

Role of glucocorticoids in the resetting of mammalian circadian clock, its implication with the biological activity of functional food

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Role of glucocorticoids in the resetting of mammalian circadian clock, its implication with the biological activity of functional food

時計遺伝子の発現リズム位相変化におけるグルコルチコイドの機能解析および
機能性食品による生体リズム同調促進作用に関する研究

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[Background and objective]

Genetic and molecular approaches have identified a basic mechanism of the circadian oscillator that is governed by inter-connected transcriptional and translational feedback loops. Gene products of *Clock* and *Bmal1* form a heterodimer that activates the transcription of *Period (Per)* and *Cryptochrome (Cry)* genes. Once PER and CRY proteins have reached a critical concentration, they attenuate CLOCK/BMAL1-mediated transactivation, thus generating circadian oscillation in their own transcription. The alternating activation and suppression of the BMAL1/CLOCK-driven positive loop and PER/CRY-controlled negative loop result in a circadian oscillation in the molecular clock, and also regulate 24-hour variations in output physiology through the periodic activation/repression of clock-controlled genes.

In mammals, the circadian clock system is composed of a master pacemaker, which is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, and subsidiary oscillators in other brain regions, as well as many peripheral tissues. The times of the day-dependent changes in the secretion of glucocorticoids from the adrenal glands are controlled by the SCN, which, in turn, synchronizes subsidiary oscillators to coordinate various biological processes, thereby generating daily rhythms in physiology and behavior.

On the other hand, a variety of foods have the ability to modify the circadian rhythms in physiology and behavior. In fact, food signal is well-recognized as dominant “Zeitgeber” for entrainment of circadian clock in peripheral tissues. Although studies on the Brand’s Essence of Chicken (BEC), a chicken meat extract containing various proteins, peptides, and amino acids, have also demonstrated its ability to facilitate the re-entrainment of the expression of clock genes in the rodent model of circadian disruption, the underlying mechanism remains to be clarified.

The objective of this thesis is to investigate the role of glucocorticoids in the resetting of the rhythmic phase of clock gene expression and also to explore whether glucocorticoids mediate the modulatory effect of BEC on the phase shift of behavioral rhythm in mice.

[Materials and methods]

Cell cultures: PERIOD2-fused luciferase-expressing C6 rat glioma cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C under a humidified 5% CO₂ atmosphere.

Bioluminescence analysis: *Per2::Luc* C6 cells were seeded on culture dishes. After treatment with 100μM forskolin for 2 h, the oscillation of bioluminescence driven from *Per2::Luc* was measured subsequently. Dexamethasone (1 nM) was added into media at the two different time points.

Animals and treatment: Mice (5-6 weeks old) were housed under a standardized light-dark cycle (lights on at Zeitgeber time (ZT) 0, off at ZT12) for two weeks before experiments. To evaluate the effect of glucocorticoid on the rhythmic phase of the expression of clock gene in the liver of mice and their behavioral rhythms, dexamethasone (2mg/kg) was administrated subcutaneously (s.c.) at four different time points. In order to evaluate the influence of BEC on behavioral rhythm of mice, they were fed with normal diet (CE-2) or the same diet containing 10% w/w BEC for 2 weeks.

Quantitative RT-PCR analysis: Total RNA was extracted from liver and adrenal glands at ZT2, ZT6, ZT10, ZT14, ZT18 or ZT22. cDNA was prepared via reverse transcription of total RNA and cDNA samples were analyzed by real-time PCR.

Western blotting: Lysates of adrenal glands were prepared from mice at six time points as outlined above. A total of 20 µg protein lysates was then resolved and probed with rabbit monoclonal antibodies against StAR or ACTIN. Specific antigen-antibody complexes were visualized using horseradish peroxidase-conjugated secondary antibodies and a chemiluminescence reagent. The band intensity was quantified and the expression levels of StAR protein were normalized by ACTIN.

Corticosterone assay: Plasma was obtained after centrifugation. Plasma corticosterone levels were measured using a corticosterone enzyme immunoassay kit according to the manufacture's protocol.

Luciferase reporter assay: The mouse StAR gene promoter-driven luciferase-expressing NIH3T3 cells were treated with L-carnosine, L-anserine, or Cyclo-(Phe-Phe). After incubation for 48 h, cell lysates were analyzed using a dual luciferase reporter assay system.

Measurement of locomotor activity rhythm: Mice were housed individually in breeding cages with food and water *ad libitum*. The cages were placed into an infrared ray area sensor, and locomotor activity was measured every 10 min. Locomotor activity was recorded under the light and dark cycles and then continuously recorded under the constant dark condition.

Statistical Analysis: Statistical significance was analyzed by one way ANOVA with Tukey's post-hoc test. A 5% level of probability was considered to be significant difference.

[Results]

Role of glucocorticoids in the resetting of rhythmic phase of clock gene expression

The ability of glucocorticoid to shift the circadian rhythms of clock gene expression was demonstrated *in vitro* and *in vivo*. *In vitro* experiment, treatment of *Per2::Luc* C6 cells with 100 µM forskolin for 2 h induced the oscillations of bioluminescence with a period length about 24 hours. When 1 nM dexamethasone was applied at 6 h after the trough point of the luminescence (in the increasing phase), the next peak was significantly phase delayed as comparison to the vehicle-treated (control) cells. On the other hand, dexamethasone was applied at 6 h after the peak point of the luminescence (in the decreasing phase). The next peak of luminescence rhythm was significantly advanced as comparison to the control cells.

In vivo experiment, phase delays of clock gene expression were observed in the liver of mice when dexamethasone (2mg/kg, s.c.) was administrated at ZT14 and ZT20, whereas administration of dexamethasone at ZT2 resulted in a phase advance. These data suggest that glucocorticoid has the ability to induce the phase shift of circadian clock gene expression in the liver of mice. The direction of phase shift of clock gene expression was dependent on its administration timing (Fig.1). On the other hand, a single administration of dexamethasone (2mg/kg, s.c.) was unable to induced obvious phase shift of behavioral rhythm in mice. These results suggested that the rhythmicity in the expression of clock genes was more sensitively responded to glucocorticoid stimuli.

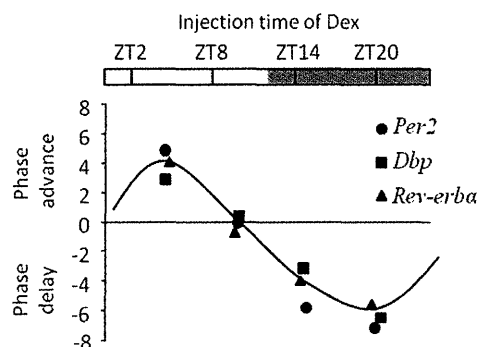


Fig. 1 Dosing time-dependent difference in dexamethasone-induced phase shift of clock gene expression. Mice received a single dosage of dexamethasone (Dex: 2mg/kg, s.c.) at the indicated time points. After injection of Dex, the amplitudes of the rhythms in clock gene expression was determined.

Dietary supplementation with essence of chicken enhances diurnal oscillation of plasma glucocorticoid levels and behavioral adaptation to the environmental lighting cycle

Dietary supplementation with BEC enhanced the diurnal oscillation in the expression of StAR mRNA levels in adrenal gland of mice without changing the expression of clock genes. Similar enhancement effect of BEC was also observed in the expression of StAR protein. Among the main components including in BEC, L-anserine and cyclo-(Phe-Phe) had the ability to enhance the StAR gene transcription. Since StAR is responsible for adrenal secretion of glucocorticoid. Prolonged feeding of mice with BEC-containing diet increased the amplitude of diurnal rhythm of plasma corticosterone levels by enhancing the oscillation in the expression of StAR in the adrenal glands.

In addition, mice fed with BEC-containing diet showed faster entrainment rate of behavior rhythm to phase advanced light-dark cycle (Fig.2), but not to phase delayed light-dark cycle. Furthermore, repetitive administration of corticosterone into mice after changing the light and dark cycle also facilitates the entrainment of their behavioral rhythm. These finding suggest that glucocorticoid has the ability to facilitate the entrainment function of circadian clock when mice were exposed to the phase-advanced light and dark cycle.

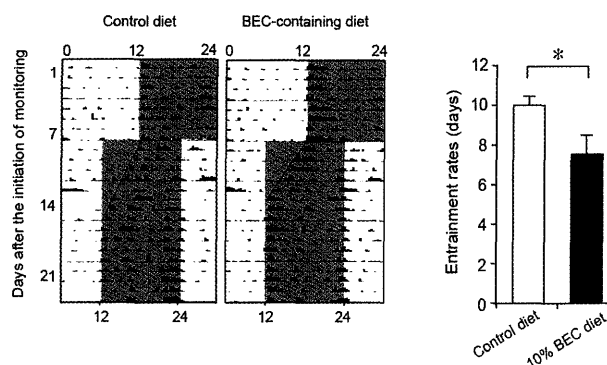


Fig. 2 Effect of BEC on the ability of mice to entrain their behavioral rhythm to the changing light-dark cycle. Mice were fed with control or BEC-containing diets for 2 weeks under normal light-dark cycle condition, and subsequently they were exposed to phase-advanced by 6h. Daily activity patterns are plotted on consecutive lines. Dark phases are indicated by grey background. Each value represents the mean $\pm$ S.E. (n=4). *, $p < 0.05$ compared between the two groups

[Discussion]

The present studies have been characterized the response of rhythmic phase of clock gene expression to glucocorticoid stimuli. The responsiveness of molecular clock was dependent on the administration timing of glucocorticoid. Some bioactivity compounds included in the specific natural product may induce the secretion of glucocorticoid. In fact, dietary supplementation with BEC increased the amplitude of diurnal oscillation of glucocorticoid secretion, accompanied by enhancement of StAR gene expression in adrenal gland. The enhancement effect of BEC on the oscillation of glucocorticoid secretion appeared to accelerate the entrainment of behavioral rhythm to phase-advanced light-dark cycle.

A large variety of functional foods and beverages are now on sale in all over the world, and the market is one of the fastest-growing sectors in the food industry today. Indeed, consumers are increasingly demanding food products fortified to provide specific health benefits. BEC is also largely consumed in Southeast Asia as traditional supplement for attenuation of physical and mental stresses. The present findings provide a new insight into the biological action of BEC, which may be a benefit for peoples disturbing the entrainment of their internal clock to daily light-dark cycle and may also attenuate the jet lag-induced fatigue and insomnia. Identification of factors responsible for physiological action of functional foods and beverages would lead to a better understanding of their significance for human health.

[Publication]

Okamura A, Koyanagi S, Dilxiat A, Kusunose N, Chen JJ, Matsunaga N, Shibata S, Ohdo S. Bile acid-regulated PPAR α activity underlies circadian expression of intestinal peptide absorption transporter PepT1/Slc15a1. *J Biol Chem* 289: 25296-25305, 2014.