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Effects of light cues on re-entrainment of the food-dominated peripheral clocks in mammals

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ABSTRACT

Although the light/dark (LD) cycle is a weak *Zeitgeber* in peripheral clocks compared with food stimuli, whether the effect of light cues on the re-entrainment of peripheral clocks can be masked by that of the dominating food cue remains unknown. In the present study, the individual reversal of LD cycle for 7 days could not obviously affect circadian patterns of core clock genes (*Bmal1*, *Cry1*, *Per1*, and *Dec1*) in the liver and heart of restricted-fed rats. In contrast, reversing the feeding schedule together with the LD cycle markedly enhanced the re-entrainment of peripheral clocks compared with reversal of the feeding regimen alone. In addition, LD reversal alone for 7 days contrarily regulated the expression levels of *Cry1*, *Per1*, and *Dec1* in the liver and heart. Moreover, daytime restricted feeding not only induced different phase shift rates of the four examined clock genes but also led to reversed phase shift direction in their resetting processes in these two peripheral clocks. Furthermore, the resetting sequences of these genes were also disparate between these two peripheral clocks. These observations suggest that the mechanisms underlying the liver and heart clocks are distinct, which may distinguish them from each other in the SCN-synchronized peripheral system.

1. Introduction

Circadian clocks are present in a variety of tissues and cells in mammals and appear to be organized in a hierarchical manner (Reppert and Weaver, 2002; Schibler and Sassone-Corsi, 2002). The master clock is localized in the hypothalamic suprachiasmatic nucleus (SCN), which controls the approximately 24-h periodicity (circadian rhythms) of mammalian behavior and physiology (Dunlap, 1999; Ishida et al., 1999; Schibler and Sassone-Corsi, 2002). Clocks in peripheral tissues, such as heart and liver, are called peripheral clocks. In fact, the expression of clock genes, including *Clock*, *Bmal1*, *Per(s)*, *Cry(s)* and *Dec(s)*, is robustly rhythmical not only in the SCN but also in the peripheral tissues (Reppert and Weaver, 2002; Lowrey and Takahashi, 2004). The SCN clock is reset mainly by the external light signal via the retinohypothalamic tract (Dunlap, 1999; Hastings, 1997; Takahashi, 1995), whereas the peripheral clocks seem to be reset mainly by food cues rather than the light cue in mammals (Damiola et al., 2000; Stokkan et al., 2001; Schibler and Sassone-Corsi, 2002). Thus, the circadian clocks presented in various tissues may be entrained differently by external time cues, including light and food.

To date, numerous studies have attempted to explain the resetting mechanism of the light-entrainable SCN clock. For example, it was reported that light pulses applied during the night can lead to rapid increased levels of *Per1* and *Per2 in the SCN* (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997), which may play important roles in the resetting of the light-entrainable SCN clock (Akiyama et al., 1999; Tischkau et al., 2003). With regard to the resetting of peripheral clocks, it has been shown that a forced daytime restricted feeding can completely synchronize peripheral clocks of nocturnal animals, independent of the master clock and the environmental light/dark (LD) cycle (Damiola et al., 2000; Stokkan et al., 2001; Schibler and Sassone-Corsi, 2002). However, the phase resetting process of peripheral clocks induced by the restricted feeding treatment is still not well known. Moreover, it has been reported that advancing the LD cycle by several days did not move the phase of the liver clock in mice if the LD shift was carried out under a fixed restricted feeding schedule (Hara et al., 2001), but it did when the mice were fed *ad libitum* (Yamazaki et al., 2000; Sakamoto and Ishida, 2000; Kobayashi et al., 2004; Sakamoto et al., 2004). Thus, it is likely that the light cue can influence the food-entrained mode (manner) of peripheral clocks. However, the mechanism by which light affects the resetting process of the food-entrainable peripheral clock has not yet been investigated. Thus, to investigate whether or how the resetting

Abbreviations: SCN, suprachiasmatic nucleus; RF, daytime restricted feeding; ZT, *Zeitgeber* time; C_t, threshold cycles; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; LD, light/dark; DL, dark/light.

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process of the peripheral clock is regulated by the food and light cues, we first investigated the individual and then the combined effects of these two external time cues on the synchronization of circadian gene expression in the rat liver and heart.

2. Materials and methods

2.1. Animals and experimental design

Male Wistar rats with a mean body weight of about 100 g were purchased from China National Laboratory Animal Resource Center (Shanghai, China). They were kept at our animal facilities (illumination with fluorescent strip lights, 200 lx at cage level; 22±1 °C) in a 12 h:12 h LD cycle. Water was available *ad libitum*; food was provided only in the dark period from the beginning of the experiments. The onset of light was defined as *Zeitgeber* time 0 (ZT0); the onset of darkness was ZT12. Rats were adapted to this lighting and feeding condition for at least 1 week before the following experiments.

For the analysis of diurnal expression patterns of circadian genes in the liver and heart (control group), rats were sacrificed at a 4-h interval of the daily cycle starting at ZT0.

In experiments in which individual effects of the light cue on circadian gene expression in the liver and heart were examined, rats were transferred to a completely reversed LD cycle without changing the previous feeding time (DL group). Four rats for each time point were killed at ZT0, 4, 8, 12, 16, and 20 on day 7 of LD reversal.

To test the phase resetting process of the peripheral circadian clock system (in the liver and heart) by the food cue with or without a change in LD cycle, rats were divided into two groups after a two day fasting. To analyze effects of daytime restricted feeding (feeding reversal only) on the peripheral clocks, the feeding time of the first group was changed from the dark phase to the light phase (starting from the beginning of day 1) for 7 days (RF group) without altering the LD cycle. To compare the individual effect of food cue, the second group was subjected to the reversal condition of both feeding schedule and LD cycle for 7 days (both reversal group) at the same time to investigate the cooperative regulation of food and light cues. In the second group, rats were transferred to darkness by extending the dark period, and thus the initial LD cycle was completely reversed to a 12 h:12 h dark/light (DL) cycle. Rats in this experiment (four per each time point) were sampled every 4 h from the start of phase changing for the first 3 days (Time 0–72 h), and then for the 5th (Time 96–120 h) and 7th days (Time 144–168 h). The purpose of fasting was to make rats consume food immediately after the feeding phase shift. Thus the impact of food stimuli can be observed immediately upon change of feeding phase. We have confirmed that fasting for 2 days did not alter the expression of circadian genes in the liver and heart.

All rats were killed after deep anaesthesia by an i.p. injection of pentobarbital sodium. Livers and hearts were quickly removed, immediately frozen in liquid nitrogen, and kept at −80 °C until use for RNA extraction. During the dark phase, dissection was carried out

Table 1
Primer sequences

Gene	Accession number	Primer sequence 5′ to 3′
Bmal1	NM024362	Forward, TGCGATGTCCCGGAAGTTAGATA
		Reverse, TCATCGGATAGAGATGTTGGCTTG
Cry1	NM198750	Forward, CGACGACCATGATGAGAAGT
		Reverse, ACAACCGAAGCGGAGATAAG
Per1	AB092976	Forward, TGGTAAAGCACCAGGGACAAC
		Reverse, GAAGGACTTTGGCCTTGAATGTAC
Dec1	AB096137	Forward, CCAGGAAACCATTGGACTCAG
		Reverse, AGAGGTCGGATACCAGCATTT
GAPDH	NM017008	Forward, GACAACTTTGGCATCGTGGA
		Reverse, ATGCAGGGATGATGTTCTGG

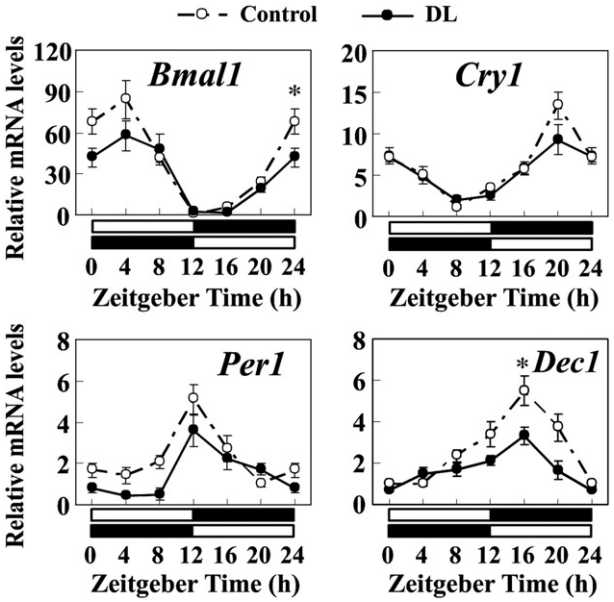

Fig. 1. The effects of light phase reversal alone on daily expression profiles of circadian genes in the liver. Rats were adapted to the 12 h:12 h light:dark (LD) cycle for at least 1 week and then divided into two groups. For the analysis of diurnal expression patterns of circadian genes in the liver (control group), rats were sacrificed at a 4-h interval of the daily cycle starting at ZT0. To examine the individual effect of light cue, rats were transferred to a completely reversed LD cycle without changing the previous feeding time (DL group). Four rats for each time point were killed at ZT0, 4, 8, 12, 16, and 20 on day 7 of LD reversal. Mean values obtained from rats in the DL group are indicated as filled circles/solid lines, and mean values of fed rats (control group) are indicated as open circles/dashed lines. The black bar at the bottom of each panel represents the duration of the dark phase of the 12 h:12 h LD cycle. The mRNA levels of all circadian genes were normalized to GAPDH mRNA. Quantitative representations of multiple results are expressed as values relative to the minimum value of the control group. The ZT24 value represents a replotting of the ZT0 value of the control group. Each value represents the mean±SEM of four animals. Differences at a given time point, determined using a one-way analysis of variance (ANOVA), were considered statistically significant when p was less than 0.05 or 0.01 (*p<0.05; **p<0.01).

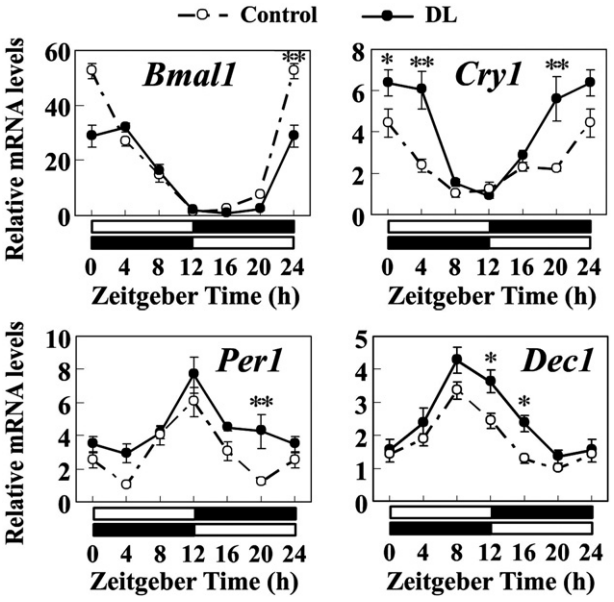

Fig. 2. The effects of light phase reversal alone on daily expression profiles of circadian genes in the heart. The experimental process and quantitative mRNA description of each gene is the same as that described in Fig. 1. Filled circles/solid lines represent data of DL group, and open circles/dashed lines indicate ones of control group. Each value represents the mean±SEM (n=4). Differences at a given time point, determined using a one-way analysis of variance (ANOVA), are considered statistically significant when p is less than 0.05 or 0.01 (*p<0.05; **p<0.01).