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## BIOLOGICAL RHYTHMS AND MEDICATIONS

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# DOES THE CIRCADIAN VARIATION OF $\beta_2$ -ADRENOCEPTOR SITES ON PERIPHERAL MONONUCLEAR LEUCOCYTES (MNL) REFLECT THE CIRCADIAN VARIATION OF DIFFERENT MNL SUBSETS?

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## ABSTRACT

The fractional distribution of lymphocyte subsets was determined in three male subjects. Its contribution to the circadian variation in the expression of  $\beta_2$ -adrenoceptor sites on peripheral mononuclear leucocytes (MNL) was calculated to be 92.7-107.3 % of the 24h-mean. This strongly favors the idea that the actually observed circadian range of 74.5-124.9 % of the 24h-mean constitutes a circadian receptor down- and up-regulation.

## KEY WORDS

circadian variation,  $\beta_2$ -adrenoceptors, peripheral mononuclear leucocytes (MNL), lymphocyte subsets, humans

## INTRODUCTION

MNL are widely used for clinical studies evaluating the expression and function of  $\beta_2$ -adrenoceptors (Haen 1987, Brodde et al 1985, Middeke et al 1985). We recently described a circadian variation in the expression of  $\beta_2$ -adrenoceptors on peripheral MNL (Pangerl et al 1986, Haen 1987). Since T-lymphocytes have been reported to bear less than half the number of  $\beta_2$ -adrenoceptor sites as B-lymphocytes (Landmann et al 1984, Krawietz et al 1982), the circadian variation in the expression of  $\beta_2$ -adrenoceptor sites may result from a circadian variation in the numerical distribution of these lymphocyte subsets.

## MATERIALS AND METHODS

Venous blood was drawn at 14h00, 18h00, 22h00, 02h00, 06h00, 10h00, and again at 14h00 from three male subjects, 24-34 years of age. Peripheral mononuclear leucocytes were immediately harvested by Ficoll-Hypaque density gradient centrifugation. The cells were incubated with a set of mouse IgG monoclonal antibodies (CD 3, CD 4, CD 8, CD 37 specific for T-, T<sub>helper</sub>-, T<sub>suppressor</sub>-, and B-cells, respectively) and in a second step with a fluoresceinisothiocyanate (FITC)-labeled goat anti-mouse IgG monoclonal antibody. After washing out exceeding amounts of antibody the cells were fixed in 0.1% paraformaldehyde and stored at 4°C. Approximately one week after sampling the fixed cells were run in a fluorescence activated cell sorter (FACS) to count the percentage of labeled cells in a cell fraction gated to contain lymphocytes and monocytes. The distribution of lymphocyte

subsets was compared to the circadian variations in  $\beta_2$ -adrenoceptor sites on peripheral MNL and in total lymphocyte count in another group of seven healthy men (Pangerl et al 1986).

The subjects were asked to follow a regular life-style for the two weeks preceding the study with bed rest between 23h00 and 07h00. On the day of the study the subjects stayed in the clinical pharmacological research unit of the institute. They continued to follow their normal daily routine. Subjects were asked to record meal times, the consumption of alcohol and coffee. All were non-smokers.

Circadian variations were statistically validated by the cosinor method (Halberg et al 1967) and by analysis of variance (anova). Significance limit was  $p < 0.05$ .

## RESULTS

The fractional distribution of T- and B-cells showed a large inter-individual variation and varied within 24 hours, but the circadian variation did not reach statistical significance (Fig. 1). The highest percentage of T-cells was observed at 18h00 (73.0 $\pm$ 0.45 % of gated cells,  $\bar{x}$  $\pm$ SE), the lowest percentage at 02h00 (56.4 $\pm$ 9.7 % of gated cells,  $\bar{x}$  $\pm$ SE). The highest percentage of B-cells occurred at 10h00 (11.2 $\pm$ 6.9 % of gated cells,  $\bar{x}$  $\pm$ SE), the lowest percentage was seen at 18h00 (6.3 $\pm$ 3.0 % of gated cells,  $\bar{x}$  $\pm$ SE).  $T_{\text{helper}}$ - and  $T_{\text{suppressor}}$ -cells also demonstrated a statistically insignificant variation with highest percentage of  $T_{\text{helper}}$  at 02h00 (51.5 $\pm$ 1.2 % of gated cells) and of  $T_{\text{suppressor}}$  at 06h00 (22.0 $\pm$ 1.0 % of gated cells). The lowest fraction was seen at 06h00 (34.6 $\pm$ 4.2 % of gated cells) and at 22h00 (12.0 $\pm$ 3.0 % of gated cells) for  $T_{\text{helper}}$  and  $T_{\text{suppressor}}$ , respectively (all  $\bar{x}$  $\pm$ SE).

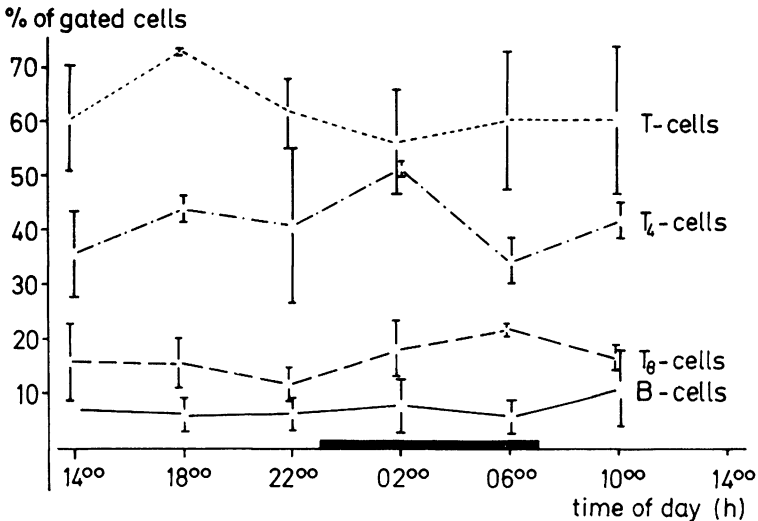


Fig. 1. Fractional distribution of human lymphocyte subsets determined over 24 hours

The total lymphocyte count demonstrated a circadian variation ( $p < 0.01$  anova,  $p = 0.07$  /  $PR = 61.3\%$  in population-mean cosinor analysis, Fig. 2 lower panel) with highest numbers at 02h00 (3367 $\pm$ 236 cells) and lowest numbers at 10h00 (2175 $\pm$ 262 cells, all  $\bar{x}$  $\pm$ SE). Highest  $\beta_2$ -adrenoceptor density occurred at the time of lowest lymphocyte count (10h00), the number of receptor sites decreased to a minimum at 02h00, when the total number of lymphocytes is highest. The circadian range was 74.5–124.9 % of 24h-mean for the expression of  $\beta_2$ -adrenoceptor sites on peripheral MNL.

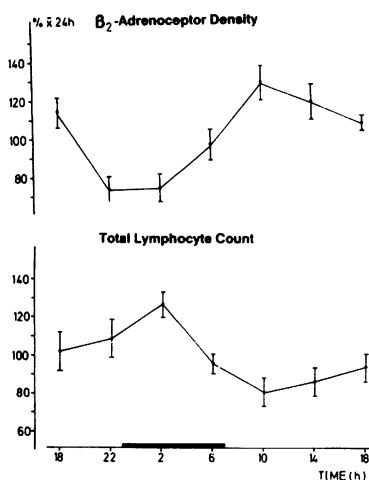


Fig. 2. Circadian variation in  $\beta_2$ -adrenoceptor density on peripheral mononuclear leucocytes (upper panel) and in total lymphocyte count (lower panel) of healthy men.

## DISCUSSION

Since the  $\beta_2$ -adrenoceptor density on MNL is expressed as sites/cell the circadian variation in the expression of these receptors is corrected for the circadian variation in total lymphocyte count. Landmann et al 1984, however, reported a different  $\beta_2$ -adrenoceptor density on T-cells (1400 sites/cell, irrespective of T<sub>helper</sub> or T<sub>suppressor</sub>) and on B-cells (3700 sites/cell). A circadian variation in the fractional distribution of lymphocyte subsets might account, therefore, for the circadian variation in the expression of  $\beta_2$ -adrenoceptor sites on peripheral MNL:

The total number of lymphocytes reaches a minimum at the time of highest  $\beta_2$ -adrenoceptor density (10h00). At that time the percentage of B-cells among peripheral MNL is highest (11.2%): approximately 243 cells of 2175 total lymphocytes may be B-cells bearing according to Landmann et al 1984 a total number of  $8.99 \times 10^5$  sites; 60.5 % of the total lymphocytes are T-cells (1316 cells) that add another  $1.84 \times 10^6$   $\beta_2$ -adrenoceptor sites resulting in an average value for all lymphocytes at that time of day of 1259 sites/cell. At the trough of the circadian variation in the expression of  $\beta_2$ -adrenoceptor sites (02h00) the total number of lymphocytes is highest. At that time 8.1 % (273 cells) of the lymphocytes (3367 cells) are B-cells participating  $1.00 \times 10^6$   $\beta_2$ -adrenoceptor sites. The fraction of T-cells is minimal at that time: 56.4 % (1899 cells) of the lymphocytes bear  $2.66 \times 10^6$   $\beta_2$ -adrenoceptors. This results in an average value for all lymphocytes of 1087 sites/cell at 02h00.

According to this very rough calculation the circadian range of the variation in the expression of  $\beta_2$ -adrenoceptor sites on peripheral MNL would be 92.7-107.3 % of the 24h-mean, which is much less than the one actually observed (74.5-124.9 % of the 24h-mean). Quite a number of incertainties are assumed in this calculation: It has not yet been established, how the extensive handling with antibody labelling and FACS-analysis affects the expression of  $\beta_2$ -adrenoceptor sites on MNL. In this context the data reported by Landmann et al 1984 have to be regarded as preliminary. Also our data on the fractional distribution of lymphocyte subsets are rather preliminary due to the small number of subjects; some cell types such as monocytes were not included yet into the analysis but may express  $\beta_2$ -adrenoceptors as well. Nevertheless the calculation yielded reasonable

adrenoceptor densities, but it appears that the fractional distribution of lymphocyte subsets does not show a relevant circadian variation at all. This study does not preclude, however, that the lymphocyte subset composition may change in response to short acting stimuli such as physical exercise. According to this study different lymphocyte subset distributions may contribute (if at all) only little to the circadian variation in the expression of  $\beta_2$ -adrenoceptor sites on peripheral MNL. As outlined elsewhere (Haen 1987) the circadian variation in the expression of  $\beta_2$ -adrenoceptor sites on peripheral MNL rather constitutes a circadian receptor down- and up-regulation in response to hormonal stimuli, such as adrenaline and cortisol concentrations in blood, respectively.

#### REFERENCES

1. Brodde OE, Daul AE, O'Hara Naoki, Khalifa AM (1985). Properties of  $\alpha$ - and  $\beta$ -Adrenoceptors in Circulating Blood Cells of Patients with Essential Hypertension. J Cardiovasc Pharmacol 7 (Suppl. 6), S162-S167
2. Haen E (1987). The Peripheral Lymphocyte as Clinical Model for Receptor Disturbances Asthmatic Diseases. Bull Eur Physiopathol Resp 22, 539-541
3. Halberg F, Tong YL, Johnson EA (1967). Circadian System Phase - An Aspect of Tempora Morphology; Procedures and Illustrative Examples. in: The Cellular Aspects of Bio-rhythms (ed H v Mayersbach), Springer Verlag Berlin/Heidelberg/New York, pp 20-48
4. Krawietz W, Werdan K, Schober M, Erdmann E, Rindfleisch GE, Hanning K (1982). Different Numbers of  $\beta$ -Receptors on Human Lymphocyte Subpopulations. Biochem Pharmacol 31, 133-136
5. Landmann RMA, Bürgisser E, Wesp M, Bühler FR (1984). Beta-Adrenergic Receptors are Different in Subpopulations of Human Circulating Lymphocytes. J Rec Res 4, 37-50
6. Middeke M, Remien J, Kirzinger S, Holzgreve H (1985). Adrenergic Hyposensitivity during Long-Term Diuretic Therapy - A Possible Explanation for the Antihypertensive Effect of Diuretics. Eur J Pharmacol 109, 401-403
7. Pangerl A, Remien J, Haen E (1986). The Number of  $\beta$ -Adrenoceptor Sites on Intact Human Lymphocytes Depends on Time of Day, on Season, and on Sex. Ann Rev Chronopharmacol 3, 331-334

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