CORTICOSTERONE RECEPTION BY ALVEOLAR MACROPHAGES REFLECTING CHANGES IN THEIR LEVEL OF FUNCTION

L. N. Shishkina, D. N. Mayanskii, G. V. Shutko, and P. V. Sergeev

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Restoration of disturbed homeostasis *in vivo* depends on sensitivity of the macrophages to antiinflammatory hormones and, in particular, to glucocorticoids (GC) [2, 4, 7]. One of the most reactive components of the mononuclear phagocyte system (MPS) is the pulmonary macrophage population [3]. However, the question of changes in the properties of receptors for GC in pulmonary macrophages after their activation remains unanswered.

The object of this investigation was to study binding of one glucocorticoid hormone (corticosterone) by alveolar macrophages of rats before and after stimulation by zymosan granules (ZG).

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats of both sexes weighing 150-200 g. To stimulate MPS a suspension of SG (0.1 mg/g) in 1 ml of 0.8% NaCl was injected intravenously 5 days before the cells were obtained. Control animals were given an injection of 1 ml of 0.85% NaCl.

The lungs were washed 3 times by the method in [3] with our own modifications. The liquid thus obtained was centrifuged for 15 min at 2000 rpm. The supernatant was poured off, and the sedimented cells were resuspended in medium 199. To 1 ml of the suspension of bronchoalveolar washing cells 2 ml of filtered native bovine serum was added, the contents were mixed without frothing, and they were applied to 3 ml of Ficoll–Verografin gradient (d = 1.078). The contents were centrifuged at 1500 rpm for 15 min. As a result of fractionation three cell fractions were obtained. The population of cells in interphase, consisting of macrophages and monocytes, was removed and washed in medium 199 to remove the gradient. Lungs of control rats yielded $(4.1 \pm 0.34) \times 10^6$ cells, those of the rats stimulated by zymosan yielded $(11.1 \pm 1.01) \times 10^6$ cells.

The cells were resuspended with 20% native bovine serum and transferred in volumes of 3 ml to bottles containing coverslips on the bottom, and incubated at 37°C for 1 h. The coverslips with adherent cells were then removed, rinsed, and incubated in medium 199 containing from 10^{-9} to 112.5×10^{-9} M [^{3}H]corticosterone (Amersham Corporation, England) for 30 sec and for 3, 10, and 30 min. In parallel experiments Tween coverslips were incubated in medium with [^{3}H]corticosterone to determine adsorption of the hormone on the side of the coverslip free from cells. One coverslip from each series, not incubated with [^{3}H]corticosterone, was dried and stained with azure II–eosin, and the number of cells on the coverslips was counted. Preliminary experiments showed that to obtain statistically significant results the number of macrophages in the monolayer must be between 2×10^5 and 4×10^5 cells. Incubation was stopped, the coverslip with cells was removed from the radioactive medium, rinsed twice in physiological saline, and placed in cuvettes with scintillation fluid for radioactivity counting (SL-30 counter, Intertechnique, France). The number of binding sites for the hormone was determined by the equation $X = N \cdot A$, where N is the number of moles bound by one cell, A the Avogadro number (6×10^{23} molecules per mole of any substance).

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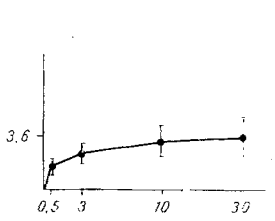

Fig. 1

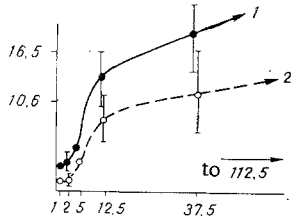

Fig. 2

Fig. 1. Uptake of [^{3}H]corticosterone into alveolar macrophages as a function of time. Abscissa, time (in min); ordinate, quantity of hormone taken up by cells (in fmoles/10^6 cells) with hormone concentration in medium of 8.3×10^{-9} M

Fig. 2. Uptake of [^{3}H]corticosterone by alveolar macrophages as a function of hormone concentration in medium. 1) Stimulation by zymosan, 2) control. Abscissa, hormone concentration ($\times 10^{-9}$ M); ordinate, quantity of hormone taken up by cells (in fmoles/10^6 cells).

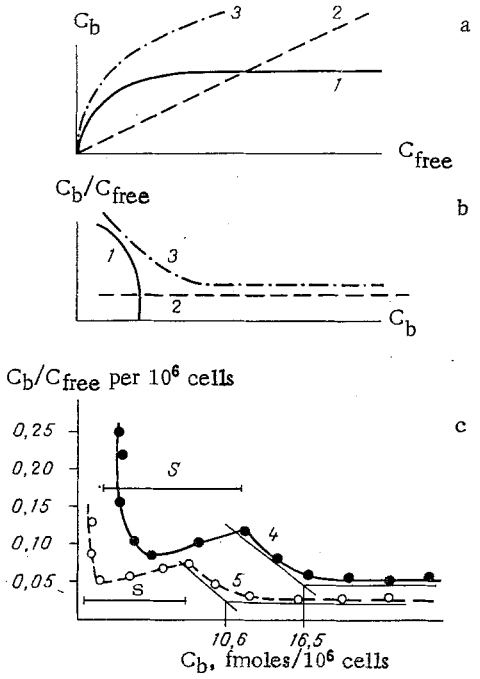

Fig. 3. Analysis of hormone binding (a) by saturable (1), unsaturable (2), and both systems together (3) on Scatchard plot (b), and binding of [^{3}H]-corticosterone by alveolar macrophages on Scatchard plot (c). C_{free}) Quantity of hormone remaining in medium after uptake by cells, C_b) quantity of bound hormone, 4) stimulation by zymosan, 5) control. s (Control) and S (stimulation by zymosan) correspond to S-shaped curve in Fig. 2.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The maximal rate of uptake of [^{3}H]corticosterone into the cells was observed during the first 30 sec of incubation. Later, between 30 sec and 10 min the intensity of uptake of [^{3}H]corticosterone by the alveolar macrophages decreased. An increase in the duration of incubation of the cells with [^{3}H]corticosterone to 30 min caused virtually no rise in the level

of hormone taken up by the cells, i.e., binding of the hormone by the alveolar macrophages had stabilized. During 30 min when the free hormone concentration in the medium was 8.3×10^{-9} M, saturation of the corticosterone binding system took place when the value of 3.64 fmoles/10^6 cells was reached (Fig. 1).

Since complete saturation of the system for binding [^{3}H] corticosterone by the alveolar macrophages took place in 30 min of incubation, accumulation of the hormone by the cells as a function of its concentration in the medium was studied during incubation for this time (Fig. 2). It will be noted that the curves reflecting uptake of [^{3}H]corticosterone by alveolar macrophages follow a S-shaped course on an increase in concentration in the medium. This is considered to be characteristic of cooperative binding, i.e., when the addition of one molecule facilitates addition of another, and so on [1]. In the region of low hormone concentrations (10^{-9} and 2×10^{-9} M) binding of [^{3}H]corticosterone by alveolar macrophages had identical values. However, an increase in hormone concentrations to 5×10^{-9} M was accompanied by a considerable rise in the level of its absorption by the cells. The sharpest increase in absorption of the hormone by the cells was observed when the free [^{3}H]corticosterone concentration in the medium was 12.5×10^{-9} M. In the region of high concentrations (37.5×10^{-9} and 112.5×10^{-9} M) the process of GC accumulation by the cells became linear in character. Analysis of the curve for uptake of [^{3}H]corticosterone by alveolar macrophages on a Scatchard plot [1, 5, 7] indicated the existence of saturable (receptor) and unsaturable (lipid) binding systems for this particular steroid hormone (Fig. 3). The capacity of the saturable system is exhausted after absorption of 10.6 fmoles/10^6 cells, equivalent to 6.38×10^3 binding sites per cell.

It will be clear from Fig. 2 that in the region of low hormone concentrations (10^{-9} and 2×10^{-9} M) its binding by activated macrophages was significantly higher than in the control ($P < 0.05$). This is evidence of an increase in sensitivity of the pulmonary macrophages to GC on stimulation of MPS with zymosan. With an increase in the [^{3}H]corticosterone concentration in the medium to 5×10^{-9} M or above, this tendency continued. In the case of stimulation of MPS by zymosan, the Scatchard plot also revealed the existence of saturable and unsaturable binding systems for corticosterone (Fig. 3). The potential of the saturable system was exhausted after uptake of 16.5 fmoles/10^6 alveolar macrophages, and the number of binding sites increased to 9.91×10^3 per cell. A further increase in binding of [^{3}H]corticosterone with an increase in its concentration in the medium took place only by the unsaturable system, and was linear in character.

An increase in receptor expression is once characteristic feature of activated macrophages. This phenomenon has hitherto been linked mainly with the Fc- and C_3-receptors of macrophages [6]. The results described in this paper confirm that binding of corticosterone by rat pulmonary macrophages depends directly on activity of the macrophages. The number of binding sites for GC, counted per pulmonary macrophage, 5 days after intravenous injection of zymosan granules into rats *in vivo* is increased on average by 1.5 times. The formation and development of foci of inflammatory infiltration in the lung depends on reactivity of the pulmonary macrophages. According to the results of the present investigation, GC bind more effectively with an activated macrophage. The increase in sensitivity of the macrophages to antiinflammatory hormones may perhaps be responsible for regression, in certain cases, of mononuclear infiltrates.

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