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REVIEW

The importance of biological rhythms in drug treatment of hypertension and sex-dependent modifications

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Abstract: The cardiovascular system is highly organized in time. Blood pressure, heart rate, peripheral resistance, pressure, and vasodilating hormones display pronounced circadian variations. New data presented here demonstrate also sex-dependent differences in vasodilating hormones, with higher NO_χ excretion in females than males and a steeper early morning rise in norepinephrine in males, whereas the 24-hour blood pressure and heart-rate profiles were not different. Various antihypertensive drugs were investigated in crossover studies – morning versus evening dosing – in hypertensive patients; however, consistent data were only described for angiotensin-converting-enzyme (ACE) inhibitors, calcium channel blockers, and angiotensin II type 1 (AT_1) receptor blockers. Whereas in dippers ACE inhibitors had a superdipping effect when dosed at night, no difference in the blood pressure lowering effect or on the 24-hour blood pressure profile was found with calcium channel blockers after morning and evening dosing. In nondippers, the calcium channel blockers isradipine and amlodipine transformed nondippers into dippers, similar after evening dosing. The effects of AT_1-receptor blockers are similar to those of ACE inhibitors. Also, diuretics are able to normalize non dipping behavior. Moreover, a circadian phase dependency in their pharmacokinetics has been demonstrated for various cardiovascular-active drugs, such as beta blockers, calcium channel blockers, oral nitrates, and ACE inhibitors, modified by the galenic formulation. There is evidence that in hypertensive dippers, antihypertensive drugs should be given during early morning hours, whereas in non dippers it can be necessary to add an evening dose or even to apply a single evening dose in order not only to reduce high blood pressure, but also to normalize a disturbed non dipping 24-hour blood pressure profile.

Keywords: chronopharmacology, hypertension, beta-blockers, calcium channel blockers, ACE inhibitors, AT_1-receptor blockers, diuretics, chronopharmacokinetics, ABPM, circadian rhythms, urine NO_χ excretion, plasma norepinephrine, sex dependency

Introduction

Impact of circadian rhythms on physiological processes

In general, it is assumed that homeostasis is a basic concept in biology and medicine. Thus, in the past, data obtained by investigators and clinicians on the pharmacokinetics, effects, and side effects of drugs were mainly restricted to daytime values. However, in the last 50 years it has been clearly evidenced that biological processes and functions are not constant in time, but that rhythmicity is the most ubiquitous feature of nature. Rhythms are found from unicellular to complex multicellular organisms such as plants, animals, and humans. Living organisms are continuously influenced by external stimuli, many of which have rhythmic patterns. Environmental rhythms in

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daily and seasonal patterns of light, food availability, and temperature are predictable in time and living creatures, including humans, which have the ability to anticipate these environmental events with periodically and predictably changing internal conditions. These rhythmic patterns of anticipation have clear advantages and survival value. The frequencies of rhythms in nature cover nearly every division of time. There are rhythms that oscillate once per second (eg, in the electroencephalogram), once every several seconds (respiratory rhythm, heart rate), up to rhythms that oscillate once per year (circannual rhythm).

The most evident environmental change that results from the regular spin of the earth around its central axis that results in the alternation between day and night seems to have induced the predominant oscillation: the circadian ("circa" = about, "dies" = day) rhythm, as proposed by Halberg.[1,2] There is sound evidence that living systems including humans are not only organized in space but are also highly organized in time. One of the first observations on a rhythmic pattern in man was presented in a self-experiment by the famous physiologist Santorio Santorio[3] in 1664, when he described daily variations in body weight due to transpiration. His famous experimental setting is shown in Figure 1.

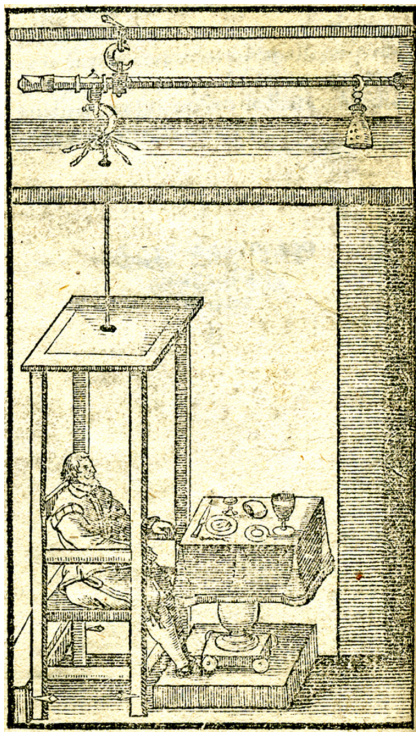

Figure 1 Day–night variations in body weight due to transpiration. Shown is the experimental setting of the author.
Note: Figure taken from the original book.[3]

Circadian rhythms have been documented throughout the plant and animal kingdom at every level of eukaryotic organization. Circadian rhythms by definition are endogenous in nature, driven by oscillators or clocks,[4–6] and persist under free-running conditions.[7,8] In various species and genera (eg, *Drosophila melanogaster, Neurospora*, mouse, golden hamster, rhesus macaque, human), the genes controlling circadian rhythms have been identified (eg, mammalian: *hper, hclock, hBmal, cry, tim, rev-erbα*; non-mammalian: *frq, clock, cycle, per, tau, tim, Rev-erbalpha*). In 1971, Konopka and Benzer[9] were able to identify on the X chromosome of *Drosophila* a region that controlled the period in the eclosion rhythm of three mutants (*per* clock gene).

Circadian clocks are believed to have evolved in parallel with the geological history of the earth, and have undergone selection pressures imposed by cyclic factors in the environment.[10] These clocks regulate a wide variety of behavioral and metabolic processes in many lifeforms. They enhance the fitness of organisms by improving their ability to efficiently anticipate periodic events in their external environments, especially periodic changes in light, temperature, and humidity.

The mammalian circadian clocks, located in the neurons of suprachiasmatic nuclei (master clock) in the brain and in cells of peripheral tissues, are driven by a self-sustained molecular oscillator, which generates rhythmic gene expression with a periodicity of about 24 hours. This molecular oscillator is composed of interacting positive and negative transcription/translation feedback loops[11–14] in which the heterodimeric transcription activator CLOCK/BMAL1 promotes the transcription of E-box containing cryptochrome (*Cry1* and *Cry2*) and Period (*Per1* and *Per2*) genes, as well as clock-controlled output genes. After being synthesized in the cytoplasm, CRY and PER proteins feedback in the nucleus to inhibit the transactivation mediated by positive regulators.[15] The mPER2 protein acts at the interphase between positive and negative feedback loops by indirectly promoting the circadian transcription of the *Bmal1* gene and by interacting with mCRY proteins.[16,17] However, this is a simplified scheme, and there are additional clock genes and transcription factors involved.

It is interesting to note that clock genes have now been found in single cells of human skin and mucosa.[18] Furthermore, it has been shown that about 8%–10% of all genes are regulated in a circadian fashion.[19] There is also circadian gene expression in mammals.[20]

In general, the human endogenous clock does not run at a frequency of exactly 24 hours, but somewhat slower.

The rhythm in human body temperature, which is timed by the biological clock, has a period of about 24.5 hours under free-running conditions, ie, without environmental time cues or Zeitgebers (eg, light, temperature). Introduced by Aschoff,[21,22] the term "Zeitgeber" is now part of the international scientific language. Mammals such as rodents or humans can entrain their activity to regular light cycles not shorter than 22 or longer than 26 hours.[23] Zeitgebers entrain the circadian rhythm to a precise 24-hour period. Zeitgebers are therefore necessary to entrain a living subject to a "normal" period of 24 hours.

In experimental animals and in humans, however, most rhythmic fluctuations still cannot be studied under free-running conditions, leaving the answer open as to what degree they are really "circadian." Purely exogenous rhythms are better termed as "24-hour" or "daily" rhythms. Thus, an overt 24-hour rhythm in a given parameter can be endogenous or predominantly exogenous in nature. Within the published clinical literature, however, the term "circadian" is not always used in the correct aforementioned sense (as used by chronobiologists). The broader term will be used here, too.

Rhythms in symptoms and onset of cardiovascular diseases

Blood pressure (BP) and heart rate display a significant 24-hour rhythm in humans, as clearly evidenced by ambulatory monitoring. The pattern can be influenced by pathological conditions (see below). In rats, these rhythms are under the control of the biological clock since they free-run in total darkness[24,25] and are abolished by ablation of the suprachiasmatic nuclei.[26] The circadian rhythms in BP and heart rate are disrupted in mice in which core clock genes have been deleted or mutated.[27] In humans, however, the question is still open whether and to what degree these rhythms are endogenous in nature.[28–30] Recent data on various circadian rhythms studied simultaneously in man indicate an endogenous component of BP rhythm with highest peak in the evening, thus not coinciding with early morning events.[31] Pathophysiological events within the cardiovascular system do not occur at random either.[32–36] Thus, the onset of nonfatal or fatal myocardial infarction predominates around 6 am to 12 pm. A similar circadian time pattern has been shown for sudden cardiac death, stroke, ventricular arrhythmias, arterial embolism, and fatal pulmonary embolism. Symptoms in coronary heart disease patients such as myocardial ischemia, angina attacks, or silent ischemia are also significantly more frequent during the daytime hours than at night, whereas the onset of angina attacks in variant angina peaks around 4 am.

During the early morning hours, not only do cardiovascular events predominate but there is also a rapid rise in BP both in normotensives[37] and primary hypertensive patients, a rapid increase in sympathetic tone and in the concentrations of pressure hormones, and the highest values in peripheral resistance.[38]

Chronophysiological aspects

In a small study, we investigated healthy young subjects aged 21–23 years (10 females, 11 males) who were active from 6 am to 12 am under natural light conditions. Plasma catecholamines every 4 hours (norepinephrine, epinephrine; high pressure liquid chromatography with electrochemical detection; Gynotec, Germering, Germany), urinary concentration of nitric oxide (NO) metabolites in 6-hour portions (NO_χ: nitrite/nitrate; Uvikon 922; Kontron Instruments, Neufahrn, Germany) together with ambulatory BP and heart rate (every 20 minutes during daytime, every 30 minutes at night; SpaceLabs 90207; Williams Medical Supplies, Rhymney, UK) were recorded over 24 hours; motility was monitored by wrist actigraphy (Actiwatch; Cambridge Neurotechnology, Cambridge, UK). Data from this study were presented only at a conference.[37]

It is of interest to note that the circadian rhythm in plasma catecholamine concentration was clearly sex-dependent, showing a more pronounced early morning rise in male than female volunteers (Figure 2), though the BP profiles (Figure 3) were not different. It should be noted that women before menopause have a much lower risk of higher BP and myocardial infarction, but catch up with the male after menopause.[41–44]

There is a likely contribution of well-known circadian rhythms in fibrinolysis, hemostasis, and thrombosis to the circadian rhythms in cardiovascular events mentioned above.[45,46] Fibrinolytic activity is reduced in the early morning hours and could favor thrombus formation. Also, the endothelium shows circadian time-dependent variations. At least in mice, thrombogenesis seems to be regulated by genetic components of the circadian clock.[47] In addition, naive aortae from Bmal1-knockout and Clock mutant mice exhibit endothelial dysfunction,[48] and circadian dysfunction contributes to hardening of arteries.[49] Endothelial cells form the interface between the circulation blood and the artery wall, and circulating blood exerts shear forces on the endothelium. The endothelium releases several vasoactive compounds (eg, NO, thrombin, adenosine diphosphate, prostacyclin). NO has been shown to be involved in the circadian rhythm of physiological BP regulation, and NO deficiency in patients with hypertension or atherosclerosis

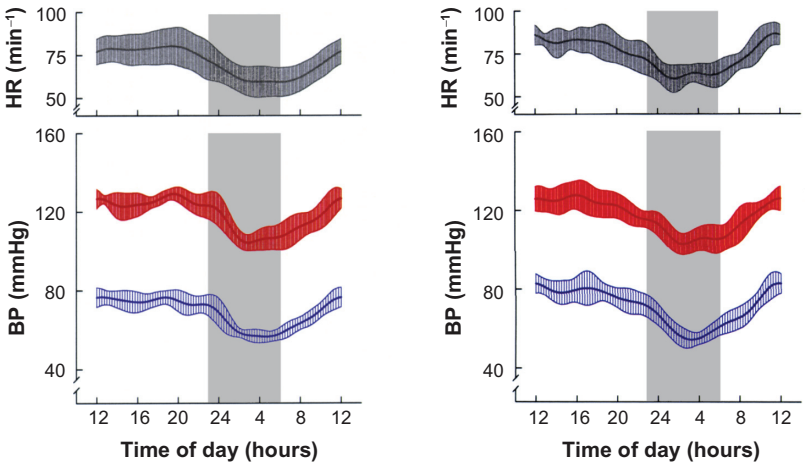

Figure 2 24-hour values in plasma norepinephrine and epinephrine concentrations and motility in healthy young (21–23 years) subjects.
Note: Shown are hourly mean values ± structural equation modeling from 11 male and 10 female (right) volunteers.[40]

contributes to disturbed BP regulation in these patients[50] and may contribute to increased cardiovascular risk. Surprisingly, Bode-Böger et al[50] provided evidence that the peak in the circadian rhythm in NO concentration coincided with the peak in BP, indicating a buffering activity of NO and BP regulation. Similar data were shown in our study in healthy young volunteers (Figures 2 and 4). It is interesting to note that normotensives without endothelial dysfunction have higher (but normal) BP and a greater day–night variation than those without endothelial dysfunction.[51] Moreover, Figure 4 demonstrates sex-dependent differences in the rhythm of NO_χ excretion, with higher values in women than in men. This could indicate that the buffering capacity on BP due to NO release is better in women than in men. Whether this also contributes to the lesser cardiovascular risk in women before menopause needs further investigation.

Chronopharmacology of hypertension

Having in mind the organization in time of living systems including man, it is easy to conceive that not only must the right amount of the right substance be at the right place but also that this must occur at the right time. This is more important when an organism or individual itself has to act or react in favorable biotic or environmental conditions, which by themselves are highly periodic. Thus, it appears that exogenous compounds including drugs may differently challenge the individual depending on the time of exposition.

These findings have greatly contributed to the fact that now "time of day" plays an increasing role in drug treatment. Therefore, a brief review on daily variations in pharmacokinetics and in chronopharmacodynamics of cardiovascular medication is presented.

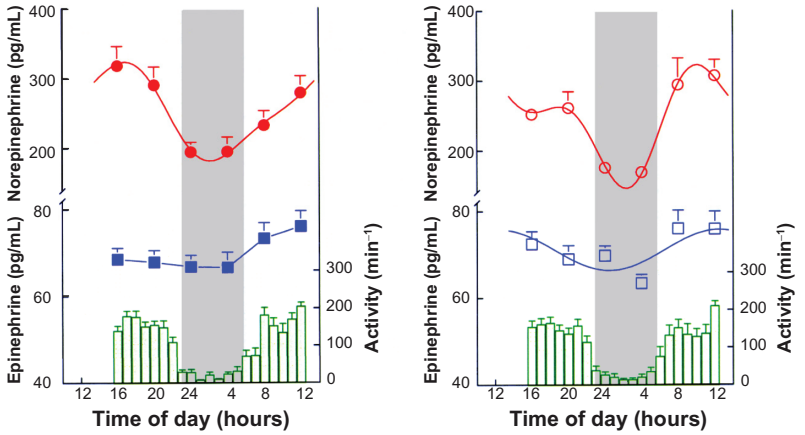

Figure 3 24-hour profiles in systolic and diastolic blood pressure and heart rate in healthy young (21–23 years) subjects.
Notes: Data were fitted with Chronos-Fit.[39] Shown are group mean values and 95% confidence limits from 11 male and 10 female (right) volunteers.[40]
Abbreviation: BP, blood pressure.

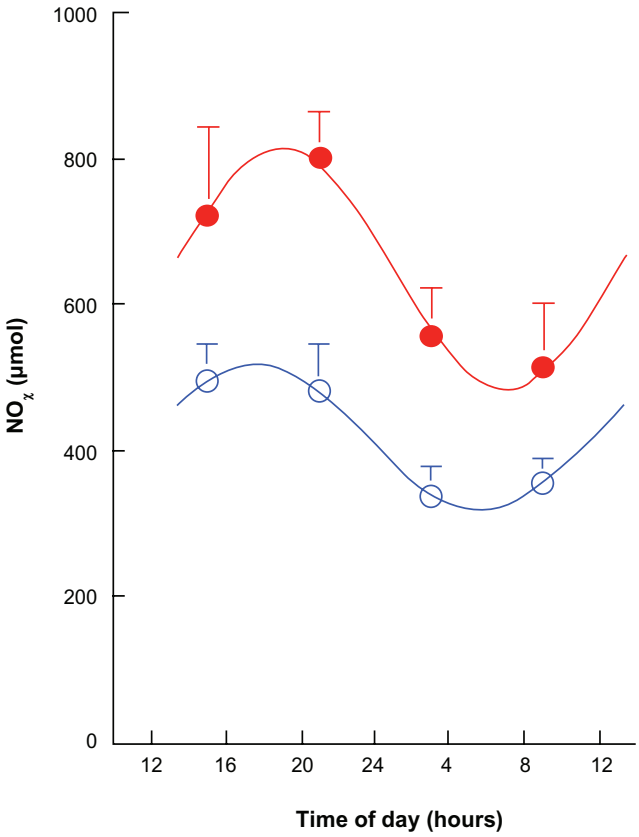

Figure 4 Amount of NO_χ (nitrate/nitrite) in 6-hour portions of urine in 10 female (filled) and 11 male (open) healthy volunteers.
Note: NO_χ is significantly higher in females than males ($P < 0.05$).

Chronopharmacokinetics

In the last decade, numerous studies in animals as well as clinical studies have provided convincing evidence that pharmacokinetics[33,52–56] and/or the drugs' effects/side effects can be modified by the circadian time and/or the timing of drug application within 24 hours of a day.[32,33,57–59]

As shown in Table 1, all functions involved in the pharmacokinetic steps – from drug absorption to drug elimination – can be circadian phase-dependent.[60]

Thus, gastric emptying of solids is faster in the morning than in the afternoon.[61] Also, the perfusion of the gastrointestinal tract varies with the time of day, being more pronounced at midnight and early morning hours than around noon and in the late afternoon.[62] Since drugs are mainly absorbed by passive diffusion, these rhythmic patterns must have implications for pharmacokinetics. These observations would nicely explain that in general drugs are more rapidly absorbed and more rapidly reach the systemic perfusion when taken in the morning. Accordingly, clinical studies showed – mainly for lipophilic drugs – that T_{max} can be shorter and/or C_{max} can be higher after morning than evening drug dosing (Table 2).

Chronomedicine

The focus in this section is on data pertinent to the field of pharmacological intervention in BP regulation.[38,58,77–90] Drug treatment of hypertension includes various types of drugs, such as diuretics, β- and α-adrenoceptor-blocking drugs, calcium channel blockers, angiotensin-converting-enzyme (ACE) inhibitors, angiotensin II type 1 (AT_1) receptor blockers, and others that differ in their sites of action as well as in half-life, galenic formulations, and, thus, in dosing interval. Despite the great number of studies published in evaluating antihypertensive drug efficacy, [91–104] time of day of drug application was only rarely a specific point of investigation. Therefore, in this section, emphasis will be put on crossover studies (morning vs evening) with antihypertensive drugs to highlight this point more clearly.

In order to adequately compare the results obtained with drugs lowering high BP, it is important to note that ambulatory blood pressure measurement is now regarded as the gold standard, ie, the method of choice, to evaluate BP profiles.[83,104–109]

Beta-adrenoceptor antagonists

Unfortunately, no crossover (morning vs evening) study with β-adrenoceptor antagonists in hypertensive patients has been published. A review of 20 "conventionally" performed studies showed that there is a tendency for β-adrenoceptor antagonists to reduce daytime BP levels and not to greatly affect nighttime values, being less/not effective in reducing the early morning rise in BP.[110] Some chronopharmacokinetic observations with beta blockers are shown in Table 2.

Calcium channel blockers

Numerous studies using a crossover design (morning vs evening) have been published.[38,80,82,89,111,112] A time-of-day effect has also been described for the kinetics of various calcium channel blockers (Table 1). The bioavailability of an immediate-release formulation of nifedipine was found to be reduced by about 40% after evening compared to morning dosing, with C_{max} being higher and T_{max} being shorter after morning dosing.[113] No such circadian time-dependent kinetics were observed with a sustained-release formulation of nifedipine.[113,114] Also, regular as well as sustained-release verapamil displayed higher C_{max} and/or shorter T_{max} values after morning dosing[75,76] (Table 2).

In primary (essential) hypertensives with a dipper profile, amlodipine, isradipine, nifedipine gastrointestinal therapeutic system, and nisoldipine did not affect the 24-hour BP profile differently after once-morning or once-evening dosing,[115–119] whereas with nitrendipine and lacidipine, the

Table 1 Biological rhythms and pharmacokinetics [oral drug application]

Liberation	Absorption GI-tract	Distribution	Metabolism liver	Elimination kidney
{Time-specified release	Perfusion	Perfusion	Perfusion	Perfusion
programmable}	Gastric pH	Blood distribution	First-pass-effect	Renal plasmaflow
	Acid secretion	Periph. resistance		Glom. filtration
	Motility	Blood cells	(Enzyme activity)	Urine excretion
	Gastric emptying	Serum proteins		Urine pH
		Protein binding		Electrolytes
	Rest – activity	Rest – activity		

Abbreviations: GI, gastrointestinal; periph, peripheral; glom, glomerular.

profile remained unaffected or slightly changed after evening dosing.[120–123] Most interestingly, the greatly disturbed BP profile in secondary hypertensives (nondippers) due to renal failure was normalized after evening but not after morning dosing of isradipine.[119,123,124] Similarly, amlodipine (Figure 5) and nisoldipine extended release transformed nondippers into dippers, but after both evening and morning dosing,[38,119,124,125] which might be due to the longer "apparent" half-life of these drugs. These findings demonstrate that time of drug dosing (mainly evening dosing) of a dihydropyridine calcium channel blocker can be advantageous in not only reducing elevated BP but also normalizing a disturbed BP profile.

ACE inhibitors and AT_1-receptor blockers

Several crossover studies (morning vs evening dosing) have been published for both ACE inhibitors[82,89,126–128] and AT_1-receptor blockers.[129–131] They demonstrated that in contrast to morning dosing, evening dosing of ACE inhibitors such as benazepril, enalapril, and perindopril resulted in a more pronounced nightly drop. In the light of a reduced cardiac reserve of patients with hypertension, a too-pronounced nightly drop in BP (ie, superdipping) after evening dosing might be a potential risk factor for the occurrence of ischemic events such as cerebral infarction.[132]

Crossover studies published on AT_1-receptor blockers in dippers – valsartan, irbesatan, telmisartan, and olmesartan – showed reduced BP after both morning and bedtime dosing.[130,133–135] In patients with chronic renal disease, olmesartan restored the nightly decline in BP.[131] In nondippers, valsartan had a slightly more pronounced effect at night.[133]

Diuretics and other antihypertensives

Antihypertensives of other classes have rarely been studied in relation to possible circadian variation. Once-daily morning

Table 2 Pharmacokinetic parameters of cardiovascular active drugs determined in crossover studies

Drug	Dose (mg) and duration	C_{max} (ng/mL)		T_{max} (h)		References
		Morning	Evening	Morning	Evening	
Digoxin	0.5, single dose	3.6*	1.8	1.2	3.2	63
Enalapril	10					
Enalaprilat	Single dose	33.8	41.9	4.4	4.5	64
Enalaprilat	3 weeks	46.7	53.5	3.5*	5.6	
IS-5-MN IR	60, single dose	1605.0	1588.0	0.9*	2.1	65–68
IS-5-MN SR	60, single dose	509.0	530.0	5.2	4.9	68
Molsidomine	8, single dose	27.0	23.5	1.7	1.7	69
Nifedipine IR[a]	10, single dose	82.0*	45.7	0.4*	0.6	70
Nifedipine SR	2 × 20, 1 week	48.5	50.1	2.3	2.8	70, 71
Atenolol	50, single dose	440.0	391.8	3.2	4.0	71
Oxprenolol[b]	80, single dose	507.0	375.0	1.0	1.1	72
Propranolol (±)[c]	80, single dose	38.6*	26.2	2.5	3.0	73
(–)-Propranolol						
Propranolol (±)	80, single dose	68.0	60.0	2.3	2.7	74
Verapamil SR	360, 2 weeks	389.0	386.0	7.2*	10.6	75
Verapamil	80, single dose	59.4*	25.6	1.3	2.0	76

Notes: At least two dosing times (around 6 am to 8 am and 6 pm to 8 pm) were studied; in some studies up to six circadian times were included. Only the parameters C_{max} = peak drug concentration and T_{max} = time to C_{max} are given. *P morning versus evening at least <0.05; [a]bioavailability significantly reduced; [b]significant difference in half-life; [c](±)-propranolol was given, kinetic data for (–)-propranolol.

Abbreviations: IR, immediate-release preparation; SR, sustained-release preparation.

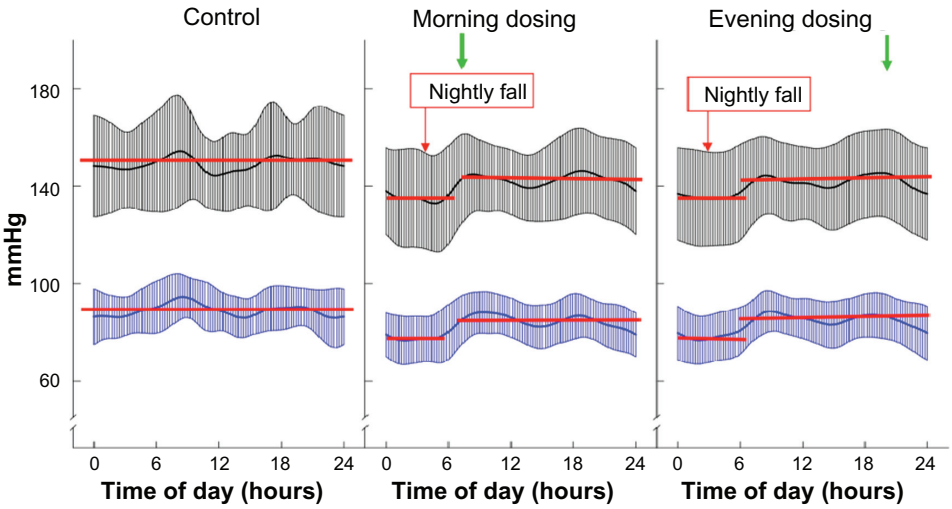

Figure 5 Effect of the calcium channel blocker amlodipine after evening or morning dosing on the blood pressure profile of hypertensive nondippers.

dosing with diuretics such as indapamide or xipamide reduced blood pressure in essential hypertensives without changing the 24-hour BP pattern.[136,137] In salt-sensitive hypertensive patients (dippers and nondippers), an interesting study was performed with diuretics: Uzu and Kimura[138] demonstrated that diuretics did not affect the circadian BP profile in dippers but transformed nondippers into dippers in salt-sensitive hypertensive patients.

Conclusion

The cardiovascular system is highly organized in time, both in health and disease. Disturbances in the physiological daily (circadian) rhythmic patterns of this system, eg, in cardiac functions and BP regulation, peripheral resistance, endothelial functions, and vessel regulation by hormones and neurotransmitters, can be regarded as warning signs of increased cardiovascular risk and can be of predictive value. There is now convincing evidence that most of these rhythms are driven by biological clocks. The aim of this paper was to review the circadian organization of the cardiovascular system in humans and to demonstrate its impact on drug treatment in hypertension. The recently published MAPEC[88] study also showed in a large population of hypertensives (both dippers and nondippers) that nightly dosing of ≥1 medication better controlled BP and reduced cardiovascular morbidity. However, the greatest benefit was seen in former nondippers, as already demonstrated in papers on crossover studies with one compound mentioned in this review. In our eyes, crossover studies with one compound can elucidate in more detail the underlying mechanisms of action of a given antihypertensive drug. In line with this assumption, the studies cited in this review clearly demonstrate that different compounds act differently on the regulation of daytime and nighttime blood pressure.

Finally, the new data presented here also demonstrate a sex dependency in vascular functions, pressure, and vasodilating hormones. These observations underline that diagnostics and treatment of cardiovascular disease must now take into account the importance of sex-dependent modifications, which have been overlooked for too long.

Disclosure

The author reports no conflicts of interest in this work.

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