EFFECT OF ADRENORECEPTOR STIMULATION ON SPONTANEOUS LYMPHOCYTE ADHESION IN VITRO

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Great importance has been attached in recent years to the study of the mechanisms of interaction between the immune and neuroendocrine systems. Experimental investigations have shown that hromones and neurotransmitters regulate the functions of the lymphoid system, and that the immune response in turn causes changes in blood hormone levels and affects electrical activity of neurons in the hypothalamus [4, 9]. Lymphocytes have been shown to express on their surface receptors to various neurotransmitters and, in particular, adrenoreceptors, which can influence blast transformation and proliferation, rosette formation, antibody production, cytotoxicity, and intracellular cyclic nucleotide levels [5, 7]. One test which can be used to characterize lymphocyte function is spontaneous lymphocyte adhesion, but the effect of adrenoreceptor stimulation on spontaneous lymphocyte adhesion has not been previously studied in vitro.

In the investigation described below the effect of adrenalin and noradrenalin, agonists of α- and β-adrenoreceptors, the α-agonist phenylephrine hydrochloride, and the β-agonist isoproterenol was investigated in conjunction with the α-adrenoblocker phentolamine and the β-adrenoblocker propranolol in vitro.

EXPERIMENTAL METHOD

Cells were isolated and spontaneous adhesion of peripheral blood lymphocytes from healthy blood donors was studied by the method developed by the writer previously to study inhibition of lymphocyte adhesion, but without the addition of the specific antigen [1, 2].

Into wells of 96-well plastic panels (No. 3040, Falcon Plastics, USA) 0.1 ml of a suspension of lymphocytes from healthy donors in a concentration of 2×10^6/ml in medium 199 with 20% embryonic calf serum, inactivated by heating to 56°C for 30 min, 0.05 ml of a solution of the drug and, if necessary, 0.15 ml of a solution of the antagonist, were introduced. The panels were incubated (37°C, 5% CO_2) in a humid chamber for 1.5 h. Nonadherent cells were then removed and counted as described previously [1, 3]. The reaction was assessed by the usual formula for studying inhibition of lymphocyte adhesion, by determining the lymphocyte adhesion inhibition (LAI) index:

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

where a is the number of nonadherent cells in the experimental samples and b the number of nonadherent cells in the control samples. Negative values of the LAI index correspond to stimulation of lymphocyte adhesion.

EXPERIMENTAL RESULTS

As Fig. 1 shows, addition of adrenalin to the test system caused no change in spontaneous lymphocyte adhesion over the range of concentrations studied. However, addition of adrenalin together with blockers of α- and β-adrenoreceptors induced marked changes in lymphocyte adhesiveness: a combination of adrenalin with phentolamine inhibited, whereas a combination of adrenalin with propranolol stimulated spontaneous lymphocyte adhesion (Fig. 1). On addition

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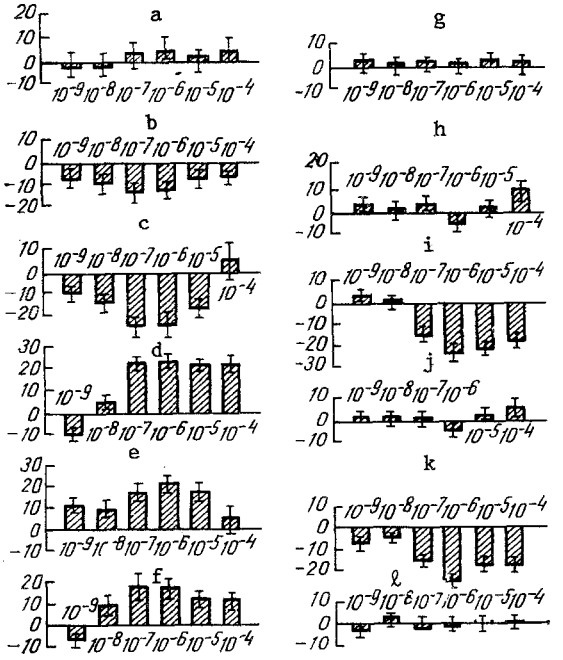

Fig. 1. Effect of various drugs on spontaneous lymphocyte adhesion. a) Adrenalin, b) noradrenalin, c) phenylephrine, d) isoproterenol, e) adrenalin + phentolamine, f) noradrenalin + phentolamine, g) phenylephrine + phentolamine, h) phentolamine, i) adrenalin + propanolol, j) isoproterenol + propranolol, k) phenylephrine + propranolol, ℓ) propranolol. Abscissa, concentration of drugs tested (in M); vertically, LAI index (in %; M ± m).

of noradrenalin to the culture, weak stimulation of lymphocyte adhesion was observed, but when noradrenalin was added in addition to the α-adrenoblocker phentolamine, lymphocyte adhesion was inhibited (Fig. 1). Stimulation of α-adrenoreceptors by phenylephrine led to significant stimulation, whereas stimulation of β-adrenoreceptors by isoproterenol caused inhibition of lymphocyte adhesion. Optimal concentrations of the drugs for the adrenergic effects were 10^{-7}-10^{-5} M. The α-adrenergic stimulation of spontaneous lymphocyte adhesion which was observed was pharmacologically specific, for it was completely abolished by phentolamine, but not by propranolol (Fig. 1). Addition of propranolol completely abolished effects induced by isoproterenol. This indicates that β-adrenergic inhibition of lymphocyte adhesion is pharmacologically specific and due to action on β-adrenoreceptors. The use of the adrenoblockers propranolol and phentolamine separately (without the corresponding agonists), incidentally, had no effect on spontaneous lymphocyte adhesion (Fig. 1).

Specific stimulation of lymphocyte α-adrenoreceptors thus enhances, whereas stimulation of β-adrenoreceptors inhibits spontaneous lymphocyte adhesion. The absence of any effect of adrenalin in the system described above is evidently due to its simultaneous action on α- and β-adrenoreceptors, stimulation of which has the opposite action on spontaneous lymphocyte adhesion.

The opposite effects of α- and β-adrenergic stimulation also have been described in other systems. For instance, IgE-mediated release of histamine and of the slowly reacting substance of anaphylaxis in the human lung is enhanced by α-adrenergic stimulation, whereas β-adrenergic stimulation inhibits this process [10]. Investigation of the effect of adrenergic drugs on plaque formation in the mouse spleen showed that the β-agonist isoproterenol gave a strong inhibitory effect, unlike the α-agonist phenylephrine [7]. Experiments in vitro showed that whereas stimulation of α-adrenoreceptors potentiates DNA synthesis in lymphocytes treated with PHA, β-adrenergic agents have an inhibitory action [8].

The results relating sensitivity of cells of the immune system to mediators of the nervous system characterize a special level of immunoregulation which is linked with neuroimmune interaction and they confirm recent data on intersystemic regulation in the body [6].

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EFFECT OF PSYCHOTROPIC DRUGS OF THE PHENOTHIAZINE SERIES ON THYMOCYTE DNA TEMPLATE ACTIVITY AND GLUCOCORTICOID-RECEPTOR INTERACTION

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Specific cytoplasmic glucocorticoid receptors, participating in the mechanism of the effect of glucocorticoids at the level of the genetic apparatus of the cell have been found in various organs and tissues of man and animals [6, 7]. Glucocorticoid receptors, like adrenoreceptors [1], are evidently targets for pharmacological intervention [8].

Many effects of drugs of the phenothiazine series, such as anti-inflammatory [4], antihistamine [9], antiserotonin [5], and inhibiting cell metabolism [3, 7] are characteristic of both natural and synthetic glucocorticoids.

It was accordingly decided to study the effect of chlorpromazine and tisercin on thymocyte DNA template activity and glucocorticoid-receptor interaction.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 80-100 g adrenalectomized 7 days before the experiments began. The effect of chlorpromazine and tizercine on thymocyte DNA template activity was evaluated on the basis of their ability to reduce incorporation of ^{3}H-uridine into the acid-insoluble fraction of thymocyte mRNA.

The antiglucocorticoid effect of chlorpromazine and tisercin was judged from the degree of weakening of the inhibitory action of triamcinolone acetonide (10^{-8} M) on incorporation of ^{3}H-uridine into the acid-insoluble fraction of thymocyte mRNA [8]. For this purppse a suspension of thymocytes (2×10^6 cells in 1 ml) was prepared in medium No. 199. The next stages of the investigation followed the description of the method in [6]. Chlorpromazine and tisercin were used in concentrations of 3.5×10^{-7}, 3.5×10^{-6}, and 3.5×10^{-5} M. To determine the

*Levomepromazine

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