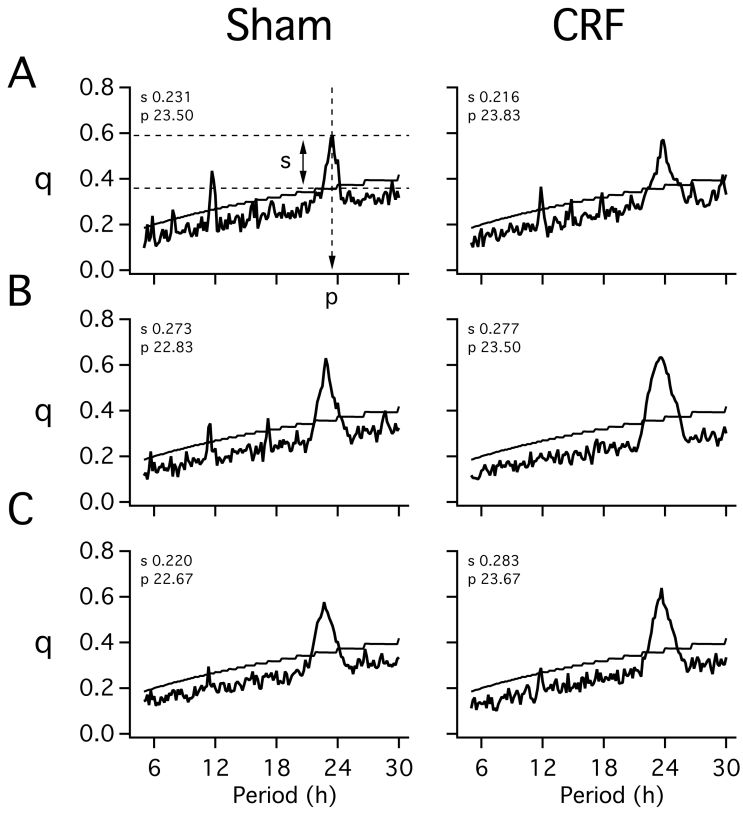

Figure 2. Periodogram analysis of the behavioral activity data. Periodograms of a Sham and a CRF animal are shown for the three 10-day windows A (early), B (middle), and C (late). Periodograms test whether data are distributed randomly in time or show cyclical patterns. In each periodogram, the standardized root mean square amplitude (*q*, thick line) is plotted with the 99% confidence limit (thin line) as a function of period. The period was varied in 10-min steps from 5 to 30 h. In all sham and CRF animals, a significant circadian (near-24 h) component was present in all three time windows. The periodogram analysis is explained in the top left panel: (1) The period (*p*) of the circadian component was quantified for each periodogram at the peak of *q* (vertical dashed line). (2) The strength (*s*) of the circadian component was determined as the difference between the peak of the circadian component and the corresponding 99% confidence limit (horizontal dashed lines show the difference between the amplitude of *q* and the corresponding confidence level).

Discussion

Sleep disturbances are common in patients with CRF (2,3,15), possibly due to disruption of the circadian system in these patients. To test this hypothesis, we performed a pilot study where we surgically induced renal insufficiency in male C57black6/J mice using a procedure that allows stable levels of urea over time, in order to avoid effects of progressive renal failure. The mice were kept under constant darkness and environmental conditions and performed long-term recordings of behavioral activity, which is the standard output parameter to investigate the circadian system. We analyzed the behavioral rhythmicity and quantified both the periodicity of the circadian system and the strength of the rhythm. Surprisingly, we found that behavioral activity remained robustly rhythmic, even after 12 weeks of CRF. The FRP was similar in both groups throughout the experiment. Furthermore, the strength of the rhythm at the end of the experiment was slightly higher in the CRF group compared to the sham group.

The results show that CRF does not induce a deterioration of circadian rhythms in behavioral activity and also does not change the endogenous frequency of the circadian oscillator. Our experiments were designed to test whether CRF affects the robustness or the frequency of behavioral circadian rhythms. As a 24-h LD cycle might drive or strengthen the endogenous rhythm generated by the circadian pacemaker, the mice were kept under constant darkness (when the period of the measured free-running rhythm accurately reflects that of the endogenous component). Therefore, using this protocol, we can exclude the possibility that light influences on the clock have compensated for the absence of a strong endogenous timekeeping signal in our mice.

Interestingly, a recent study in CRF patients found a correlation between glomerular filtration rate (GFR) and the amplitude of the melatonin rhythm, whereas no correlation was found between GFR and the rhythms in cortisol or core body temperature (5). These observations indicate that circadian rhythmicity in physiological parameters is differentially affected by CRF. In conjunction with our present results, the data suggest that the central circadian pacemaker in the suprachiasmatic nucleus is relatively unaffected by uremia, and the observed rhythm deterioration in CRF patients likely originates downstream from the SCN. The mechanisms regulating sleep are complex and involve many brain regions. While the SCN is essential for regulating the timing of sleep, it is not essential for the presence of sleep (16). In the context of the present results, sleep problems in patients could be explained, for example, by a decrease in sensitivity of sleep-regulating brain

functions to circadian signals from the SCN rather than a decrease in rhythmicity in the SCN itself.

The resilience of sleep–wake rhythms to disturbance by CRF indicates that the circadian pacemaker is not impaired by uremic toxins. Our results suggest therefore that the deleterious effect of conventional HD on sleep quality in patients is a side-effect of the treatment and not a consequence of the disease itself (2,3,5,17). This view is consistent with recent studies showing that nocturnal methods such as automated peritoneal dialysis and nocturnal HD lead to the restoration of the nocturnal melatonin peak, correlating with improved sleep quality (5). In conclusion, our data show that the circadian pacemaker is not impaired by CRF, raising the prospects for optimized dialysis treatment protocols that are expected to improve the circadian sleep–wake regulation and thereby lessen the additional burden of chronic sleep insufficiency.

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