Klin Wochenschr (1988) 66:579–582

Klinische Wochenschrift

Circannual Variation in the Expression of β_2-Adrenoceptors on Human Peripheral Mononuclear Leukocytes (MNLs)

E. Haen, I. Langenmayer, A. Pangerl, B. Liebl, and J. Remien
Walther-Straub-Institut für Pharmakologie und Toxikologie, München

Summary. Peripheral mononuclear leukocytes (MNLs) are widely used as a tissue model in studies of β-adrenoceptor disturbances in hypertension and asthmatic diseases. The β_2-adrenoceptor density (B_{max}), however, depends not only on the gender of the person under study and on the time of day the blood specimens are obtained. Evidence is now reported for a circannual variation in the expression of β_2-adrenoceptor sites on peripheral MNLs. In male volunteers the 24-h mean was found to be highest in the men studied in April/May (1135 ± 10 sites/cell) and decreased to 891 ± 16 sites/cell in August and to 712 ± 90 sites/cell in December ($\bar{x} \pm$ SE, $P < 0.01$ April/May compared to December). Concomitantly the circadian amplitude increased from $17.3\% \pm 6.4\%$ of 24-h mean in April/May to $28.2\% \pm 1.4\%$ of 24-h mean in August and to $34.2\% \pm 4.2\%$ of 24-h mean in December ($\bar{x} \pm$ SE, $P < 0.05$, April/May compared to December). The circadian acrophase remained constant ($190° \pm 30°$ equivalent to 12 h 40 min $\pm$ 2 h 00 min, $\bar{x} \pm$ SE).

Key words: Circannual variation – Circadian variation – β_2-Adrenoceptor sites – Peripheral mononuclear leukocytes – Humans

Mononuclear leukocytes (MNLs) are widely used as a tissue model in studies of β-adrenoceptor disturbances in hypertensive [2, 6, 7] and asthmatic diseases [4, 12]. We recently reported a *circadian* variation in the expression of β_2-adrenoceptors on MNLs in humans [10]. Here we report evidence of a *circannual* variation in the expression of these receptors on MNLs.

Abbreviations: MNLs = Peripheral mononuclear leucocytes; B_{max} = β_2-Adrenoceptor density; ^{125}ICYP = ^{125}Iodo-cyanopindolol; PEF = Peak expiratory flow; SE = Standard error; ANOVA = Analysis of variance; M = Circadian mesor; A = Circadian amplitude; ϕ = Circadian acrophase

Materials and Methods

Venous blood was drawn from the antecubital vein of 10 male volunteers, 19–34 years old, every 4 h. Immediately thereafter MNLs were harvested in each of the 68 specimens by density centrifugation using Ficoll-Hypaque [8]. Cells were incubated for 120 min at 37° C with 10–12 concentrations of ^{125}ICYP in the range of 1.8–600 pmol (total binding T). Nonsaturable binding of the radioligand was determined in parallel incubations with 10^{-6} mol/l unlabelled (−)-timolol (unspecific bindung U). Specific binding (B) was calculated as the difference between total and unspecific binding ($B = T - U$). The data were iteratively fitted to a model of two independent classes of binding sites (a saturable high-affinity and a nonsaturable low-affinity binding site) using a computer programm named MULTI [9, 15]. The total number of high-affinity binding sites (B_{max}) and their equilibrium dissociation constant (K_d) were obtained from the curve fit as parameters of the β_2-adrenoceptor on MNLs [1].

Eight apparently healthy subjects were studied in April/May ($n = 4$), August ($n = 1$), and December ($n = 3$). In August two additional subjects were studied, who complained of mild nocturnal asthma. The 24-h mean of their peak expiratory flow (PEF), however, was within the normal range (597 ± 33 l/min and 498 ± 29 l/min, $\bar{x} \pm$ SE) with a nocturnal dip of 30% of the 24-h mean at 0600 h and of 26% of the 24-h mean at 0200 h, respectively. None of the subjects took any medication.

The circadian variations were statistically validated by cosinor analysis [3] and by an analysis

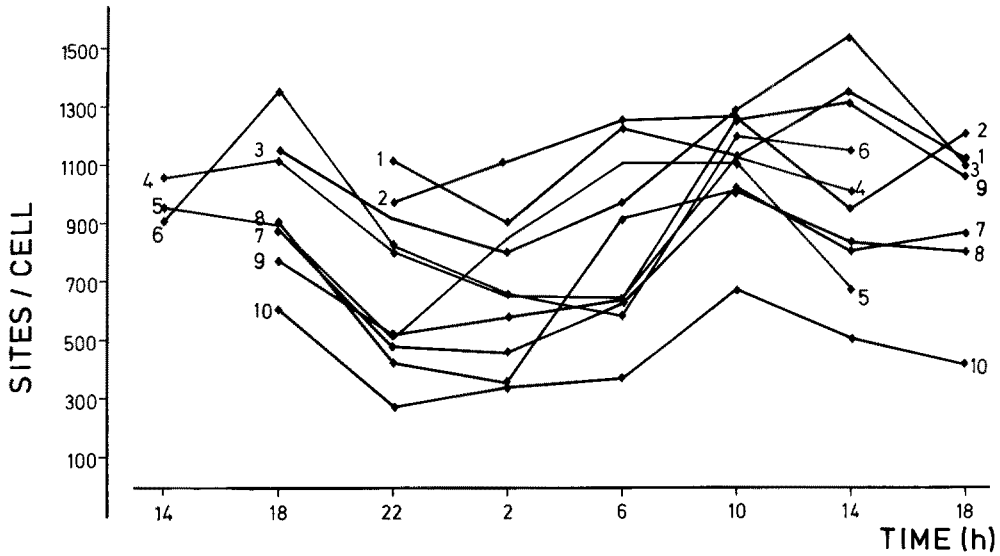

Fig. 1. Circadian variation in the expression of β_2-adrenoceptor sites on mononuclear leukocytes in each of the 10 male subjects studied at different times during the year (April/May: numbers 1, 2, 3; August: numbers 4, 5, 6; December: numbers 7, 8, 9, 10). Subject no. 2 was restudied as no. 8 in December

of variance (ANOVA); annual differences were tested using Student's t-test. $P<0.05$ was regarded as significant. The cosinor analysis also yielded the circadian mesor, the circadian amplitude, and the circadian acrophase as parameters of a circadian variation [5]: The circadian mesor (M) is defined as the midline of a cosine function with a period of 24 h fitted to the seven time-specified B_{max} values of each individual circadian variation; with equidistant data as in this study the mesor is equivalent to the 24-h mean. The circadian amplitude (A) is defined as the maximal deviation of the fitted cosine function from its midline. The circadian acrophase (ϕ) is defined as the time lag of the maximum of the fitted cosine function in reference to local midnight.

Results

A circadian variation in the expression of β_2-adrenoceptors on peripheral MNLs was detected in all individuals with maximal values around noon and minimal values around midnight (Fig. 1). The equilibrium dissociation constant for the high-affinity binding site showed neither a significant circadian nor a significant circannual variation. The arithmetic mean of the 68 determinations was 4.5 ± 0.5 pmol/l ($\bar{x} \pm$ SE).

When the circadian variation parameters for the β_2-adrenoceptor density of all healthy subjects were compared to those of the men complaining of nocturnal asthma no differences could be found (Table 1). Neither was there any difference, when the two men complaining of nocturnal asthma, who were both studied in August, were compared to the apparently healthy man studied in the same month (Table 1).

Table 1. Parameters for the circadian variation in the expression of β_2-adrenoceptor sites on human mononuclear leukocytes as determined in male subjects ($\bar{x} \pm$ SE)

Subjects	n	24-h mean (sites/cell) $\bar{x} \pm$ SE	Circadian amplitude (% of 24-h mean) $\bar{x} \pm$ SE
All apparently healthy men	8	892 ± 98	25.8 ± 8.5
Mild nocturnal asthma in August	2	893 ± 27	29.0 ± 2.0
Apparently healthy man in August	1	890	28.5

If the same data were analyzed according to the months the experiments were performed, a circannual variation became apparent in the expression of β_2-adrenoceptors on MNLs. The 24-h mean was highest in the men studied in April/May (1135 ± 10 sites/cell) and decreased to 891 ± 16 sites/cell in August and to 712 ± 90 sites/cell in December ($\bar{x} \pm$ SE, $P<0.01$ April/May compared to December, Fig. 2). Concomitantly the circadian amplitude increased from $17.3\% \pm 6.4\%$ of 24-h mean in April/May to $28.2\% \pm 1.4\%$ of 24-h mean in August and to $34.2\% \pm 4.2\%$ of 24-h mean in December ($\bar{x} \pm$ SE, $P<0.05$, April/May compared to December, Fig. 2). The circadian acrophase re-

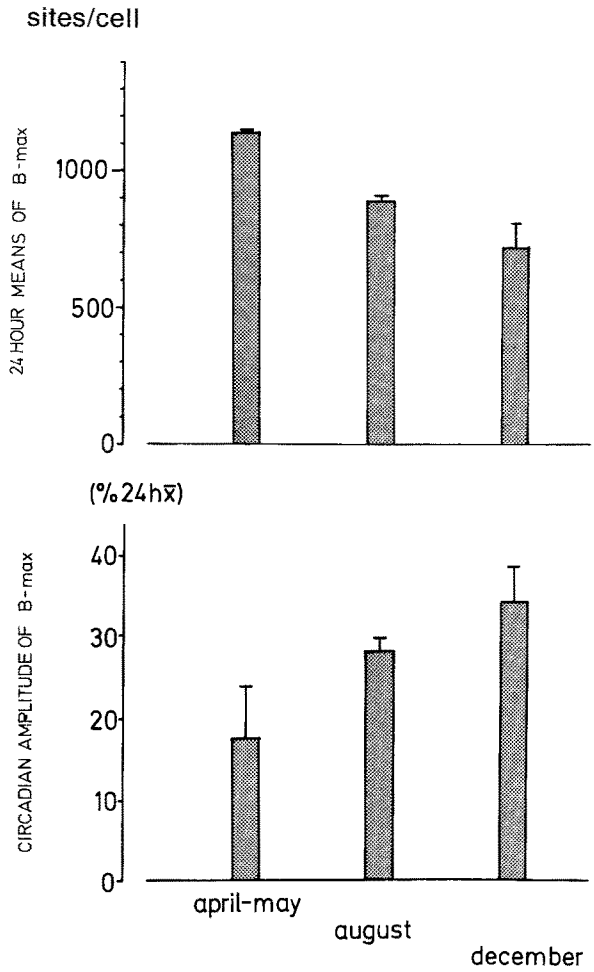

Fig. 2. 24-h means (*upper panel*) and amplitudes (*lower panel*) of the circadian variation in the expression of β_2-adrenoceptor sites on human mononuclear leukocytes as determined in male subjects at different times during the year ($\bar{x} \pm$ SE). (% 24 h $\bar{x}$: data expressed as a percentage of the 24 h mean)

mained constant (190° ± 30° equivalent to 12 h 40 min ± 2 h 00 min, $\bar{x} \pm$ SE).

Discussion

Disturbances in the expression and function of adrenoceptors have been discussed as pathophysiological mechanisms in hypertension [2, 6, 7] and bronchial asthma [4, 12, 13]. Usually peripheral MNLs are used as tissue models in in vivo studies in humans. In our own investigations there is no evidence so far that a pathological alteration of the expression of β_2-adrenoceptors due to mild nocturnal asthma may be detected by studying MNLs of those subjects. If there should be such an alteration, it will be definitely less pronounced than physiologically occurring variations in the number of β_2-adrenoceptor sites expressed on MNLs.

We recently demonstrated the influence of the time of day and of the subjects' gender on the expression of β_2-adrenoceptors on MNLs [4, 10]. Additional studies of apparently healthy men and individuals complaining of mild nocturnal asthma now provide evidence that also the time of year influences the β_2-adrenoceptor density. As pointed out elsewhere, the circadian variation in the expression of β_2-adrenoceptors on peripheral MNLs is correlated to the circadian variation of plasma adrenaline and cortisol concentrations resulting in an endogenous down- and up-regulation, respectively [4]. We assume, therefore, that circannual variations in circulating catecholamine and glucocorticoid concentrations trigger the circannual variation in the expression of β_2-adrenoceptor sites on MNLs. Data that are in agreement with such an assumption have been reported by Reinberg et al. [11] and Touitou et al. [14] for serum cortisol with a circannual acrophase in March and June, respectively. Data on a circannual variation of plasma catecholamine concentrations are still lacking.

The evidence reported in this paper is a rather casual finding observed during the evaluation of experiments designed to study circadian variations. To firmly establish the suggested circannual variation it must be demonstrated intraindividually in a sufficient number of subjects. Such studies are now in progress in our laboratory, and the results will be reported in about 2 years.

Even now, however, we believe the evidence for a circannual variation in the expression of β_2-adrenoceptors on MNLs is strong enough that this factor should be taken into account in clinical studies on β_2-adrenoceptors, together with the circadian variation and the differences between males and females already reported earlier [4, 10].

Acknowledgements: The authors wish to thank Christina Lemmermann, Iris Reithmeier, and Sybille Kirzinger for their skillfull technical assistance; without their readiness to work extra hours and night shifts this study could not have been performed. Irmgard Langenmayer and Andreas Pangerl are medical students; part of this work was done in fulfillment of their medical thesis (Dr. med.)

References

1. Anhäupl T, Liebl B, Remien J (1988) Kinetic and equilibrium studies of $(-)^{125}$iodocyanopindolol binding to β-adrenoceptors on human lymphocytes: evidence for the existence of two classes of binding sites. J Rec Res 8:47–57
2. Brodde OE, Prywarra A, Daul A, Anlauf M, Bock KD (1984) Correlation between lymphocyte β_2-adrenoceptor density and mean arterial blood pressure: elevated β-adrenoceptors in essential hypertension. J Cardiovasc Pharmacol 6:678–682
3. Cornelissen G, Halberg F, Stebbings J, Halberg E, Caran-

dente F, Hsi B (1980) Data aquisition and analysis by computers and pocket calculators. La Ricerca Clin Lab 10:333–385
4. Haen E (1987) The "peripheral lymphocyte" as clinical model for receptor disturbances: asthmatic diseases. Bull Europ Physiopath Respir 22:539–541
5. Haen E, Halberg F (1985) Chronopharmakologie und Chronotherapie – Von der experimentellen Forschung zur praktisch klinischen Anwendung. Dtsch Ärzteblatt 82:3837–3848
6. Landmann R, Bürgisser E, Bühler FR (1983) Human lymphocytes as a model for beta-adrenergic receptors in clinical investigation. J Rec Res 3:71–88
7. Middeke M, Remien J, Block LH, Kirzinger S, Landrock A, Holzgreve H (1983) Beta$_2$-adrenoceptor density on membranes and on intact mononuclear cells in essential hypertension. Res Exp Med 183:227–232
8. Middeke M, Remien J, Holzgreve H (1984) The influence of sex, age, blood pressure, and physical stress on β_2-adrenoceptor density of mononuclear cells. J Hypertension 2:261–264
9. Naar R (1986) Über die Wirkung amphiphiler Pharmaka auf die mechanische Belastbarkeit und Verformbarkeit intakter Erythrozyten. Dissertation, München, pp 1–154
10. Pangerl A, Remien J, Haen E (1986) The number of β-adrenoceptor sites on intact human lymphocytes depends on time of day, on season, and on sex. Ann Rev Chronopharmacol 3:331–334
11. Reinberg A, Lagoguey M, Cesselin F, Touitou Y, Legrand JC, Delasalle A, Antreassian J, Lagoguey A (1978) Circadian and circannual rhythms in plasma hormones and other variables of five young human males. Acta Endocrinol (Kbh) 88:417–427
12. Scarpace PJ, Littner MR, Tashkin DP, Abrass IB (1982) Lymphocyte beta-adrenergic refractoriness induced by theophylline or metaproterenol in healthy and asthmatic subjects. Life Sci 31:1567–1573
13. Szentivanyi A (1968) The beta adrenergic theory of the atopic abnormality in bronchial asthma. J Allergy 42:203–232
14. Touitou Y, Sulon J, Bogdan A, Reinberg A, Sodoyez JC, Demey-Ponsart E (1983) Adrenocortical hormones, aging and mental condition: seasonal and circadian rhythms of plasma 18-hydroxy-11-deoxy-corticosterone, total and free cortisol and urinary corticosteroids. J Endocrinol 96:53–64
15. Yamaoka K, Tanigawara Y, Nakagawa T, Uno T (1981) A pharmacokinetic analysis program (Multi) for microcomputers. J Pharm Dyn 4:879–885

Received: November 26, 1987
Returned for revision: January 21, 1988
Accepted: April 20, 1988

Dr. Ekkehard Haen
Walther-Straub-Institut für Pharmakologie und Toxikologie
Nussbaumstr. 26
D-8000 München 2