rhythms. This model starts by assuming the existence of one circadian pacemaker (the SCN) consisting of gated pacemaker circuits in which 'on-cells and off-cells excite themselves via positive feedback, inhibit each other via negative feedback, and are tonically aroused'. However, experimental data exclusively favoring this and/or other one-oscillator models [17–19] are still lacking. Results obtained from experimental manipulations of split activity rhythms in hamsters, such as differing phase responses of the two activity components to dark pulses [11], more likely support a two-oscillator or multi-oscillator hypothesis.

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Number of glucocorticoid receptors in lymphocytes and their sensitivity to hormone action

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Summary. The study demonstrated a decreased level of glucocorticoid receptors (GR) in peripheral blood lymphocytes from hypercholesterolemic subjects, and an elevated level in patients with acute myocardial infarction. In the lymphocytes with a high GR number, dexamethasone inhibited [^{3}H]-thymidine and [^{3}H]-acetate incorporation into DNA and cholesterol, respectively, in the same manner as in the control cells. On the other hand, a decreased GR number resulted in a less efficient dexamethasone inhibition of the incorporation of labeled compounds. These data showed that the sensitivity of lymphocytes to glucocorticoids changed only with a decrease of GR level.
Key words. Glucocorticoid receptors; lymphocytes; hypercholesterolemia; acute myocardial infarction.

The cellular receptor system provides for high efficiency and selective action of glucocorticoid hormones in target tissues. Opinions as to whether the response of somatic cells to hormone action depends on the number of receptors are contradictory [1–4]. Human peripheral blood lympocytes are well-known target cells for glucocorticoids. It has been demonstrated that glucocorticoids have a direct, specific inhibiting effect on metabolic processes in these cells [3]. The aim of this work is to find out whether the number of lymphocyte glucocorticoid receptors (GR) on a cell determines its sensitivity to hormone action.

Materials and methods

Lymphocytes were separated from the blood of 40–55-year-old men: (1) normolipidemic healthy volunteers (Ch $\leq$ 270 mg/dl, Tg $\leq$ 200 mg/dl, HDL Ch > 35 mg/dl); (2) normolipidemic patients with acute myocardial infarction (no more than 24 h since the onset of anginal attack and appearance of abnormal Q wave) (3) hypercholesterolemic subjects (Ch > 270 mg/dl, with coronary atherosclerosis angiographically verified). Patients with diabetes mellitus and other endocrine pathologies were excluded from the study.

Fifteen ml of blood was drawn from the ulnar vein into dry test-tubes with EDTA (1 mg/ml) and separated over a Ficoll-Pague [5]. The mononuclear cell layer was removed and suspended in medium 199. This cell suspension was incubated in plastic tissue culture flasks at 37 °C for 45 min to remove monocytes. After this procedure differential cell counts showed that the number of monocytes or polymorphonuclear leukocytes was less than 3% of the cells remaining after adherence. The lymphocytes were washed with phosphate buffer saline (PBS) pH 7.2 and resuspended in medium 199 at a density of $5-6 \times 10^6$ cells/ml.

The number of specific binding sites for glucocorticoids in the lymphocytes was assessed with a synthetic glucocorticoid analoque [1,2,4,6,7-^{3}H]-dexamethasone (Amersham, UK, 82.4 Ci/mmol) according to Scatchard[6], using a modification of this method[7]. 500 µl of cell suspension were added to glass tubes containing 50 µl of [^{3}H]-dexamethasone and 50 µl of unlabeled dexamethasone. The final concentration of [^{3}H]-dexamethasone was 1 nM and the final concentration of unlabeled dexamethasone ranged from 0.5 to 50 nM. The unlabeled steroids were dissolved in ethanol and the final concentration of ethanol was always less than 0.01%. The specific binding of [^{3}H]-dexamethasone on lymphocytes of different origin was linearly dependent on the number of cells (from 2 to 10×10^6 cells/ml) in a sample. After additions, the tubes with lymphocytes ($5-6 \times 10^6$ cells) were vortexed gently and incubated at 37 °C, 5% CO_2, 80% humidity for 1.5 h. After the incubation the cells were twice washed in cold phosphate buffer, pH 7.2. Finally, 1.5 ml of 70% ethanol was added to each sample, and the samples transferred to counting vials with scintillator.

A 1000-fold excess of unlabeled steroid was used to determine the nonspecific binding. Specific binding was calculated as the difference between total and nonspecific binding. The number of binding sites/cell, and dissociation constants, were estimated by computer-assisted analysis of the binding data.

The intracellular cholesterol synthesis was measured by [^{3}H]-acetate incorporation into a sterol fraction precipitated with digitonin[8]. For this purpose, lymphocytes were incubated with 10 µCi of [^{3}H]-acetate over 1 h. To measure [^{3}H]-thymidine incorporation into DNA, lymphocytes were incubated with 2 µCi [^{3}H]-thymidine over 1 h. After the incubation, the cells were collected on GF/C filters, (Whatman, UK), and consequently washed with 20 ml PBS buffer, 10 ml trichloroacetic acid, 10 ml ethanol. Then the filters were dried and assessed for radioactivity[9]. To study the inhibiting effect of glucocorticoids on the synthesis of cholesterol or DNA, the cells were preincubated with various dexamethasone concentrations for 6 and 4 h before adding [^{3}H]-acetate or [^{3}H]-thymidine, respectively.

Significance of differences was evaluated using Student's t-test.

Results

The study of peculiarities of the specific [^{3}H]-dexamethasone binding in lymphocytes of patients with coronary heart disease revealed two categories of patients with abnormal cellular receptor levels compared to the group of healthy volunteers ($n = 15$, $N = 4800 \pm 460$ binding sites/cell). An elevated level of [^{3}H]-dexamethasone binding sites in lymphocytes was found in subjects with acute myocardial infarction ($n = 21$, $N = 12400 \pm 1241$ b.s./cell, $p < 0.001$), and a low level in subjects with hypercholesterolemia ($n = 17$, $N = 3000 \pm 384$ b.s./cell, $p < 0.01$).

In specially designed experiments it was shown that a 1.5-h incubation of lymphocytes with the hormone provided for a complete saturation of all highly specific [^{3}H]-dexamethasone binding sites in different lymphocyte preparations. The Scatchard analysis of the binding revealed linear plots, consistent with a single class of binding site of uniform binding affinity in all lymphocyte preparations under study (fig. 1).

Glucorticoids are known to inhibit DNA and cholesterol synthesis in lymphocytes[10]. As can be seen from figure 2,

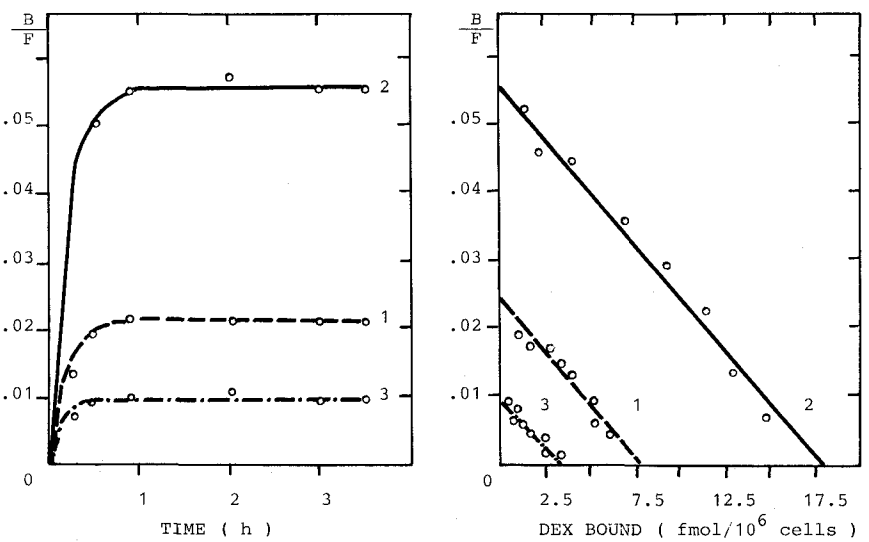

Figure 1. Binding of [^{3}H]-dexamethasone to human lymphocytes. Lymphocytes were separated from a healthy volunteer (1), and from patients with acute myocardial infarction (2) and hypercholesterolemia (3). On the left, time-dependent binding of [^{3}H]-dexamethasone to human lymphocytes. Cells were incubated with 1 nM [^{3}H]-dexamethasone in the presence or absence of 1000-fold excess of unlabeled dexamethasone. On the right, Scatchard plot of binding data. Cells were incubated with 1 nM [^{3}H]-dexamethasone and varying concentrations of unlabeled dexamethasone for 1.5 h. The line of best fit was computed by regression of least square. The points represent the means of duplicate samples.

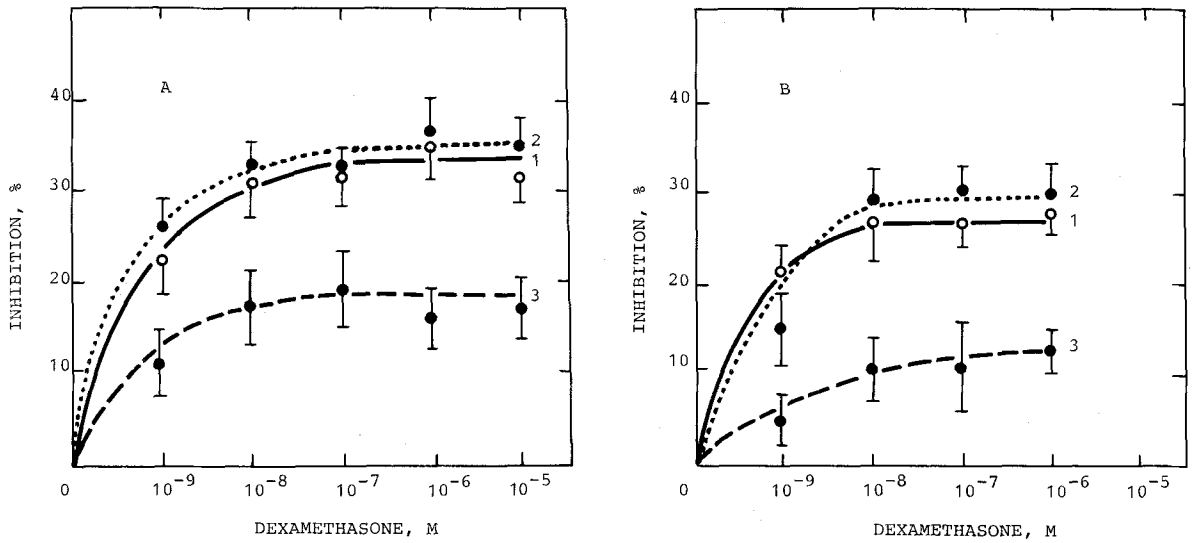

Figure 2. Inhibition of [^{3}H]-thymidine (*A*) and [^{3}H]-acetate (*B*) incorporation by dexamethasone in lymphocytes with different levels of GR (in %). Lymphocytes with the following number of receptors: 1 - 5010 ± 302 b.s./cell (n = 6); 2 - 9560 ± 795 b.s./cell (n = 4); 3 - 3180 ± 240 b.s./cell (n = 7). The cells were preincubated for 4 h (*A*) and 6 h (*B*) at 37 °C in the presence of various concentrations of dexamethasone and then received the label for an additional 1 h (see Materials and methods). Values are expressed as mean ± SEM of duplicate samples from n experiments, n = number of patients.

inhibition of [^{3}H]-thymidine incorporation in DNA and [^{3}H]-acetate incorporation in the sterol fraction by dexamethasone were less pronounced in lymphocytes with a low GR level if compared to lymphocytes with a normal receptor level. This means that a decrease in the number of GR results in a decrease of lymphocyte sensitivity to glucocorticoids.

Inhibition of [^{3}H]-thymidine and [^{3}H]-acetate incorporation by dexamethasone in lymphocytes with elevated receptor levels was similar to that in lymphocytes with normal number of GR. Thus, the rise of the number of GR in lymphocytes does not increase cellular sensitivity to the hormone. These data were in agreement with the findings of Munk et al.[3] who failed to find any changes in hormonal sensitivity in the lymphocytes with an increased number of GR, resulting from mitogen stimulation.

Thus, lymphocyte sensitivity to glucocorticoids changed only when the number of cellular receptors declined, and did not alter with an increase. Two explanations for the mechanism for this phenomenon can be suggested; either that the normal receptor level ensures maximal cellular sensitivity to hormone, or that abnormal receptors are present in lymphocytes with an elevated number of GR[11].

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