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BRAIN RESEARCH

Research Report

rPer1 and rPer2 induction during phases of the circadian cycle critical for light resetting of the circadian clock

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ABSTRACT

Photic resetting of a biological clock is one of the fundamental characteristics of circadian systems and allows living organisms to adjust to a particular environment. Nocturnal light induces the *Per1* and *Per2* genes, which leads to a resetting of the circadian clock in the suprachiasmatic nucleus (SCN), the mammalian circadian center. In our present study, we investigated whether light differentially induces the rat *Per1* (r*Per1*) and *Per2* (r*Per2*) genes to enable resetting of their circadian clocks. In a 24-hour LD cycle (12 h light:12 h dark), which is shorter than the normal free-running period for rats, *Per1* alone showed strong induction in the ventrolateral region of the SCN (VLSCN) during the early day. In contrast, in a 25 hour LD cycle (12.5 h light:12.5 h dark), which is longer than the free running period for these animals, r*Per2* alone was strongly induced in the VLSCN, at the end of the light phase and during the early dark periods. Our current findings therefore suggest that *Per1* and *Per2* are differentially regulated for daily entrainment to the LD cycle.

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1. Introduction

Circadian rhythms are generated by endogenous timekeeping mechanisms that have been conserved throughout evolution. In mammals, the suprachiasmatic nucleus (SCN) is the center of these circadian rhythms and within the SCN, individual neurons are autonomous oscillators that generate circadian firing rhythms (Herzog et al., 1998; Liu et al., 1997; Welsh et al., 1995). Molecular dissection of these processes has further demonstrated that the generation of the circadian rhythm is produced by a transcription/translation feedback loop that regulates clock genes (Chang and Reppert, 2001; Dunlap, 1999; Ripperger and Schibler, 2001; Wager-Smith and Kay, 2000).

The endogenous circadian clock of the mammalian SCN can be adjusted by light exposure, which can in turn synchronize the biological clock in the SCN with the external environment. Although the molecular mechanisms underlying this are not yet fully understood, a number of recent findings have now suggested that the induction of *Per1*/*Per2* expression by light during the night is the principal event that produces long term state changes, i.e. the phase shift of the circadian rhythm. *Per1* and *Per2* are induced in the SCN after light exposure during the night (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997; Takumi et al., 1998; Yan and Silver, 2002; Zylka et al., 1998) and the PER1 protein has also been shown to respond to light during the night,

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suggesting that it plays a role in photic entrainment (Field et al., 2000; Yan and Silver, 2004). Furthermore, antisense oligonucleotides against either m*Per1* or m*Per2* have been shown to attenuate the extent of the phase shift produced by a light exposure event (Akiyama et al., 1999; Tischkau and Gillette, 2005; Wakamatsu et al., 2001). These findings together suggest that *Per1* and *Per2* are fundamentally involved in the resetting of the circadian clock.

Since the period of the endogenous circadian clock is not an even 24 h, biological organisms have to adjust their internal clock to the environmental LD cycle on a daily basis. Morning and evening light is particularly important for the resetting of the central circadian clock. Living organisms with circadian periods of less than 24 h require exposure to clock-delaying light at dusk and organisms with periods that are greater than 24 h need to be exposed to clock-advancing light at dawn (Refinetti, 2006). Hence, and Assuming that *Per1* and *Per2* activation is fundamental to phase resetting, these genes will need to be induced during the critical period in which the central clock is reset.

In our present study, we examined whether *Per1* and *Per2* in the rat are transactivated both at dawn and at dusk during the daily resetting of the SCN clock to steady LD conditions. We utilized a T-cycle experimental system that compels the animals to advance or delay their entrainment to environmental LD cycles and then determined whether *Per1* and *Per2* are differentially induced.

2. Results

2.1. *Time course analysis of rPer1/rPer2 expression during a 24 hour period under LD and DD conditions*

We examined the expression of the r*Per1*/r*Per2* genes at around dawn and dusk. These timepoints corresponded to periods from 30 min before (−30 min: ZT23.5) to 120 min after (+120 min: ZT2) the onset of the light phase and 90 min before (−90 min: ZT10.5) to 60 min after (+60 min: ZT13) the end of the light phase (Figs. 1 and 2), respectively. A cluster of intensely-labeled r*Per1*-expressing neurons was detectable in the VLSCN at 30 min after the onset of the light phase (+30 min: ZT0.5; Fig. 1B) under LD conditions and increased in number over the course of the following 2 h (Figs. 1A, C). In the DMSCN, a similar linear increase was found in the r*Per1*-positive cell number at around the equivalent of dawn under both LD and DD conditions (Fig. 1C). Under LD conditions, we observed a significant increase in the number of r*Per1* mRNA-positive cells (two way ANOVA; $p<0.01$) in the VLSCN, but not in the DMSCN (two way ANOVA; $p=0.27$), from 30 min before to 120 min after the onset of the light phase (Figs. 1A, C). In the VLSCN, a Tukey's multiple comparison test revealed significant differences from 30 min (+30 min) to 120 min (+120 min) after lights on ($p<0.01$; Fig. 1C).

In the LD experiment, r*Per2* mRNA-positive neurons were also found to increase shortly after light onset. Weakly labeled r*Per2*-expressing neurons appeared in the VLSCN by 30 min after the onset of the light phase (+30 min: ZT0.5; Fig. 1E) and increased in number over the course of the next 2 h (Figs. 1D, F). In the DMSCN, there was also a similar linear increase in the r*Per2* cell number at lights on under both LD and DD conditions (Figs. 1D, F). The morning light in the LD experiment significantly increased the number of r*Per2* mRNA-positive cells (two-way ANOVA; $p<0.01$) in the VLSCN, but not in the DMSCN (two-way ANOVA; $p=0.15$; Figs. 1D, F). By analysis with Tukey's multiple comparison test, significant differences were again revealed from 30 min (+30 min) to 120 min (+120 min) after lights on ($p<0.01$; Fig. 1F).

At around dusk, both r*Per1*- and r*Per2*-expressing cells were found to increase in the VLSCN but more modestly when compared with the dawn conditions (Fig. 2). The number of r*Per1* mRNA-positive neurons under LD conditions increased and peaked at 30 min before the end of the light exposure (−30 min: ZT11.5; Fig. 2B), and these numbers gradually decreased until 60 min after the end of the light phase (+60 min: ZT13; Figs. 2A–C). However, under DD conditions, no significant differences were observed (Figs. 2A, C). Light exposure at around dusk significantly increased the number of r*Per1* mRNA-positive cells (two-way ANOVA; $p<0.01$) in the VLSCN but not in the DMSCN (two-way ANOVA; $p=0.36$) (Figs. 2A, C). In the VLSCN also, a Tukey's multiple comparison test revealed significant differences at 30 min before lights off (−30 min) and also at 0 min (0 min) ($p<0.01$; Fig. 2C).

r*Per2* was found to be induced in the VLSCN also during the late day (Figs. 2D, F). From CT and ZT0 before the end of the light phase (0 min: ZT12), r*Per2*-positive neurons had increased in the VLSCN (Figs. 2D, F) in LD compared with DD, apparently as a result of light exposure. Light exposure at around dusk significantly increased the number of r*Per2* mRNA-positive cells in the VLSCN (two-way ANOVA; $p<0.01$) and DMSCN (two-way ANOVA; $p=0.045$; Figs. 2D–F). In the VLSCN, analysis using Tukey's test revealed significant differences from 0 to 60 min after lights off ($p<0.05$ at 0 and 30, $p<0.01$ at 60; Fig. 2F).

2.2. *Time course analysis of rPer1/rPer2 expression over a 25 hour period under LD and DD conditions*

To examine the expression of r*Per1* and r*Per2* at times when animals need to delay their circadian clock to enable entrainment to environmental lighting conditions, we utilized the T-cycle paradigm. The rats were housed under LD 12:12 hour conditions for 2 weeks, and then exposed to LD 12.5:12.5 hour conditions. Under both of these sets of conditions, the rats were entrained to the LD cycles (Fig. 3). As the rat is known to

Fig. 1 – Time course analysis of rPer1 and rPer2 expression at around dawn under 24 h LD and DD conditions. Dashed lines indicate the SCN boundaries and denote the dorsomedial and ventrolateral subdivisions within the SCN. Scale bar, 200 μm. (A, D) Representative coronal sections of the SCN at around dawn subjected to r*Per1* (A) or r*Per2* (D) in situ hybridization. The top black–white bar indicates an LD cycle and the black bar indicates a DD cycle. (B, E) Higher magnification images of the panels shown in A and D, respectively. (C, F) Graphs showing the number of SCN cells expressing r*Per1* (C) or r*Per2* (F) mRNA in the VLSCN or DMSCN under entrained conditions or in the constant dark. *$p<0.05$, **$p<0.01$, Tukey's test. Each data series represents the mean ± SEM.

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