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Importance of Circadian Rhythms for Regulation of the Cardiovascular System – Studies in Animal and Man

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Abstract – **In all mammals the cardiovascular system is highly organised in time. Pathophysiological cardiovascular events also do not occur at random (e.g. sudden cardiac death, stroke, ventricular arrhythmias, arterial embolism, symptoms of coronary heart disease, myocardial infarction). Radiotelemetry allows to get more insight into the circadian regulation of the cardiovascular system in unrestrained freely-moving animals. We monitored by telemetry blood pressure, heart rate (also be ECG-recordings), motility and body temperature in various strains of normotensive and hypertensive rats as well as in wildtype and knock-out mice. Our data gave evidence that the circadian rhythms in blood pressure and heart rate are controlled by the biological clock(s), since in rats and mice the rhythms persisted under free-running conditions in total darkness and were abolished in rats by lesioning of the "master clock" located in the suprachiasmatic nulcei.**

I. Introduction

The cardiovascular system is highly organised in time; blood pressure, heart rate, peripheral resistance, and the release/activity of vasodilating and pressure hormones all display circadian variations. Pathophysiological events are also not random, as shown for sudden cardiac death, stroke, ventricular arrhythmias, arterial embolism, symptoms of coronary heart disease and myocardial infarction [1]. To get more insight into the circadian regulation of the cardiovascular system various strains of rats were studied as animal models of human primary and secondary hypertension. Experiments were performed under highly controlled environmental conditions under a light:dark (LD) schedule of 12:12 h. To test for the contribution of the biological clock(s) rats and mice were monitored under free-run conditons in total darkness (DD) as well as after a shift of theLD cycle simulating a westward jet-lag.

II. Methods

The availability of implantable radiotelemetric devices made it possible to study various cardiovascular functions in freely-moving, unrestrained animals [2-5]. There is a clear-cut advantage of radiotelemetry over the conventional tail-cuff method since radiotelemetry does not induce stress reactions in experimental animals. The telemetric devices from Data Sciences Inc., St. Paul, MN, were used in rats and mice allowing to measure simultaneously systolic and diastolic blood pressure, heart rate and motility by implanting the blood pressure transmitter into the abdominal aorta (rats) [5] or into the carotic artery (mice) [6] or an ECG-transmitter was used (Arraj & Lemmer, unpublished) to allow continuous monitoring of the heart rate as well as temperature and activity in mice.

M[1]ost of the animal experiments were performed under a light:dark schedule of 12:12 hours (LD) under highly controlled environmental conditions [5]. In order to test the hypothesis of an involvement of circadian clock(s) in the regulation of cardiovascular rhythms additional experiments were performed under free-running conditions in total darkness (DD), after lesoning of the master clock in the central nervous system (suprachiasmatic nuclei, SCN) or after shifting the light phase by -6 hours (simulation of jet-jag) in order to test for adaptation to the new LD schedule. For data analysis the Chronos-Fit program [7] was used

Aside from normotensive control rats (Sprague-Dawley [SPD], Wistar-Kyoto [WKY]), spontaneously-hypertensive rats [SHR] were used as a model of human primary hypertension and transgenic TGR(mRen2)27 rats [TGR] as a model for human secondary hypertension [8, 9]. Experiments were also performed in wildytype (C57) mice and several strains of transgenic or knock-out mice (eNOS-/-) are under investigation.

III. RESULTS

In telemetric studies not only was the severe hypertension confirmed but surprisingly an inverse circadian BP pattern was found with peak values in the resting phase of the rats, i.e. during the light phase, whereas the rhythms in heart rate and motility were not disturbed with peak values in the dark phase [4]. Thus, there was an internal desynchronisation between blood pressure and heart rate. Transgenic rats are normotensive up to about 7 weeks after birth and also display a normal circadian profile in blood pressure (BP), heart rate (HR), and motility with peak values in the activity period during darkness [8-11]. Thereafter, TGR develop hypertension simultaneously with an about 12 hour shift of the peak in BP from the dark into the light/rest phase [8, 11]. This seems due to an ontogenic regulation of the mouse renin gene [12].

The circadian rhythms in blood pressure and heart rate of WKY, SDR and SHR - but not of TGR(mRen-2)27 - mirrored their activity patterns in that peak values were

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observed in the rats' activity phase [5]. The circadian patterns in blood pressure and heart rate of WKY, SDR and SHR are similar to patterns observed in normotensive and primary hypertensive humans with the blood pressure dipping in the resting phase.

Interestingly, hypertensive TGR have a normal circadian pattern in kidney function, including renal plasma flow [13], and in the cerebral blood flow [14] both peaking in the dark span. Thus, in TGR renal and cerebral blood flow are internally desynchronized from the systemic flood flow, the underlying mechanisms are under investigation.

In order to get further insight into the disturbed mechanisms of regulation of the blood pressure rhythm in TGR we studied processes of signal transduction of the sympathetic nervous system and the renin-angiotensin-system [RAS] [15] since TGR have an overexpressed RAS system. The overactive renin-angiotensin system in TGR lead to activation of the sympathetic nervous system with subsequent increase in BP. Although BP was significantly elevated in TGR, plasma catecholamine concentrations were reduced rather than increased in TGR, however, the rhythmic pattern and nocturnal peak was unaltered [16].

In a recent study we were able to demonstrate in both non-hypertensive (4 weeks of age) and hypertensive (10 weeks) TGR lower norepinephrine (NE) concentrations and a reduced expression of the tyrosine-hydroxylase in cardiac tissue and adrenal glands (RT-PCR/Western blot). In the hypothalamus NE concentrations were not different between the strains, however, tyrosine-hydroxylase mRNA was significantly higher in TGR than SPD [8, 9]. The turnover of NE was also reduced in hypertensive TGR in heart tissue and increased in the hypothalamus, both in the light and the dark phase. In adrenal glands of TGR the mRNA of NE reuptake$_1$-transporter was also reduced, in the hypothalamus NE reuptake$_1$-transporter could not be detected [9]. These data indicate that the transgene in TGR leads to an increased central stimulation of the sympathetic nervous system and a consequent down-regulation in the peripheral organs.

In wild-type mice also a circadian rhythm in heart rate (a slo by ECG) and blood pressure was found by telemetry [17, 18], which persisted under DD. This observation was confirmed by radiotelemetric ECG-registration in mice . Both in wildtype C57 and in eNOS-/- mice we could simulate jet-lag by shifting the LD cycle by -6 hours (westward flight), reentrainment of the rhythms in HR and body temperature occurred within 4-5 days [19].

Most behavioural and physiological parameters in mammals display at least some evidence of a 24 hr temporal structure, reflecting an innate temporal programme provided by biological clocks. The suprachiasmatic nucleus (SCN) of the hypothalamus serves as the main zeitgeber for such circadian rhythms. Light induction of clock genes might be a general factor through which the body clock is brought into synchronisation with the external environment [20-23]. In rodents light-induced phase shifts of behavioural rhythms are known to be positively correlated with the induction of the transcription factor Fos in the SCN and it has been suggested that Fos itself mediates these light-induced phase shifts [24-28]. As described above, alterations of endogenous rhythms, including severe changes in the rhythmic pattern of blood pressure have been detected in transgenic hypertensive TGR(mRen2)27 rats [5, 8, 9]. Both in normotensive rats [29, 30] and in TGR ablation of the suprachiasmatic nucleus eliminated 24-h blood pressure variability [28] and abolished the rhythm in motility as well as in heart rate and blood pressure [29]. This observation gives evidence that - at least in the rat - cardiovascular rhythms must also be under the control of the central clock(s) located in the SCN.

Another important feature of circadian rhythms is that they free-run under constant environmental conditions, i.e during constant darkness, indicating that they are really governed by an internal clock. Both in rats [31] and mice [17] we were able to demonstrate that the rhythms in cardiovascular functions (blood pressure, heart rate) persisted under free-run with a period deviating from 24-hours. In normotensive rats an increase and in normotensive mice a shortening of the periods were found (Lemmer, unpublished). Interestingly, in DD blood pressure and heart rate in TGR persisted but with no increase in period length, indicating that light perception must be disturbed in this transgenic rat strain [1, 31].

Immediate early genes, especially c-fos, are thought to play an essential role in photic entrainment of circadian rhythms. Assessment of c-fos mRNA expression by microdissection and RT-PCR in the suprachiasmatic nucleus showed that, in contrast to normotensive Sprague-Dawley rats, the 24 hr rhythm of c-fos mRNA expression in TGR(mRen2)27 rats is abolished [28]. Moreover, light-induced c-fos expression within the nucleus could be found in the normotensive controls, but was absent in transgenic hypertensive rats [9, 28]. When a one hour light pulse was applied during their subjective night to the transgenic rats housed in total darkness (DD) it had no effect on blood pressure and heart rate rhythm, only the activity rhythm showing a slight phase shift. In normotensive control SPD rats such a light pulse resulted in a significant phase-delay of about 2 hours [28].

Thus, this data suggest that the transgene in TGR leads not only to a disturbance of the cardiovascular system but also influences the light entrainment response, which is accompanied by an altered c-fos mRNA expression in the suprachiasmatic nucleus [8, 28].

IV. Discussion and Conclusion

In conclusion, the data obtained in various strains of rodents can help to better understand the rhythmic regulation of blood pressure and heart rate and the underlying mechanisms involved [1].This is of special importance for the evaluation of various forms of human primary and secondary hypertension or “dippers” and “non-dippers”. In addition, many persons have to work in night shifts which has been associated with an increased risk of cardiovascular disease or they travel across several time zones. Both of these conditions lead to disturbances

of the biological clock and the internal rhythmic organisation of the body with symptoms of intolerance to shift work or jet lag. With the use of radiotelemetry one can simulate theses conditions in rodents (changing light:dark conditions, inducing light shifts) and finally study the effects of drugs, which might be helpful to ameliorate the symptoms.

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REFERENCES

[1] B. Lemmer, "The importance of circadian rhythms on drug response in hypertension and coronary heart disease - from mice to man," *Pharmacol Ther*, vol. Epub ahead of print Febr 8, 2006.

[2] J. Lange, B. Brockway, and S. Azar, "Telemetric monitoring of laboratory animals: an advanced technique that has come of age," *Lab Anim*, 1991.

[3] R. Brockway and B. Brockway, "A new method for chronic measurements of respiratory rate in conscious freely moving rats," *Chronobiol Int*, vol. 13 Suppl 1, pp. 14, 1996.

[4] K. Kramer, H. P. Voss, J. A. Grimbergen, P. A. Mills, D. Huetteman, L. Zwiers, and B. Brockway, "Telemetric monitoring of blood pressure in freely moving mice: a preliminary study," *Lab Anim*, vol. 34, pp. 272-280, 2000.

[5] B. Lemmer, A. Mattes, M. Bohm, and D. Ganten, "Circadian blood pressure variation in transgenic hypertensive rats," *Hypertension*, vol. 22, pp. 97-101, 1993.

[6] B. Lemmer, M. Arraj, M. Thomas, and P. Zuther, "eNOS-knock-out mice display a disturbed 24-h rhythm in heart rate but not in blood pressure," *Am J Hypertens*, vol. 17, pp. 79A/P-127, 2004.

[7] P. Zuther and B. Lemmer, "Chronos-Fit. Version 1.02," 2004.

[8] B. Lemmer, K. Witte, H. Enzminger, S. Schiffer, and S. Hauptfleisch, "Transgenic TGR(mREN2)27 rats as a model for disturbed circadian organization at the level of the brain, the heart, and the kidneys," *Chronobiol Int*, vol. 20, pp. 711-38, 2003.

[9] B. Lemmer, S. Schiffer, K. Witte, and S. Gorbey, "Inverse blood pressure rhythm of transgenic hypertensive TGR(mREN2)27 rats: role of norepinephrine and expression of tyrosine-hydroxylase and reuptake1-transporter," *Chronobiol. Int.*, vol. 22, pp. 473-488, 2005.

[10] K. Witte, W. Grebmer, E. Scalbert, P. Delagrange, B. Guardiola-Lemaitre, and B. Lemmer, "Effects of melatoninergic agonists on light-suppressed circadian rhythms in rats," *Physiol Behav*, vol. 65, pp. 219-224, 1998.

[11] K. Witte and B. Lemmer, "Development of inverse circadian blood pressure pattern in transgenic hypertensive TGR(mREN2)27 rats," *Chronobiol Int*, vol. 16, pp. 293-303, 1999.

[12] Y. Zhao, M. Bader, R. Kreutz, M. Fernandez-Alfonso, F. Zimmermann, U. Ganten, and R. Metzger, "Ontogenetic regulation of mouse Ren-2d renin gene in transgenic hypertensive rats, TGR(mREN2)27," *Am J Physiol Endocrinol Metab*, vol. 265, pp. E699-E707, 1993.

[13] M. Pons, A. Schnecko, K. Witte, B. Lemmer, J. M. Waterhouse, and J. Cambar, "Circadian rhythms in renal function in hypertensive TGR(mRen-2)27 rats and their normotensive controls," *Am J Physiol*, vol. 271, pp. R1002-8, 1996.

[14] C. A. Wauschkuhn, K. Witte, S. Gorbey, B. Lemmer, and L. Schilling, "Circadian periodicity of cerebral blood flow revealed by laser-Doppler flowmetry in awake rats: realtionship to blood pressure and activity.," *Am J Physiol Heart Circ Physiol*, vol. Epub ahead of print, May 13, 2005.

[15] B. Lemmer, K. Witte, A. Schanzer, and A. Findeisen, "Circadian rhythms in the renin-angiotensin system and adrenal steroids may contribute to the inverse blood pressure rhythm in hypertensive TGR(mREN-2)27 rats," *Chronobiol Int*, vol. 17, pp. 645-58, 2000.

[16] S. Schiffer, S. Pummer, K. Witte, and B. Lemmer, "Cardiovascular regulation in TGR(mREN2)27 rats: 24h variation in plasma catecholamines, angiotensin peptides, and telemetric heart rate variability," *Chronobiol Int*, vol. 18, pp. 461-74, 2001.

[17] K. Witte, S. Engelhardt, B. J. Janssen, M. Lohse, and B. Lemmer, "Circadian and short-term regulation of blood pressure and heart rate in transgenic mice with cardiac overexpression of the beta 1-adrenoceptor," *Chronobiol Int*, vol. 21, pp. 205-16, 2004.

[18] B. Lemmer, M. Arraj, S. Gorbey, and T. Siebert, "Radiotelemetry in mice: circadian rhythms in wild-type and eNOS knock-our mice.," presented at UK Telemetry User Group Meeting, Manchester, 2005.

[19] M. Arraj and B. Lemmer, " Circadain rhythm in heart rate, motility and body temperature of wildtype C57 and eNOS knock-out mice under light-dark, free-run and after time zone transition," *Chronobiol Int*, in press, 2006.

[20] U. Albrecht, Z. S. Sun, G. Eichele, and C. C. Lee, "A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light," *Cell*, vol. 91, pp. 1055-1064, 1997.

[21] Y. Shigeyoshi, K. Taguchi, S. Yamamoto, S. Takekida, L. Yan, H. Tei, T. Moriya, S. Shibata, J. J. Loros, J. C. Dunlap, and H. Okamura, "Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript," *Cell*, vol. 91, pp. 1043-1053, 1997.

[22] K. Spoelstra, U. Albrecht, G. T. van der Horst, V. Brauer, and S. Daan, "Phase responses to light pulses in mice lacking functional per or cry genes," *J Biol Rhythms*, vol. 19, pp. 518-29, 2004.

[23] U. Albrecht, "The mammalian circadian clock: a network of gene expression," *Front Biosci*, vol. 9, pp. 48-55, 2004.

[24] J. M. Kornhauser, K. E. Mayo, and J. S. Takahashi, "Light, immediate-early genes, and circadian rhythms " *Behav Genet*, vol. 26, pp. 221-240, 1996.

[25] K. Shimomura, J. M. Kornhauser, J. P. Wisor, T. Umezu, S. Yamazaki, N. L. Ihara, J. S. Takahashi, and M. Menaker, "Circadian behavior and plasticity of light-induced c-fos expression in SCN of tau mutant hamsters," *J Biol Rhythms*, vol. 13, pp. 305-314, 1998.

[26] B. Rusak, L. McNaughton, H. A. Robertson, and S. P. Hunt, "Circadian variation in photic regulation of immediate-early gene messenger RNAs in rat suprachiasmatic nucleus cells," *Brain Res Mol Brain Res*, vol. 14, pp. 124-130, 1992.

[27] E. L. Sutin and T. Kilduff, "Circadian and light-induced expression of immediate early gene messenger RNAs in the rat suprachiasmatic nucleus," *Brain Res Mol Brain Res*, vol. 15, pp. 281-290, 1992.

[28] B. Lemmer, S. Hauptfleisch, and K. Witte, "Loss of 24 h rhythm and light-induced c-fos mRNA expression in the suprachiasmatic nucleus of the transgenic hypertensive TGR(mRen2)27 rat and effects on cardiovascular rhythms," *Brain Res*, vol. 883, pp. 250-7, 2000.

[29] B. J. A. Janssen, C. M. Tyssen, H. Duindam, and W. J. Rietveld, "Suprachiasmatic lesions eliminate 24-h blood pressure variability in rats," *Physiol Behav*, vol. 55, pp. 307-311, 1994.

[30] K. Witte, W. Grebmer, E. Scalbert, P. Delagrange, B. Guardiola-Lemaitre, and B. Lemmer, "Effects of melatoninergic agonists on light-suppressed circadian rhythms in rats," *Physiol Behav*, vol. 65, pp. 219-24, 1998.

[31] K. Witte and B. Lemmer, "Free-running rhythms in blood pressure and heart rate in normotensive and transgenic hypertensive rats," *Chronobiol Int*, vol. 12, pp. 237-47, 1995.