EFFECT OF STIMULATION OF DOPAMINE AND HISTAMINE RECEPTORS ON SPONTANEOUS LYMPHOCYTE ADHESION IN VITRO

D. D. Kharkevich

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Advances in immunopharmacology in recent years have necessitated further research into the mechanisms of regulation of immunity, connected in particular with the system of biogenic amines [1]. There is evidence [1, 5, 7] that dopamine and histamine act on several parameters of the immune system, but the effect of these biogenic amines on spontaneous lymphocyte adhesion has not hitherto been studied.

The aim of this investigation was to study the effect of dopamine and histamine on spontaneous adhesion of human lymphocytes in vitro, with the aid of specific blockers: of dopamine receptors — haloperidol, of H_1 receptors — diphenhydramine, and H_2 receptors — cimetidine.

EXPERIMENTAL METHOD

The same method as the writer used previously to study inhibition of lymphocyte adhesion was used on this occasion to isolate the cells and to study spontaneous adhesion of peripheral blood lymphocytes from healthy blood donors, but without addition of the specific antigen [2, 3, 6].

Into the wells of No. 3040 96-well plastic plates (Falcon, USA) were poured 0.1 ml of lymphocyte suspension from healthy blood donors (in a concentration of $2 \cdot 10^6$ cells/ml), 0.05 ml of medium 199 with 20% embryonic calf serum (Gibco, England), inactivated by heating to 56°C for 30 min, 0.05 ml of a solution of the preparation in the concentration to be tested, and 0.05 ml of the corresponding antagonist (medium 199 in the control) The plates were incubated (37°C, 5% CO_2) in a humid chamber for 1.5 h. They were then accurately inverted [4] and incubated in the horizontal inverted position for a further 30 min. In that way nonadherent cells were separated from those adherent to the bottom of the wells. Nonadherent cells were then transferred from the wells by means of an automatic micropipet into test tubes containing 3% acetic acid solution and counted in a Goryaev's chamber. The reaction was evaluated by the usual formula for studying inhibition of lymphocyte adhesion with the aid of the index of inhibition of lymphocyte adhesion (ILA):

$$\text{IILA} = \frac{a - b}{b} \cdot 100\%,$$

where a is the number of nonadherent cells in the experimental samples, and b the number of nonadherent cells in the control samples. A negative value of IILA corresponds to stimulation of lymphocyte adhesion.

EXPERIMENTAL RESULTS

Dependence of spontaneous adhesion of human lymphocytes on the molar concentration of dopamine in vitro is shown in Fig. 1. Dopamine potentiates the adhesive properties of lymphocytes; the greatest stimulating effect of dopamine is observed, moreover, in concentrations of 10^{-4}-10^{-6} M. The absence of effect of dopamine on spontaneous lymphocyte adhesion in low concentrations (10^{-8} M) is evidently due to the fact that lymphocytes have a threshold of sensitivity to that substance, whereas diminution of the adhesive properties with an increase

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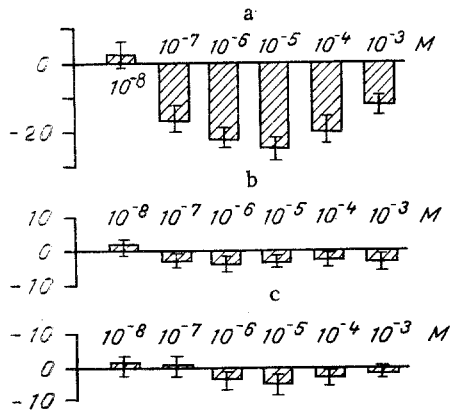

Fig. 1

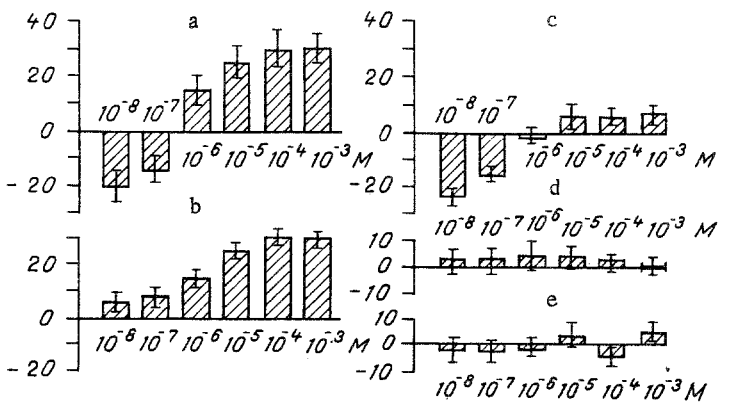

Fig. 2

Fig. 1. Dependence of spontaneous lymphocyte adhesion on concentration of dopamine (a), dopamine and haloperidol in equal concentrations (b), and haloperidol alone (c). Here and in Fig. 2: abscissa, molar concentrations of substances; ordinate, IILA (in %).

Fig. 2. Dependence of spontaneous lymphocyte adhesion on concentration of histamine (a), histamine and diphenhydramine in equal concentrations (b), histamine and cimetidine in equal concentrations (c), and diphenhydramine (d) and cimetidine (e) alone.

in concentration from 10^{-5} M to 10^{-3} M indicates that an opposite, inhibitory action of high concentrations of dopamine can develop if physiological concentrations are exceeded.

To study the pharmacological specificity of dopaminergic intensification of spontaneous lymphocyte adhesion discovered in this way, haloperidol, a blocker of dopamine receptors, was added simultaneously with equal concentrations of dopamine to the cultural wells. It will be clear from Fig. 1 that haloperidol abolished the stimulating effect of dopamine in all concentrations studied; addition of haloperidol, moreover, gave rise to no other effects. Incidentally haloperidol, when added without dopamine did not affect spontaneous lymphocyte adhesion (Fig. 1). The dopaminergic intensification of spontaneous lymphocyte adhesion is thus due to the stimulating action of the compound on dopamine receptors.

Dependence of spontaneous lymphocyte adhesion of the molar concentrations of histamine in vitro is illustrated in Fig. 2. Depending on the concentrations used, histamine may have opposite effects on lymphocyte adhesion: in low concentrations (10^{-8}-10^{-7} M) it has a stimulating action, in higher concentrations an inhibitory action on the adhesive properties of lymphocytes. Investigation of the effect of histamine in a concentration of 10^{-6}-10^{-7} M in some cases showed the total absence of an effect of the drug on spontaneous adhesion.

To study the pharmacological specificity of action of histamine, diphenhydramine, a blocker of H_1 receptors, and cimetidine, a blocker of H_2 receptors, were added simultaneously with histamine to the cultural wells. It was found that diphenhydramine, used in concentrations equal to those of histamine, abolished only the intensification of spontaneous lymphocyte adhesion, whereas inhibition persisted at the previous level. Addition of cimetidine, on the other hand, abolished the inhibition of lymphocyte adhesion, whereas the stimulating effect remained at its previous level (Fig. 2).

Data on the effect of cimetidine and diphenhydramine in cultures without histamine on adhesion also are given in Fig. 2. The histamine receptor blockers did not act, in the concentration range studied (10^{-8}-10^{-3} M), on spontaneous lymphocyte adhesion. Consequently, the intensifying effect of histamine in low concentrations on spontaneous lymphocyte adhesion is due to stimulation of H_1 receptors, for it was abolished by diphenhydramine but not by cimetidine, whereas the inhibitory action of the compound in higher concentrations is connected with predominant excitation of H_2 receptors, for it was completely abolished by cimetidine but not by diphenhydramine.

The data showing intensification of spontaneous lymphocyte adhesion by dopamine are in agreement with information in the literature on the stimulating effect of the dopaminergic system on different components of the immune response: stimulation of lymphocyte migration

into the spleen and an increase in the number of helper cells in it, an increase in the number of helper cells in the bone marrow, activation of amplifiers [1], and increased activity of T cells and macrophages [5]. Histamine, on the other hand, has a suppressive action on many immunologic phenomena, including inhibition of lymphocyte proliferation in response to stimulation by an antigen or mitogen, antibody formation, and lymphocytotoxicity, and it depresses cutaneous delayed-type hypersensitivity, release of lymphokines, T-helper cell generation, and effector functions, followed by a decrease in polyclonal activation of B cells [5, 7]. It has been suggested [8] that the suppressor action of histamine is due to stimulation of H_2 receptors on the surface of the lymphocytes and it is mediated by a raised intracellular cAMP level. The possibility that manifestation of the opposite effects of histamine may depend on its concentrations in vitro has been described [8] in the lymphocyte blast transformation reaction.

The results confirm data in the literature on the possible effect of the biogenic amines system on immunity and they indicate that the monoaminergic system contains components capable of exerting opposite effects (both stimulating and inhibitory) on lymphocyte function.

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CHANGES IN FUNCTION AND KINETICS OF MACROPHAGES AND LYMPHOCYTES CAUSED BY RETINOIC ACID

V. I. Nozdrin, V. G. Kvachev, and T. I. Galenko

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Along with retinol esters, when it enters the human or animal body in high doses, retinoic acid (RA) has a positive immunomodulating action [4, 6, 7]. It has been suggested that, when present in the blood unbound with transport protein, RA causes damage to the blood cells and, in particular, erythrocytes. Subsequent stimulation of the immune response immediately after such an event develops like the response to antigenic conversion of erythrocyte membrane proteins [2]. On experimental testing of this hypothesis several features of erythrocyte damage, increased phagocytosis of altered erythrocytes by macrophages, and activation of interaction between macrophages and lymphocytes were found [1, 3]. However, observations in vivo give only indirect evidence of the mechanisms of the immunomodulating action of RA. Experiments in vitro in this respect have several advantages, relating primarily to the possibility of studying the effect of RA on the composition and functions of individual populations and types of immunocompetent cells.

The aim of this investigation was to study the action of RA in vitro on quantitative parameters and function of human macrophages and T and B lymphocytes.

Department of Histology, Cytology, and Embryology, I. M. Sechenov First Moscow Medical Institute. Ukrainian Veterinary Research Institute, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.)

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