Biological Rhythms

(a review of general endocrinology)

- **Neuroendocrine control:** homeostatic responses and biological rhythms. A role for anticipation or feed-forward mechanisms or scheduled events.

- **Biological rhythms and SCN:** what are they and how have been approached (what are oscillators, entrainment, light and melatonin, central and peripheral clocks). SCN, running activity rhythm and ovulatory LH surge. SCN signal transduction.

Today’s lecture

control and “story lines”
Take Home Message (THM)

It is thought that circadian regulation of physiology and behavior imparts survival advantages to organisms that use clocks. In mammals, a master clock resident in the SCN synchronizes other central and peripheral oscillators to evoke this regulation. This master oscillator consists of interlocking transcriptional-translational feedback loops, and it regulates both core clock genes necessary for oscillator maintenance as well as specific output genes that directly or indirectly mediate physiology under circadian control. It is now clear that both neuroanatomic and molecular outputs of the clock are necessary for proper circadian clock function.

It’s all in the timing: many clocks, many outputs.

Neuroendocrine control

Throughout the course I have hinted that feedforward control helps to anticipate needed homeostatic responses (e.g. GLP-1).
A similar anticipation mechanism is based on the presence of biological clocks, central and peripherals, at cellular and sub-cellular levels.
Multiple systems integrator

Hypothalamic nuclei / areas
- PVN, paraventricular nucleus
- ARC, arcuate nucleus
- VMH, ventro-medial nucleus
- DMH, dorso-medial nucleus
- LH, lateral hypothalamus
- ME, median eminence

Extra-hypothalamic nuclei / areas
- A, amigdala
- AP, area postrema
- DMN, dorso motor nucleus of vagus
- NTS, nucleus tractus solitarius
- PBN, para braquial nucleus
- MFB, medial forebrain bundle

from a central hypothalamic integrator to a diffused brain integrator

Neuroendocrine control

the PVN or “integrator” concept and the SCN or “anticipation” concept

hierarchy

CS

- Fb receptor

error signal

controlled variable
Neuroendocrine control

**if glucose is detected in the blood ???**

- Glycemia up
- Insulin up
- Glycemia down

**example**

- Glycemia
- Insulin
- "homeostatic control"
- e.g. glucose on βcells

Neuroendocrine control

**if glucose is detected in the GI tract ???**

- Duodenal glucose up
- GLP-1 up
- βcell "primed"

**example**

- GLP-1
- βcell
- "anticipation"
- e.g. GLP-1 on βcells
Theoretically, a feedforward mechanism anticipates the logistics needed to carry on a specific physiological effect.

So what ??? ... and the rhythms ???

Neuroendocrine control

Biological rhythms

Chronobiology, or the study of biological rhythms, concerns itself with the timing of events within and external to animals.

Single cells and animals have evolved timing systems that are important for every type of behaviors and physiology.

examples

Migration and hibernation versus day and night ???

hours of anesthesia
electrical current
plasma prolactin
plasma melatonin
memory score
The golden hamster is the chronobiologists' favorite animal subject because of their impressive daily rhythms in their use of a running-wheel kept in their cage. They sleep during the day and run all night.
Biological rhythms - a clock

But, what happens if you do not know if it is day or night???

Effect of constant darkness or constant light. Both conditions are free running conditions.
Biological rhythms - a clock

splitting rhythm after prolonged exposure to constant light

(each horizontal line represents two days of data)

activity rhythms Effect of constant darkness or constant light. Both conditions are free running conditions.

Biological rhythms - a clock

Evening (E) / morning (M) oscillators in mammalian photoperiodism.

A, oscillators entrained to a short night rhythm
B, as nights get longer (PRC) the oscillators drift apart since their free running rhythms differ
C, in long nights oscillators adopt an entrainment pattern where they overlap minimally

entrainment (zeitgebers)

A, oscillators entrained to a short night rhythm
B, as nights get longer (PRC) the oscillators drift apart since their free running rhythms differ
C, in long nights oscillators adopt an entrainment pattern where they overlap minimally

entrainment (zeitgebers)

... if light pulses here ...

one clock vs. two clocks entrainment vs. resetting (hold this model until the examples at the end of this lecture)
Biological rhythms - PRC

Effect of a light pulse

Entrainment (zeitgebers)

All individuals are running in constant darkness, a free running condition.

Biological rhythms - Melatonin

Melatonin is derived from the amino acid tryptophane the same as serotonin
Melatonin is released from the pineal gland into the CSF and blood during the night.

**Biological rhythms - Melatonin**

- **CSF**
- **plasma**

**L:D Summer condition**
- e.g. long day vs short night

**L:D Winter condition**
- e.g. short day vs long night

**Summer vs Winter condition**
**Biological rhythms - Melatonin**

**Summer condition** induced by exogenous melatonin infusion

**Winter condition** induced by exogenous melatonin infusion

- e.g. long day vs short night
- e.g. short day vs long night

**Melatonin**

- exogenous melatonin induced Summer vs Winter condition

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**Biological rhythms - Melatonin**

**Free running rhythm of drinking behavior in rats**

- Melatonin
- Vehicle

- Exogenous melatonin entrained rats to the period of the injection regime
Biological rhythms - SCN

Blinded rat with intact SCN

All individuals are blind, thus they are running in constant darkness, a free running condition

So, light may not be necessary ...

Suprachiasmatic nucleus

Blinded rat with intact SCN

Blinded rat with a lesioned SCN

All individuals are blind, thus they are running in constant darkness, a free running condition
So, what might be the relationship among biological rhythms, light, the SCN and melatonin?? ...
So, what might be the relationship among biological rhythms, light, the SCN and melatonin???

Melatonin is released from the pineal gland into the CSF and blood during the night.

... and what might be their relationship with a PVN integration center or with diffused centers?
Theoretically, a feedforward mechanism anticipates the logistics needed to carry on a specific physiological effect.

The PVN or “integrator” concept and the SCN or “anticipation” concept.
SCN - repro rhythm to clock

Blinded rat with intact SCN
(have repro rhythm)

Blinded rat with a lesioned SCN
(do not have repro rhythm)

individuals are blind, thus they are running in constant darkness, a free running condition

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SCN - repro rhythm to clock

The SCN is responsible for the precise timing of the LH surge:

1. SCN lesions eliminates the ovarian cycle (behavior & LH surge).
2. The LH surge maintains its exact relationship to the locomotor activity in a constant light environment, suggesting they are controlled by the same circadian mechanism.
3. Lengthening / shortening of activity cycle by pharmacological treatment or light entrainment alters the estrous behavior and the LH surge.
4. Pentobarbital in proestrous AM temporarily blocks the LH surge, which occurs at precisely the normal time the next day.

... and how would you use this knowledge to make a clock ??? ...
DNA

Steroid S + R ----> SR

Protein synthesis

cAMP ----> PKA ----> channel / enzyme

Na / K pump

Cellular response

5 XX1 HRE 3

RNA

mRNA

... a hint ... how would you use this knowledge to make a clock ??? ...

e.g. repro rhythm to clock

SCN - repro rhythm to clock

... and what the hell is this supposed to mean ??? ...

e.g. repro rhythm to clock
**SCN - repro rhythm to clock**

- SCN - repro rhythm to clock
- SCN - repro rhythm to clock

**Diagram:**
- SCN - repro rhythm to clock
- SCN - repro rhythm to clock

**Text:**
- SCN - repro rhythm to clock
- SCN - repro rhythm to clock

... a negative feedback

**Diagram:**
- SCN - repro rhythm to clock
- SCN - repro rhythm to clock

**Text:**
- SCN - repro rhythm to clock
- SCN - repro rhythm to clock

... a negative feedback
SCN - repro rhythm to clock

- Photoreceptor
- SCN clock neuron
- Retinal ganglion cell
- Glutamate
- Ca++
- Nuclear responses
- Target cell
- Neurogenic or paracrine

Signal transduction:
- Light
- SCN neuron
- Ca++ - Kinase cascades
- CREB/ATF-1
- Clock-controlled gene

Other terms:
- SCN - repro rhythm to clock
- SCN neuron
- CCGs
- CCG
- S
- E
Phosphorylation of CREB

SCN - repro rhythm to clock

Oscillators (SCN & others)

SCN times rhythmic events:

1. SCN lesions eliminates rhythmic events. Fetal SCN transplantation to SCN lesioned rats restore rhythmic activities.

2. The restored rhythm depends on donor SCN (exp: 22h vs 24h rhythm).

3. In some cases, this restoration does not depend on re-establishment of synaptic connections since encapsulated SCN (allows diffusion of small chemicals but not neuronal outgrowth) is able to restore activity rhythms.

4. However, in some other cases, neuronal connections may be necessary to restore rhythmic events.
Oscillators (SCN & others)

Circadian rhythms in isolated brain regions: in vitro studies

Per luciferase transgene expression

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Oscillators (SCN & others)

Circadian rhythms in isolated brain regions: in vitro studies

<table>
<thead>
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<th>Per luciferase transgene expression</th>
<th>% Rhythmic</th>
<th>Amythynic area</th>
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<tr>
<td>Pn</td>
<td>100(10)</td>
<td>BMSTp</td>
<td>3</td>
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<td>HS</td>
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<tr>
<td>AN</td>
<td>90(19)</td>
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Circadian rhythms in isolated brain regions: in vitro studies

Per luciferase transgene expression in pineal gland

extra SCN oscillators

Oscillators (SCN & others)

the PVN or "integrator" concept and the SCN or "anticipatory" concept

and ... how do oscillators get entrained or reseted (zeitgeber) ? ...
Oscillators (SCN & others)

Light resetting kinetics

Delayed induction of Per in shell by peptidergic signals from core

Rapid induction of Per in core by retinal innervation

Retina

Oscillators (SCN & others)

Eye

SCN CLOCK

Humoral cues?

Peripheral clocks

Anticipation of and adaptation to food processing

Anticipation of and adaptation to food processing

Eye

SCN CLOCK
Experimental endpoint: PRC studies using neuronal firing rate in SCN slices

Experimental results: Signaling in the SCN is selectively responsive and integrative

Example of 

entrainment or Zeitgeber pathways

Its all in the timing
Its all in the timing

Take Home Message (THM)

It is thought that circadian regulation of physiology and behavior imparts survival advantages to organisms that use clocks. In mammals, a master clock resident in the SCN synchronizes other central and peripheral oscillators to evoke this regulation. This master oscillator consists of interlocking transcriptional-translational feedback loops, and it regulates both core clock genes necessary for oscillator maintenance as well as specific output genes that directly or indirectly mediate physiology under circadian control. It is now clear that both neuroanatomic and molecular outputs of the clock are necessary for proper circadian clock function.
The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system

R M Buijs, C G van Eden, V D Goncharuk and A Kalsbeek
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Abstract
The biological clock, the suprachiasmatic nucleus (SCN), is essential for our daily well-being. It prepares us for the upcoming period of activity by an anticipatory rise in heart rate, glucose and cortisol. At the same time the ‘hormone of the darkness’, melatonin, decreases. Thus, the time-of-day message penetrates into all tissues, interestingly not only by means of hormones but also by a direct neuronal influence of the SCN on the organs of the body. The axis between the SCN and the paraventricular nucleus of the hypothalamus (PVN) is crucial for the organization/synchronization of the neuroendocrine and autonomic nervous system with the time of day. This SCN-neuroendocrine PVN axis takes care of a timely hormonal secretion. At the same time, the SCN-autonomic PVN axis fine-tunes the organs by means of the autonomic nervous system for the reception of these hormones. Finally, the similar organization of the projections of the human SCN as compared with that in the rodent brain suggests that these basic principles of neuroendocrine-autonomic interaction may also be true in the human. The physiological data collected in humans thus far seem to support this hypothesis, while pathological changes in the SCN of humans suffering from depression or hypertension indicate a role for the SCN in the etiology of these diseases.

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Figure 1: The provides a detailed anatomical scheme of demonstrated and putative connections of the SCN in the rat brain that is used by the biological clock to entrain its rhythm into the neuroendocrine and autonomic neural projections of the PVN. Three major neurotransmitters of the SCN are indicated here: GABA, glutamate and VIP. VIP, which is released during the daytime (Gallone & Rocci 1997) is proposed to inhibit CRH neurons via GABAergic interneurons in the subPVN and DMH. At the same time, it is proposed that the activity of the sympathetic projecting neurons in the PVN e.g. projecting to the spinal is suppressed, by means of a GABAergic modulation, resulting in the release of melatonin secretion. It is proposed that parasympathetically projecting neurons will not receive a direct GABAergic input from the SCN but rather a stimulatory VIP or GABAergic activating input. During the dark period, when the animal is active, the network should lead to an activation of the sympathetically projecting neurons of the PVN and to an inhibition of the parasympathetically projecting ones. The opposite is proposed to be true for the daytime period, when the animal is inactive.
Suggest Reading

Figure 2: This represents the daily peak in plasma corticosterone and melatonin as observed in intact male rats (solid horizontal bars and shaded areas represent nighttime). (Upper figure) Spaced infusions of VP antagonists over the 24 h clearly revealed a stimulatory peak of corticosterone for which an as-yet-unknown SCN transmitter (green) is responsible. In addition, it revealed that a strong endogenous inhibitory action of VP (red line in upper figure) is the strongest in the middle of the inactive period. The stimulatory action on corticosterone secretion is indicated by the green curve. (Lower figure) Infusions of GABA agonists inhibited melatonin secretion at night and the GABA antagonist bicuculline infusions into the PVN resulted in a daytime secretion of melatonin. Consequently, we propose that the secretion of GABA from SCN terminations in the PVN and line in lower figure) shapes the nightly melatonin peak. The three colored lines indicate at least three temporal separated actions of individual SCN neurons.

Suggest Reading

Figure 3: This illustrates the main pathways by which the SCN communicates its circadian signal to the body. Via its connections with the PVN, three different output systems are affected: 1) the neuroendocrine neurons of the PVN responsible for the control of the pituitary hormones, 2) parasympathetically projecting neurons in the PVN that specifically target the dorsal motor nucleus of the vagus (DMN) and 3) sympathetically projecting neurons in the PVN that specifically target the spinal cord preganglionic neurons located in the IML. The peripheral organs are reached via these three mechanisms. The neuronal message, in fact, prepares and sets the sensitivity of the organ for the arrival of the hormones. In return, the neuronal and hormonal message of the organs will feed back to the CNS and SCN as indicated by the three arrows directed to the brain.
Suggest Reading

Figure 4. This illustrates the postmortem staining for VP in the SCN of a control person and of a hypertensive person. Both sections were chosen because they represent the mid-portion of the SCN with the highest number of neurons stained for VP. The figure demonstrates a clear reduction in immunoreactivity in VP neurons in the human SCN in patients who had a long history of hypertension. This dramatic decrease in VP staining, coinciding with a similar decrease in neurotensin or VIP staining, indicates that either these changes are the result of hypertensive feedback or one of the causes of hypertension. (Bar=100 μm). (Reprinted with permission from Goncharuk et al. 2001.)

In conclusion, the present evidence suggests that the SCN utilizes three major mechanisms to synchronize the physiology of the organism to the daily change in activity. These mechanisms activate or inhibit (1) hypothalamic centers that facilitate certain behavior that can be associated with activity or inactivity, (2) neuroendocrine hypothalamic centers that are responsible for hormone secretion and (3) preautonomic hypothalamic neurons that affect parasympathetic and sympathetic autonomic centers in brain stem and spinal cord. This action of the SCN on these target areas is of course not isolated but instead is highly synchronized in order to prepare the whole organism, brain and organs for the coming period of activity. The first postmortem analyses of the SCN in human physiological disorders suggest that indeed the functionality of the biological clock may be affected in diseases such as depression and hypertension (Zhou et al. 2001, Goncharuk et al. 2002).
Suggest Reading

The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system

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