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Chapter 24

Chronotherapy

DANIEL P. CARDINALI[1], GREGORY M. BROWN[2], AND SEITHIKURIPPU R. PANDI-PERUMAL[3]*

[1]*Faculty of Medical Sciences, Pontificia Universidad Católica Argentina, Buenos Aires, Argentina*
[2]*Department of Psychiatry, Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada*
[3]*Somnogen Canada Inc, Toronto, ON, Canada*

Abstract

The objective of chronotherapy is to optimize medical treatments taking into account the body's circadian rhythms. Chronotherapy is referred to and practiced in two different ways: (1) to alter the sleep–wake rhythms of patients to improve the sequels of several pathologies; (2) to take into account the circadian rhythms of patients to improve therapeutics. Even minor dysfunction of the biological clock can greatly affect sleep/wake physiology causing excessive diurnal somnolence, increase in sleep onset latency, phase delays or advances in sleep onset, frequent night awakenings, reduced sleep efficiency, delayed and shortened rapid eye movement sleep, or increased periodic leg movements. Chronotherapy aims to restore the proper circadian pattern of the sleep–wake cycle, through adequate sleep hygiene, timed light exposure, and the use of chronobiotic medications, such as melatonin, that affect the output phase of circadian rhythms, thus controlling the clock. Concerning the second use of chronotherapy, therapeutic outcomes as diverse as the survival after open-heart surgery or the efficacy and tolerance to chemotherapy vary according to the time of day. However, humans are heterogeneous concerning the timing of their internal clocks. Not only different chronotypes exist but also the endogenous human circadian period (τ) is not a stable trait as it depends on many internal and external factors. If any scheduled therapeutic intervention is going to be optimized, a tool is needed for simple diagnostic and objectively measurement of an individual's internal time at any given time. Methodologic advances like the use of single-sample gene expression and metabolomics are discussed.

INTRODUCTION

Biological rhythmic oscillations are ubiquitous in behavior and metabolism over a 24-h span. Environmental factors such as light: dark cycles (LD), nutrient availability, temperature, and exogenous exposure to toxins can influence those oscillatory processes (Bass and Takahashi, 2010; Buhr et al., 2010; Wright et al., 2013). Most species contain a molecular clock that coordinates transcriptional and/or biochemical rhythms with strong implications for physiology and health (Hastings and Goedert, 2013). Thus, the mammalian circadian clock has been shown to influence immune responses, variation of inflammatory responses as a function of time of day, and susceptibility to infections (Keller et al., 2009; Scheiermann et al., 2013; Chen et al., 2020). A disrupted circadian clock is a risk factor for various disorders including neurodegeneration, metabolic syndrome, diabetes, and cancer (Turek et al., 2005; Marcheva et al., 2010; Hastings and Goedert, 2013; Shilts et al., 2018). Circadian misalignment has become pervasive in modern 24/7 society as a result of increased exposure to light at night, shift work, air travel, social jet lag, and lack of sleep (Akerstedt, 2003; Wittmann et al., 2006; Wyse et al., 2014; Potter et al., 2016). Moreover, a disturbed

*Correspondence to: S.R. Pandi-Perumal, Somnogen Canada Inc, College Street, Toronto, ON, M6H 1C5, Canada. E-mail: pandiperumal2021@gmail.com

circadian clock not only increases the risk of pathophysiology, but most diseases can, in turn, alter circadian rhythmicity.

The goal of chronotherapy is to optimize medical treatments taking into account the body's circadian rhythms. Chronotherapy is referred to and practiced in two different ways: (1) to alter the sleep–wake rhythm of patients to improve pathologies and (2) to take into account the circadian rhythms of patients to improve therapeutics. We will briefly discuss both approaches of chronotherapy and how the field can be further expanded by the use of single-sample gene expression assays and metabolomics to measure individual body's time.

BIOLOGICAL RHYTHMS: AN OVERVIEW RELEVANT FOR CHRONOTHERAPY

Circadian clock universality, from cyanobacteria to angiosperms and from protozoa to mammals, including *Homo sapiens*, has now been documented (Foster, 2020). Circadian rhythms are powered by endogenous pacemakers that have periods that, in the absence of appropriate time signals, are approximately 24 h long. The external and internal signals that achieve entrainment of the endogenous oscillator to an exact period of 24 h are called Zeitgebers. In humans, the most important external Zeitgeber is the LD cycle (Foster, 2020). In mammals, the circadian system is composed of many individual, tissue-specific cellular clocks. To generate coherent physiological and behavioral responses, the phases of this multitude of cellular clocks are orchestrated by a master circadian pacemaker residing in the hypothalamic suprachiasmatic nucleus (SCN) (Buijs et al., 2017). In mice, the SCN comprises approximately 20,000 neurons, and in humans 50,000 neurons (Abrahamson and Moore, 2001; Goncharuk et al., 2001). The SCN contains locally projecting neurons that communicate with each other and with other hypothalamic structures (Hastings et al., 2020). The axons of many SCN neurons end within the nucleus itself, thus forming local and/or collateral circuit connections from longer-range projections. The SCN core projects thickly into the SCN shell, which has scattered projections back to the core. The neuronal cell bodies in the SCN are small, have simple dendritic arbors, and are very close (Van den Pol, 1980).

Neurons in the SCN core and shell regions differ depending on their neurochemical content (Ma and Morrison, 2020). Vasoactive intestinal peptide (VIP) is expressed in approximately 10% of all SCN neurons, while arginine vasopressin (AVP) is expressed in approximately 20% of SCN neurons. VIP positive neurons are located mainly in the ventral and central part of the SCN. In addition to VIP, neurons in the SCN core also contain substance P, gastrin-releasing peptide, calretinin, and calbindin. The largest proportion of AVP-positive neurons is found in the dorsomedial part of the SCN (i.e., the shell). Neurons containing cholecystokinin and prokineticin 2 are found in this region in addition to AVP neurons. In most SCN neurons, neuropeptides are colocalized with γ-aminobutyric acid (GABA), and almost all synapses between SCN neurons are GABAergic (Ono et al., 2020). It has been reported from electrophysiologic data that glutamate is also present in the efferent pathways of the SCN. The greater electrical activity has been observed in the SCN during the day, in both nocturnal and diurnal mammals.

Concerning SCN efferents, AVP and VIP fibers arising from the SCN branch extensively to innervate the SCN itself and the central and medial part of the anteroventral hypothalamic area, the area below the paraventricular nucleus (sub-PVN), the ventral part of the PVN, and the dorsomedial nucleus of the hypothalamus (Dai et al., 1997, 1998). The observation that AVP fibers, and to some extent VIP fibers pass between the SCN and the PVN, indicates that the human SCN and the PVN have also a direct anatomical connection. The SCN and the arcuate nucleus have mutual projections, being a circuit of major importance for the association between time and metabolism (Buijs et al., 2017). It may explain metabolic disruption and obesity in shift workers, or jet lag, light at night, and short sleep conditions in terms of circadian disruption. Some SCN neurons of unknown identity reach neuropeptide Y neurons in the arcuate nucleus, while SCN VIP neurons project to α-MSH neurons in the arcuate nucleus and also innervate the area in this nucleus close to the median eminence. SCN AVP neurons contact kisspeptin neurons in the arcuate in female rats, and SCN prokinecitin 2 fibers also project to the arcuate nucleus. In turn, the arcuate nucleus sends projections from agouti-related peptide neurons to the ventrolateral region of the SCN, which is also a target of for kisspeptin projections (Buijs et al., 2019).

At a molecular level, circadian clocks are based on clock genes, some of which encode proteins able to feedback and inhibit their transcription. These cellular oscillators consist of interlocked transcriptional and post-translational feedback loops that involve a small number of core clock genes (about 12 genes identified currently) (Welz and Benitah, 2019). The negative and positive transcriptional/translational feedback loops to form the core clockwork have been characterized in rodents by transgenic gene deletion methodology. Clock gene expression oscillates because of the delay in the feedback loops, regulated in part by phosphorylation of the clock proteins that control their stability, nuclear reentry, and transcription complex formation (Welz and Benitah, 2019).

The reader is referred to other chapters in this section for a detailed description of this hierarchy. We will mainly deal with the major synchronizing outputs of the circadian system, namely environmental light, melatonin, food intake, locomotor activity, and cortisol, which are all relevant to the subject of chronotherapy.

Circadian rhythms can be altered in terms of their three main components, i.e., period (τ), amplitude, and phase, by a variety of stimuli including photic and nonphotic ones, as well as a large number of chemical disturbances that can influence the biological clock. Indeed, in almost every pathologic condition, acute or chronic, a significant chronodisruption occurs (Golombek et al., 2013). An entraining agent can reset, or phase shift, the internal clock. Depending on when an organism is exposed to such an entraining agent, circadian rhythms can be advanced, delayed, or not shifted at all. Therefore, adjusting the daily activity pattern to the appropriate time of day involves a rhythmic variation in the influence of the Zeitgeber as a resetting factor (Duffy et al., 2015).

Light impinging on a particular population of photosensitive retinal ganglion cells is a major Zeitgeber modifying the activity of the SCN (Hastings et al., 2020). The primary photoreceptor pigment involved in the effect of the LD cycle on circadian rhythmicity is melanopsin, present in a minute group of uniquely photosensitive retinal ganglion cells. These cells form the specialized retinohypothalamic tract, which has efferent connections to the SCN and other hypothalamic nuclei (Foster, 2020). Rod and cone cells in the retina play a relatively minor role in circadian photic input. In addition, the SCN receives direct innervation from many sensory areas, such as the nucleus of the solitary tract, the circumventricular organs, and the arcuate nucleus. This information is important for adjusting circadian physiology to changes in food availability, locomotor behavior, and other environmental stressors.

Through autonomic nervous system projections involving the superior cervical ganglia, the SCN controls the release of the major internal synchronizer melatonin (Cardinali, 2017). The SCN drives and controls nocturnal synthesis and secretion of the pineal hormone melatonin, which in turn interacts with melatonin receptors on SCN neurons. The SCN master clock regulates secondary oscillators present in most of the body's organs via changes in melatonin and cortisol levels and the activity of the sympathetic nervous system. Consequently, most physiological functions display rhythmic changes. Furthermore, this action extends to the cyclic, ebb-and-flow activity of most mental and emotional functions, e.g., stupor, depression, elation, and excitement (Zaki et al., 2018).

Concerning food intake as a Zeitgeber, it should be stressed that circadian rhythms have a strong influence on the timing of food intake and metabolic processes. As an example of predictive homeostasis, circadian rhythms set up bodily physiology for optimal use and storage of energy. In turn, food-related signals are internal Zeitgebers providing temporal order to organs involved in metabolic regulation as well as to the SCN itself (Buijs and Guerrero-Vargas, 2017). Thus food intake should be synchronized with the SCN to construe efficient responses to environmental challenges. In humans, a loss of synchrony between mealtime and the SCN promotes obesity and metabolic disorders. The feedback of peripheral signaling to the SCN includes hormone-sensitive brain areas that project to the SCN, as well as sensory feedback from the autonomic nervous system (Espitia-Bautista et al., 2017).

The circadian system is involved at many levels in the regulation of energy metabolism. This includes synchronization of behavior such as food intake and locomotor activity with homeostatic responses in body temperature, heart rate, hormone secretion), as well as with cellular and molecular fluctuations like the uptake and production of metabolites, gene expression, and activity of metabolic enzymes (for reference, see Méndez-Hernández et al., 2020).

In preparation for the metabolic demands that occur during a 24-h cycle, information about time is preserved in skeletal muscle through the cellular circadian clocks (Gutierrez-Monreal et al., 2020; Kemler et al., 2020). Indeed, the skeletal muscle can be considered one of the largest collections of peripheral clocks, with an important contribution to energy metabolism. Gene expression of the muscular circadian clock in rodents and humans reveals common diurnal patterns based on rest/activity cycles rather than on LD cycles. Experimental studies in which the circadian clock is disrupted in skeletal muscle demonstrate impaired glucose management and insulin resistance. Circadian misalignment in humans modifies skeletal muscle clocks and leads to impaired energy metabolism and insulin resistance. Exercise is a powerful modulator of skeletal muscle metabolism and is considered a crucial preventive and therapeutic intervention strategy for sarcopenia (Choi et al., 2020; Mirizio et al., 2020).

Concerning glucocorticoids, changes in circulating cortisol are driven by the PVN (Minnetti et al., 2020). This is mediated by the stimulation of the hypothalamic–hypophyseal–adrenal axis via the release of PVN corticotropin-releasing hormone into the portal system to stimulate ACTH secretion from the adenohypophysis. In rats, the PVN also controls via a direct projection of the sympathetic autonomic nervous system to the adrenal cortex, the release of corticosterone (the physiological corticoid in this species) (Buijs et al., 2019). The feedback control by glucocorticoids is given

by glucocorticoid receptors located in the pituitary, arcuate nucleus, PVN, and hippocampus (McEwen et al., 1968; de Kloet et al., 1998). Remarkably, PVN neurons projecting to the adrenal gland do not express glucocorticoid receptors but are strongly affected by projections from the arcuate nucleus that detect and produce fast adjustments of circulating corticosterone (Leon-Mercado et al., 2017). Moreover, mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) agonists in the arcuate nucleus prevent the increase of corticosterone after stress, indicating the fundamental role of the arcuate nucleus to modify glucocorticoid secretion in stress or time of day (Leon-Mercado et al., 2017).

THE SLEEP/WAKE CYCLE, A TARGET FOR CHRONOTHERAPY

Although the study of circadian rhythms has flourished in recent decades, the application of chronobiologic principles to clinical medicine and, more specifically, to therapeutic medicine is still in development. As an integrative discipline in physiology and medical research, chronobiology makes possible the discovery of new therapeutic tools that address central mechanisms in various diseases.

The sleep/wake cycle reliably reflects the function of the circadian clock in various animal species, including humans (Foster, 2020). Its endogenicity was demonstrated by its persistence under constant environmental conditions. This rhythm is controlled by the genes of the molecular clock in mammals. In humans, the sleep/wake cycle is considered and has been used as a marker of the circadian synchronization system in isolation studies and in psychiatry. The relative ease of monitoring the sleep–wake cycle has further supported its use as a reference rhythm for circadian time of medications and for evaluation of circadian clock function (Zaki et al., 2018).

Circadian disruption by sleep loss affects every major system in the human body. Chronic changes in sleep have been associated with a plethora of serious medical problems from obesity and diabetes to neuropsychiatric diseases. Several epidemiologic studies have reported associations between sleep/wake cycle disruption and cardiometabolic disease, including reports studying the effects of shiftwork (Chellappa et al., 2019; Rijo-Ferreira and Takahashi, 2019; Grandner, 2020). Short sleep duration has further been associated with incident diabetes and weight gain, as well as impaired appetite control. Shortened sleep and poor sleep quality have also been identified as risk factors for cognitive decline, neurodegenerative disease, mood changes and depression, as well as other neuropsychiatric conditions (Brownlow et al., 2020; Ferini-Strambi et al., 2020). There is also mounting evidence linking sleep to both immune function and cancer (Shilts et al., 2018; Daniel et al., 2020; Haspel et al., 2020; Iranzo, 2020).

Sleep-inducing drugs, including hypnotics such as benzodiazepines (BDZ) and nonbenzodiazepine BDZ receptor agonists (zolpidem, zaleplon, eszopiclone, also known as *Z*-drugs), have clear chronopharmacologic effects that result in temporary changes in their pharmacologic profiles (Golombek et al., 2015). Also, since the side effects of various drug families include sleep-inducing properties (for example, in asthma and other medications for respiratory diseases), prescriptions should take into account the timing of administration to take full advantage of such side effects.

In the case of human sleep, its duration and organization depend fundamentally on its circadian phase and is regulated by the interaction of homeostatic and circadian processes that are carried out independently, but in a complementary way. The homeostatic component (process S, for "sleep") leads to sleep approximately one-third of each 24-h cycle, and the circadian component (process C) links the desire to sleep with the daily fluctuations of hormones programmed by the body clock. This two-process model of sleep, first proposed by Borbély in 1982, explains how homeostatic and circadian factors regulate the quantity and timing of sleep (Borbély et al., 2016). According to this view, the requirement for sleep increases during wakefulness because of homeostatic process S in the brain, while circadian process C reflects circadian modification of vigilance. Borbély's theory states that the likelihood of wakefulness and sleep are traded off against one another in a circadian mode. Homeostatic process S is defined as a homeostatic sleep-promoting process, which continuously escalates during wakefulness. Process S is related to a decrease in intellectual performance and vigilance and an increase in sleepiness/fatigue while awake. During sleep, particularly slow wave sleep, process S continuously decreases (i.e., sleep pressure disintegrates). In contrast, the circadian scheduled process C (also known as the circadian pacemaker) is best seen as a nearly 24-h endogenous oscillatory variation for sleep propensity.

The circadian rhythm in synthesis and secretion of pineal melatonin is closely associated with the sleep rhythm in both normal and blind subjects (Emens and Eastman, 2017). The onset of nighttime melatonin secretion is initiated approximately 2 h in advance of an individual's habitual bedtime and has been shown to correlate with the onset evening sleepiness. Several studies implicate endogenous melatonin in the physiological regulation of the circadian mechanisms ruling sleep propensity (Gobbi and Comai, 2018).

The two-process model of sleep regulation is also useful to understand how chronobiological treatments differ

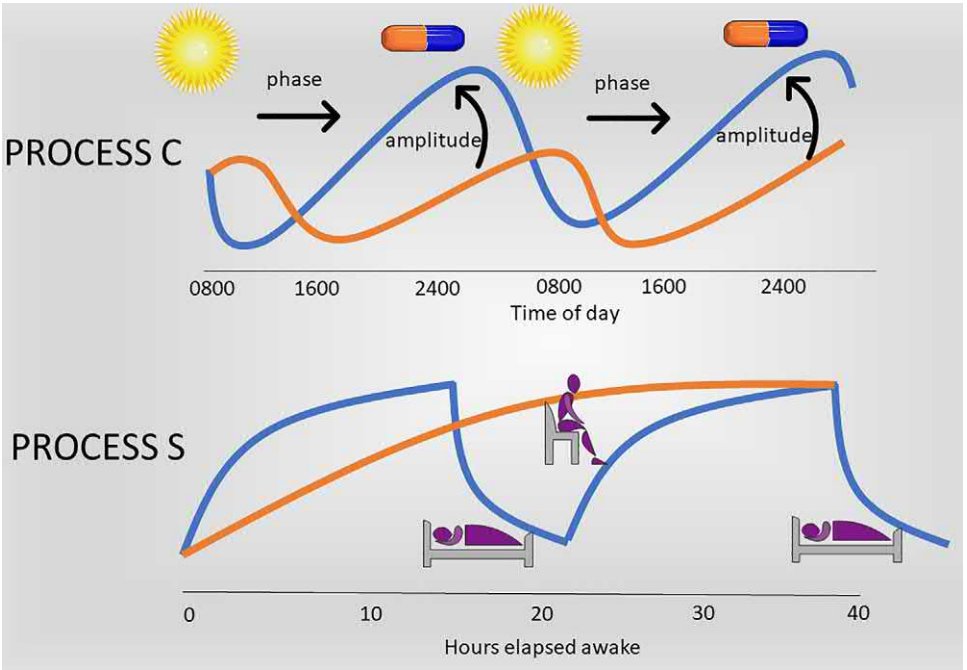

Fig. 24.1. Effect of chronotherapy in depressed patients. A schematic representation of the two-process model of sleep regulation over 2 days is shown (*blue lines*: normal; *red lines*: depressed patients). The homeostatic process S represents the increase in sleep pressure with elapsed time awake, and its dissipation during sleep. The circadian process C follows time of day and is characterized by its endogenous period, phase, and amplitude. Chronotherapy (light in the morning, melatonin at the evening) acts primarily on C, not S. The low build up of process S can be increased by sleep deprivation (lower panel).

in their mode of action, by targeting either the sleep homeostat or the circadian system (Fig. 24.1). Manipulations of the sleep–wake cycle have profound and rapid effects on depressed mood in 60% of all diagnostic subgroups of affective disorders (Wirz-Justice and Benedetti, 2020). The therapeutic effect of sleep deprivation is associated with an increase of homeostatic sleep pressure (lower panel. Fig. 24.1). An increase in amplitude and a phase advance of sleep alone, without sleep deprivation, also improves mood (top panel, Fig. 24.1) (Wirz-Justice and Benedetti, 2020).

Many studies have shown that sleep/wake disruption is an important contributor to neuropathology. Sleep deprivation (Ooms et al., 2014) or slow wave sleep disruption (Ju et al., 2017) has been shown in healthy subjects to increase cerebrospinal fluid (CSF) levels of amyloid β (Aβ)1–42 and Aβ1–40, respectively. This reduction was reported to be prevented by a single night of total sleep deprivation. Similar findings were obtained in mice, in which sleep deprivation caused increases of Aβ peptides in the interstitial fluid of the brain (Kang et al., 2009). A “glymphatic” hypothesis (Iliff et al., 2012) holds that an active, lymphatic-like movement occurs in brain extracellular space driven by perivascular astrocytes, which are strongly enriched in aquaporin-4 and by changes in the vascular lumen. Water release via aquaporin-4 would be responsible for an actively driven fluid exchange between para-arterial and para-venous spaces. The exchange of solutes between the CSF and the interstitial fluid occurs mostly during slow wave sleep when the cortical interstitial space increases by more than 60% and provides a low-resistance path for the movement of CSF and interstitial fluid in the brain parenchyma (Iliff et al., 2012; Wardlaw et al., 2020). This is impaired in the aging human brain. Various neurologic disease states such as stroke, traumatic brain injury, and AD have been interpreted in terms of the contribution by glymphatic dysfunction (Boespflug and Iliff, 2018).

In recent years, there has been a significant expansion in the development and use of multimodal sensors and technologies to monitor sleep and circadian rhythms. These developments make accurate sleep monitoring at scale a possibility for the first time. Vast amounts of multisensor data are being generated with potential applications ranging from large-scale epidemiologic research linking sleep patterns to disease to wellness applications, including the sleep coaching of individuals with chronic conditions (Perez-Pozuelo et al., 2020).

SOME BASIC CONCEPTS ABOUT CHRONOPHARMACOLOGY

Traditionally, drug delivery has meant getting a simple chemical absorbed predictably from the gut or from the site of injection. A second-generation drug delivery goal has been the perfection of continuous constant rate (zero-order) delivery of drugs. However, living organisms are not "zero-order" in their response to drugs. Rather, they are predictable resonating dynamic systems, which require different amounts of drug at predictably different times within the circadian cycle in order to maximize desired and minimize undesired drug effects.

Medical chronobiology is concerned with the mechanisms of periodic influences on health and disease (Foster, 2020). Chronopathology is the study of biological rhythms in disease processes and in morbid and mortal events; most medical conditions are affected by circadian rhythms. Chronopharmacology is the discipline that investigates the effects of a drug as a function of biological time (Dallmann et al., 2016; Ballesta et al., 2017; Winter and Soehnlein, 2018; Ohdo et al., 2019).

Two concepts must be considered when dealing with day-related changes of drug efficacy: (1) circadian changes in drug bioavailability (chronopharmacokinetics) and (2) circadian changes in the susceptibility to the drug (chronesthesia). Clinical chronopharmacology (or chronotherapy) is the purposeful alteration of drug level to match rhythms to optimize therapeutic outcomes and minimize size effects.

As the body's primary defense against metabolic poisoning, and the target of many toxic substances, the liver is continuously exposed to relatively high amounts of ingested drugs or toxins. Being a major organ of metabolism and detoxification of drugs, knowledge of circadian effects on transcriptional activities that govern daily biochemical and physiological processes in the liver is key for pharmacological and toxicological studies. A circadian variation in relative expression levels of enzymes responsible for phase I and phase II categories of drug metabolism was found (Zmrzljak and Rozman, 2012; Ballestri et al., 2016). In phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics. In phase II, the modified compounds are then conjugated to polar compounds by transferase enzymes such as glutathione S-transferases.

Therefore it is essential to consider time of day effects on drug administration and animal sacrifice when designing and interpreting toxicology studies.

Chronopharmacology involves both investigating the pharmacological effects based on biological timing mechanisms and investigating the pharmacological effects on body rhythms. In terms of the drug's effects, temporal variations may affect its pharmacokinetics (i.e., chronopharmacokinetics) due to underlying changes in absorption, distribution, metabolism, and overall bioavailability, or its pharmacodynamics, reflected by changes in expression of drug receptors or signal transduction mechanisms (chronopharmacodynamics). In psychotropic drug terms, chronopharmacodynamics is attributed to a rhythmic neurotransmission system, including temporal changes in neurotransmitter levels, receptors, and second messengers. In addition, time-related variations in toxicity and unwanted side effects should also be considered (chronotoxicity) (Dallmann et al., 2016). All these concepts converge in the definition of chronopharmacology, which deals with the design and evaluation of drug delivery systems that release a bioactive agent at a rate that ideally coincides with the biological requirement of a given disease therapy. As a consequence, chronotherapy advocates the use of temporal characteristics of the patient and the disease process to optimize the therapeutic response and minimize undesirable side effects of a drug, e.g., treatment of sleep and psychiatric disorders with light therapy or hormonal intervention (Ballesta et al., 2017; Ohdo et al., 2019).

Several diseases with established oscillatory rhythm in their pathogenesis have been identified. In the case of asthma, chronotherapy has been extensively studied (Thakur et al., 2019; Waggoner, 2020). Airway resistance increases progressively at night in asthmatic patients. Circadian changes are seen in normal lung function, which reaches a low point in the early morning hours. This dip is particularly pronounced in people with asthma. Chronotherapies that have been employed for asthma include oral corticosteroids, theophylline, and β_2-adrenoceptor agonists (Thakur et al., 2019). All of them are target to correct airway resistance increase at night.

The chronobiology, chronopharmacology, and chronotherapeutics of osteoarticular pain have also been extensively reviewed (Whibley et al., 2019). Patients with osteoarthritis tend to have less pain in the morning and more at night, while those with rheumatoid arthritis have pain that usually peaks in the morning and decreases throughout the day (Thakur et al., 2019). In addition, a number of drugs used to treat rheumatic diseases have varying therapeutic and toxic effects based on the time of day of administration. Chronotherapy for all forms of arthritis should be timed to ensure that the highest blood levels of the drug coincide with peak pain. For osteoarthritis sufferers, the optimal time for a nonsteroidal antiinflammatory drug such as ibuprofen would be around noon or midafternoon. The same drug would be more effective for people with rheumatoid arthritis when taken after the evening meal (Galbo and Kall, 2016; Whibley et al., 2019).

Many of the functions of the gastrointestinal tract exhibit circadian rhythms (Orr et al., 2020; Parasram and Karpowicz, 2020). Gastric acid secretion is highest at night, while gastric and small bowel motility and gastric emptying are all slower at night. These 24-h rhythms have important implications in the pharmacokinetics of orally administered drugs: at nighttime, when gastric motility and emptying are slower, drug disintegration, dissolution, and absorption are slower. Suppression of nocturnal acid secretion is an important factor in duodenal ulcer healing. Therefore for active duodenal ulcer, once daily at bedtime is the recommended dosage regimen for H_2-antagonists (Jamali et al., 1995).

Cardiac events occur with a circadian pattern (Chellappa et al., 2019). Numerous studies have shown an increase in the incidence of early morning myocardial infarction, sudden cardiac death, stroke, and episodes of ischemia. This is because several functions in the cardiovascular system (blood pressure, heart rate, stroke volume, cardiac output, blood flow) show circadian rhythmicity (Chellappa et al., 2019; Dredla and Castillo, 2019; Grimaldi et al., 2019). For example, platelet aggregability increases and fibrinolytic activity decreases in the morning, leading to a state of relative hypercoagulability of the blood (Budkowska et al., 2019). Blood pressure is at its lowest during the sleep cycle and rises steeply during the early morning awakening period. In addition, circadian changes in lipid fractions in patients and normal subjects may contribute (Otamas et al., 2020). A circadian rhythm of hepatic cholesterol synthesis occurs; studies with β-hydroxy β-methylglutaryl-CoA reductase inhibitors (statins) indicated that evening dosing was more effective than morning dosing (Kouhpeikar et al., 2020). The circadian variations of glucose and insulin in diabetes have been also extensively studied and their clinical relevance in case of insulin substitution have been discussed (Bass and Takahashi, 2010; Garfield, 2019). This is of particular importance for devices including real-time continuous glucose monitoring and continuous subcutaneous insulin infusion (sensor-augmented insulin pumps).

In the case of cancer, human and animal studies indicate that chemotherapy may be more effective and less toxic if cancer drugs are administered at carefully selected times that take advantage of tumor cell cycles while less toxic to normal tissue (Dallmann et al., 2016). The blood flow to tumors and tumor growth rate are each up to threefold greater during daily activity phase of the circadian cycle than during the daily rest phase. This chronotherapy strategy offers further promise for improving current cancer-treatment options, as well as for optimizing the development of new anticancer or supportive agents (Dallmann et al., 2016). Concerning brain tumors, chronotherapeutic drug sensitivity can be important for gliobastoma treatment. In the case of DNA alkylating agents like temozolomide, recent studies have shown that its cytotoxicity is modulated by cell-intrinsic circadian rhythms in glioblastoma cells in both humans and mice. Likewise, after the adjuvant usage of curcumin with chemotherapeutic drugs like cisplatin or doxorubicin glioblastoma, cell death was attained upon the temporal administration of curcumin several hours prior to the rhythmic peak expression of mPER2 protein (for a discussion, see Arafa and Emara, 2020).

As far as other drugs that affect the CNS, information on their chronopharmacology has long been available. Several chronopharmacologic studies have been performed on the effects of antipsychotic and antidepressants drugs (for reference, see Zaki et al., 2018). The timing of drug efficacy along the circadian cycle differed among drugs, even when the same endpoints were compared. For example, when the effects of dosing time on the pharmacological activity of several antidepressants acting on serotonergic, noradrenergic, and/or dopaminergic neurons were investigated, all antidepressants reduced immobility, but their activities varied according to the dosing time (Kawai et al., 2019). Moreover, the peak time often varied with the variable measured for a given drug.

MELATONIN, A CHRONOBIOTIC PROTOTYPE

Drugs that directly affect the circadian phase, and thus the output of the biological clock, are called chronobiotics and, in fact, they represent a promising line of research for the treatment of circadian disorders, particularly when they lack undesirable side effects. The term chronobiotic was introduced in the early 1970s and has been used to broadly define a drug that affects the physiological regulation of the structure of biological time and, specifically, is capable of therapeutically recovered desynchronized circadian rhythms in the short or long term, or prophylactically avoiding its interruption after an environmental attack (Dawson and Armstrong, 1996). The magnitude and direction of phase changes depend on the circadian phase in which these compounds are administered, which in turn produces pronounced phase changes in behavioral rhythms. For example, melatonin given in the morning delays the phase of circadian rhythms, while when given in the evening it advances the phase of circadian rhythms. For most part of the day, melatonin administration is unable to modify the phase of the clock (phase response curve). The requirements for an ideal chronobiotic are summarized in Fig. 24.2.

Melatonin is a prototype chronobiotic that plays a major function in the coordination of circadian rhythmicity

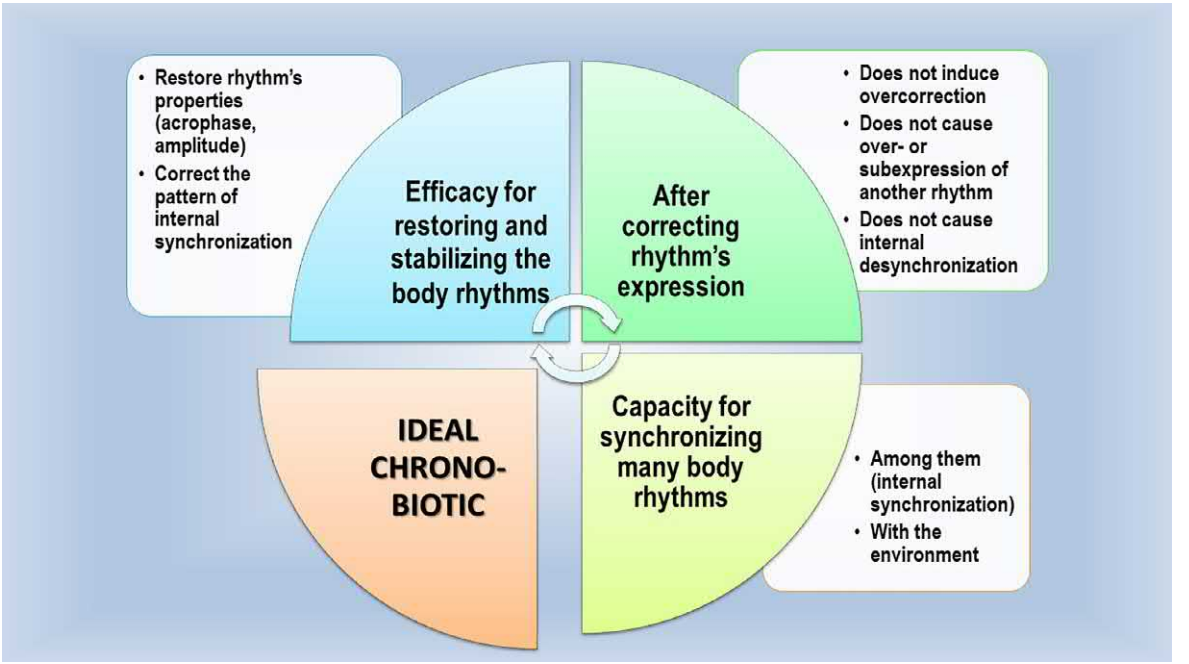

Fig. 24.2. An ideal chronobiotic drug must display efficacy for restoring and stabilizing the body rhythms, must not induce over- or subcorrection of rhythms and must have the capacity to keep internal synchronization as well as synchrony with the environment. Melatonin shares most of these properties.

(Pevet et al., 2017). Melatonin secretion is an "arm" of the biologic clock in the sense that it responds to signals from the SCN and that the timing of the melatonin rhythm indicates the status of the clock, both in terms of phase (i.e., internal clock time relative to external clock time) and amplitude (Arendt, 2018). From another point of view, melatonin is also a chemical code of night: the longer the night, the longer the duration of its secretion. In most mammalian species, this pattern of secretion serves as a time cue for seasonal rhythms (Clarke and Caraty, 2013).

Pineal melatonin production is controlled by a complex neural system originating in the hypothalamic PVN and terminating in the high levels of the thoracic spinal cord—the superior cervical ganglion sympathetic system. The postganglionic sympathetic nerve terminals of the superior cervical ganglion release norepinephrine into the pineal gland that triggers melatonin synthesis by its interaction with β- (mainly) and α-adrenoceptors on the membrane of pineal cells. Melatonin, due to its high diffusibility, is not stored inside the pineal and is released as soon as it is produced (Tan et al., 2018). The structures which regulate circadian rhythms have been described as the SCN-melatonin loop (Tan et al., 2018). This loop includes melanopsin-containing retinal ganglion cells, the retinohypothalamic tract, SCN, PVN, intermediolateral cell column, the sympathetic cervical ganglia, the pineal gland, and the melatonin rhythm which feedback impacts the SCN.

As a result, the melatonin production and consequently its CSF and blood levels are circadian in nature and tightly synchronized with the environmental LD cycle. Indeed, the circadian pineal production of melatonin is restricted to the dark phase of the LD cycle in all mammalian species. It is noteworthy that melatonin is always produced during the night independent of the daily pattern of activity/rest of the species, indicating its strong relationship with the external photoperiod. Additionally, melatonin is produced during the night provided there is no light. Given the regularity of the daily melatonin production that is associated with high and low or absent blood concentrations during the night and day, respectively, melatonin is able to synchronize the circadian rhythms of several organs and their functions (Arendt, 2019).

Like the effects induced by the external Zeitgeber light, effects by the internal Zeitgeber melatonin on the circadian clock are time-dependent. Daily timed administration of melatonin to rats shifts the phase of the circadian clock, and this phase shifting may explain the effect of melatonin on sleep in humans. Indirect support for such a physiological role derived from clinical studies on blind subjects (who show free running of their circadian rhythms) treated with melatonin (Skene and Arendt, 2007). More direct support for this hypothesis was provided by the demonstration that the phase response curve for melatonin was opposite (i.e., about 180 degrees out of phase) to that of light (Lewy, 2010).

The direct effects in regions containing high densities of melatonin receptors, such as the circadian pacemaker SCN, or the anterior pituitary pars tuberalis (PT), a site of particular relevance for photoperiodically controlled reproduction (Clarke and Caraty, 2013) strongly supported the premier significance of melatonin's physiological role. Although the control of circadian and seasonal rhythmicity represents melatonin's main physiological function, the actions of the methoxyindole are by no means restricted to areas of high receptor density (Hardeland et al., 2011).

The pleiotropy of melatonin has to be analyzed at different levels, from the sites of synthesis and local dynamics, distribution of receptors and other binding sites in target organs, cell-specific differences in signaling as related to the presence of G protein variants, and intracellular effects—with a particular focus on mitochondrial actions—to numerous secondary changes induced by influencing other hormones, neurotransmitters, neurotrophins, and further signal molecules (Cardinali, 2019b). In functional terms, melatonin exerts a host of effects that can be under the control of the SCN and, in seasonal breeders, the premamillary hypothalamus and the PT, and may also have direct effects in numerous peripheral organs. In particular, melatonin is involved in sleep initiation, vasomotor control, adrenal function, antiexcitatory actions, immunomodulation including antiinflammatory properties, antioxidant actions, and energy metabolism, influencing mitochondrial electron flux, the mitochondrial permeability transition pore, and mitochondrial biogenesis (Hardeland et al., 2011; Cardinali, 2019b).

The chronobiotic action of melatonin is mediated via the melatonin receptors, which have been identified both in the CNS and in the periphery (Dubocovich et al., 2010). MT_1 and MT_2 receptors all belonging to the superfamily of membrane receptors associated with G-proteins (G-protein-coupled receptors, GPCR) have been cloned. More recently, another member, GPR50, was included in the melatonin receptor subfamily. GPR50 shows high-sequence homology to MT_1 and MT_2 but does not bind to melatonin or any other known ligand. Ligand-independent functions for GPR50 such as the allosteric regulation of other proteins/receptors through their interaction with GPR50 in common protein complexes have been proposed. In the case of the molecular complex of GPR50 with the melatonin MT1 receptor, GPR50 negatively regulates the function of MT1.

An interesting feature of GPR50 receptors is their capacity to form homo- and heteromers between each other and also with other GPCRs (Cecon et al., 2017) among them the serotonin 5-HT_{2C} receptor. The heteromers display functional properties different from those of the corresponding homomers. For example, acting on the MT_2/5-HT_{2C} heteromer, melatonin binding induces the activation of Gq signaling through a transactivation of the serotonergic receptor caused by conformational changes of the MT_2, which is normally not coupled to a Gq.

Circulating melatonin is loosely bound to albumin and in the liver, it is first hydroxylated and then conjugated with sulfate and glucuronide (Claustrat and Leston, 2015). In human urine, 6-sulfatoxymelatonin has been identified as the main metabolite. In the brain, melatonin is metabolized into kynurenine derivatives. As already mentioned, in mammals, circulating melatonin is derived almost exclusively from the pineal gland. In addition, melatonin is synthesized locally in most cells, tissues, and organs, including lymphocytes, bone marrow, thymus, gastrointestinal tract, skin, and eyes, where it can play an autocrine or paracrine role (Acuña-Castroviejo et al., 2014). Indeed, there is now strong evidence that melatonin is produced in every animal cell that has mitochondria (Tan and Reiter, 2019). In both animals and humans, melatonin participates in diverse physiological functions that indicate not only the duration of the night but also improve the elimination of free radicals and the immune response, showing relevant cytoprotective properties (Cardinali, 2019a).

Melatonin is a powerful chronobiotic with very slight hypnotic capacity. Daily doses of 2–5 mg melatonin timed to advance the phase of the internal clock by interaction with MT_1 receptors in the SCN, maintains synchronization of the circadian rhythms to a 24-h cycle in sighted persons who are living in conditions likely to induce a free-running rhythm (Lewy, 2010). Melatonin synchronizes the rhythm in persons after a short period of free-running. In blind subjects with free-running rhythms, it has been possible to stabilize, or entrain, the sleep/wake cycle to a 24-h period by giving melatonin, with resulting improvements in sleep and mood (Skene and Arendt, 2007). In normal-aged subjects and in demented patients with disturbed synchronization of the sleep/wake cycle (Riemersma-van der Lek et al., 2008) melatonin administration is helpful to reduce the variation of onset time of sleep. In demented patients, melatonin improved the circadian rhythm, cognition and mood, and diminishes nocturnal restlessness. The phase-shifting effect of melatonin is also sufficient to explain its effectiveness as a treatment for circadian-related sleep disorders, such as jet lag or delayed phase sleep syndrome (Arendt, 2018; Burgess and Emens, 2018).

Several meta-analyses support the view that the chronobiotic/hypnotic properties of melatonin are useful in aged patients with primary sleep disorders to decrease sleep onset latency and to increase total sleep time, with

little if any effect on sleep efficiency (Ferracioli-Oda et al., 2013; Auld et al., 2017; Li et al., 2018). At least two expert consensus reports support such a role of melatonin in adult insomnia (Wilson et al., 2010; Geoffroy et al., 2019).

FUTURE OF CHRONOTHERAPY

A key barrier to the widespread adoption of chronotherapy is that humans are heterogeneous with respect to the timing of their internal clocks. In other words, humans have different "entrainment phases," a concept that underlies different "chronotypes" whose physiological and behavioral rhythms range from early (morning chronotypes) to late (evening) chronotypes (Roenneberg et al., 2007). Furthermore, the endogenous human circadian period (τ) is not a stable trait but depends on many internal and external factors, including genetics (Stothard et al., 2017), dependence on age and sex (Roenneberg et al., 2007), season (Hsu et al., 2015), exposure to light (Allebrandt et al., 2014; Dallmann et al., 2016; Stothard et al., 2017), and time zone (Roenneberg et al., 2007). Therefore if any scheduled therapeutic intervention is going to be optimized, a tool is needed for simple diagnostic and objectively measurement of an individual's internal time at any given time.

Current approaches to evaluating internal time are questionnaire based and are therefore not objective, or are cumbersome and expensive, and require multiple measurements under controlled conditions such as dim light melatonin onset (DLMO). Indeed, DLMO is the current gold standard for evaluating the circadian phase (Facer-Childs et al., 2020). Because light suppresses melatonin secretion, sampling should be done under controlled low-light conditions and with a frequency (every 30–60 min over a period of 5–6 h) that makes the protocol difficult for use in daily clinical routine.

Two interesting technical papers have substantially advanced this scientific field. Wu and coworkers used a hybrid design combining epidermis samples from both deep phenotyping of 20 individuals and snapshot data from more than 200 other human subjects to find biomarkers of the human circadian phase (Wu et al., 2018). By using an algorithm (cyclic ordering by periodic structure, CYCLOPS) to reconstruct the temporal order of all samples, the authors identified hundreds of genes cycling at population level and found that phase relationships of human skin epidermis output genes were conserved. After measuring global mRNA expression, computational approaches define 188 diurnal epidermal genes and a set of 29 biomarker genes whose combinatorial expression could accurately determine the circadian phase within 3 h from a single gene (Wu et al., 2018).

Wittenbrink et al. followed a three-stage biomarker development strategy that began with the circadian transcriptomics of blood monocytes from 12 individuals in a consistent routine protocol combined with machine learning approaches to identify biomarkers for internal time (Wittenbrink et al., 2018). These biomarkers were then migrated to a clinically relevant gene expression profiling platform that was externally validated by an independent study with 28 early or late chronotypes (Wittenbrink et al., 2018). Therefore these two papers point out to the epidermis and blood monocytes as sources of single-sample gene expression assays that can determine the phase of the body clock.

Another recent approach to measure internal time is circadian metabolomics (Minami et al., 2009; Brown, 2016; Malik et al., 2020). Current high-throughput technologies allow the simultaneous monitoring of multiple dynamic cellular events over time, ranging from gene expression to metabolite abundance and subcellular localization. These fundamental temporal and spatial perspectives have allowed a more comprehensive understanding of how various dynamic cellular events and biochemical processes are related in health and disease. With advances in technology, metabolomics has become a more routine "omics" approach to studying metabolism, and several groups have recently embarked on "circadian metabolomics" (i.e., studying the 24 h metabolome). To date, circadian metabolomes have been reported for human serum, saliva, breath, and urine, as well as tissues of various species under specific disease or mutagenesis conditions (Minami et al., 2009; Brown, 2016; Malik et al., 2020). Importantly, these studies have consistently revealed that 24-h rhythms prevail in almost all tissues and metabolic pathways. Furthermore, these circadian rhythms in tissue metabolism are ultimately linked and driven by internal 24-h biological clocks. This redundant network can transmit metabolic and bidirectional timing information for optimal timing of metabolic processes. They have also helped illuminate individual variation in these mechanisms that could prove important in personalized therapy for metabolic disease. Finally, these technologies have provided platforms with which to detect potential drugs that affect clock-modulated metabolic function.

CONCLUDING REMARKS

In addition to posing scientific and technical challenges, chronotherapy runs counter to the current in much of the developed world, which is designed to free people from natural biological rhythms. Many can enjoy the comfort and encouragement that the so-called 24/7 Society offers. Night lighting makes the streets safer, and in a culture where people consider time to be a

precious commodity, sleep can be interpreted as a waste. Specific to the patient experience, the hospital care process is designed around the rapid delivery of diagnostic and medical intervention services, and therefore circadian rhythms and sleep quality are inevitably ignored. However, biological sleep and established circadian rhythms are essential for health, and a greater effort is urgently needed to communicate scientific findings to the general public and the medical community (Haspel et al., 2020).

An important technical need to facilitate chronotherapy studies is a fast and robust way to determine the individual's circadian phase (biological time of day versus external time). The reason for this is that in our society, almost everyone is at risk of being out of sync due to modern infrastructure (i.e., artificial light, social media, irregular sleep–wake schedules, time zone changes, etc.). As an example, analysis of human circadian transcriptomes from biopsy samples suggests that approximately 20% of the population is significantly lagged relative to most, perhaps reflecting the prevalence of shift work in developed countries, also the effect of light, its intensity, color and timing, as well as behavioral activity, are important as inputs to the clock (Anafi et al., 2017; Lucassen et al., 1995; van Oosterhout et al., 2012; Opperhuizen et al., 2017; Itzhacki et al., 2018; Molcan et al., 2019; Nagai et al., 2019; Phillips et al., 2019).

Any chronotherapy trial in which an intervention is tied to a certain time of day must deal with the fact that the internal biological time of the patients involved can be quite different. Future attempts to translate circadian biology to the clinic should focus on ways to define each participant's circadian phase in advance so that therapy is delivered at the optimal time for each individual. To this end, it would be valuable to include a run-in period in which sleep hygiene and uniform circadian training are pursued as a goal before attempting a chronotherapy intervention. However, requiring this initial period for chronotherapy trials could discourage future research due to the higher cost and complexity of the study this practice would incur. Fortunately, recent work on circadian biomarkers suggests that spot analysis of gene expression in blood samples can determine the internal circadian time of patients, which may offset the need for a run-in period (Wittenbrink et al., 2018; Wu et al., 2018). Circadian metabolomics could also be helpful (Minami et al., 2009; Brown, 2016; Malik et al., 2020). Ultimately, societies may or may not be prepared to make changes to, for example, how hospitals are run or the adoption of extended-release drug preparations that are more expensive in the short term but confer superior medical and long-term economic utility (Haspel et al., 2020).

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