Dose-response relationships in clinical pharmacology.
L. Lasagna, S. Erill, C.A. Naranjo, editors

CIRCADIAN CHRONOPHARMACOLOGIC MODULATION OF DOSE-RESPONSE RELATIONSHIPS IN RODENTS AND IN MEN

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ABSTRACT

Both desired (chronoeffectiveness) and/or undesired effects (chronotolerance) of at least 140 drugs depend upon their dosing-time along the 24-h scale (circadian) rhythms) among other time-scales. Circadian rhythms in dose-response relationships have been documented for more than 20 drugs in rats, mice and human beings with regard to their effectiveness, tolerance or other pharmacologic variables.

Circadian changes of dose-response in survival rate (chronotolerance) of rodents have been shown for amphetamines, barbiturates; anticancer agents, etc. Dose-response relationships characterized by chronopharmacologic changes have been shown for tremorine, propranolol, ACTH, sodium pentobarbital and THP-adriamycine in laboratory animal experiments. In humans, dose-response relationships have been demonstrated for lidocaine, bronchodilators, antihistamines (both H1 and H2) as well as heparin.

The dose-response relationship is usually steeper at the acrophase (peak time of the effect) than 12 h earlier or later when raw data are considered. This means that within a given range of doses a) the dose-response may be nil or small at a certain time of day with regard to some drugs (e.g. propranolol) and b) the relative amplitude of dosing time-dependent effects remains large whatever the dose.

Mechanisms of the circadian modulation in dose-response relationships seem to involve chronopharmacodynamic changes rather that chronopharmacokinetic ones.

INTRODUCTION : PROPERTIES OF BIOLOGICAL RHYTHMS. CHRONOPHARMACOLOGY. DEFINITIONS & CONCEPTS

Almost all biological phenomena that can be physically and chemically quantified are not constant but vary periodically and thus predictably as a function of time (1). This is true for all living beings from eukaryote single cell organisms which possess neither a nervous nor endocrine system to the human species. Biological rhythms include pharmacologic processes.

Biological rhythms are usually described and quantified by four parameters :
- The period τ, which is the span of time between characteristics of regularly recurring events, e.g. peaks, troughs, etc. With regard to the period τ, one usually considers circadian ($\tau \simeq 24$ h), circannual ($\tau \simeq 1$ year) and ultradian (or pulsatile $\tau < 20$ h) rhythms.

The other 3 parameters pertain to the descriptive characteristics of a biperiodicity when the period is known. They are : - The acrophase ϕ, which is the

peak time of the changes with regard to a phase reference ϕ_o , e.g. can be expressed in hours and minutes for a 24-hr rhythm with midnight taken as ϕ_o . - The amplitude A is one-half of the total variability between peak (Ø) and trough. - The mesor M is the rhythm-adjusted mean (equal to the arithmetic average when data are collected at equidistant intervals).

Biological rhythms in plants and animals including man have similar properties. 1. Such rhythms are genetically inherited. As a consequence, they persist in constant conditions (e.g. continuous darkness with constant temperature, humidity, etc.) 2. Biological rhythms are governed at several levels of organization by a set of oscillators (so-called biological clocks or pacemakers). The suprachiasmatic nucleus appears to be one of them. 3. Acrophases of biological rhythms do not randomly occur over time (e.g. 24 h). In fact, their temporal distribution describes a time-structure referred to as an "anatomy in time". 4. Organisms rely on external signals, such as dawn (onset of light) and/or dusk (onset of darkness), to cue or reset their biological clocks. These external signals, which consist of periodic changes in the natural environment (e.g. light/dark alternation related to the Earth's rotation), are called synchronizers or Zeitgebers (time givers). Syrchronizers do not create rhythms although, they are able to phase shift Ø and/or to influence the τ within certain limits. In most plant and animal species, the light/dark alternation with τ = 24 h is the most powerful synchronizer. However, other cyclic factors (e.g. alternation of noise/silence, heat/cold, etc.) also have a synchronizing effect. In man, the socioecologic synchronization (e.g. activity/rest schedules governed by imperatives of our social life as well as factors of our ecologic niche) plays a major role. 5. Biological rhythms are currently viewed as adaptative phenomena of both species and individuals to predictable changes in environmental factors linked to the Earth's rotation around its axis in 24 h as well as around the Sun in 365 days.

Due to the organism's time structure, metabolic pathways for both nutrients and drugs are neither open permanently nor with the same patency. Therefore, toxic as well as pharmacologic effects of medications may vary as a function of biological time, for example over 24 hours or the year (1).

Chronopharmacology designates rhythmic (predictable-in-time) differences in pharmacologic effects of a substance. This means that effects of drugs are dosing time-dependent, e.g. in the 24 h scale. Chronotoxicology designates rhythmic (predictable-in-time) changes in toxic effects of a potentially noxious agent (physical as well as chemical). The reverse (antinomy) is chronotolerance and refers to temporal changes of the organism's resistance to potentially harmful treatments. Illustrative examples are given in table 1.

Chronopharmacokinetics or chronokinetics designate biologic time-related changes in the pharmacokinetics of an agent quantified by parameters of one or several

TABLE 1 DOSING TIME-DEPENDENCE IN DOSE-RESPONSES OF RODENTS TO VARIOUS DRUGS. ANIMALS SYNCHRONIZED WITH 12 H OF LIGHT (L) ALTERNATING WITH 12 H OF DARKNESS (D) I. CHRONOTOLERANCE : SURVIVAL RATES (SR) etc..

Animal	Drug;Dose;Route * (No of tested dosing times/24 h)	Tested effect	Circadian peak time location	Time of major dose-response	Authors
Rats	Amphetamine 26 to 30 mg/kg IP (7)	24 h-survival rate (SR)	Best SR at onset of L	Onset of L ≈ onset of rest span	Scheving et al, 1968
Rats	Pentobarbital 75;85; & 90 mg/kg IP (4)	24 h - SR	Best SR ∿ onset of L	Onset of rest span	Pauly et al,1964
Rats	Tremorine 45 & 80 mg/kg IP (4)	24 h - SR	Best SR ∿ late-L span	Late rest span	Pauly et al, 1964
Rats	Cis-diammine dichloroplatinum 5 & 11 mg/kg IP (2)	Day-3 Spleen weight Blood urea	Best tolerance Late D with regard to late L***	Late activity span	Lévi, 1982
Mice	Arabinosyl cytosine 400 & 200 mg/kg IP (6)	Day 1 to 13 Survival rate	Best SR Late D span in winter ***	Late activity span	Scheving et al, 1974
Mice	THP-Adriamycin 18; 25 & 32 mg/kg IV & IP (6)	Day-60 SR	Best SR ∿ late L span	Late rest span	Lévi et al, 1985
Mice	Mercuric chloride 4;5; 6 mg/kg IP (4)	Day-10 Survival rate	Best SR : mid D span in winter ***	Mid activity span	Cal et al, 1984
Mice	Gentamicin 250;275;300;325 mg/kg IM (4)	Day-10 survival rate	Best SR early to mit D span	Mid activity span	Pariat et al, 1984
Mice	Dibekacin 320;355;390; 425; 460 mg/kg IM (4)	Day-10 survival rate	Best SR : early to mid D span	Mid activity span	Pariat et al, 1984
Mice	Netilmicin 120;130;140 & 145 mg/kg. IM (4)	Day - 10 survival rate	Best SR early D span	Early activity span	Pariat et al, 1984

* IP : intra peritoneal; IV : intravenous; IM : intra muscular routes.
*** : A seasonal change in circadian peak time of SR has been validated.

curve patterns (models). Illustrative examples are given in table 2.

Chronesthesy designates rhythmic (predictable-in-time) differences in the susceptibility or sensitivity of a biological target (i.e. receptors, membrane permeability, cells, tissues, organs, organ systems, etc.) to an agent. Chronesthesy emphasizes predictable,rather than randomly distributed, biologic time-related differences of such a target. When healthy organisms are concerned and metabolic (molecular) processes are documented, the term chronopharmacodynamics is used by certain authors instead of chronesthesy. Illustratives examples are given in table 2. Chronoeffectiveness refers to temporal changes in the desired therapeutic effects of drugs.

Chronotherapy or chronoptimization is enhancement of desired effects and/or reduction of undesired toxic effects by determining the best (biological) time

for drug dosing including the use of programmable-in-time pumps.

In a set of experimental and clinical chronopharmacologic investigations both dosing time and dose have been manipulated. Thus obtained data have been summarized in tables and analysed in order to better understand the modulation of dose-response relationships as a function of dosing time(s) in the circadian (24 h) domain.

CIRCADIAN CHANGES OF DOSE-RESPONSE RELATIONSHIPS APPEAR TO BE A RATHER COMMON PHENOMENA

The statement summarized by the above title is based on many illustrative examples which have been gathered in 3 tables. The first one (2-8) deals with the dose-response relationship in survival rate (chronotolerance) of rats and mice with regard to 10 well known potentially noxious chemical agents.

Among similar groups of animals, each one treated with a fixed dose of a given agent, the mortality appears to be dosing time-dependent in the 24 h scale. Expressed as a percent of the mesor (24 h adjusted mean) the survival rate may be as hight as 75-85% at a certain clock hour and zero 12 h earlier or later. Within certain limits changes in the fixed dose are associated with changes in mortality rate at each documented test time; the higher the dose, the greater the rate (figure 1). However, the curve pattern is maintained whatever the dose which means that respective peak and trough locations in the 24 h scale are dosing time-dependent but not dose dependent. The qualification regarding limits is related to the fact that obviously no chronotolerance phenomenon is observed when the fixed dose is either too small (no lethality) or too large (no survivor).

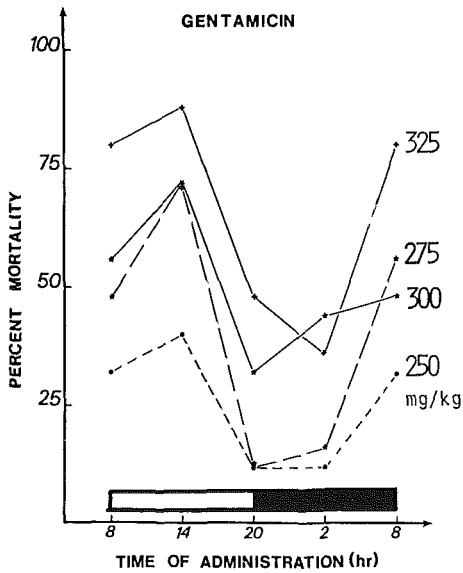

Figure 1. Dosing time-dependent survival rate (day 10) of mice to 4 different fixed doses of gentamicine (I.M.) Redrawn from Pariat et al 1984

TABLE 2 DOSING-TIME DEPENDENCE IN DOSE-RESPONSES OF RODENTS TO VARIOUS DRUGS. ANIMALS SYNCHRONIZED WITH 12 H OF LIGHT (L) ALTERNATING WITH 12 H OF DARKNESS (D). II. CHRONOPHARMACOLOGY INCLUDING CHRONOKINETICS, CHRONESTHESY, IN VITRO EXPERIMENTS, ETC

Animal	Drug.Doses.Route* (no of tested dosing times/24 h)	Tested effects	Circadian peak time location	Time of major dose response	Authors
Rats	Tremorine 64 & 100 mg/kg S.C. (4)	Time (t) to onset of constant tremor	Longuest t ~ mid D span	~ mid activity span	Pauly et al, 1964
Rats	(±) Propranolol 6 & 30 µMoles/kg S.C. (2)	Volume of distribution. Total body clearance	Values during L span greater than during D	Rest span	Lemmer et al, 1981
Rats	(±) Propranolol 10; 30; 100 µmoles/kg. S.C. (2)	Decrease of heart rate	Major effectiveness during D span	Activity span	Lemmer et al, 1983
Mice	ACTH 0.04; 0.4 & 4 IU (7)	Response of incubated adrenals to ACTH added in vitro	Max corticosterone output : late D span	Late activity span	Ungar et al, 1962
Mice	Pentobarbital 65; 71.5;78.7; 86.5 mg/kg I.P. (6)	Duration of general anaesthesia	Longuest duration early to mid L span	Mid rest span	Nelson et al, 1973
Mice	THP-Adriamycin 0.05;0.03;0.06 & 0.125 µg/ml (6)	Bone marrow culturated in agar in vitro exposure	Best tolerance mid L span	Mid rest span	Ferlé-Vidovic et al, 1983

*SC : sub cutaneous; IP : intra peritoneal routes

TABLE 3 DOSING TIME-DEPENDENCE IN DOSE-RESPONSES OF MAN TO VARIOUS DRUG. HEALTHY SUBJECTS (HS) AND/OR PATIENTS (PS) SYNCHRONIZED WITH A DIURNAL ACTIVITY FROM ~ 0700 TO ~ 2300 AND A NOCTURNAL REST

Drug; dose; route *	Tested effect (no of dosing times/24 h)	Circadian peak time location	Time of major dose/ response	Authors
Lidocaïne 2 & 4 mg.ID	Duration of local (skin) anaesthesia HS (5)	Longuest duration : mid-activity span (1500 h)	1500 h	Reinberg A. & M.A. 1977
Ipratropium bromide 80 & 200 µg. Inhaled	Bronchodilation HS (4)	Most effective during night time	2230 h	Gaultier et al. 1977
L. Epinephrine 0.01. 0.03 & 0.075 µg/ml/kg I.V.-CRI	Bronchodilation Ps with nocturnal asthma (CRI)	Most effective during night time	0400 h	Barnes et al, 1982
Sustained-release theophylline SRT. P.O. chronic	Bronchodilation. Control of nocturnal dip in asthmatic PS(2)	More effective during night time e.g. evening dosing	Bronchial patency correlates only at night with plasma concentration of SRT	Reinberg et al, 1987
"	"	More effective with a large dose at 1900 h	"	Bruguerolle, 1987
Terfenadine (anti-H1) 20 & 60 mg P.O.	Depth of antihistamine effect. Skin test H.S. Cmax (2)	Evening hours (1900 h)	1900 h	Reinberg et al, 1978
" "	Time to Cmax : tmax (2)	Shorter at 1900 h than at 0700 h	No dosing time-related difference	" "
Ranitidine (anti H2) 6.5 & 10 mg/h I.V. - CRI	Increase of intragastric pH. 15PS with healed duodenal ulcer (CRI)	More effective during night time than day time	Night time	Sanders et al, 1988
Heparin 0.2; 0.4 & 0.6 IU/ml	Anticoagulant effect tested in vitro in blood samples HS; cancer etc (2)	Sensitivity to heparin greater at midnight than at 1000 h	Midnight	Scully et al, 1987

* ID : intradermal injection; IV : intravenous infusion; PO : per os; CRI : constant rate infusion.

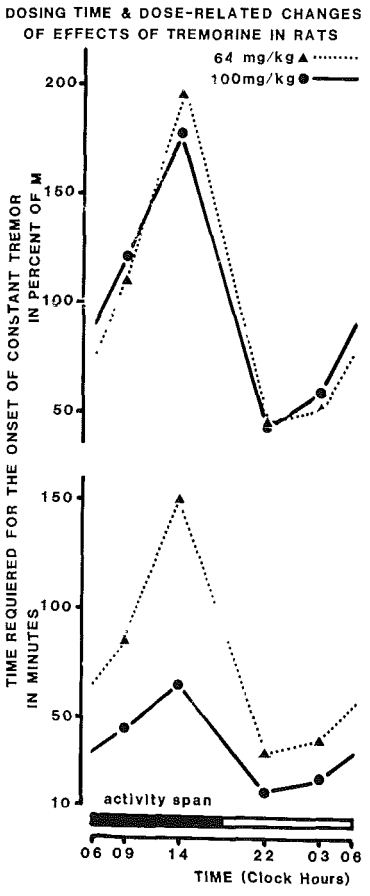

Figure 2. Time required for the onset of constant tremor (ordinate) expressed in minutes (bottom) and as percent of the 24h mean (top). Redrawn from Pauly et al. 1964.

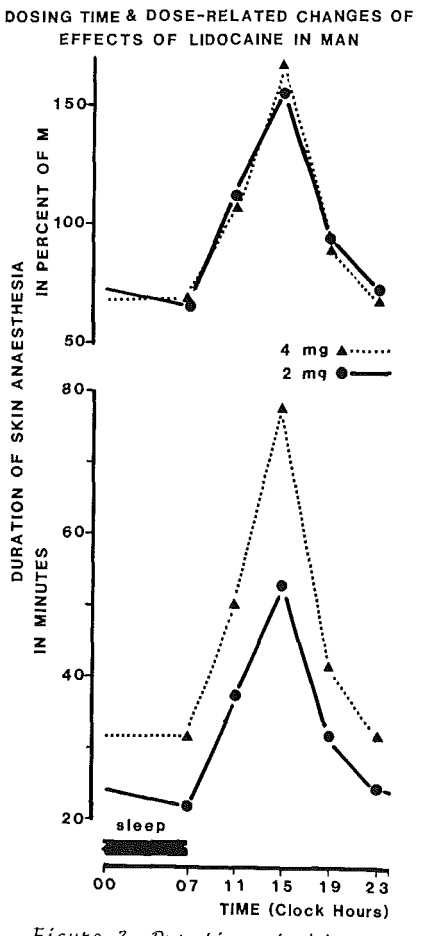

Figure 3. Duration of skin anaesthesia (ordinate) expressed in minutes (bottom) and as percent of the 24h mean M (top). Redrawn from Reinberg et al. 1977.

Tables 2 (3, 9-13) and 3 (14-21) summarize chronopharmacologic phenomena, respectively documented in rodents and men. Whatever, the tested pharmacologic effect, agent or animal species, dosing time-dependent circadian changes were reported; for a given agent, in a given species, it persisted from one experiment to the next when the fixed dose was manipulated. Again, this is true within acceptable limits commonly used to quantify the investigated pharmacologic phenomenon.

THE 24 H MEAN LEVEL OF A CHRONOPHARMACOLOGIC EFFECT IS DOSE-DEPENDENT

Illustrative examples of this phenomenon are given in figures 2 and 3. The time required for the onset of constant tremor resulting from administration of tremorine in rats (3), the duration of lidocaine-induced local anaesthesia in human (14) exhibited steeper dose-response differences at the peak time than at any other time when units of measurement were considered. However, when for a given dose, dosing time-related changes are expressed as a percent of the 24 h mean, curve patterns are similar for both large and small doses (figures 2 & 3). In other terms, what is dose-dependent in circadian changes of a chronopharmacologic effect is the 24 h mean level of the curve rather than its pattern as shown by the analyses of relative changes. As a consequence, the dose response may be nil or very small at certain clock hours (e.g. racemic propranolol on the heart rate of conscious rat during the day) and may exhibit large differences 12 h later or earlier (fig. 4 ↕10).

The relative circadian amplitude is not dose-dependent for certain drugs (figs. 2 & 3) while some changes of this parameter may by observed for others. This is the case for dosing time-dependent effects of L-adrenaline on the human bronchial patency (16) (Raw data figure 5). Peak (20 h) to trough (04 h) differences expressed as a per cent of the 24 h mean vary from 18% (0.01 g/ml/kg L-adrenaline) to 15% (0.075 g/ml/kg).

MECHANISMS INVOLVED IN THE CIRCADIAN MODULATION OF DOSE-RESPONSE RELATIONSHIPS

One of the first factors to be considered is the circadian(dosing time-dependent) change in parameters characterizing the pharmacokinetics of a drug. Actually, dose-response relationships can be modulated by the latter as demonstrated by Lemmer (9, 10) for both the volume of distribution and total body clearance of propranolol (table 2). However, despite the fact that this factor may play a role for certain drugs and/or circumstances, it is not likely to be a major one. For example, a positive correlation ($r = 0.86$; $p < 0.01$) can be established between the plasma concentration of theophylline and the effectiveness of the drug as a bronchodilator (peak expiratory flow- PEF-measurements) only when a sustained release preparation is given in the evening (17, 18). This dose-response relationship cannot be found with a morning dosing (no correlation : $r = 0.45$; $p > 0.05$) as demonstrated by Reinberg et al (17) and Bruguerolle et al (18).

The major factor seems to be the chronesthesy as illustrated by two sets of experiments. Regarding the first one, drugs [adrenaline (16) or the anti-histamine H2 ranitidine (20)] were administered to maintain plasma concentration at a constant level over the 24 h span. Comparison of different constant levels shows that dose-response relationships remain modulated (table 3 and figure 5), suggesting that circadian changes in target biosystems are concerned. Evidence again supporting this hypothesis was provided by a second set of experiments conducted in vitro (fig. 6 and 7). Ferlé-Vidovic et al (13) and Lévi et al (22) have documented cir-

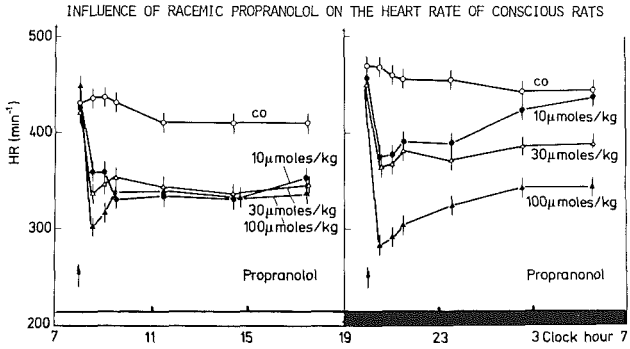

Figure 4. Propranolol (10 to 100 µ moles/kg,S.C.) injected at either 0730h or at 1930h to rats synchronized with L:07-19h and D:19-07h. Redrawn from Lemmer et al. 1981.

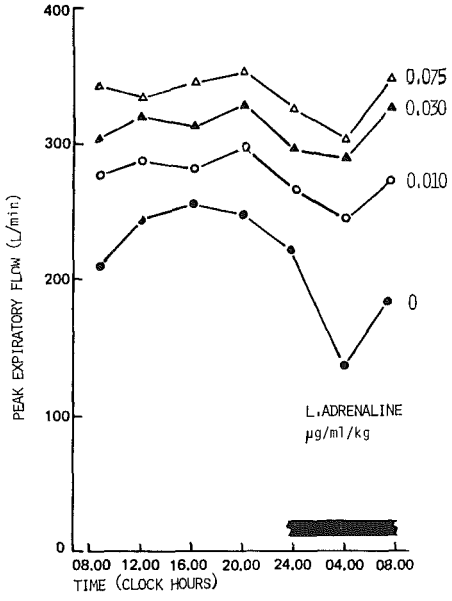

Figure 5. Effect of L-adrenaline infusion on bronchial patency (PEF) as a function of dosing time in a group of 5 males with nocturnal asthma. The increase in PEF, from control values, was the greatest when adrenaline was infused at 04h with regard to other time points. Too, the slope of the dose-response was significantly steeper at 04h in comparison to 16h. Redrawn from Barnes et al. 1982.

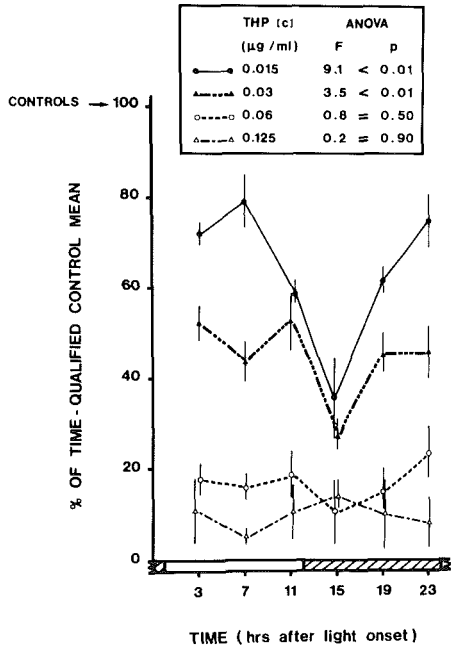

Figure 6. Circadian changes in the proliferative activity of committed hematopoietic stem cells quantified by the number of granulocyte macrophage colony forming units (GM-CFUc in ordinate as % of the control mean). Bone marrow cells were exposed for 1h to THP-ADR (doses on top) then washed and cultivated. GM-CFUc were counted after 6 days. From Ferlē-Vidivic et al 1983, and Lēvi et al. 1988.

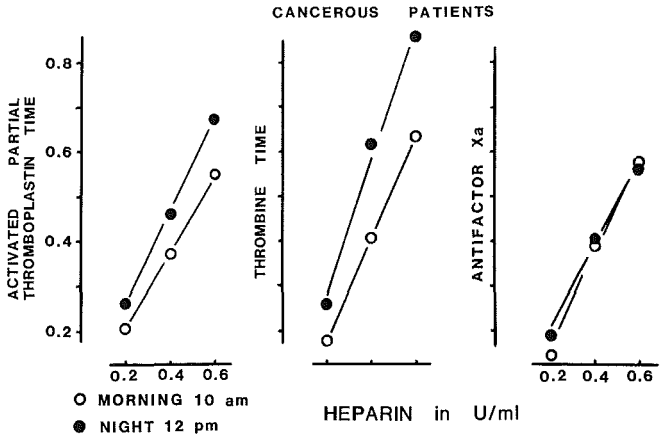

Figure 7. Morning (10h) v. night (00h) variation in sensitivity to heparin measured in vitro by APTT, thrombine time and anti-factor Xa methods. Heparin was added to achieve a final concentration of 0.2, 0.4 and 0.6 U/ml. Heparin units were calculated with reference to standard curves prepared using pooled normal plasma. Drawn from data published by Scully et al. 1987.

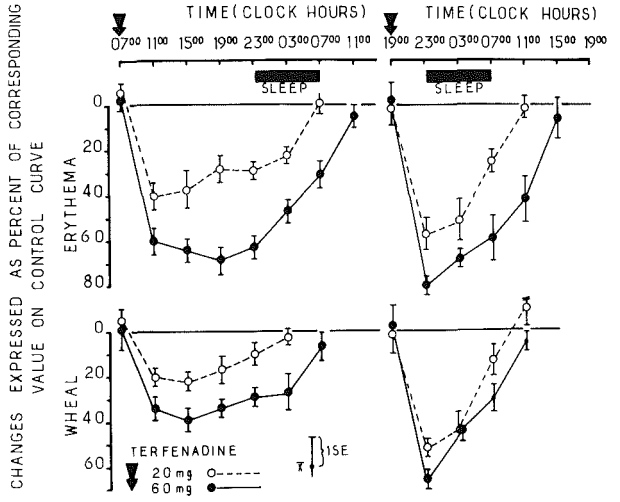

Figure 8. Dosing time-dependent (07h v. 19h) changes in terfenadine-induced inhibition of local skin reactions to histamine I.D. 2 μg/0.1 ml). Surface area of skin reactions (wheal and erythema) were measured before and at fixed intervals after each dose of drug or placebo (control curve). Changes were expressed as percent of corresponding value on control curve for each of the 10 healthy volunteers and averaged. From Reinberg et al. 1978.

cadian changes in the tolerance of mouse bone marrow cultured in vitro after exposure to 4'-tetrahydropyranyladriamycin (THP-ADR) during one hour at different test times and concentrations. A circadian rhythm was detected in addition to dose-response relationships (fig. 6). This was also the case for the anticoagulant effect of heparin tested in vitro in blood specimens of healthy subjects, cancer patients (fig. 7) etc. sampled at midnight and at 10 h (21). The sensitivity to heparin was greater at 00 h than at 10 h. However, the slope of the dose response curve exhibited time related differences with regard to anticoagulant effects (activited partial thromboplastine time and thrombine time) but not with regard to a test close to the molecular concentration (antifactor Xa).

The number of receptors (rather than their affinity) is presumably involved in the circadian modulation of dose-response to antihistamine, both H1 (19) and H2 (20), to β-blockers (9, 10) and to bronchial β-stimulants (15, 16).

PRACTICAL IMPLICATIONS

Since dose-response relationships are dosing time-dependent in the 24 H scale, time of day and subjects' synchronization must be known in both animal experiments and clinical investigations. Dramatic misunderstanding of data may result from the ignorance of a circadian modulation in dose-response relationships. A 12 h change in the dosing time of a drug is usually associated with changes in its dose-response magnitude ranging from small and even nil differences to impressive ones. This was the case for drugs listed in tables 1, 2, 3.

Dosing time-related changes in effects of a drug can be used to enhance both its tolerance and effectiveness without increasing the dose used as clinically demonstrated for local anaesthetics, bronchodilators, H1 and H2 antihistamines, as well as heparin (table 3).

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Discussion - Chronopharmacological effects on dose-response curves

D.G. Grahame-Smith

For a series of reasons, among them to improve compliance or to reduce side effects, some drugs are given in single big daily doses, either in the morning or at night. This is the case of tricyclic antidepressants, betablockers and corticosteroids, for instance. I quite see the effects of circadian rhythms in the case of acute effects, but I would like to hear your comments about the times of doses in the case of drugs that act through a chronic effect, as may happen with tricyclics in depression or betablockers in hypertension.

A. Reinberg

It is very difficult to say anything because of the lack of human data with regard to antidepressants and antihypertensives. In the case of corticosteroids it is obvious that when they are given in the morning their tolerance and effectiveness are improved, except if you specifically want to inhibit the adrenal, in which case it is best to give the drug in the evening. The beneficial optimization resulting from morning dosing of corticoids can be maintained for many years (3 to 13) in asthmatics.

L. Lemberger

You mentioned that there is practically no dose-response relationship for the effects of propranolol on heart rate of conscious rats during the day, whereas large differences between doses were seen during the night. Don't you think that this is simply due to the higher level of sympathetic activity in the rat during the night? Perhaps when we try to establish dose-response relationships for an agent that blocks something and the results are deceptive we should resort to the use of a stimulating drug, such as a cathecolamine if we are studying the effects of betablockers or pentagastrin if we are evaluating an H_2 antihistamine.

A. Reinberg

I agree with your suggestion, circadian changes in the sympathetic tone can be one the factors involved, but we cannot

dismiss the possibility of circadian variation of other factors e.g. that of receptor sensitivity. Bjorn Lemmer $Frankfurt), the author of these studies, has demonstrated large amplitude presynaptic level as well as circadian rhythms of the adenylate cyclase-phosphodiesterase system at the post-synaptic level. These factors are also involved.

B.P. du Souich

Can you comment on the problem posed by slow release formulations? In this case, there could be a circadian variation in drug kinetics plus a circadian variation in effect.

A. Reinberg

This is the case as far we can judge from data so far available on particular clases of drugs. For instance, it has been shown by Hervé Decousus et al (Saint-Etienne) that there is a circadian rhythmicity in the anticoagulant effects of heparin even if the drug is infused at a constant rate. Even with a chronic administration, a circadian variation in both the kinetics and the effects of sustained release theophyllines or anti-inflammatory drugs is very well documented. I think that the main point to stress is that one can no longer be sure that if over the 24 h span a constant plasma concentration is achieved one will get constant effects. Many more examples can be found in articles published in the Annual Review of Chronopharmacology.

L.F. Prescott

You have been referring to circadian rhythms, but I presume that the sleep pattern, independently from the underlying circadian rhythm may have some effect, per se, on drug responses.

A. Reinberg

Indeed. Sleep is associated with posture-dependent changes in liver blood flow, as well as with changes in the rate of gastric emptying, and this may represent a variability factor. The problem is that the changes that may be attributed to posture are usually due to the circadian rhythms, so the situation is even more complicated. Again, we are dealing here with a multifactorial system and sleep is only one factor.