Annu. Rev. Pharmacol. Toxicol. 1992. 32:51–66

CONCEPTS IN CHRONOPHARMACOLOGY

Alain E. Reinberg

Chronobiology-Chronopharmacology Unit, Fondation A. de Rothschild, 29 rue Manin, 75940 Paris Cedex 19, France

KEY WORDS: chronopharmacology, chronotherapy, circadian rhythms, dosing time dependencies

INTRODUCTION

Chronopharmacology is the study of how the effects of drugs vary with biological timing and endogenous periodicities. The goal is to improve our understanding of periodic and thus predictable (e.g. circadian) changes in both desired effects (chronoeffectiveness) and tolerance (chronotolerance) of medications. Dosing time-dependent changes also include quantification of parameters characterizing endogenous circadian rhythms (CR), in terms of pharmacologic effects, e.g. the 24-h adjusted mean (M), the period (τ), the amplitude (A, the peak-to-trough difference), and the acrophase (ϕ, the peak time location in the 24-h scale). Chronopharmacology became recognized as a scientific domain of investigation only in the early 1970s (1–3).

For conventionally trained pharmacologists, it was not clear that predictable temporal variations of effects and disposition of agents (e.g. medications, hormones, and toxic substances) are governed by endogenous biological rhythms rather than by changes of external factors. On the 24-h scale (as well as on the yearly scale) there are peaks and troughs of physiological variables that are not randomly distributed; their respective locations correspond to a temporal organization controlled by a set of pacemakers (so-called biological clocks) that presumably are interconnected and hierarchized (3–6).

Periodic changes of environmental factors (zeitgebers or synchronizers) are used by organisms as signals and cues to reset the circadian clocks. For many

0362-1642/92/0415-0051$02.00

plants and animals, prominent zeitgebers (time givers) are the light–dark (LD) cycle, alternation of rest and activity related to constraints of work schedules, and/or habits of social life in humans (4, 6–8).

Zeitgebers do not create rhythms (including periodic changes in the metabolic fate of an agent and its effects), but they are able to synchronize them. Therefore, both the subject synchronization (e.g. the LD cycle of an experiment with rodents) and dosing time of a drug must be known for the correct interpretation of pharmacologic data (1, 4, 6).

Chronopharmacologic phenomena can be viewed as resulting from an adaptation of the organism to cyclic environmental changes, e.g. those associated with the Earth's rotation in ca. 24 h. Von Mayersbach (9) demonstrated that the circadian cytological reorganization in the liver of the rat was associated with functional readjustment. At the end of the activity span (onset of light), the liver cells contain the largest amount of glycogen and the smallest amount of proteins. At the end of the rest span (onset of darkness), only a few glycogen granules are apparent, but the albumin level in the liver is at its highest. These circadian changes are not related to food intake because they persist during fasting; they are under the control of the temporal organization of enzyme activities involving glycogen-synthetase I and phosphorylase *a* (10). Many other activities (e.g. mitosis, DNA and RNA synthesis) and enzyme functions, including drug-metabolizing enzymes (2, 11), exhibit large circadian changes in the liver cells. Moreover, the latter is one of several examples showing coordinated ultrastructural, enzymatic, and functional circadian rhythms at the subcellular and molecular level (4, 6, 9, 12, 13).

It seems that the many different functions of a cell are programmed in time, presumably because there is limited energy available. Therefore, energy is spared in favoring a certain function at a certain time (e.g. mitotic processes, rebuilding of subcellular fractions, and storage of energy) and in shifting to another function at another time (e.g. specific activities of the cell such as hormone production and/or release, secretion of metabolites, and production of enzymes) (4, 9). Enzyme networks are likely to participate in the temporal organization in a sense that a given metabolic pathway is used at a certain time of the day whereas a different one is used 12 h later or earlier.

From a practical point of view, the expression of the effect of a drug will depend on what stage of a programmed function is featured when the agent reaches the target cell. Thus, chronopharmacologists are confronted with new facts that must be explained to and understood by traditionally schooled physiologists and pharmacologists. Concepts such as chronokinetics, chronesthesy, chronergy, and chronotherapy (4) must be redefined to include present knowledge. In addition, chronopharmacology must include new aspects dealing with genetic-, sex-, and age-related differences.

CHRONOKINETICS

Chronokinetics are defined as dosing time–dependent and predictable (rhythmic) changes in parameters used to characterize the pharmacokinetics (or the bioavailability) of a drug, e.g. maximum concentration (C_{max}), span of time to reach C_{max} (t_{max}), area under the concentration–time curve (AUC), and half-life ($t_{1/2}$) (14; Figures 1 and 2).

By 1991, chronokinetic circadian changes of more than 100 different molecules had been documented (15), with 31 articles devoted to dosing-time dependencies in the bioavailability of theophyllines alone (16). However, chronokinetic changes may be restricted not only to a single-compartment model but also to changes in the model itself; such is the case for mequitazine, an H1 antihistamine. Therefore, chronokinetics of certain drugs may involve changes from a mono- to a multicompartmental model as a function of drug dosing time (4). Chronokinetics of drugs have been validated for many animal species including humans, with both acute and chronic administration even for sustained release preparations having a half-life as long as 84 h.

Mechanisms Underlying Chronokinetic Changes

With regard to physical properties of drugs (e.g. hydrophilicity, lipophilicity, and solubility), circadian changes in biosystems have been explored and even modulated with regard to their absorption (e.g. speed of gastric emptying and intake from gut, lung, and skin), distribution (e.g., blood flow through an organ and binding capacity of plasma proteins) metabolic rates relative to liver functions, and excretion relative to kidney functions (e.g. glomerular filtration, tubular reabsorption, and urinary pH) (4, 11, 13, 15, 17, 18). Proper mathematical formulas have been used to calculate the absorption and excretion rate constants, the clearance, and the volume of distribution from pharmacokinetic data to validate dosing time–related changes (Figure 1). The dominant factor underlying circadian rhythms (CR) in kinetics may differ from drug to drug; the major role is played by gut absorption for theophylline (16) and by renal clearance rate for ketoprofen, a nonsteroid anti-inflammatory drug (NSAID) (19), and 5-fluorouracil (5-FU), an anticancer agent (20). However, multifactorial process must always be considered in order to fully understand the chronokinetics of a drug. Entire chapters of books have been devoted to these questions (4, 6, 15, 21). Here we discuss two examples: One is selected with regard to its critical importance (CR of liver enzymes), and the other selected for its interest is not usually taken into account (CR in plasma protein binding of drugs).

CIRCADIAN RHYTHMS OF LIVER ENZYMES Radzialowski & Bousquet (2) demonstrated CR in hepatic drug-metabolizing enzymes of rodents. Temporal

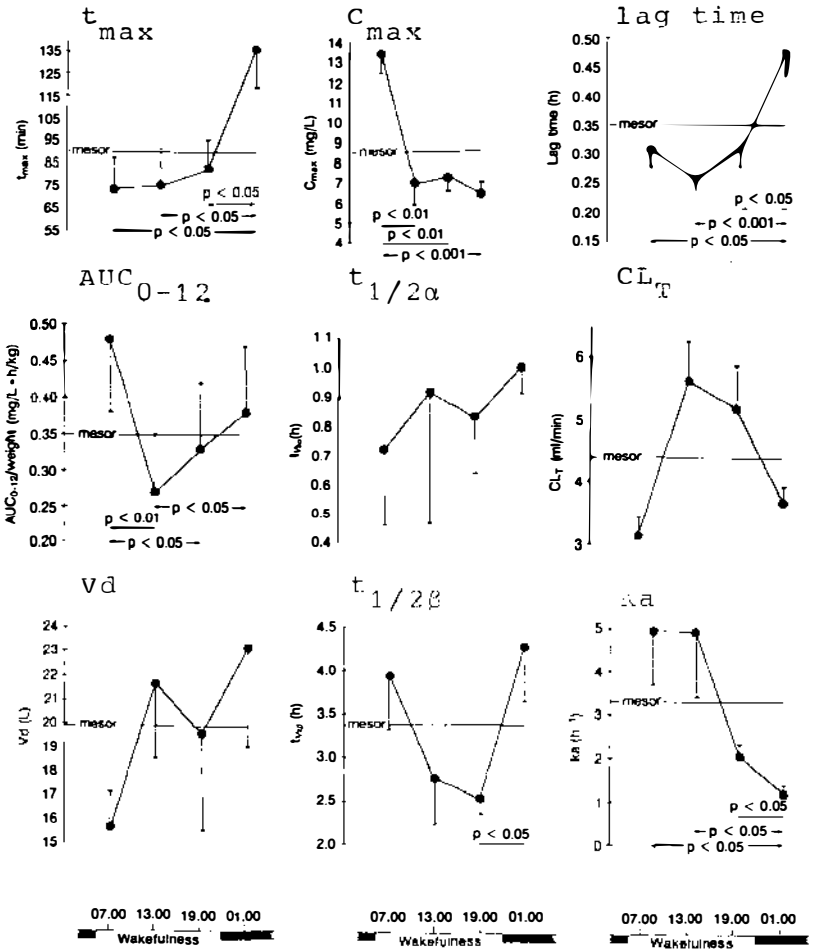

Figure 1 Dosing time–dependent changes of pharmacokinetic parameters ($X \pm$ SEM) of oral ketoprofen (NSAID). This randomized crossover study consisted of a single oral dose of ketoprofen (100 mg) administered orally to eight healthy young male volunteers, at 07.00, 13.00, and 01.00 h at least 1 week apart. Abbreviations and symbols: mesor, 24-h adjusted mean; horizontal arrows, statistical comparison of the values by Student's t test. C_{max}, maximum plasma concentration (mean ± SEM); t_{max}, time to reach C_{max} (mean ± SEM). AUC_{0-12}, area under the curve observed up to 12 h; trapezoidal method, values corrected with regard to body weight. V_d, apparent volume of distribution. $t_{1/2\alpha}$, half-life of the distribution phase; $t_{1/2\beta}$, elimination half-life; lag time, time of appearance of the drug in plasma; CL_T, total clearance. Ka, absorption rate. Reprinted from (29).

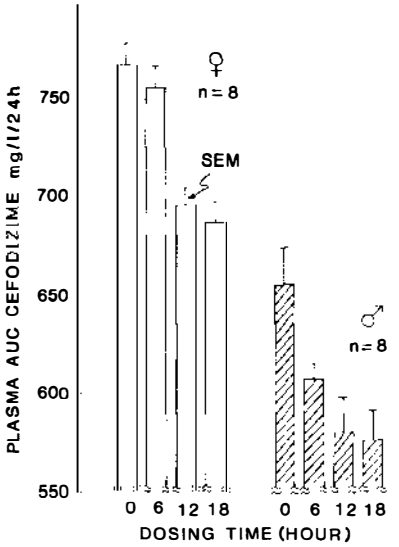

Figure 2 Dosing time–dependent differences in plasma AUC for cefodizime, an antibiotic agent of the cephalosporin group. Cefodizime (2 g) was injected intravenously at 00.00, 06.00, 12.00, and 18.00 h (1 week apart) in eight male and eight females young healthy volunteers. AUC ($X \pm$ SEM) shows dosing time–related differences. The figure shows that for both male (white bars) and female (shaded bars) subjects, the largest AUC corresponded to dosing at 00.00 h and the smallest to dosing at 18.00 h, with $P < 0.01$ (by analysis of variance). The figure also shows an important sex-related difference, with the AUC for female subjects being higher at each dosing time than the AUC for male subjects ($P < 0.001$), with no interaction between sex and dosing time. Reprinted from (31).

variations of various oxidative reactions catalyzed by the monoxygenase system have been reported for substrates such as aminopyrin, paranitroanisole, hexobarbital and 4-dimethyaminobenzene, aniline, benzphetamine, benzpyrene, imipramine, etc (22). The oxidative reaction was peaking in the middle of the (nocturnal) activity span. Moreover, it has also been demonstrated that CR of corticosterone adrenal secretion control CR of involved enzymes. More recently, Bélanger et al (11, 22) have validated the presence of diurnal variations in the transferase and hydrolases involved in glucuronide and sulfate conjugation of rat liver. The K_m (Michaelis constant) and V_{max} (maximal reaction rate) of the soluble fraction of sulfotransferase were, respectively, four times greater and twice as great during (diurnal) rest than during (nocturnal) activity. In contrast, conjugation catalyzed by UDP-glucuronosyltransferases of microsomes was greater during activity than during the rest phase, whether or not the animals were fed. CR in the concentra-

tion of cytochrome P-450 in liver cells and in the enzymatic induction of some drugs with regard to microsomal systems has been quantified by Bélanger et al (22). CR in liver perfusion, resulting from CR in blood flow, is also a factor to be taken into account, e.g. in dosing time–related changes in clearance of propranolol, a β-blocking agent (23). It seems that drug metabolism resulting from oxidative microsomal reactions reaches its zenith during the activity span and its nadir during the rest span. Conversely, sulfate conjugations are much faster during rest than during activity.

CIRCADIAN RHYTHMS IN PLASMA PROTEIN BINDING OF DRUGS The phenomenon of CR in plasma protein binding of drugs was first demonstrated for cortisol and thereafter for its synthetic analogs (24). CR in plasma total proteins, albumin, globulins, etc, related to liver activity, induces circadian changes in the free (active) fraction of drugs and hormones. In the rat the peak time of protein binding occurs during the (nocturnal) activity spans for disopyramide, lidocaine, and carbamazepine (15, 21), which also correspond to the peak time of plasma proteins. As a result the circadian amplitude of the free fraction of carbamazepine is 18% (binding range from 90 to 72%). CR in plasma protein binding has been demonstrated for valproic acid, carbamazepine, diazepam, lidocaine, prednisone, and *cis*-diamindichloroplatinum (*cis*-DDP) (15, 21, 25–27). Most human plasma protein concentrations including albumin and α_1-acid glycoprotein reach their nadir during the nocturnal rest and their zenith in the morning. Touitou et al (28) have shown that in young healthy adult subjects the circadian amplitude of plasma protein was rather small (8 to 15%) compared with that of healthy elderly subjects (ca 75 years). An impressive nocturnal fall was observed for the latter (circadian amplitude of ca 20%), a result which suggests that the free fraction of drugs usually bound to plasma proteins increases during the nocturnal rest as a function of aging. Moreover, a large-amplitude CR of *cis*-DDP protein binding has been demonstrated in vitro by Hecquet et al (27), using plasma samples collected round the clock.

Some Drugs Delivered at a Constant Rate Do Not Provide Constant Levels Over the 24-h Span

It has been shown for ketoprofen (NSAID) and two anticancer agents, 5-FU and adriamycin, that delivery at a constant rate does not provide constant levels over the 24-h span (19, 20, 29, 30). Large-amplitude circadian changes in drug concentrations in plasma resulted from a venous infusion at a constant rate. These changes were highly predictable with either ketoprofen (19, 29) or 5-FU (20) but exhibited important, unpredictable, and interindividual differences with adriamycin (30). The rapid renal clearance of both ketoprofen and 5-FU may help to explain this phenomenon.

Chronokinetic Changes Can Be Either Sex or Age Related, as Well as Phenotype Related

A statistically significant sex-related difference in the chronokinetics of cefodizime, an antibiotic, has been shown in humans (31; Figure 2). In both sexes, large-amplitude CR of plasma AUC were found in studies with four different dosing times. However, both the amplitude and the 24-h mean were larger in women than in men. Dosing time–dependent changes in t_{max}, C_{max}, and AUC of sustained-release indomethacin (NSAID) have been demonstrated in young adults but not in elderly subjects (15). The polymorphism in the human potency to acetylate some drugs (e.g. isoniazid, procainamide, and hydralazine) was shown to be genetically determined. With regard to these genetic factors, comparison between slow and rapid acetylor types of healthy young subjects has shown statistically significant differences in the chronokinetic pattern of isoniazid (32).

CHRONESTHESY

The term *chronesthesy* was used first to designate rhythmic (temporally predictable) changes in the susceptibility or the sensitivity of a target system to a drug, which cannot be explained by chronokinetic changes. Later on, taking into account many experimental findings, we proposed, with Labrecque and Smolensky (4, 6, 17), that chronesthesy be considered the chronopharmacologic counterpart of the pharmacodynamic concept. Apart from the fact that chronopharmacodynamics is a rather cumbersome and long word, it deals basically with mechanisms of time-related variation in effects and metabolism of drugs in healthy organisms. Since metabolic processes have already been taken into account when referring to chronokinetics, chronesthesy, chronoeffectiveness, and chronotolerance, the term chronopharmacodynamics does not add further precision or specification. It can be used, however, if it helps understand the meaning of chronesthesy.

Therefore, chronokinetics and chronesthesy are complementary concepts (17), and chronesthesy itself designates circadian and other rhythmic changes in the susceptibility of a target system. In human subjects the target can be a tissue, e.g. the skin (CR in epidermal skin reaction to intradermal injection of histamine with a nocturnal peak) (4, 6), the bronchial tree (CR of the bronchial response to acetylcholine as well as beta-agonists [Figure 3], among other agents, inhaled in the form of aerosol) (4, 6, 33), or the stomach (number of mucosal lesions quantified by fibroscopy is twice greater after morning [10.00 h] than evening [20.00 h] ingestion of 1 g of acetylsalicylic acid) (34). Experiments in laboratory rodents have also shown that the target biosystem may be located either at the molecular level of the receptors or in different subcellular systems (e.g. membrane structure and enzyme systems).

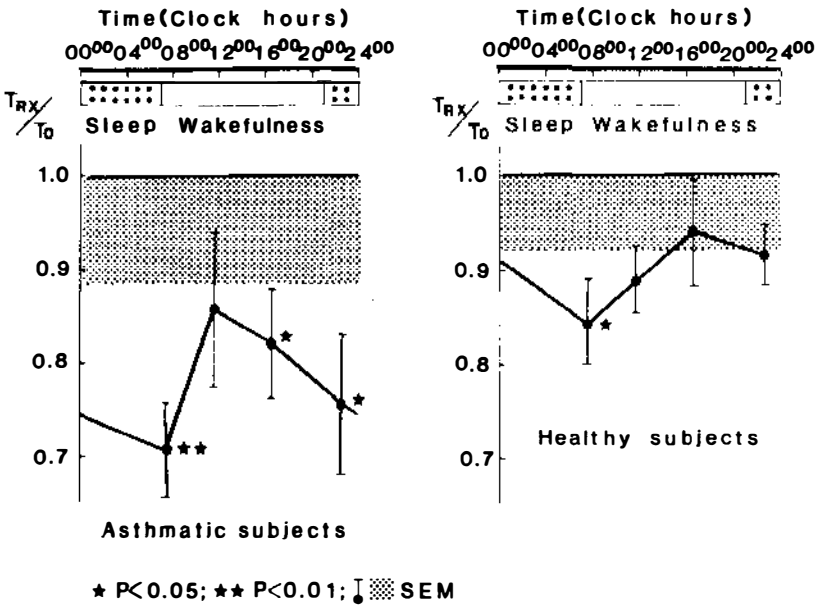

Figure 3 Dosing time–related (circadian) changes in effectiveness of inhaled (aerosol) orciprenaline (β-agonist agent) expressed as T_{RX}/T_0 ratio. T_0 and T_{RX} are the measurements of total pulmonary resistance (Rl) (esophagal balloon technique) before and 10 min after inhalation of 2 mg of orciprenaline, respectively. Boys and girls, 8 to 13 years old, including seven healthy subjects and six suffering from nocturnal asthma, had their Rl measured at four fixed times (07.30, 11.30, 16.30, and 22.30 h). Patients had no asthma attacks and had received no medication for 8 to 15 days. A drug-induced decrease of Rl (an increase of airway patency) is associated with a decrease of the T_{RX}/T_0 ratio. The smaller the ratio, the larger the patency. Shaded areas represent lower (from $T_{RX}/T_0 = 1$) confidence limits (−SEM) of control values obtaines without the β-agonist agent (e.g. placebo control). In both healthy and asthmatic subjects a statistically significant decrease (t test, $^*P < 0.05$ or $^{**}P < 0.01$) was observed with dosing at 07.30 h but not with dosing at 11.30 h. The bronchodilating effect of orciprenaline also occurred in asthmatics with dosing at 22.30 h. The chronesthesy of the bronchial target to orciprenaline led us to conclude that bronchi are sensitive to this agent during the night but not during the day. Reprinted from (33).

The CR parameters of many specific variables and/or biosystems can be altered at this level in response to a particular drug at a given time, e.g. acrophase (ϕ) shift or drift (so-called phase-response curve), changes of the period (τ) length, and/or the 24-h rhythm-adjusted mean (M).

Circadian Rhythms of Receptors

CR have been documented and quantified in various receptors and organs, e.g. brain and heart in rats (35–37) and blood cells in humans (38). In all cases a CR was observed for the number of binding sites rather than for

binding capacity of sites. Statistically significant 24-h rhythms occurred in all receptors studied by Wirz-Justice and coworkers (35), as well as by others (36, 37) using homogenates from the whole rat forebrain.

In addition, waveform, amplitude, and acrophase of these circadian changes vary with the time of year, even though rats have been kept synchronized with a defined and constant LD cycle. These rhythms are endogenous because they persist under constant environmental conditions without a known zeitgeber and also when animals are deprived of sleep. However, they can be altered after the destruction of a circadian pacemaker in the suprachiasmatic nuclei.

The pattern of a receptor rhythm may change from one brain region to another. For example, the pattern can be different from the same ligand in different nuclei of the hypothalamus. In rats, receptor rhythms vary according to strain and even within the same strain from different breeding lines. Binding to a given ligand in a defined brain region varies with age, leading to changes in CR parameters such as amplitude, acrophase shift, and/or 24-h mean (35–38).

However, the circadian amplitude in the number of certain receptors is sometimes not large enough to explain fully the impressive magnitude of an observed change in chronesthesy. Large-amplitude CR in the adenyl cyclase-phosphodiesterase system can coexist with small or no amplitude in rat brain receptors (12, 36). It is likely that this system, which is activated after the specific molecular binding, amplifies the circadian changes of the response to a greater extent than do receptors. The existence of a biological rhythm amplifier involving the CR of cyclic AMP (cAMP) at the target level helps to explain circadian changes in certain drug effects, even if a steady state in the bioavailability of the drugs is achieved by some technical means.

Practical Implications of Chronesthesy

It is conventionally assumed, without experimental evidence, that a constant level of a drug in plasma over time should result in a constant effect(s). Much experimental evidence has shown that this hypothesis must be rejected.

As a first consequence of chronesthesy, the effects of a drug vary over the 24-h scale even if the concentration of the agent in plasma remains constant. Let us consider three illustrative examples. Unfractionated heparin was delivered by intravenous continuous infusion at a constant rate during a 48-h span (39) to six patients. The time course of activated partial thromboplastin time (APTT), thrombin time (TT), and factor Xa inhibition assay (fac.Xa) were obtained by analysis of blood samples taken every 4 h for 48 h. Statistically significant CR were validated (Figure 4). The anticoagulant effect was at its trough around 08.00 h (with a risk of thrombosis) and at its peak around 03.00 h, i.e. the middle of the nocturnal rest span (with a risk of bleeding) (Figure

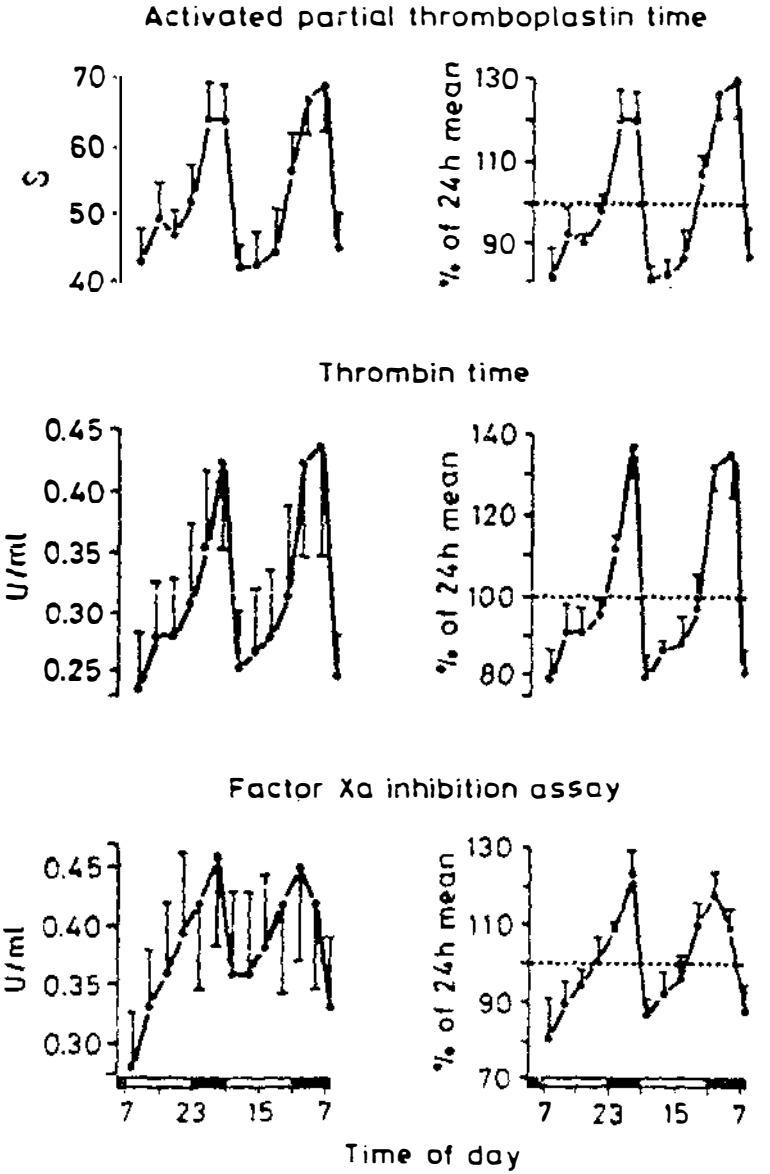

Figure 4 Time course of APTT, TT, and fac.Xa measured every 4 h for 48 h in six patients receiving continuous intravenous unfractionated heparin treatment delivered at a constant rate. Results (±1 SE) are expressed in raw values (on the left) and as a percentage of the individual 24-h mean (on right). Statistically significant CR (24 h) and ultradian rhythms (12 h) were validated by several statistical methods (cosinor, *t* test, analysis of variance) for three coagulation tests. The anticoagulant effect peaked in the middle of the night, around 03.00 h, and was at its lowest value around 08.00 h. A 40% difference occurred between peak and trough levels in anticoagulant effects despite the continuous delivery at a constant rate. Reprinted from (39).

4). Ranitidine (an H2 antihistamine) was also infused intravenously at a constant rate over time (24 h) in 15 patients with healed duodenal ulcer (40). The tested effect was the drug-induced increase in intragastric pH continuously measured with a probe. The same dose of ranitidine (6.5 and 10 mg h^{-1}) proved to be more effective during the night than during the day. A third example relates to mequitazine (an H1 antihistamine) with a steady-state level in plasma achieved by an 8-day administration. In subjects receiving mequitazine chronically during an 8-day span, the CR in cutaneous response to intradermally injected histamine persisted (17, 41). Other illustrative examples showing the importance of chronesthesy relate to, e.g. bronchodilation respectively induced by theophylline, β-adrenergic agents, and corticosteroids (4, 6).

As a second consequence of chronesthesy, the dosing time of a medication is at least as important as the dose. Dose–response relationships in both experimental and clinical chronopharmacology have been reviewed by Reinberg & Lévi (42). With regard to drugs quoted above (unfractionated heparin, ranitidine, mequitazine, and bronchodilators), it appears that to obtain a quantified similar effect, the dose must be changed as a function of the dosing time.

CHRONERGY

The term *chronergy* was introduced to designate rhythmic (temporally predictable) differences in effects of drugs on the organism as a whole (4, 6, 17). Chronergy has a neutral meaning that includes rhythmic changes of both desired (effectiveness) and undesired (toxicity and its counterpart, tolerance) effects.

Obviously, temporally predictable dependencies in either effectiveness or tolerance of a drug are based on both its chronokinetics and the chronesthesy of the target system. Let us consider again studies of unfractionated heparin (19, 42). The anticoagulant effect was at its trough around 08.00 h and at its peak around the middle of the nocturnal rest span, with a circadian amplitude of 40%. The chronokinetics of the drug were documented by Decousus et al (39), using labeled heparin. A statistically significant CR in plasma clearance was observed, with a peak at 14.00 h and a trough at 08.00 h and a rather small amplitude with regard to that of the anticoagulant effect. Therefore, chronokinetic changes are not likely to explain observed CR in anticoagulant effects.

Further studies (43) have shown time dependencies in effects of heparin in vitro, e.g. coagulation tests between blood specimens sampled at 10.00 h and at midnight from the same subjects. Statistically significant differences were observed in APTT and TT with greater anticoagulant effect at midnight than at 10.00 h.

In this case, as in many others, the chronesthesy of the target system rather than the chronokinetics of the drug appears to be the key phenomenon.

CHRONOTHERAPY

The term *chronotherapy* refers to the use of a chronopharmacological approach to clinical treatment so as to enhance both effectiveness and tolerance of a drug by determining the best biological time for its dosing. Therefore, not only the chronoeffectiveness of a drug but also its potential chronotoxic effects must be explored.

In fact, chronotoxicology was the first and remains the best-documented domain of chronopharmacology (1, 4, 6, 8, 13, 18, 42). Not only are medications a concern, but so are pollutants and toxicants in the workplace in several industries. The concept also questions the conventional definition of a lethal dose (LD_{50}), which still does not include reference to dosing time. If death is the endpoint of a toxic effect, it is commonplace to find a change from 0 to 70% survival, depending on the time at which animals were exposed (1, 6, 8, 18, 42, 44–46). Problems raised by chronotoxic effects of drugs are of major interest, especially when rather toxic substances such as anticancer agents are concerned. How to increase the tolerance for an anticancer agent is a critical question for oncologists. Large-amplitude circadian changes in murine tolerance for at least 20 anticancer agents have been documented (6, 46, 47). Animal experiments are used to find the time when the toxic drug is best tolerated. To transfer the information thus gained to patients, a set of chronopharmacologic requirements must be respected. (*a*) Animal synchronization must be known (e.g., hours of LD alternation), especially if rats and mice are concerned, since they are nocturnal species. (*b*) Circadian changes in toxic effects with regard to, e.g., hematological, renal, hepatic, cardiac, and central nervous system (CNS) functions must be similar or very close between the animal model and the human situation. This means that the use of certain strains of mice (or rats) must be recommended, since there are well-documented genetic differences with regard to the chronotolerance of anticancer drugs (48, 49). (*c*) Mechanisms of chronotoxic CR must be investigated. CR in the output of enzymes known as indices of toxic effects have been documented in chronotoxic experiments for both the kidney (18) and the liver (50, 51). Circadian changes in the tolerance of mouse bone marrow cultured in vitro after exposure to 4'-tetrahydropyranyl-adriamycin (THP-Adr, an anticancer agent) for 1 h at six different test times and three concentrations have been documented (52, 53). A CR was validated in the proliferative activity of committed hematopoietic stem cells (10^5 per agar dish) quantified by the number of granulocyte-macrophage colony-forming units counted after 6 days of culture. The peak of tolerance occurred during

the activity span. Chronotoxic effects (e.g. body weight loss) of oxaliplatin (an anticancer analog of *cis*-DDP) can be phase shifted with regard to placebo control, in rats treated with *p*-chlorophenylalanine (*p*-CPA), a serotoninergic system–blocking agent which obliterates the CR of suprachiasmatic nuclei (54). In *p*-CPA-treated rats, the CR of the white blood cell count and corticosterone levels in plasma were not altered (54).

Chronotherapeutic studies have already been performed with conventional administration (e.g. oral) of agents such as corticoids, theophylline, and beta-agonists in nocturnal asthma; NSAIDs in rheumatoic diseases; H2 antihistamines in gastroduodenal ulcers; H1 antihistamine in allergic rhinitis; β-blocking agents in hypertensive patients; anticoagulants in cardiovascular diseases; and 6-mercaptopurine as maintenance chemotherapy in children with acute lymphoblastic leukemia (6). Impressive results were obtained when administration times were based on pertinent chronopharmacologic findings. In addition, programmable-in-time pumps have been used to treat cancer patients with adriamycin, 5-FU, oxaliplatin, etc. Since the administration time of these agents greatly influenced the extent of human tolerance, the need for a circadian administration time–related therapy appears inescapable (6, 17, 55).

Chronopharmacologists must now face a new challenge, viz. the search for reliable markers of rhythms: the circadian rhythm parameters (e.g. acrophase) of variables to guide chronotherapy on the one hand and to estimate the chronophysiological status of a patient, on the other. Marker rhythms can be circadian changes of self-measured peak expiratory flow for asthmatics, self-rated pain for rheumatic patients, blood pressure for hypertensive patients, and so on. But problems remain to be solved, e. g. for patients suffering from cancer or infectious diseases.

SUMMARY

Circadian and other rhythmic changes in biological susceptibility and response of organisms to a large variety of physical and chemical agents including medications and foods are rather common phenomena. Time-related differences in drug effects depend upon endogenous circadian rhythms, which include metabolic pathways. In addition, chronopharmacology investigates drug effects on parameters (e.g. circadian period, peak time, amplitude, and adjusted mean) used to characterize biological rhythms.

A better understanding of periodic and thus predictable changes in drug effects can be attained by consideration of complementary concepts: (*a*) The chronokinetics for a drug, i.e. dosing time–dependent and predictable (rhythmic) changes in parameters used to characterize the pharmacokinetics (or the bioavailability) of a drug, e.g. C_{max}, t_{max}, AUC, and $t_{1/2}$; (*b*) the

chronesthesy, i.e. rhythmic changes in susceptibility of the target biosystem to this drug, including CR in pharmacodynamic processes; and (c) the chronergy, i.e. the drug-integrated overall effect. One of the aims of chronopharmacology refers to the use of a chronopharmacological approach to clinical treatment so as to enhance both effectiveness and tolerance of a drug by determining the best biological time for its administration.

Literature Cited

1. Reinberg, A., Halberg, F. 1971. Circadian chronopharmacology. *Annu. Rev. Pharmacol.* 11:455–92
2. Radzialowski, F. M., Bousquet, W. F. 1968. Daily rhythmic variations in hepatic drug metabolism in the rat and mouse. *J. Pharmacol. Exp. Ther.* 163:229–38
3. Moore-Ede, M. C., Sulzman, F. M., Fuller, C. A. 1982. *The Clocks That Time Us*. Cambridge, MA: Harvard Univ. Press. 448 pp.
4. Reinberg, A., Smolensky, M. H., eds. 1983. *Biological Rhythms and Medicine*. New York: Springer. 305 pp.
5. Arendt, J., Minors, D. S., Waterhouse, J. M., eds. 1989. *Biological Rhythms in Clinical Practice*. London: Wright. 299 pp.
6. Reinberg, A., Labrecque, G., Smolensky, M. H. 1991. *Chronobiologie-Chronothérapeutique*. Paris: Flammarion Méd.-Sci. 200 pp.
7. Aschoff, J., Wever, R. 1981. The circadian system of man. In *Handbook of Behavioral Neurobiology*, ed. J. Aschoff, 4:311–48. New York: Plenum. 488 pp.
8. Halberg, F. 1969. Chronobiology. *Annu. Rev. Physiol.* 31:675–700
9. von Mayersbach, H. 1983. See Ref. 4, pp. 47–78
10. Shimazu, T. 1979. Hypothalamic regulation of the circadian rhythm of liver metabolism. In *Biological Rhythms and Their Central Mechanism*, ed. M. Suda, O. Hayashi, H. Nakagawa, pp. 309–18. Amsterdam: Elsevier. 453 pp.
11. Bélanger, P. M., Lalande, M., Labrecque, G., Doré, F. 1985. Diurnal variations in the transferase and hydrolases involved in glucuronide and sulfate conjugation of rat liver. *Drug Metab. Dispos.* 13:386–89
12. Lemmer, B., Bärmeier, H., Schmidt, S., Lang, P. H. 1987. On the daily variation in the beta-receptor-adenylate cyclase-phosphodiesterase system in rat forebrain. *Chronobiol. Int.* 4:469–76
13. Lemmer, B., ed. 1989. *Chronopharmacology*. New York: Marcel Dekker. 720 pp.
14. Reinberg, A., Smolensky, M. 1982. Circadian changes of drug disposition in man. *J. Clin. Pharmacokinet.* 7:401–20
15. Bruguerolle, B. 1989. See Ref. 13, pp. 3–13
16. Smolensky, M. H., McGovern, J. P., Scott, P. H., Reinberg, A. 1987. Body time-dependent differences in the kinetics and effects of bronchodilator medications. *J. Asthma* 24:91–134
17. Reinberg, A., Smolensky, M., Labrecque, G. 1986. New aspects in chronopharmacology. *Annu. Rev. Chronopharmacol.* 2:3–26
18. Cal, J. C., Dorian, C., Catroux, P., Cambar, J. 1989. See Ref. 13, pp. 655–81
19. Decousus, H., Ollagnier, M., Cherrah, Y., Perpoint, B., Hocquart, J., Queneau, P. 1986. Chronokinetics of ketoprofen infused intravenously at a constant rate. *Annu. Rev. Chronopharmacol.* 3:321–22
20. Petit, E., Milano, G., Lévi, F., Thyss, A., Bailleul, F., Schneider, M. 1988. Circadian-varying plasma concentration of 5-fluorouracil during 5-day continuous venous infusion at constant rate in cancer patients. *Cancer Res.* 48: 1676–79
21. Bruguerolle, B. 1984. *Chronopharmacologie*. Paris: Ellipse-Marketing. 160 pp.
22. Bélanger, P. M., Labrecque, G. 1989. See Ref. 13, pp. 15–34
23. Lemmer, B., Bathe, K. 1982. Stereospecific and circadian phase dependent kinetic behavior of *dl*-, *l*-, and *d*-propanolol in plasma, heart and brain of light-dark-synchronized rats. *J. Cardiovasc. Pharmacol.* 4:635–43
24. Angeli, A., Frajria, R., De Paoli, R., Fonzo, D., Ceresa, F. 1978. Diurnal variation of prednisolone binding to serum corticosteroid binding globulin in

man. *Clin. Pharmacol. Ther.* 23:47–53
25. Levy, R. H. 1982. Time-dependent pharmacokinetics. *Pharmacol. Ther.* 17:383–97
26. Nakano, S., Watanabe, H., Nagai, K., Ogawa, N. 1984. Circadian stage-dependent changes in diazepam kinetics. *Clin. Pharmacol. Ther.* 36:271–77
27. Hecquet, B., Meynadier, J., Bonneterre, J., Adenis, L., Demaille, A. 1985. Time dependency in plasmatic protein binding of cisplatin. *Cancer Treat. Rep.* 69:79–83.
28. Touitou, Y., Touitou, C., Bogdan, A., Reinberg, A., Auzeby, A., et al. 1986. Differences between young and elderly subjects in seasonal and circadian variations of total plasma and erythrocyte counts. *Clin. Chem.* 32:801–4
29. Ollagnier, M., Decousus, H., Cherrah, Y., Lévi, F., Queneau, P., Reinberg, A. 1987. Circadian changes in the pharmacokinetics of oral ketoprofen. *Clin. Pharmacokinet.* 12:367–78
30. Lévi, F., Bailleul, F., Metzger, G., Misset, J. L., Vannetzel, J. M., et al. 1986. Adriamycin chronotherapy of advanced breast cancer with a programmable implantable drug administration device. *Annu. Rev. Chronopharmacol.* 3:237–40
31. Jonkman, J. H. G., Reinberg, A., Oosterhuis, B., de Noord, O. E., Kcrkhof, F. A., et al. 1988. Dosing-time and sex-related differences in the pharmacokinetics of cefodizime and in the circadian cortisol rhythm. *Chronobiologia* 15:89–102
32. Ollagnier, M., Gay, J. P., Cherrah, Y., Decousus, H., Reinberg, A. 1988. Is acetyl of phenotype distribution dependent on circadian rhythms? *Annu. Rev. Chronopharmacol.* 5:257–58
33. Gaultier, L., Reinberg, A., Motohashi, Y. 1988. Circadian rhythm effects in total pulmonary resistance of asthmatic children, of a β-agonist agent. *Chronobiol. Int.* 5:285–90
34. Moore, J. G., Goo, H. 1987. Day and night aspirin-induced gastric mucosal damage and protection by ranitidine in man. *Chronobiol. Int.* 4:111–16
35. Wirz-Justice, A. 1987. Circadian rhythms in mammalian neurotransmitter receptors. *Prog. Neurobiol.* 29:219–59
36. Lemmer, B. 1989. See Ref. 13, pp. 477–508
37. Wirz-Justice, A., guest ed. 1986. Circadian rhythms of receptors. *Chronobiol. Int.* (Special issue) 3:87–146
38. Reinberg, A. 1988. New concepts in Chronopharmacology. *News Physiol. Sci.* 3:84–88
39. Decousus, H. A., Croze, M., Lévi, F. A., Perpoint, B. M., Jaubert, J. G., et al. 1985. Circadian changes in the anticoagulant effect of heparine infused at a constant rate. *Br. Med. J.* 290:341–44
40. Sander, S. W., Moore, J. G., Buchi, K. N., Bishops, A. L. 1988. Circadian variations in the pharmacodynamic effect of intravenous ranitidine. *Annu. Rev. Chronopharmacol.* 5:335–38
41. Reinberg, A. 1989. See Ref. 13, pp. 115–36
42. Reinberg, A., Lévi, F. 1990. Dose-response relationships in chronopharmacology. *Annu. Rev. Chronopharmacol.* 6:25–46
43. Scully, M. F., Decousus, H. A., Ellis, V., Parker, C., Girard, P., Kakkar, V. V. 1987. Measurement of heparin in plasma: influence of inter-subject and circadian variability in heparin sensitivity according to method. *Thromb. Res.* 46:447–55
44. Haus, E., Halberg, F., Kühl, J. F. W., Lakatua, D. J. 1974. Chronopharmacology in animals. *Chronobiologia.* 1(Suppl. 1):122–56
45. Scheving, L. E. 1980. Chronobiology—a new perspective in pharmacology and chemotherapy. *Trends Pharmacol. Sci.* 1:303–7
46. Lévi, F., Boughattas, N. A., Blazsek, I. 1988. Comparative murine chronotoxicity of anticancer agents and related mechanisms. *Annu. Rev. Chronopharmacol.* 4:283–332
47. Hrushesky, W. 1984. Chemotherapy timing: an important variable in toxicity and response. In *Toxicity of Chemotherapy*, ed. M. C. Perry, J. W. Yarbo, pp. 449–77. New York: Grune & Stratton. 596 pp.
48. Ashkenazi, I. E., Peleg, L., Cohen-Kitay, Y., Carlebach, R. 1990. Chronotoxicity and phenotype variability. *Annu. Rev. Chronopharmacol.* 7:241–44
49. Lévi, F., Boughattas, N. A., Blazsek, I. 1988. Comparative murine chronotoxicity of anticancer agents and related mechanisms. *Annu. Rev. Chronopharmacol.* 4:283–331
50. Lavigne, J. G., Belanger, P. M., Doré, F., Labrecque, G. 1983. Temporal variations in chloroform-induced hepatoxicity in rats. *Toxicology* 26:267–73
51. Brukner, J. V., Luthra, R., Kyle, G. M., Muralidhara, L., Ramanathan, R., Acosta, D. 1984. Influence of time of exposure to carbon tetrachloride on toxic

liver injury. *Annu. Rev. Chronopharmacol.* 1:373–76
52. Ferlé-Vidovic, A., Blazsek, I., Lévi, F. 1986. Circadian time dependence of murine bone marrow tolerance for the anticancer agent 4'O-tetrahydropyranyl adriamycin. *Annu. Rev. Chronopharmacol.* 3:195–98
53. Lévi, F., Blazsek, I., Ferlé-Vidovic, A. 1988. Circadian and seasonal rhythms in murine bone marrow colony forming cells affect tolerance for the anticancer agent 4'O-tetrahydropyranyl adriamycin. *Exp. Hematol.* 16:696–701
54. Boughattas, N. A. 1989. *Rythmes de la pharmacocinétique et la pharmacodynamie de 3 agents anticancéreux (cisplatine, oxaliplatine et carboplatine) chez la souris: approche de leur régulation.* D.Sc. thesis. Univ. Paris VII. 240 pp.
55. Lévi, F. 1987. Chronobiologie et cancer. *Pathol. Biol.* 35:960–68