GLUCOCORTICOID RECEPTORS IN NORMAL HUMAN LYMPHOCYTES

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Glucocorticoid (GC) receptors (GCR) are cytosol proteins. A particular feature of these receptors is that they have high affinity for GC, limited binding capacity (saturability), and high specificity for various steroid hormones [12]. Like any protein structure, GCR are genetically determined.

It has been shown that specific GCR are present in most organs and tissues [4, 9]. The presence of specific GCR in human blood cells was demonstrated by Lippman in 1973. It has also been shown that their number is about equal in poly- and mononuclear cells, T and non-T lymphocytes, and in Tμ and Tγ subpopulations of lymphocytes [11], and that the nonspecific affinity for GC is identical in neutrophils and eosinophils [13].

Data in the literature on correlation between the number of GCR in human peripheral blood lymphocytes and sensitivity to GC therapy are few in number and highly contradictory [5]; most of these investigations, moreover, have been conducted on cancer patients.

It has been shown that the parameters of binding of GC with receptors in human skin fibroblasts in culture are a stable feature and that sensitivity to GC therapy depends on the number of corresponding receptors. Both patients and normal subjects have been shown to be distributed, according to the number of these receptors, into two nonoverlapping classes, N and 2N, and that the group of patients was indistinguishable as regards parameters of binding of GC with receptors from the group of normal blood donors as a whole [2, 3]. Investigation of GCR in skin fibroblasts in culture is a very laborious process, very time consuming, and so cannot find extensive application in clinical practice.

Because of the importance of this problem for practical purposes, further attempts to devise simpler methods of studying GCR are perfectly justified. In the USSR attempts to undertake research of this kind have been made, and in particular, GCR in human lymphocytes have been studied in the cytosol fraction of destroyed leukocytes [1]. This technique (and its various modifications) requires multistage processing of biological material and, in the writers' opinion, this greatly reduces the reproducibility and reliability of the experimental results. It has been shown, in particular, that the purity of isolation of the cytosol varies considerably from one experiment to another, and that GCR in the isolated cytosol fraction are highly unstable. In the writers' view, the comparative quantitative study of the first and most important step of interaction with cells can be performed more rationally on intact lymphocytes.

The aim of the investigation described below, using a high level of standardization of the experiments, was to assess the reproducibility of the results and the character of distribution of normal blood donors with respect to the number of GCR in their lymphocytes, which was compared with their distribution in cultures of skin fibroblasts, i.e., to assess the possibility of using lymphocytes as an object for comparative studies of the number of GCR in human cells.

EXPERIMENTAL METHOD

The method of Homo [8] and Kontula [10] was used with certain modifications. Eleven normal healthy subjects (seven men and four women) aged from 19 to 43 years took part in the in-

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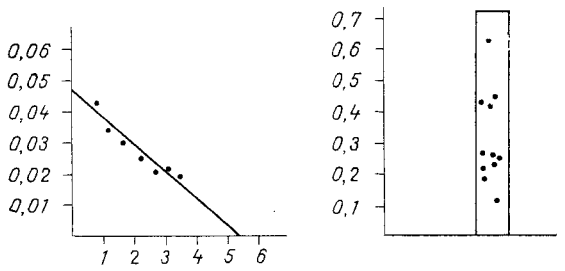

Fig. 1. Parameters of GC binding with receptors of volunteers' lymphocytes on Scatchard plot. Abscissa, specific binding of [^{3}H]dexamethasone (in cpm/mg protein × 10^3); ordinate, specific binding of [^{3}H]dexamethasone (free hormone × 10^3). Maximal number of binding sites per milligram protein (B_m) 0.29 × 10^{-12} M. K_d = 6.3 × 10^{-9} M.

vestigation. Blood (20 ml) was taken from the fasting subjects from the cubital vein (heparin was added in a dose of 40 IU/ml blood). The blood was transported to the laboratory at a temperature of 20-22°C and the experiment began within the first hour (not later!) after the blood was taken. The blood was diluted in the ratio 1:2 with Versene solution, pH 7.5, at room temperature. Lymphocytes were isolated by Boyum's method [6]. After centrifugation for 30 min at 400g the lymphocytes were drawn off and washed 3 times in Hanks' solution, buffered with HEPES (pH 7.35) at 400g for 10 min each time. The yield of lymphocytes in films stained with azure-eosin was 89-95%. Dilutions of labeled [^{3}H]dexamethasone in Hanks' solution with HEPES from 1 to 10 nM in a volume of 5 ml were prepared. The labeled dexamethasone was obtained from Amersham Corporation (England) and had activity of 19.1 μCi/mmole. In some parallel dilutions of the labeled hormone, a 500-fold excess of unlabeled dexamethasone (from Sigma, USA) was added. All dilutions and the control were treated with 0.3 ml of carefully resuspended lymphocyte suspension in Hanks' solution with HEPES, after which the samples were incubated at 37°C for 45 min. The samples were shaken every 15 min.

The experiment was then continued at a temperature of 4°C. After centrifugation for 5 min at 400g, 0.5-ml samples from the supernatant were decanted into flasks, each containing 10 ml of dioxan scintillator, to determine free radioactivity. The residual supernatant was poured off and the sedimented lymphocytes were washed twice to remove any free radioactivity with Hanks' solution (pH 7.4; 4°C). The intact lymphocytes thus obtained were made up to 0.5 ml with distilled water, after which the cells were destroyed by ultrasound. Considering that the protein content was about equal in all samples (this was tested in a few experiments), protein was determined only in the control, by Flores' method [7]. The samples were poured into scintillation flasks and radioactivity was measured on a counter (from Intertechnique, France). Specific binding was calculated as the difference between total binding and binding in the presence of a 500-fold excess of unlabeled hormone. A Scatchard plot [14] was prepared from the results (Fig. 1). The results were expressed in picomoles/mg protein.

EXPERIMENTAL RESULTS

GCR were found in the peripheral blood lymphocytes of all the blood donors tested. The important finding was that the number of lymphocytes varied considerably in different donors. This parameter must evidently be taken into account when the necessary and sufficient amount of biological material for an experiment is estimated. One of the most important and, admittedly, as yet unsolved problems was and still is standardization and reproducibility of experiments to study steroid hormone receptors. The experimental results obtained in the study of steroid receptors in different laboratories vary considerably. Our own data are evidence that practically any disturbance of standardization of the experimental technique — the time and conditions of taking the blood (fasting or after a meal), the time of arrival of the blood and at the beginning of the experiment, the pH of the salt solutions used, temperature and conditions of centrifugation, and so on — all have a marked influence on the results.

Dilution of the heparinized blood with Versene solution is considered to be preferable to dilution with phosphate buffer, because the solution used prevents lymphocyte aggregation,

so that a more homogeneous cell suspension can be obtained. Labeled dexamethasone is known [12] to bind exclusively with cell receptors and not to interact with corticoid-binding globulin. This fact also was taken into consideration.

With observance of all the experimental conditions listed above, good reproducibility of the parameters of GC-receptor binding was obtained in repeated experiments on the blood donors.

On the basis of the results showing variability in the number of GCR in human skin fibroblasts, in which all the individuals tested were divided into two classes depending on the number of receptors, namely N (relatively resistant to glucocorticoids) and 2N (sensitive to glucocorticoids) [3], the existence of the same subdivision into classes, based on number of GCR, also was postulated in the lymphocytes of normal individuals, although the size of the sample was too small to allow strict proof of this hypothesis. All the donors studied were therefore divided conventionally into persons with a relatively small number of GCR (under 0.4 picomole of bound [^{3}H]dexamethasone/mg protein) and those with a relatively high content of receptors in their lymphocytes (above 0.4 picomole of bound [^{3}H]dexamethasone/mg protein).

The most important qualitative characteristics of receptors, namely the dissociation constant (K_d), was practically identical in the two conventionally distinguished groups: 6.2×10^{-9} and 5.5×10^{-9} M, respectively. Fluctuations of K_d within the groups also were very small. This is further evidence of the stability of the results and of the definite standardization achieved by conducting the experiments in this manner.

If the binding activity of GC with the receptors is used as a guide to the number of receptors, the existence of two groups of individuals with different numbers of GCR in their lymphocytes can thus be postulated; the number of donors with relatively few receptors is the greater. This may be of definite importance in the understanding of resistance to GC therapy encountered in clinical practice. Further investigation of this phenomenon in patients with internal diseases and, in particular, those whose treatment requires relatively large doses of GC (systemic lupus erythematosus, the nephrotic variant of glomerulonephritis, and so on) may be of great interest.

Under the conditions of isolation of peripheral blood lymphocytes and determination of the parameters of GC-receptor binding described above satisfactory reproducibility of the experimental results can be achieved and, accordingly, lymphocytes can provide a suitable object with which to analyze one of the important mechanisms of the response of sensitivity to GC treatment and to study genetic control of the number of GCR in the cell.

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