of the disease PEFR values are steadily low during the acute phase submitted to intensive care; then large swings appear during the recovery stage, with a further decrease in the amplitude of oscillations as the patient progressively improves. In such patients wide PEFR variations are connected with an increased risk of ventilatory arrest [5]: therefore in this condition a marked morning dip must be regarded as the hallmark of a dangerous airway instability.

In stable outpatients, the recurrence of some morning decrease of PEFR is very common (up to 91% in our experience), however when a definitely high threshold of variation is looked for, a marked drop in the prevalence rate (to 15%) is noticed. In these patients the amplitude of morning dipping seems linked to its frequency, since the deepest falls have been reported in subjects in whom the phenomenon was noticeable on the majority of the monitored days [1]. In a similar sample we observed that, although morning dipping is initially found in subjects with more severe functional picture, it does not imply any worse prognosis; in addition, unlike the hospitalized patients, where morning dipping may be persistent and refractory to therapy, in stable outpatients the pattern may be easily reversible in response to treatment or even spontaneously [4].

Finally it must be emphasized that the absence of a morning dip does not always imply bronchial stability and absence of risk: in fact we observed high degrees of diurnal variability, even in the presence of negligible morning dips (fig. 2). This finding corresponds to the possible, though rare, occurrence of patterns of diurnal variation other than the circadian one so far discussed. Since exaggerated variability, whenever noticed, indicates hyperreactivity, it may be recommended that besides the magnitude of morning dips, some index of overall variability (such as the coefficient of variation) be included among the output of all the programmes of PEFR monitoring.

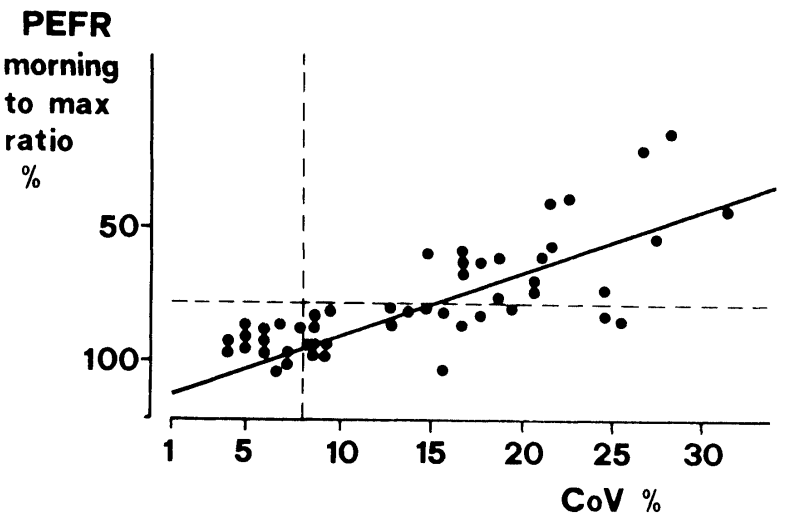

Fig. 2. – Relationship between the coefficient of variation (CoV) in percent (on the abscissa) and the morning-to-maximum ratio of PEFR in percent (on the ordinate). The regression line is represented ($p < 0.01$). The points in the right lower quadrant are relevant to cases of high variability of PEFR in the absence of a significant morning dip.

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THE PERIPHERAL LYMPHOCYTE AS CLINICAL MODEL FOR RECEPTOR DISTURBANCES: ASTHMATIC DISEASES

E. Haen*

In 1968 Szentivanyi published a theory relating the pathophysiology of asthmatic disorders to receptor disturbances in the sympathetic system [9]. Since then much interest had been focused on the cell structures that translate external stimuli into intracellular biochemical functions. Two major problems had to be solved, however:
1. A methodology was needed to follow changes at the receptor sites.
2. A clinical model for human lung tissue had to be developed, since it is impossible to obtain human lung tissue for screening studies, especially if a chronobiological design is considered.

The methodological development went hand in hand with the establishment of the 'Peripheral Lymphocyte' as clinical model. These white blood cells may be easily and repeatedly collected in large numbers without greatly disturbing the patient's comfort. Studying insulin receptors on peripheral lymphocytes both Archer *et al.* and Soll *et al.* demonstrated, in 1974, a malfunction of the insulin receptor in diabetes. This observation prompted studies on other receptor systems. Using a radio-immuno-assay (RIA) Conolly and Greenacre [5] showed that the cyclic adenosine-monophosphate (cAMP) in peripheral lymphocytes of asthmatic patients could be stimulated to the same extent as in healthy controls, unless the patients were treated with β-sympathomimetic drugs. Although this study could

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not confirm β-adrenoceptor disturbances in asthma, it suggested a biochemical explanation for a clinica phenomenon called 'tachyphylaxis', a decreasing effectiveness of β-sympathomimetic drugs under continuous therapy of asthmatic symptoms. This situation was obviously caused by either a decreased β-adrenoceptor number or a decreased responsiveness of the tandem-arranged intracellular biochemical events.

At about the same time radio-receptor-assays (RRA) became available, that allowed the determination of the number of adrenoceptor sites and their affinity for certain ligands. In 1976 Williams used tritiated dihydroalprenolol in the first RRA for β-adrenoceptors. With iodinated hydroxy-pindolol AARONS *et al.* [1] demonstrated a decrease in receptor density under treatment with β-sympathomimetic drugs, a condition that is known today as 'receptor down-regulation'. Both mentioned radioligands, however, showed a high amount of unspecific binding. Therefore, ^{125}Iodo-cyano-pindolol (^{125}I-CYP) is most often used today in β-sympathetic RRAs, a radioligand developed by ENGEL *et al.* [6].

In displacement experiments with ^{125}I-CYP BRODDE *et al.* [3] detected a selectivity of the β-adrenoceptors on peripheral lymphocytes for β_2-ligands and a stereospecificity for their (−)-forms, in other words: The 'Peripheral Lymphocyte' bears β-adrenoceptors of the same type found primarily on bronchial smooth muscle tissue.

For many years now Professor Remien has used the 'Peripheral Lymphocyte' as a clinical model for hypertensive disorders. Based on the numerous reports of circadian variation of asthmatic symptoms (*e.g.* BARNES *et al.* [2]) we set out to investigate the receptor sites in chronic obstructive pulmonary diseases.

METHODS AND RESULTS

In a first set of experiments we studied the circadian variation of adrenoceptor density in 11 healthy subjects (7 males, 4 females, aged 22–34 yr). As outlined by PANGERL *et al.* [7], we found a marked circadian variation in all subjects with peak values around noon and a trough around midnight. This is in good agreement with the nocturnal dip reported for bronchial patency in asthma. However, we were suprised to observe pronounced differences between males and females. Nocturnal asthma should be found, therefore, more consistently in males, a phenomenon not explicitly mentioned in the literature. Moreover, for organizational reasons, we had to study three of our male subjects in early summer, another four in November/December. Despite the small number of cases the differences in mesor (1135 ± 10 sites/cell in summer, 712 ± 90 sites/cell in winter) and amplitude ($17.3 \pm 6.4\%$ of mesor in summer, $34.3 \pm 4.2\%$ of mesor in winter) were significant ($p < 0.01$ and $p < 0.05$, respectively; $\bar{x} \pm SE$).

We now investigated asthmatic patients. Like Conolly and Greenacre in their cAMP stimulation test, SCARPACE *et al.* [8] were unable to detect any significant differences between asthmatic and healthy people when studying adrenoceptor numbers in 1982. Under treatment with a β-sympathomimetic drug, however, they observed a down-regulation of 41% in their normal subjects. This is in the range reported by Aarons, whereas the receptor number decreased to undetectable values (0%) in what they called 'asthmatic responders'. In our chronobiological investigations one patient presented the same reaction to a β-sympathomimetic dosier aerosol (fig. 1). After one week of theophylline treatment his receptor number had recovered and showed the known circadian variation with a marked nocturnal dip.

In another patient, who was completely untreated at the time of investigation we could demonstrate a good correlation between β-adrenoceptor density and peak expiratory flow (PEF, $r = 0.907$), which further validates the 'Peripheral Lymphocyte' as clinical model for asthmatic diseases. PEF seemed to be 4 h phase-delayed to the β-adrenoceptor density (fig. 2), the single cosinor method detected a time delay of 71° equivalent to 4h44. This patient's circadian variation

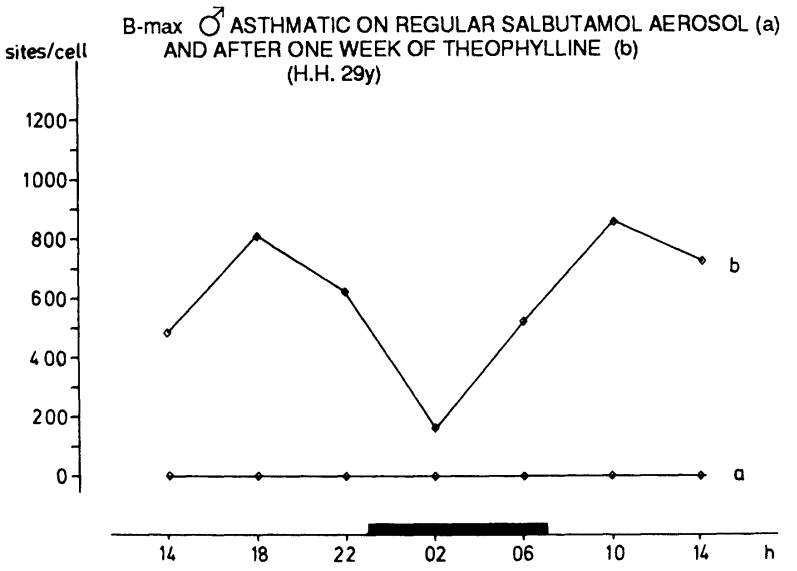

Fig. 1. – β-adrenoceptor density (B_{max}) on intact peripheral lymphocytes in a male asthmatic patient (29 yr) after coninuous aerosol therapy with a β-sympathomimetic drug (a) and after one week on oral theophylline (b). (From: Langenmayer I, Haen E, Emslander H, Remien J: Circadian variation in airway obstruction. 1986/87, unpublished).

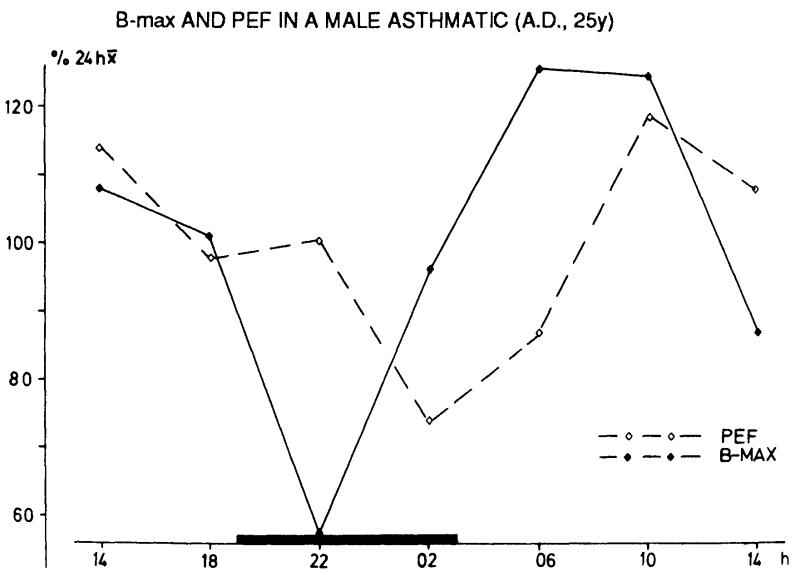

Fig. 2. – β-adrenoceptor density (B_{max}) on intact peripheral lymphocytes and peak expiratory flow (PEF) in a male asthmatic patient (25 yr) who was never treated for asthmatic complaints before the time of investigation. Data are expressed as % of the 24h-mean (24h-mean ± SE B_{max}: 884 ± 78 sites/cell, PEF: 8.4 ± 0.4 l/s). (From: Langenmayer I, Haen E, Emslander H, Remien J: Circadian variation in airway obstruction. 1986/87, unpublished)

of the β-adrenoceptor density fitted well into the range observed in our healthy subjects, confirming the results of Scarpace and Conolly, but in contrast to Szentivanyi's theory. However, taking into account the suggested seasonal variation, our patient, who was studied in August impressed with a lowered mesor (884 compared to 1135 ± 10 sites/cell, $\bar{x} \pm SE$) and an increased amplitude (26.9% of mesor and a range of $+25.7/-42.4\%$ of the 24h-mean compared to 17.3% of mesor and a range of $+14.0/-13.7\%$ of the 24h-mean).

DISCUSSION

There is a circadian variation of β-adrenoceptor density, that may be explained as physiological down-regulation by endogenous plasma catecholamine concentrations. The trough in β-adrenoceptor density (as determined by PANGERL *et al.* [7]) is about 6 h phase-delayed to the peak in plasma catecholamine concentrations (as determined by BARNES *et al.* [2]) and in our studies on asthmatics about 4 h phase-advanced to the trough in PEF. Nocturnal asthmatic complaints might then be regarded as hypersensitivity of β_2-adrenoceptors to down-regulating stimuli. In healthy people the down-regulated β-adrenoceptors do recover due to the increasing plasma cortisol concentrations in the morning. In our control group both circadian variations were in phase ($r = 0.935$, $p < 0.01$). Such an up-regulation of down-regulated β-adrenoceptors by glucocorticoids was described by BRODDE *et al.* [4] in a pharmacological experiment.

The clarification of the time relationships between the endocrine and receptor variables involved, as well as of the seasonal differences in the circadian variation of β-adrenoceptor density, will be the most important task in the future for uncovering any disturbances on the sympathetic receptor level in asthma.

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CONTRIBUTION TO THE CHRONOBIOLOGY OF LUNG FUNCTION: CHANGES OF BASELINE VALUES OF FOUR LUNG FUNCTION INDICES BETWEEN 8 H AND 17 H IN PATIENTS WITH BRONCHITIC COMPLAINTS WITHOUT ASTHMATIC COMPONENTS

A. Minette*

This contribution deals with the results of a longitudinal comparison of forced expiratory volume in one second (FEV_1), forced expiratory flow when 25–75% forced vital capacity has been exhaled (FEF_{25-75}%FVC), peak flow rate (PFR) and residual volume (RV) at different hours during the day. The study was originally made with a view to studying airway-responses to drugs [5] and of epidemiological surveys on respiratory health problems [6]. The question was posed as to whether significant biological differences exist throughout the day, which would necessitate the investigation of all subjects at the same hour daily to make valid comparisons.

The subjects were in-patients with bronchitic complains but no asthmatic components. The diagnosis was made according to the definitions of the Ciba Guest Symposium [1], the symptoms being recorded by means of the European Coal and Steel Community (ECSC)-questionnaire 1967 [3].

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