발생과 생식 제 7권 제 1호, 1~7 (2003)
Dev. Reprod. Vol. 7, No. 1, 1~7 (2003)

Rhythms and Biological Clock

Donchan Choi

Division of Environmental and Biological Sciences, College of Natural Science, Yong-In University, Yongin, Gyeonggi-do 449-714, Korea

리듬과 생체시계

최 돈 찬

용인대학교 자연과학대학 환경생명학부

ABSTRACT : Most animals, including human beings, live in a cyclic pattern of life that is influenced by the ambient changes of environment. The regular changes occurred by rotation of the Earth itself, its revolving around the Sun, and the local environment, are reflected by the distinct behavior in the living organisms. These regular changes of environment have been imprinted into the genes within the living organisms through the evolutionary process over a long period of time. The genes are expressed by rhythms during the process of fetal development followed by growth. The environmental modifications ultimately are settled in genes, serving as a biological clock that is located putatively in the hypothalamus. Thus the biological clock governs a large number of rhythms and affects the time of birth and death, life expectancy, behavior, physiology, cell division, biochemical reaction, etc. The rhythms are readjusted to the changes of environmental cues. The biological clock has the great advantage of predicting and preparing the regular changes of environment.

Key words : Rhythm, Phase response curve, Suprachiasmatic nucleus, Reproduction, Biological clock.

요 약 : 사람을 비롯한 대부분의 동물들은 주변 환경의 영향을 받아 주기적인 생활을 영위하고 있다. 지구의 자전 및 공전으로 그리고 국소적으로 발생되는 주기적인 변화는 생명체의 행동으로 뚜렷하게 표출된다. 이러한 환경의 주기적인 변화는 오랜 기간의 진화과정을 통하여 생명체 내의 유전자로 각인되었으며, 이 유전자들은 발생 과정을 포함하는 성장 과정에서 리듬으로 발현된다. 환경의 변화는 결국 유전자로 정착하여 생체 시계로서 작용하며, 시상하부의 시신경교차상핵(suprachiasmatic nucleus, SCN)이 유력한 해부학적 위치이다. 따라서 생체 시계는 지구상에 살고 있는 생명체의 각종 리듬을 지배하여, 출생 및 사망, 수명, 행동, 생리, 세포 분열, 생화학적 반응 등등에 영향을 미친다. 리듬은 서서히 변화하는 환경의 변화에 맞도록 재조정된다. 생체 시계는 규칙적인 환경 변화를 예측하고 이에 미리 대비하게 하는 장점을 지니고 있다.

INTRODUCTION

The living organisms are under the influence of environment which is determined by the Sun and the Moon. The Earth circulates the Sun on a basis of about one year and the Earth itself rotates in a cycle of about 24 hours. The former generates annual rhythm and the latter daily rhythm. The former results in seasonal changes and the latter circadian rhythm, such as sleep-wake cycle.

† Correspondence: Division of Environmental and Biological Sciences, Yong-In University, 470 Samga-dong Yongin-Shi Gyeonggi-do, 449-714 Korea. Tel: 82-31-330-2781, Fax: 82-31-330-2886, E-mail: dcchoi@yongin.ac.kr

The biological clock is an intrinsic internal clock that reflects the changes of natural environmental cues. The clock shows various cyclicity from many cycles to nearly one cycle in a day, to several cycles in a year(Palmer, 2002; Waterhouse et al., 2002; Binkley, 1990).

Humans, like other animals, have been endlessly fighting with against the reluctant phenomena of our ambient environment. In spite of external severe ambient forces, humans have survived due to their sophisticated methods for controlling internal environment. The methods are related to the behavior, physiology, and biochemistry of the organisms that have evolved from the beginning.

The annual cycling of seasons and its effect on nature are well known. It has a range from the comparative dormancy in the winter to the activity of summer. There are clear differences in the ambient temperature and amount of daylight between the seasons, accompanied by such as the migrations of birds, the opening of blossom and leaves, the mating seasons, and the waking up from hibernation. It seems clear that such changes, and its importance to farming and to cultural activities, have long been recognized.

In addition to these annual changes, there are daily rhythms caused by mainly the rotation of the Earth. Living things have evolved through the reflection of the daily changes and the responses.

The distinction between day and night is one of the most pervasive rhythms being experienced. The time taken for the Earth to complete on revolution is about 24 hours. Many aspects of our environment show a daily rhythm with a period of 24 hours(called circadian rhythm, circa = about; dian = a day) as a result of this solar day. Some of circadian rhythms observed in humans are considered here, and where appropriate, some animals.

It is not only the environment but also the living organisms that display rhythms(Wollnik, 1989). The rhythmic phenomena can be seen most clearly when consider their behavior. Human as well as many animals are normally diurnal. they are active in the daytime and inactive at night. Whereas nocturnal organisms show the opposite orientation. Humans are also more flexible than other animals, so that in the modern society many people work at night and sleep during the day. Artificial lighting has enabled us to behave independently of the natural environment. Even so, we normally synchronize our times of sleep, leisure, and meals.

There are not many normal people who are willing to endure the loneliness for the experiment required. Thus using those who are willing to volunteer often has limitation because a good experiment should involve only subjects who are representative of the population as a whole. Working with patients, nonvolunteers hospitalized, is very difficult because of sickness, settings, and the people(doctors, nurses, and medical students) who are taking care of the patient. Despite the difficulties associated with using human volunteers, there is a positive side also that is not available when other animals are used. After the experiment is over each subject can verbally relate all his experiences.

CIRCADIAN RHYTHMS

As mentioned earlier, humans are diurnal, waking up in the morning and sleeping at night. They have a cyclicity of activity time, which is repeated at about 24 hours. When a man was restricted in a cave with everything needed where the environmental cues were totally absent, astounding results were witnessed (Pierpaoli et al., 1996). His biological clock seemed to ignore his mental confusion and guided his body functions in a fairy orderly manner, measuring off days that averaged 24.5 hours in length. It means that the biological clock, when no longer forced into synchrony with, and by, the day/night cycle, may run slightly slower. His rhythm, which is longer than the natural day length, remained out of synchrony with the day/night cycle at the cave entrance for the whole stay underground. Consequent data with more subjects showed circadian sleep/wakefulness rhythms that averaged 24 hours and 40 minutes in length.

The first sleep/wake rhythm has been observed to emerge in the first week of post-uterine life. However, the rhythm is so weak that it can be discerned only by measuring the total minutes awake versus those sleep. By 10 weeks of age, the daytime sleep had dropped to about 3.5 hours, while the nighttime average stretched to 10 hours. It suggests that the neonate began to adapt to the light/dark cycle following an environmental cue.

Blind people who have no idea whether the lights are on or off are thought to live in constant darkness. One of them showed that each night he fell asleep closer and closer to dawn. In usual life at home he fell asleep at 5 am and awoke at 1 pm by 10 days. On the next day he was admitted to the hospital where he was allowed to sleep any time he wished. Without any worry he followed these directions. Each day he fell asleep 48 minutes later on average. He returned home with instructions to force himself to sleep at night only and the rhythm was sustained. During the social activity, even though he tried to follow the instruction with a full effort and got some shut-eye at night, he still tended to fall asleep during daytime. When the onset of the beginning of sleep was extrapolated during the first 36 days, the trend of sleep intervals was 24.8 hours. There was no changes in hospital, but his social activity modified the life-lasting rhythms. This unfortunate man appeared to be in a lifetime struggle with his living clock. He wanted to exist on a 24-hour basis and be in synchronization with the rest of humanity, while his clock was equally obsessed with guiding him along at the 24.8 hour period.

Human body temperature also undergoes a daily rhythm. The body temperature curve in usual life peaked from 7 to 8 pm and was lowest sometime around 5 am. This temperature rhythm persists the whole time, even in the state of sickness, such as flu or polio.

Height is also changed on the basis of a day, due to sleep-wake rhythm. Adult humans show about 2 centimeters taller on getting up in the morning compared with when we go to the bed the last night. It is due to upright posture, loosing stature by the gravity during active daytime. Urine production is higher in the daytime than at night.

Thus, there are many rhythms in the body whose cyclicity is not usually synchronized. Each of the body's many rhythms holds a particular phase relationship with every other rhythm, meaning that all the rhythms do not peak at the same time. When a person is subjected to a constant conditions, his rhythms usually adopt a period slightly longer or shorter than 24 hours in length, and not everyone of them assume the same length. In the longer cyclicity, average sleep/wake interval was 33.6 hours and the cyclicity of temperature rhythm was an average period of only 24.6 hours. The other shorter case showed an average of 12.5 hours in sleep/wake interval and an average period of only 24.5 hours in the cyclicity of temperature rhythm. Thus these two cases present internal desynchronization, indicating that different rhythms are governed by specific clocks that each runs at their own speed.

In addition to the rhythms mentioned above, circadian rhythmicity was also shown in heart rate, cell division, alcohol metabolism, pain feeling, even birth and death time, etc.

PHASE RESPONSE CURVE

The phase response curve is a response of organisms to the constant environmental cue(Binkley, 1990). So the data in relation to human beings are not available because putting human to experiments is impossible. Most of the experiments have been performed with some animals with marked behavior. Golden hamsters are one the mammals who show clear wheel running activities mainly at night(Binkley, 1990; Chesworth et al., 1987). When the animals were put in normal light/dark cycle mimicking natural cycle in animal room, they run the wheel for the full length of darkness. Even though they were put in constant darkness, they still run the wheel along with the cycle, called free running activity, as if they were put in the light/dark cycle. Then the rhythms are modified slowly to adjust to the intrinsic biological clock. The phenomenon was interpreted as an imprinted rhythm both inherited from the consequence of evolution and learned from the beginning of life. The phase response curve is determined by measuring the onset of wheel running activity, by giving a short time(i.e. 1 hour) of light to the animals in the absence of environmental stimulus(light in this case of golden hamsters). By the exposure of animals to light the degree of changes of the onset of wheel running activity is measured by the time period in terms of advance or delay(Fig. 1).

Because the time period of cycle of the animal in the constant darkness is changed, one cycle is determined by the sum of the time period of both non-activity and running activity. The full length of the sum is regarded as 24 hours and the time of the onset of running activity 12 o'clock(the beginning of subjective night). As results, the light delayed the running activity up to 1 hour during from 2 hours prior to the beginning of subjective night to about 3 hours after the subjective night. But the light

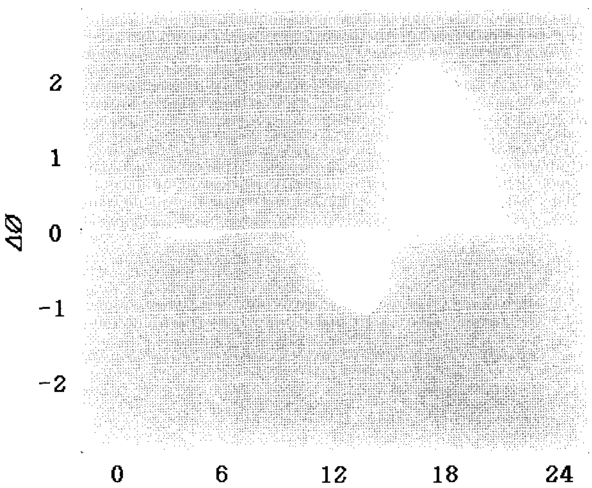

Fig. 1. A profile of phase response curve of golden hamsters measured by wheel running activity. The animals were kept in constant darkness and light were exposed for 1 hour at different time of a day. The changes of the onset of the wheel running activity was put on Y-axis by hours(ΔØ) and exposure time on X-axis(time). Positive numbers show phase advance and - phase delay.

advanced the running activity up to 2 hours during from 3 hours after the subjective night to the beginning of the subjective day. In summary, light exposed to late evening and early night lengthens the cyclicity but light during from middle to late night shortens it. There is no effects of light during the subjective day. The phenomenon is likely to happen to human beings. The phase response curve to light enables the body clock to be adjusted to run in synchrony with our light/dark schedule.

LOCATION OF BODY CLOCK

Although the exact site of the body clock is not known in human because of unethical to perform experiments, there are good evidences to indicate its whereabouts in several mammals. Suprachiasmatic nucleus(SCN) in the hypothalamus, which is located at the base of the brain, is a good candidate(Stetson & Watson-Whitmyre, 1976). Rhythmical electrical activity can be recorded from the nerve cells that make up the SCN and this rhythm has a period of about 24 hours. But it can be also thought that, instead of being the clock itself, these cells merely receive a rhythmic input from other region of the brain which is the clock. Removing the SCN abolishes the rhythms of feeding, drinking, body temperature, and activity. These observations are also not conclusive because of the reason mentioned above. Neural connections between the SCN and most other regions of the brain have been cut, and yet the SCN have continued to be rhythmic. But this approaches, severing all the inputs to the SCN, are not technically possible. Slices of the brain have been removed and incubated in special tissue-culture chambers. When this chambers have been maintained in constant conditions, the electrical and secretory activities from slices of the SCN have continued to show a daily rhythm. Moreover, transplants of brain tissue containing SCN generated rhythmicity in animals that has previously been made arrhythmic by removal of its SCN. Thus the cells of the SCN are sufficient by themselves to produce daily rhythmicity. As far as humans are concerned, there is an anatomical evidence for a group of cells in the same region of the brain as the SCN in other mammals. This strongly suggests that the SCN is the site of the biological clock in human as well.

The hypothalamus controls body temperature, food and water intake, sexual drive, and the secretion of several hormones. The hypothalamus is close to those regions of the brain which are involved in controlling the autonomous nervous system(which controls breathing, heart rate, and blood pressure), sleep, and alertness. Taken together, it can be seen that the biological clock can influence the body as a whole, not only by modifying behavior but also by acting on variables(hormones, the autonomous nervous system, and body temperature) whose effects permeate the entire body.

The information about the clock resides as clock genes in the nucleus. The first such clock gene was found in the fruit fly and named 'per'(short for 'period') and another gene was then discovered and called 'tim'(short for 'time'). These genes have also been found in several animal species, indicating evolutionary conservation. The genes exert their effect by producing proteins that is normal process in the living organisms. If mutations in the clock genes are occurred, changes in the properties of the clock genes are followed by interacting the clock protein with other substances(Ralph & Menaker, 1988). This results in the period of free-running rhythms in these organism being much shorter or longer than the normal.

The cells of the SCN are situated immediately above the optic chiasm, an area where the two nerves which carry visual information from the eyes cross over each other on their way into the brain. Considering the importance of the light/dark cycle as a zeitgeber, an environmental or other periodic influence, is intuitively appropriate, since there will be link between the eyes and the body clock. Indeed, it has been shown in mammalian species that there is a nerve, the retinohypothalamic tract, that runs directly from each eye to one of the SCN. This nerve branches from the optic nerve when the optic nerve enter the brain, the main part of the brain that are involved in the sensation of the vision. This suggests that the sense of vision is separate from the process by which the light/dark cycle acts as a zeitgeber and adjust the body clock.

In rodents, cyclic phenomenon is modified by the administration of melatonin. Melatonin is secreted into the bloodstream from the pineal gland only at night. It also adjusts the body clock, but its phase-shifting effects are opposite to those of light, since melatonin in the morning delays the body clock, and advances it in the evening. There are receptors for melatonin in the SCN, and it is thought that melatonin acts as a zeitgeber.

The secretion of melatonin is suppressed by light, and this means that light and melatonin normally act together to adjust the biological clock. Thus, light on waking in the morning will advance the biological clock, not only by the direct phase-dvancing effect of light at this time, but also by suppressing melatonin secretion and so removing the phase-delaying effect that melatonin would exert at this time. Melatonin receptors were identified and under the intensive investigation(Roca et al., 1996; Reppert et al., 1995a, 1995b; Reppert et al., 1994; Ebisawa et al., 1994; Morgan et al., 1994).

When the possible role of activity as a zeitgeber is considered, it has been shown that introducing a hamster to a new running wheel at different times of the day adjust the timing of the biological clock. The direction of adjustment depends upon the time that the running wheel has been introduced to the animal.

Exercise in human seems to be less effective. Whether this is because we have not exercised long or hard enough, or do not find exercise sufficiently exciting, or are not as sensitive as hamsters to the effects of exercise, is not known. Certainly, our own studies have shown that even when sports science students exercise for half an hour at a vigorous rate, it has no effect on the human clock. Nevertheless, there are input pathways to the SCN through which information about activity could pass.

RHYTHMS AND REPRODUCTION IN HAMSTERS

Although there are a large number of seasonally breeding animals, considerably more effort has been invested in the role of the pineal and its hormone melatonin in controlling repoductive function in a few species of hamsters than other species. The golden hamsters(*Mesocricetus auratus*) are far and away the most well studied. As for most other seasonally breeding rodents, photoperiodic cues are important environmental factors in timing reproduction(Choi, 1996; Stetson & Watson-Whitmyre, 1986). Despite of lacking of field studies, laboratory investigations using both natural photoperiodic conditions and controlled lighting have shown that the golden hamsters are long-day breeders; days of greater than 12.5 hours of light in summer promote and maintain gonadal activity, while short days in winter induce gonadal regression(Elliott, 1976; Gaston & Menaker 1967). prolonged exposure to short days results in a spontaneous resumption of gonadal activity that is preceded by those endocrine changes necessary to induce maturation of the accessory reproductive organs. At this point the hamster is refractory to short photoperiod: even continuous darkness will not cause a second bout of gonadal regression. Refractoriness is terminated by prolonged exposure to long days, and following recovery from refractoriness the animal can once again respond to short daylength with gonadal regression. In relation to the natural photoperiod, gonadal regression commences in late summer; the gonads remain regressed until late winter when spontaneous recrudescence occurs. recrudescence is complete by late spring. Following that time, gonads regain the reproductive activity. Thus reproductive activities are photoperiod-dependent in the golden hamster. To generate the annual reproductive cycle, the golden hamster must distinguish between long and short daylength to correlate gonadal response with season. That is, the animal have a system for measuring daylength and translating this information into neural and neuroendocrine signals to drive the hypothalamic-hypophyseal-gonadal axis. To this end, the pineal of the golden hamsters has been shown to play an integral role. As mentioned above, the photic receptors are in the retina. Removal of the eye results in gonadal regression in reproductively mature hamsters. In a photoperiodic species light has two functions: the most obvious function is the induction of the photoperiodic response(maintenance of gonadal function, stimulation of gonadal recrudescence); less obvious, but every bit as important, is the entrainment of the circadian clock(Liu et al., 1997; Gaston & Menaker, 1967). Therefore, the photic information must be transmitted from the receptor to the clock. There are many evidence showing SCN as a circadian clock regulating wheel-running activity, photosensitivity, and estrous cyclicity in golden hamsters. SCN lesions abolish not only locomotor activity, drinking rhythms, rhythms of adrenal corticosterone but also the rhythms of pineal N-acetyltransferase that is an enzyme involved in producing pineal hormone melatonin. Pinealectomized golden hamsters do not experience short day-induced gonadal regression, indicating roles of melatonin in reproductive activity. Moreover, reproductive functions are modified by the administrations of melatonin, by injections, infusions, and implants, and in a single or multitude times, disrupting natural production of melatonin, which are accompanied by the changes of behavioral parameters (Maywood et al., 1991; Stetson &, Watson-Whitmyre, 1986; Stetson & Tay, 1983; Watson-Whitmyre & Stetson, 1983).

CONCLUSION

Human body has been preparing for waking up in the morning, so that by the time we are better prepared for the rigors of a new day. In the evening, our body clock begins to 'tone down' in preparation for sleep. It has been suggested that there are advantages to possessing a body clock. When humans respond to a change of environment, the response can take place only after the change has begun to exert its effect. it enables clock possessor to predict a regular change and be ready when it takes place. The biological clock governing circadian rhythms is imprinted as a consequence of a long period of time in putatively SCN which is a place of hypothalamus of a brain. But the rhythms are endlessly readjusted to the changes of environmental cues, leading to an evolutionary basis. As far as human beings live on the Earth and are influenced by the changes of the environment, the rhythms will be generated and persisted with fine readjustment forever and ever.

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