molecule, but also with polar groups of membrane lipids, with a consequent increase in viscosity of the membrane and its stabilization. Coupling of BAR, G-protein, and adenylate cyclase does not take place under those conditions.

On the basis of the foregoing facts BAR can be regarded as a lipoprotein membrane complex, and the difference between the action of adrenomimetics and adrenoblockers can be explained by the different character of the influence of these substances on the lipid components of the β-adrenoreceptor complex.

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SOME CHARACTERISTICS OF SEROTONIN BINDING BY CELLS OF IMMUNOCOMPETENT TISSUES AND BY SYNAPTOSOMES

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KEY WORDS: serotonin; imipramine; immunocompetent cells; synaptosomes.

The writers showed previously that cells of immunocompetent tissues have affinity for the biogenic amine, serotonin (5-HT), which has some of the basis features of receptor interaction: high specificity of binding (nonspecific binding for blood leukocytes and peritoneal cells was 5.8 and 4.5% respectively), reversibility, saturation, and the presence of temperature of pH optima. The localization of 5-HT-binding structures both on the membrane surface and inside the cell [4, 11] was determined with the aid of imipramine, which inhibits the passage of predominantly 5-HT through the plasma membrane [18, 19]. There is also information in the literature on the character of 5-HT binding by the coarse membrane fraction obtained from blood leukocytes [2]. As later investigations showed, activity of 5-HT binding is changed by immunization [5].

In the investigation described below relations between surface and intracellular adsorption of 5-HT by cells of immune and nonimmune animals were compared in order to determine which type of adsorption is functionally linked with immunogenesis, with the aim of using the information thus obtained to shed light on the effector mechanisms of serotoninergic immunomodulation and subsequently to use it for deliberate intervention in immunogenesis.

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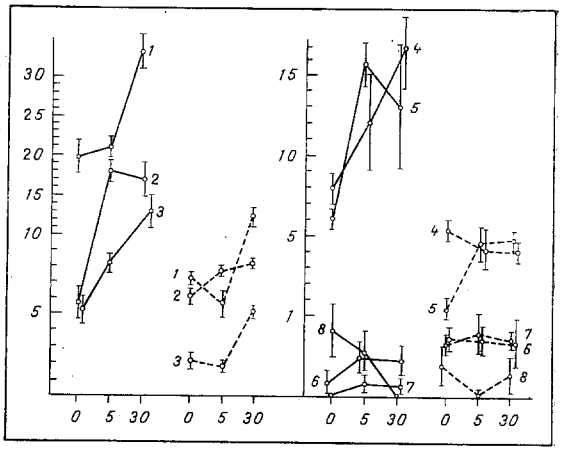

Fig. 1. Imipramine-sensitive (intracellular) and imipramine-resistant (surface) binding of ^{3}H-5-HT by cells of immunocomponent tissues and synaptosomes. Abscissa, time after immunization (in min); ordinate, binding of 5-HT: 1) by synaptosomes (in cpm · 10^{-3}/mg protein); 2) by blood leukocytes; 3) by peritoneal cells (in counts/min · 10^{-3}/10^6 cells; 4-8) binding of 5-HT by suspensions of bone marrow, spleen, thymus, Peyer's patches, and Lymph nodes respectively (in counts/min · 10^{-2}/10^6 cells). Continuous line shows imipramine-sensitive binding, broken line – imipramine-resistant binding.

EXPERIMENTAL METHOD

Male CBA mice aged 3-5 months were used. Suspensions of cells from peritoneal exudate, bone marrow, lymph nodes, thymus, spleen, and Peyer's patches, of blood leukocytes [3], and brain synaptosomes [15] were obtained from them. The cells were washed three times at 4°C with Hanks' solution containing 0.001 M HEPES, pH 7.2, by centrifugation in siliconized test tubes (150 g, 10 min). The number of dying cells estimated by staining them with 0.04% solution of trypan blue. The resulting suspensions contained 90-99% of living cells. The synaptosomes were washed, sedimented at 600 g (15 min), and the required doses were obtained on the basis of their protein content. Cells ($0.5 \cdot 10^6$-$2 \cdot 10^6$/ml) and synaptosomes (0.5-1.5 mg/ml) were suspended in Eagle's medium with the addition of 0.01 M HEPES, pH 7.2, and incubated (0.5 ml) in the presence of 2 μCi/ml of ^{3}H-5-HT (5-hydroxytryptamine creatine sulfate, 12 Ci/mmole, from Amersham Corporation, England) in siliconized tubes in an atmosphere with 5% CO_2 at 37°C for 30 min. The cells and synaptosomes were then washed in the same tubes four times with 10 ml of 0.85% NaCl with 1 mM Tris-HCl, pH 7.2, and transferred into flasks with dioxane scintillator. The quantity of bound radioactivity was determined on a Mark III counter (Nuclear Chicago, USA), with a counting efficiency of 60%.

Intact mice were immunized by intravenous injection of sheep's red blood cells (SRBC, 10^7) in 0.4 ml of 0.85% NaCl. Control animals received an injection of 0.4 ml of 0.85% NaCl. Imipramine (10^{-6} M) was added to the cell suspension immediately before addition of ^{3}H-5-HT to the culture medium. The dose of imipramine was chosen on the basis that, as the writers showed, an increase in its concentration from 10^{-6} to 10^{-4} M reduced the degree of 5-HT binding by the test cells only negligibly [4]. The concentration of the substance used was thus minimal, to give near-maximal inhibition of 5-HT binding.

The results were subjected to statistical analysis by Student's t test. Arithmetic mean values (of four determinations) with 95% confidence limits are given in Figs. 1 and 2. The difference between total and imipramine-resistant binding of ^{3}H-5-HT was attributable to the imipramine-sensitive component.

EXPERIMENTAL RESULTS

Data obtained during culture of suspensions of cells and synaptosomes with ^{3}H-5-T and when its passage through plasma membranes was inhibited by imipramine, are given in Figs. 1 and 2. Choice of imipramine for this purpose for the cells to be studied was based on preliminary experiments, in which imipramine-sensitive binding was sharply inhibited by ouabain and dinitrophenol, which are known to inhibit active transport

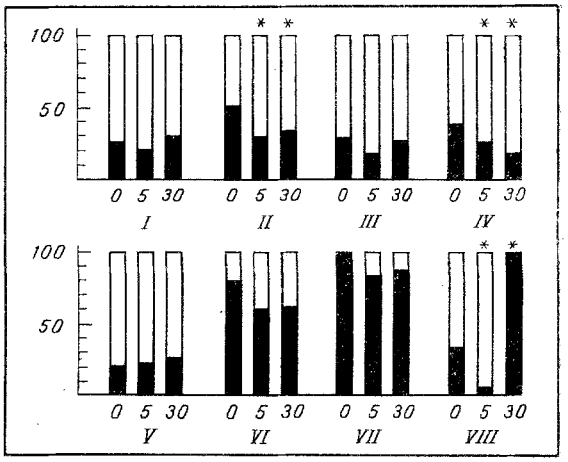

Fig. 2. Relative percentages of imipramine-sensitive and imipramine-resistant binding of ^{3}H-5-HT. Abscissa, time after immunization (in min); ordinate, percentage of total binding. Black columns – imipramine-resistant binding, white columns – imipramine-sensitive binding by synaptosomes (I), by blood leukocytes (II), and by cells of peritoneal exudate (III), bone marrow (IV), spleen (V), thymus (VI), Peyer's patches (VII), and lymph nodes (VIII). *P < 0.05 compared with cells of nonimmune mice.

of materials into the cell. Comparison of imipramine-sensitive (intracellular) and imipramine-resistant (surface) binding of the ligand in nonimmune animals revealed considerable differences in specific activity (Fig. 1) of imipramine-sensitive and imipramine-resistant binding, depending on the type of suspension studied. For instance, maximal specific imipramine-resistant binding was a feature of synaptosomes and blood leukocytes, but minimal binding of this type was found with lymph node cells; maximal imipramine-sensitive binding was associated with synaptosomes, a high level with blood leukocytes and peritoneal cells, and absence of imipramine-sensitive binding was found in cells of Peyer's patches. As regards the ratio between these two forms of binding (Fig. 2), the distribution of the label in blood leukocytes was uniform, in thymocytes and cells from Peyer's patches binding was predominantly imipramine-resistant, and in the remaining suspensions 61-79% of the label was accounted for by imipramine-sensitive binding. After injection of the antigen both the specific activity of the two forms of binding and the ratio between surface and intracellular ligand changed.

Imipramine-sensitive binding increased in suspensions with high and average activity immediately after immunization (after 30 min in the suspension of synaptosomes). Imipramine-resistant binding changes less uniformly: It decreased in the suspension of synaptosomes 5 min after injection of the antigen, a small decrease was observed in bone marrow cells until 30 min, and in other cases it increased. As regards suspensions with weak adsorption activity a certain trend could be detected in 5-HT binding after immunization in thymus and lymph node cells, whereas cells of Peyer's patches did not react under these conditions (Fig. 1). Despite significant changes in the levels of intracellular and surface binding of 5-HT, the ratio between them was changed as a result of injection of the antigen only in the case of leukocytes and bone marrow and lymph node cells (Fig. 2). The disturbance of proportion and absence of a parallel in the changes in imipramine-sensitive and imipramine-resistant binding were most probably due to heterogeneity of the 5-HT-binding cells.

The increased intensity of 5-HT binding after immunization in some organs may be the result of an inflow of 5-HT binding cells into them on account of cellular migration induced by the antigen [14, 16]. However, since 5-HT-binding by cells of the immune system as a whole was increased, this phenomenon could also have depended on qualitative changes in the cells themselves. For instance, antigen-induced transformation of the cell membranes could be the cause of changes in capacity for interaction [17], or after immunization some cells became sensitive to 5-HT, as has been demonstrated for glucocorticoids [12]. On variation of the physiological conditions, moreover, the concentration of receptors in the target cells changes, and so also, evidently, does the sensitivity of the cells to the corresponding ligand [1]. The increase in the ability of immunocytes and synaptosomes to bind 5-HT in response to immunization may also be linked with a fall in its level in the blood and brain in the early stage of immunogenesis [8]. Dependence of the binding capacity of the disclosed target cells on the extracellular 5-HT level, which we postulate, is confirmed by reduction of its

adsorption by spleen and peritoneal exudate cells and by synaptosomes 2 min after immunization [5], which coincides with release of 5-HT into the blood stream immediately after arrival of the antigen there [6], whereas the increase in extracellular concentration of 5-HT may in turn reduce the number of binding sites [13] or may lead to competitive inhibition of binding of the labeled ligand. The results are evidence of a functional association of both surface and intracellular 5-HT with the immune process. The surface fraction of the amine is perhaps essential for more rapid action on the cell, whereas the intracellular fraction is essential for slower action, of the polypeptide hormone type [13]. Considering the important role of the inner surface of the plasma membrane in the control of lymphocyte behavior [9] and the more intensive penetration of 5-HT into the lymphocytes immediately after antigenic activation (Fig. 1), by analogy with certain hormones [1, 7, 10] we can postulate that 5-HT penetrates into the cells we are studying, not for degradation, but for action.

The role played by the changes revealed in imipramine-sensitive and imipramine-resistant 5-HT binding by the test suspensions is not yet clear. However, it is evident that they are caused by injection of the antigen, and are thus evidence in support of interdependence between the response of immunocompetent tissues to immunization and the process of adsorption of 5-HT by the cells of these tissues and by synaptosomes. Differences in the level of binding, its character, its direction, and the rate of changes in response to the antigenic stimulus depend, it must be considered, on the multistage character of the immune response and the heterogeneity of the immunocytes, and they are evidence of the complexity of the effector mechanism of serotoninenergic immunomodulation.

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