GLUCOCORTICOID CELL RECEPTION IN MICE OF DIFFERENT STRAINS WITH NATURAL KILLER CELL ACTIVITY DEPRESSED DURING IMMOBILIZATION STRESS

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Glucocorticoids (GC) have a marked depressive action on activity of natural killer cells (NKC) *in vitro* [4, 7], and also when injected directly into animals [8]. These hormones are evidently the most powerful natural immunodepressants. Other stress mediators, catecholamines in particular, also cause definite biochemical changes and functional disturbances of lymphoid cells [5], although the effects of GC are more profound and more generalized in character, and they may be regarded as a dominant factor determining the development of the stress reaction at the immunocompetent cell level.

Genetically determined differences in sensitivity to GC are known in mice and man, and above all, to pharmacological doses of GC [2, 9]. These differences are linked primarily with the number of specific glucocorticoid receptors (GCR), for they are the key element of the system of GC–cell interaction, limiting all subsequent stages of this interaction.

The stress reaction is one of the main components of the adaptation process in animals and man in response to a change in external environmental factors, and a stable, genetically determined variety of this reaction determines not only the direction and effectiveness of formation of mechanisms of adaptation, but also the formation ultimately of intraspecific variation of constitutional types.

Meanwhile the problem of genetic differences in sensitivity to stress and correlation of these differences with genetically determined variation of GCR has not been studied. Estimation of the degree of depression of NKC during exposure of the animal to stress factors is evidently a promising approach to the quantitative assessment of reactivity of the animal to stress and, in particular, of the contribution of GC to individual differences of this reactivity in animals of different strains. The reason is that during analysis of NKC activity a certain definitive cell function is evaluated, and this distinguishes this particular test advantageously from the use of individual, even multiple, biochemical parameters, which do not give a picture of the definite final effect, and also from integral parameters, which because of their multiplicity of components, are difficult to analyze.

For the reasons given above, the aim of the present investigation was to study differences in stress-induced depression of NKC activity in mice of different inbred lines, depending on parameters of GC binding with GCR of spleen cells and on the hormonal status of the animals.

EXPERIMENTAL METHOD

Male CBA, C57BL/6, BALB/c, and A/Sn mice were kept on a standard diet, with 10 animals per cage, for 3 weeks before the experiment. The animals were obtained at the age of 8-12 weeks from the Stolbovaya and Svetlye Gory nurseries of the Academy of Medical Sciences of the USSR. The level of stress-induced depression of NKC was estimated from the degree of lowering of the cytotoxic index of mouse splenocytes in a culture of cancer cells during stress, as a percentage of the control [3].

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TABLE 1. Correlation of Stress-Induced Depression of NKC and Parameters of GCR Binding and Plasma Corticosterone Level at Rest

Strain of mice	B_{max}, pmoles	K_d, nM	N, %	Resting corticosterone level (pm)	NKC activity after stress, in percent of normal
C57Bl/6	14,9±0,42	8,82±0,61	31±4	313	90±5
CBA	11,5±0,29	4,50±0,32	30±3	266	70±7
BALB/c	7,12±0,54	3,10±0,24	29±2	131	55±5
A/Sn	14,49±0,46	4,90±0,37	31±5	213	40±7

Legend. For B_{max} all paired differences between strains, except between C57BL/6 and A/Sn, are highly significant ($P < 0.001$); For K_d all paired differences between strains except CBA and A/Sn are highly significant ($P < 0.01$).

Serum corticosterone levels were determined in the Laboratory for Hormone Study, Institute of Prophylactic Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

Parameters of GC binding were determined on intact splenocytes from mice aged 11-13 and 28-32 weeks by the method described previously for human lymphocytes, with minor modifications [2]. Aliquots of a suspension of washed splenocytes were incubated with tritium-labeled dexamethasone (40-43 Ci/mmole, Amersham International, England), in concentrations of 1 to 15 nM in Hanks' solution for 40 min at 37°C. After incubation the cells were washed three times with buffer consisting of 0.05 M Tris-HCl and 0.32 M sucrose (pH 7.5) at 4°C for 10 min to remove unbound dexamethasone, followed by centrifugation for 10 min at 800g. The washed cells were disrupted by ultrasound and radioactivity was counted in dioxan scintillator. To separate nonspecific binding from total binding of the label, the procedures described above were carried out after addition of a 500-fold excess of unlabeled to labeled dexamethasone. The quantity of bound ^{3}H-dexamethasone was calculated per liter of splenocyte suspension with a cell concentration of 10^6/ml and expressed in picomoles (pmoles) in order to observe dimensionality in the subsequent calculations. The maximal number of binding sites (B_{max}) and the equilibrium dissociation constant (K_d) of GC with GCR were calculated by means of a Scatchard plot [10].

Accumulation of GC-GCR complexes in the nucleus (N) was estimated with a saturating concentration of labeled hormone of 40 nM, by the method used on fibroblast cultures [6]. After incubation of the labeled hormone and washing, the cells were disrupted in a final volume of 1 ml of hypotonic buffer (10 mM Tris-HCl, 2 mM MgCl, 1 mM $CaCl_2$, pH 0.8 at 4°C) to isolate the nuclei. The purity of the isolated nuclei was verified by fluorescence microscopy after staining with acridine orange. The isolated nuclei were sedimented by centrifugation at 1500g for 10 min at 4°C, after which the quantity of radioactivity was counted separately in the nuclear and cytoplasmic fractions.

Nonspecific binding was estimated in the presence of a 500-fold excess of unlabeled dexamethasone. Accumulation of GC-GCR complexes with the nucleus was calculated by the equation:

$$N = \frac{\text{nulear binding}}{\text{nuclear + cytoplasmic binding}} \times 100\%.$$

EXPERIMENTAL RESULTS

The principal functional characteristics of the GCR (the number of binding sites and the constant of interaction of GC with GCR) were unchanged by the stress reaction and were stable over the age range from 11 to 32 weeks studied in mice of all four strains. Thus the principal functional characteristics of GCR of mice are independent of physiological variation in GC concentration and the age of the animals within the range stipulated.

The principal parameters of the hormone–cell system investigated and the results of the study of depression of NKC activity under the influence of stress are summarized in Table 1. Differences in the number of GCR in the different strains evidently do not correlate with the degree of depression of NKC activity, and at first glance this contradicts modern views according to which there is strict correlation between the sensitivity of cells to GC and the number of GCR. This apparent disparity between sensitivity to stress and, consequently, to GC and the number of GCR is connected with the considerable excess of GCR which, unlike when GC is used pharmacologically, is not completely utilized during physiological or even pathophysio-

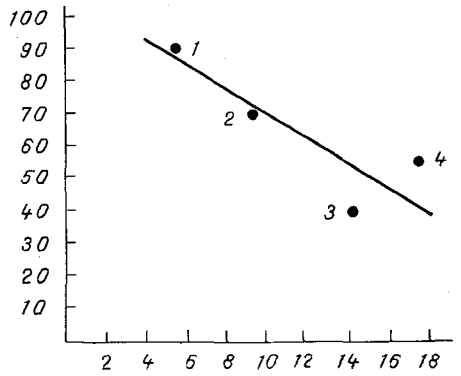

Fig. 1. Dependence of level of post-stressor NKC activity on efficiency of saturation of nucleus with hormone–receptor complexes at the "basal" corticosterone level. Abscissa, saturation efficiency (SE) of nucleus with hormone–receptor complexes (× 10 M^{-1}); ordinate, NKC activity after stress (in percent of control). 1) C57BL/6; 2) CBA; 3) A/Sn; 4) BALB/c.

logical variations of the GC level. Under conditions when the maximal GC concentration is determined by the power of their secretion by the adrenal cortex in response to stress, but the corticosterone concentration at the height of the stress reaction, according to our results, was virtually the same in mice of different strains, it is affinity of the hormone for GCR (K_d) which assumes essential importance, and the level of depression of NKC activity, as the results showed, correlates closely with a decrease in K_d (or with an increase in the affinity of GC for GCR). Under these circumstances, despite the evident negative correlation between the value of K_d and the degree of stress-induced depression of NKC activity, this one parameter alone cannot determine the potential sensitivity of the cells to the action of physiological fluctuations in the GC level, since with equal values of K_d the sensitivity of the cells to GC must be directly dependent on B_{max}, which in the situation under analysis is by no means evident, on account of the marked differences in both K_d and B_{max} between the different strains. In the modern view the biological effect of GC ultimately depends on the proportion of GC-GCR complexes which have accumulated in the nucleus (N); the value of N, moreover, is independent of the GC concentration and, consequently, of the absolute number of GC-GCR complexes formed [9]. It will be clear from Table 1 that the value of N for mice of all strains studied was virtually equal, but this does not rule out the probability of genetically determined variations of N in man, for example.

The potential sensitivity of the cells to physiological fluctuations of the GC level must thus evidently be determined by the saturation efficiency (SE) of the nucleus with GC-GCR complexes at the given concentration of GC, in this case of corticosterone. The concentration of GC not at the height of stress, but at rest, must evidently be taken as the guide, for it is in animals at rest that the level of this vital hormone that is necessary and sufficient for effecting the "basal" metabolism of the cells and, consequently, the "basal" level of saturation of the nucleus with GC-GCR complexes, is recorded in animals. It is therefore evident that the lower the GC concentration at rest, the higher the saturation efficiency (SE) of the nucleus with GC-GCR complexes must be during physiological fluctuations in the GC concentration and, in particular, during stress.

Incidentally, by the term "saturation" in this case is meant not the saturation of all nuclear acceptors of GC-GCR complexes, the number of which is much greater and the number of GCR in the cells, but the efficiency of the process of chromatin saturation with GC-GCR complexes in cells of different genotypes, at a given resting GC concentration characteristic of each genotype. Although this resting GC concentration depends on parameters of GC reception by the cells, at the same time it is qualitatively additional to them, for it is evidently determined also by the particular features of the subsequent stages of interaction of the GC-GCR complex with chromatin and regulation of cell metabolism at rest, which cannot be described by means of B_{max}, K_d, and N.

Since hormone–receptor interaction obeys the law of mass action, the complex quantitative characteristic of the simplest hormone–cell system can be represented in a general form as $SE = \frac{B_{max} \cdot N}{K_d \cdot [GC]}$, where B_{max} is the maximal number of binding sites of GC with the cells (in pmoles), K_d is the equilibrium constant of dissociation of GC from GCR (in nM), N accumulation of GC-GCR complexes in the nucleus (in percent), and [GC] the resting GC concentration (in nM).

Correlation between the value of SE and the level of poststressor NKC activity within each strain of animal was found to be stable and statistically significant ($p < 0.001$), but the high correlation ($r = -0.84 \pm 0.17$, $p < 0.05$) of interlinear variation in the level of NKC activity after stress with the value of SE (Fig. 1) suggests that the connection between SE and stress-induced depression of NKC activity is sufficiently universal in character.

It can be concluded from the results that the level of stress-induced depression of NKC is decided by the genetically determined parameters of GC binding with the cell GCR, and by the definite resting GC level for each strain of mice. Parameters of binding of GC with GCR are stable, with definite characteristics of each strain of animal, independent of exposure to stress of the animals' age.

The mechanism of differences in stress-induced depression of NKC activity in different strains of mice thus involves the existence of differences in the genetically determined potential sensitivity of the lymphoid cells to the action of physiological fluctuations of the GC level, which in turn is determined by the efficiency of formation of GC-GCR complexes and their accumulation in the nucleus of the presence of a "basal" GC concentration.

Considering the dimorphism for GCR content discovered previously in human somatic cells and the stable differences between the constant of interaction of GC with GCR in animals of different strains over a series of cell generations, and also the absence of ontogenetic variation of GCR in the period from the 5th week of embryonic development until the 50th year of postnatal development [1], it can be postulated that the SE parameter provides a useful approach to the study of genetically determined variations of sensitivity to GC at the cellular level, its contribution to the formation of stable human constitutional features, formed against the background of fluctuations of the GC level acting throughout ontogeny, and also the role of this variation in pathology.

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