Endocrine Rock 'n Roll: Rhythms and Secretion

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Contents

Although rhythms during hormonal release have fascinated endocrinologists for a long time, many questions—especially regarding their physiological purpose—have not been answered. The origin and relevance of secretory episodes (pulses) from the hypothalamus and pituitary in their 1–3-h intervals are not known. The mechanisms, for example, of regular gonadotropin-releasing hormone (GnRH) release during the fertile years in men and women as well as in many animals defy analysis. Many elements, however, have been defined which determine in isolated neurons or larger tissue aggregates the pulse frequency and pulse amplitude.

Secretory maxima can be observed from active organs. Peak levels, with their rhythmic repetitions, are analyzed in serum. Concentration maxima (peaks or pulses) alternate periodically with minimal secretion (nadirs). Short intervals are defined as peaks with spans of 2–3 h or shorter. Serum-level oscillations during a whole day (circadian pulses), for example, are exemplified by cortisol, with a peak in the morning and a nadir in the evening. Furthermore, serum concentrations of growth hormone or melatonin increase during the night and are dependent on as well as independent of sleep. The annual or seasonal animal fertility cycles and hibernation phases have ultralong rhythms. Complex networks of regulations are at the origin of these periodic changes, and hormones are major parameters. The analysis of the causal zeitgeber of these rhythms is beyond the grasp of endocrinology.

Circadian light–dark cycles and annual temperature oscillation are periodic parameters which act extrinsically on any organism. There is, however, in plants and

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animals a conserved pacemaking mechanism with similar principles in protozoans and metazoans.

12.1 A Universal Pacemaker

The control of the generation and degradation of seven proteins is sufficient to generate a rhythm of about 24 h (circadian). The major functions of these proteins are described in Table 12.1. Decisive is the feedback inhibition exerted by the protein period (PER) on the transcription of PER RNA and thus on its own synthesis. Further important aspects of this autonomous oscillation are as follows:

- Phosphorylation of PER by casein kinase 1 epsilon (CK1ϵ), which is required for PER to get into the cellular nucleus
- Binding of phosphorylated PER to cryptochrome (CRY), allowing transport of the PER–CRY dimer into the nucleus
- Constitutive transcriptional activation of *PER* by promoter binding of the dimer formed between brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like protein 1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK)
- Blocking of any PER transcription by binding of PER–CRY to promoter-bound BMAL1–CLOCK
- Ubiquitin-induced protein degradation—for example, by modification with ubiquitin and subsequent protease digestion in proteasomes

Translation of proteins other than PER—CRY, BMAL1, and CLOCK also occurs periodically, potentially blocked by CRY. Forger and Peskin (2003) developed a model from biochemical data and some assumptions which simulates a periodic almost 24-h autonomous oscillation. Their model takes into account the seven proteins, the transcriptional activity, nuclear–cytosol transport, translation, phosphorylation, cytosol–nuclear transport, and ubiquitin-mediated degradation.

More recently, the effect of PER–CRY on *PER* transcription has been found to result from circadian changes of histone acetylations of *PER* and *CRY*. Rev-ERBa transcribed from the antisense strand of thyroid hormone receptor controls circadian expression of BMAL1 and is itself controlled in a circadian manner, as is the protein ROR (an orphan nuclear receptor similar to retinoic acid receptor) binding to the same DNA site.

The major components of the circadian clock have thus been found: proteins with basic helix–loop–helix (bHLH) structures (Fig. 12.1) and PER–ARNT–single-minded homolog (SIM) (PAS) domains (see Table 12.1), whose transcription, translation, degradation, phosphorylation, and alternate cellular localization in the nucleus and cytosol oscillate in a circadian rhythm. The exact regulation of the cellular rhythm is not yet understood. We recognize the how, understand marginally the biochemistry, and ignore the purpose for which these rhythms are generated.

Table 12.1 Proteins of the zeitgeber

Name/OMIM entry[a]	Function/comment
BMAL1/602550	BMAL1 dimerizes to CLOCK. The dimer interacts with E-boxes: CACGTG DNA motifs with five E-boxes in the PER promoter and one in the CRY promoter. Dimer binding to the E-box activates transcription of *PER* and *CRY*. BMAL1–CLOCK levels are enough to permanently cover all E-boxes. By binding of PER–CRY dimer to a single E-box, PER and CRY transcription is inhibited. ARNT is the nuclear transport mediator for the aryl hydrocarbon receptor (with which dioxin or DDT interacts) and which stimulates *CYP1A1* and activation of other CYP enzymes in response to foreign substances. BMAL1 is very similar to ARNT. BMAL1 has bHLH and PAS domains
CLOCK/601851	CLOCK dimerizes to BMAL1 and binds as a dimer to E-boxes (see above). In 1997, 25 years after the discovery that the supraoptic nucleus plays a role in circadian oscillations, the mouse CLOCK protein was described by two groups as required for maintenance of circadian rhythms. CLOCK has, like PER and BMAL1, bHLH and PAS domains
PER1/602260	Transcription of *PER*[b] and the presence of PER obey a 24-h rhythm. *PER* is transcribed when no PER protein is present. After the cytosolic synthesis of PER, it is phosphorylated by CK1ϵ and CK2ϵ. Phosphorylated PER is co-transported with CRY into the nucleus and blocks the transcription of its own gene: classic feedback inhibition. PER proteins consist of bHLH and PAS domains, the former for DNA interaction (see Fig. 12.1), the latter for dimerization
CRY1/601933	CRY binds cytosolically phosphorylated PER. Thereafter, the complex enters the nucleus. Therein by it binding to BMAL1–CLOCK dimers, *PER* transcription is blocked. Binding by CRY makes the protein TIM (for "timeless") ready for degradation in proteasomes. Photolyases help to eliminate UV defects from DNA. They contain flavin adenine dinucleotide and pterin groups. CRY proteins are such photolyases; their role, however, appears to be to act as blue light photoreceptors. For their involvement in the circadian rhythm, the photolyase function is not important
CK1ϵ/600863	CK1ϵ cytosolically associates with PER and phosphorylates PER several times. This phosphorylated PER then couples to CRY and enters the nucleus. Casein kinases are widely distributed. They phosphorylate serine or threonine residues. CK1ϵ mutations change the circadian rhythm of flies and hamsters. The hamster mutant *tau* inhibits autophosphorylation of CK1ϵ and reduces binding to PER. Thus, nuclear availability of PER is diminished and the circadian rhythm is changed. The same applies for CK2ϵ

ARNT aryl hydrocarbon receptor nuclear translocator, *bHLH* basic helix–loop–helix, *BMAL1* brain and muscle aryl hydrocarbon receptor nuclear translocator like protein 1, *CK1ϵ* casein kinase 1 epsilon, *CK2ϵ* casein kinase 2 epsilon, *CLOCK* circadian locomotor output cycles kaput, *CRY* cryptochrome, *CYP* cytochrome P450, *PAS* period–aryl hydrocarbon receptor nuclear translocator–single-minded homolog, *PER* period

[a]Very detailed descriptions of the identification, cloning, and history of proteins can be found on the Internet: http://omim.org/entry/*number in column 1*

[b]For simplicity PER1 and PER2 are treated as one entity like CRY1 and CRY2. "PER" thus means PER1 and/or PER2

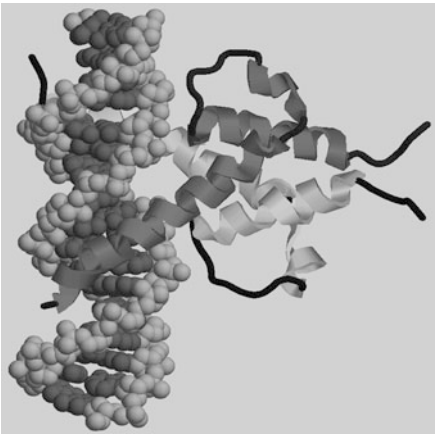

Fig. 12.1 Binding of two basic helix–loop–helix (bHLH) proteins to DNA. The long helix of the first bHLH (*dark gray*) interacts with the major DNA groove, and its short helix interacts with the second bHLH, which sinks its large helix into the next major groove (Produced with RasMol using Protein Data Bank entry 1A0A)

Neurons of the suprachiasmatic nucleus (nucleus suprachiasmaticus) are capable of maintaining, without any optical stimulation from an external light–dark cycle, a circadian rhythm for weeks. In that regard they differ from other cells where a circadian rhythm can be observed which, however, is stopped once supraoptic nucleus (nucleus supraopticus) input has ended. Thus, neurons of the supraoptic nucleus constitute the self-sufficient, superior, light-independent *zeitgeber*.

Light-independent circadian rhythms determined when animals are kept in permanent darkness differ individually in length and are about 22.5–23.5 h long. In a usual daily course with light–dark phases from the sun, the circadian rhythm is set by specialized optical nerves at sunset (reset!). Most probably all other endocrine secretory rhythms are generated with input from the supraoptic nucleus and thus accept the externally controlled day–night rhythm. A shift of the so-called biorhythm from the external day–night rhythm thus, although often stated, cannot be confirmed by endocrine analysis. Arctic winters and summers where the day–night rhythms are interrupted and permanent night shifts are exceptional conditions for circadian endocrine secretion. The endocrinology of subpolar life with very long days in summer and very short days in winter might provide valuable research themes.

12.2 Circadian Rhythms (Pulse Frequency 24 ± 2 h)

In Fig. 12.2, two different pulse rates can be distinguished: a short one with intervals of about 1 h (circhoral or ultradian) and a longer one with a 24-h oscillation (circadian). A circadian oscillation can be overlaid by an ultradian rhythm. In the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), we notice circadian variability from the shift of pulse amplitudes. For glucocorticoid hormone release, a circadian rhythm is apparent with peak secretion during the night and a nadir during the day.

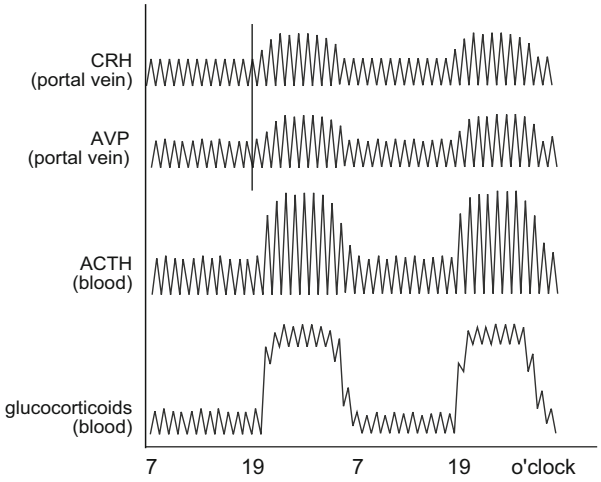

Fig. 12.2 Circadian rhythm of corticotropin-releasing hormone (*CRH*)/arginine vasopressin (*AVP*)/adrenocorticotropic hormone (*ACTH*) pulse amplitude and cortisol release. In daily rhythm, CRH and AVP pulse amplitudes oscillate under control of the suprachiasmatic nucleus. This influences, in turn, ACTH pulses, where nadirs still occur in short intervals. Adrenal glucocorticoids show these short oscillations; however, the baseline is shifted after onset of darkness (Redrawn from Chrousos 1998)

From the scheme one can conclude that circadian glucocorticoid pulses appear as a consequence of enhanced CRH amplitudes. The circadian rhythm of glucocorticoid secretion has its origin in the brain: neurons of the suprachiasmatic nucleus project to neurons of the paraventricular nucleus (nucleus paraventricularis) and trigger CRH release.

12.3 Ultradian Rhythms (Pulse Frequencies Below 22 h)

CRH is secreted as shown in Fig. 12.2 in a hourly rhythm, GnRH is secreted at 60–90-min intervals, and insulin secretion peaks every 4 min. For understanding the regularities in the generation of secretory episodes, we should regard neurosecretory cells not as hormone-producing ones, but as neurons. Nunemaker et al. (2003b) demonstrated that GnRH neurons possess several kinds of action potentials—that is, activities of ion channels and coupled calcium oscillations are at the origin of pulsatile hormone release. Most probably a coordinated influx of calcium into cells occurs, and these are discharged and therefore secrete hormone simultaneously (Fig. 12.3). With regard to insulin secretion from β cells, similar electric pulses and calcium oscillations precede the insulin secretions. Enhancing the intracellular calcium will trigger fusion of secretory granules to the cell membrane, where the granular content is freed (Sect. 11.4, Fig. 11.6).

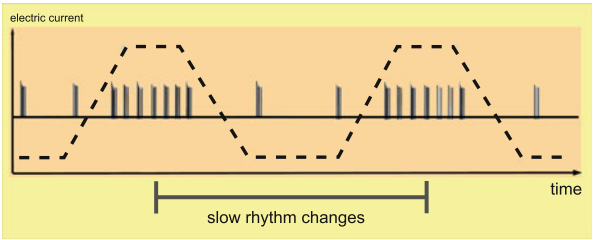

Fig. 12.3 Rhythm of autonomic episodic channel openings in gonadotropin-releasing hormone (GnRH) neurons. The opening of ion channels causes the membrane potential to change. If the membrane potential is measured for long periods, episodes become apparent where rapid openings and closures occur (so-called bursts). The frequency of bursts follows a slower rhythm depicted by the *dashed line*. In the same rhythm, secretory granules fuse to the cell membrane and thus release hormones, here GnRH, in a periodic manner (Redrawn from Nunemaker et al. 2003b)

With use of GnRH tumor lines (GT1-7) it was shown that sodium and calcium channels are mainly responsible for transmembrane ion currents; these effects could also, however, be generated by coordinated potassium ion influx. Different calcium channel types were involved (Nunemaker et al. 2003a).

Insulin-producing β cells as well as GnRH neurons occur together with many other cells of similar type. When pulses of insulin or GnRH are generated, secretion has to be coordinated. For this, the slower rhythm—that is, the burst frequency (Fig. 12.3)—has to be kept in time. A mechanism for this has yet to be found. In GnRH neurons, axonal interplay using GABAergic and noradrenergic neurons can be assumed, whereas in β cells, no mechanism has yet been proposed. Since the pancreas and the islets are intensively innervated, a noradrenergic control might perhaps coordinate insulin release.

The pulsatile GnRH release during the menstrual cycle is modulated by estradiol. Again, the slow rhythmic frequency of burst episodes is changed, not their intensity or the variation of rare versus frequent bursts. It appears that estradiol interacts where the coordination is controlled.

Current knowledge suggests that GnRH neurons generate their burst rhythm autonomously. Single cells or cell lines from GnRH neurons demonstrate rhythmic channel openings and secret GnRH in an episodic manner. What is missing is information about the biochemical origin of these episodes. For other hormone-producing cells, any proteins or genes have been found generating regular rhythms in second or minute intervals.

We do know, however, that permanent application of GnRH or its synthetic derivatives blunts any episodic GnRH release. The GnRH receptor is found not only on luteinizing hormone/follicle-stimulating-hormone-producing gonadotropic cells of the pituitary, but also in the hypothalamus. This makes GnRH when it is secreted hypothalamically in a paracrine way or as neurotransmitter a candidate for the signal that coordinates release from GnRH neurons in the median eminence (eminentia mediana). A permanently elevated GnRH level would, in turn, result

in hypothalamic GnRH receptors being occupied, induce their internalization and degradation, and thus mediate a potential GnRH receptor blockage of GnRH synthesis. If we assume a GnRH–GnRH receptor interaction results in periodic stimulation of GnRH synthesis, permanently elevated levels of GnRH (or GnRH agonists as well) would disturb this rhythm. For the explanation of the effects of a GnRH receptor antagonist, receptor blockage suffices.

12.4 Seasonal (Annual) Rhythms

The length of the light–dark phases of a day changes with the season. Simultaneously, the median temperature varies. Existential functions such as collecting food, hibernation, and reproduction are coupled to these rhythms. All wild animals are subject to these rhythms, whereas domestic animals are no longer fully dependent on warm–cold phases or summer–winter rhythms.

Endocrine adaption of sexual activity with the seasonal rhythm has been analyzed in only a few examples (Fig. 12.4). These examples exhibit so many species characteristics that we restrict ourselves to basic determination of seasonal rhythm.

In recent years a new role of the upper part of the anterior pituitary, the pars tuberalis, was found. Pars tuberalis cells play a role in seasonal endocrine control.

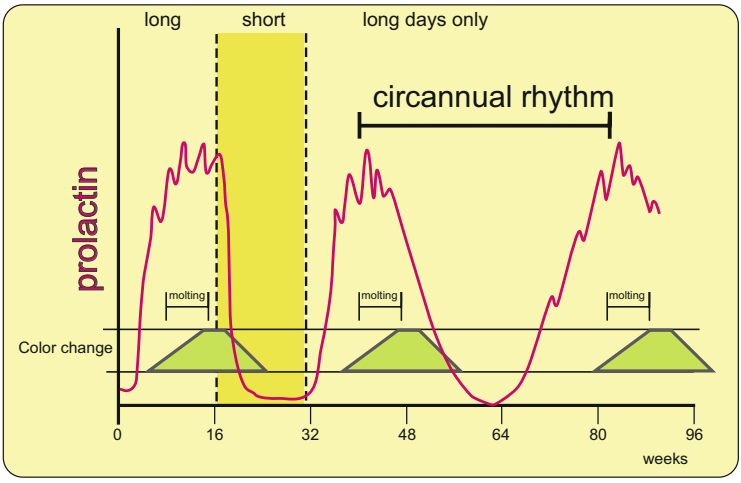

Fig. 12.4 Annual rhythm of prolactin release and coat color changes in the Soay sheep. Under the influence of clock genes and the seasonly determined melatonin, pituitary prolactin release is triggered in the light season. This, in turn, controls color coat changes and the start of the molt. The basic rhythm is prolonged when the day length (experimentally maintained) is not shortened (*right*). The level of prolactin in blood still decreases, and a coat color change occurs. Yet, a new rise in prolactin level followed by another coat color change and molt occurs without changes in the light–dark rhythms, but with longer intervals than in daylight-controlled phases (Redrawn from Lincoln et al. 2003)

These cells, called calendar cells, express the clock genes (see earlier) *PER*, *CRY*, *BMAL1*, and *CLOCK*. In the pars tuberalis, however, their expression, in contrast to that in the suprachiasmatic nucleus, appears to be controlled by melatonin. One premise for this is melatonin receptor expression in these cells. Melatonin serum levels are strongly dependent on the length of darkness (see Sect. 7.3), and thus levels are low in summer and high in winter. Calendar cells integrate the amount of melatonin present and differentiate short (6–10-h) from long (12–16-h) melatonin receptor interaction episodes.

As a consequence, in seasons with short nights, the calendar cells secrete a prolactin-stimulating hormone, tuberalin, whose identity has not yet been established. As described in Sect. 4.5.2, a prolactin-stimulating hormone in sensu stricto has not been found in the hypothalamus of animals and humans; in contrast, prolactin release is strongly blocked by dopamine. Such a tuberalin might be a de facto prolactin-releasing hormone or an endogenous dopamine antagonist, binding to but not activating the dopamine receptor.

In contrast to the suprachiasmatic nucleus, the amplitude of PER expression and that of inducible cyclic adenosine monophosphate (cAMP) early repressor (ICER) are seasonally controlled: PER and ICER are synthesized mainly on long days; on short days very little if any PER or ICER is synthesized. By injection of melatonin, PER and ICER expression is delayed. PER and ICER expression starts when melatonin is removed. With high levels of melatonin removed (dark season), more PER and ICER are made than in light seasons. Mice lacking the pineal gland or those unable to synthesize melatonin or with defective melatonin receptors do not show morning PER and ICER peaks. As long as melatonin receptors are occupied, cAMP-dependent signal transduction is blocked. With melatonin lacking in daylight, these signals can be switched on and PER and ICER can be made (Messager et al. 1999).

We stress that these phenomena of secretion rhythms are become better understood with respect to their generating mechanisms. The physiological relevance of these findings has not been unraveled yet. It may be that these ultradian, circadian, and seasonal periodicities serve to maintain receptor sensitivities. Another possibility might be that by secretion in pulses, signal detection is facilitated with easier distinction of a signal from serum noise.